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Gibberellic Acid Inhibits Floral Formation and Delays Flower Differentiation in '0900 Ziraat' Sweet Cherry Cultivar

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Summary

This paper reports on the effects of gibberellic acid (GA_3) on floral formation and the potential for GA_3 to reduce flower primordia formation in sweet cherry (*Prunus avium* L.). GA_3 was applied to 4-year-old '0900 Ziraat'/'Gisela 5' trees at two growth stages (early flowering and beginning of fruit development) at the doses of 0, 25, 50, 100, and 250 mg L^{-1}, for a 2-year period. Bud samples were taken from the beginning of July to the end of September in both years in six sequential dates. During the dormant seasons, buds were collected to determine number of flowers per bud. Samples were fixed and stored in a solution of formalin, 70 % ethanol and glacial acetic acid (10:50:5, v/v). Flower formation in the buds was examined using a stereo zoom microscope and the morphological changes in each sample were recorded using a camera mounted on the microscope. Number of flowers per bud was negatively and linearly related to GA_3 concentration. Floral formation in '0900 Ziraat' sweet cherry (untreated control) started in early-July and continued until mid-September. The progress during which the sepal, petal, stamen and pistil primordia were differentiated was considerably slower at GA_3 applications. Imperfection of the floral buds was observed as the floral organs being replaced by leaf-like appendages or as none of the flower buds having one normally-developed flower primordia or as floral organs or flower primordia being much smaller than the other.

Key words. bud – flower primordial – GA_3 – *Prunus avium*

Introduction

Increasing demand by the world markets for high fruit quality has brought about some unavoidable applications in orchards. Orchard growers apply GA_3, possibly the most widely used plant growth regulator, to fruit trees in order to increase fruit weight and firmness, to prolong storage life, to prevent physiological disorders and fruit softening (Looney 1983; Buban et al. 1993). Horvitz et al. (2003) and Cline and Trought (2007) reported that the application of GA_3 sprays on the sweet cherry cultivars 'Napoleon', 'Sweetheart' and 'Bing' increased fruit firmness and weight. Effects of GA_3 were also evaluated in 'Van' cv. in reducing the risk of crop loss by making fruit more resistant to physiological disorders such as deep suture, fruit cracking and double fruit (Engin et al. 2009).

Multiple applications of gibberellic acid at early flowering and at the beginning of fruit development are currently accepted as a standard application among sweet cherry growers worldwide for improving fruit set and quality. On the other hand, it is known that gibberellins inhibit floral bud development in some species and significantly decrease number of flowers per tree the following season (Southwick and Yeager 1991; Gonzalez-Rossia et al. 2007). This effect might cause an additional lost in cherry cultivars, especially in '0900 Ziraat', an inherently lower yielding cultivar with excellent fruit quality.

Timing of GA_3 application is important since the processes of bud development can be affected during a limited period each year. Engin and Ünal (2007) reported that flower bud formation in cv. '0900 Ziraat' starts at the beginning of July, during which gibberellin applications are mostly performed in orchards. GA_3 application during this period might be the cause of variation in the progression of flower differentiation. Therefore it is imperative to pinpoint the period of floral induction and differentiation for each cultivar (Southwick and Glozer 2000).

The objective of this research was to evaluate the potential effects of different GA_3 concentrations on reducing flower numbers per bud the following season and on progression of flower differentiation in '0900 Ziraat' sweet cherry.

Materials and Methods

Plant material

'0900 Ziraat' sweet cherry (*Prunus avium* L.) grafted on 'Gisela 5' (*P. cerasus* 'Schattenmorelle' × *P. canescens*), a common rootstock for new sweet cherry orchard in Turkey, was used. '0900 Ziraat' is a late blooming and cultivar with high quality big fruits (medium weight 9.5 g). It has low productivity. Four-year-old trees were contained in 25 pots filled with a granite soil: bark compost (2:1, v/v) and grown under field conditions at the experimental field of Çanakkale Onsekiz Mart University, Turkey. Standard orchard management strategies (pruning and pesticide application) were carried out every year.

Gibberellic acid (GA_3) prepared separately in a tank was sprayed on the trees with a small sprayer at two growth stages (early flowering and beginning of fruit development) at the doses of 0, 25, 50, 100, 250 mg L^{-1} respectively, for a 2-year period. The trees were sprayed to run off, using an average of 1.2 ± 0.22 L of solution per tree. Pure water was sprayed on the control.

Twenty-five buds uniform in size and vigor were collected every ten days from the trees from the beginning of July to the end of September in both years. The buds were dissected by removing each bract or leaf using a scalpel under a stereo zoom microscope (SZ61, Olympus, Southend-on-Sea, Essex, UK). Each axillary structure within each bud was examined and identified. Collections were terminated when more than 50 % of the dissected buds had their pistils initiated. The morphological changes in flower primordia using a camera mounted on the microscope (Olympus C7070) were observed and the developmental stage of the flower buds was classified as described by Engin and Ünal (2007) for sweet cherry. For each collecting time, the percentage of flowers at a distinct stage was registered. The number of buds observed varied depending on availability and necrotic or aborted flowers were neglected.

During the dormant season in the following December, twenty buds per tree, similar in diameter, were collected for determination of the number of flowers per bud.

The samples were stored and fixed in a mixture of formalin, 70 % ethanol and glacial acetic acid (10:50:5, v/v) (McLaughlin and Greene 1991).

Statistics

The experiment was designed as randomized parcels in five replications with 5 trees in each. Data for the number of flowers per bud were evaluated with Minitab (Release 13.1, Minitab Inc.) statistical package program and significance between the means was determined by Duncan's multiple range tests. Effects of GA_3 applications in each stage during the progression of flower bud development were tested with z-proportion test after data normalization. The number of observations was 25.

Results

The progression of flower bud formation in '0900 Ziraat' sweet cherry was revealed by the use of stereomicroscope. The first sign of floral initiation occurred when the meristematic apex of the bud had a dome-like shape. After that, floral plateau developed in the order of flower primordia, sepal, petal, stamen and pistil, and these organs obtained their normal morphology by mid-September.

Development of flower bud was impaired when exposed to GA_3 applications. It was more pronounced in higher GA_3 applications. In a normally developed flower bud, pistil primordia initiated within the floral cup (Fig. 1a) in which they could be seen with removed sepals and petals. Our observations in the bud sample showed that some of the flowers were still at stage of sepal-petal differentiation (Fig. 1b). In the extremest form, the floral organs involved were replaced by leaf-like appendages. In some buds, this was so high that none contained one normal-developed flower (Fig. 1c), suggesting that the buds during sepal-petal formation were affected by GA_3 and floral organs had not been formed, but they reverted back to the sepal- or petal-like appendages. In the control buds, all flowers had equally sized and developed organs. In the samples collected at the end of August, the flowers were unequally developed and some flowers were much smaller than the other when exposed to GA_3 applications (Fig. 1d).

Effects of different GA_3 treatments on the development of the floral organs in 2008 and 2009 vegetative season were shown in Table 1 and 2, respectively. Early signs of stamen primordia were already visible on the trees by July 24th in 2008 (Table 1). In the samples were obtained at the beginning of August, 50 % of the flower buds in the control were already in the stage of stamen primordia. More than 37 % of the flower buds contained petal primordia. All the applications, except for the highest GA_3 concentration, had around 20 stamen primordia formed. On August, 14th in 2008, clear differences were observed between the lowest gibberellin concentration and the higher ones during the stamen primordia development. It seemed that the percentage of flowers with pistil primordia started was not statistically significant between the control and 25 mg L^{-1} treatment. As the time progressed, the effects of the gibberellin applications were significant, generally lagging behind the control flowers. On Sept.4, the amount of flowers with pistil primordia in the 100 mg L^{-1} treatment was 8 to 10 times lower than in the lower concentrations. By the end of the experiment in 2008, it was evident that control trees had their flowers complete with all the floral parts, while GA_3 treated buds, on the other hand, had still contained fewer buds with incomplete organs in the decreasing order with the increasing application doses.

In 2009, there were some retarding effects of gibberellin on floral organ development compared to those in

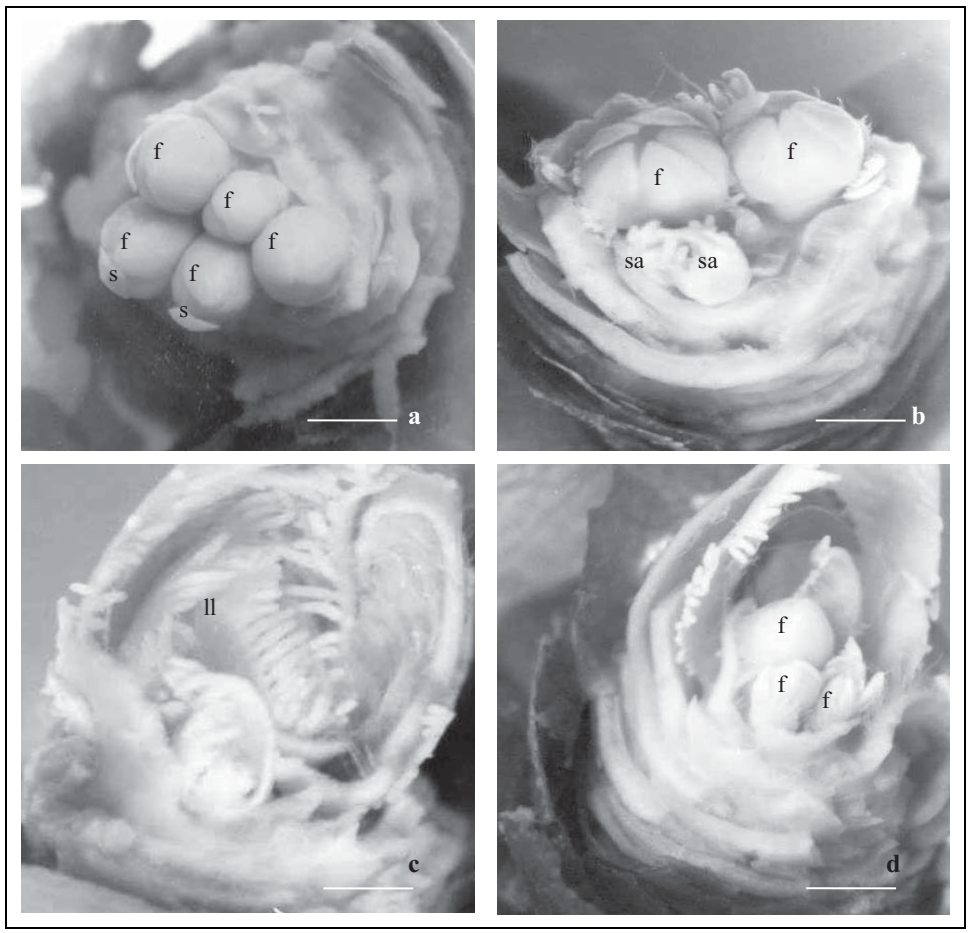


Fig. 1. Micrographs of '0900 Ziraat' sweet cherry buds, showing developmental changes. Bar = 100 µm. a. All floral organs differentiated, including sepal (s), petal, stamens and pistil. Stamens and pistil are completely covered by the sepals. Inflorescences containing five flowers (f). Sampled Aug. 28th, 2009; b. Inflorescences containing two developed flowers, sepal-like appendages (sa). Sampled Aug. 28th, 2009; c. Bud had none of normally developed flower. Floral organs replaced by leaf-like (ll) appendages. Sampled Sep.14th, 2009; d. Inflorescences containing three unequally developed flowers, two flowers much smaller than the other. Sampled Aug. 24th, 2009.

2008 (Table 2). For instance, in 2008, some of the flowers had differentiated into petal primordia. However, in 2009, only one-fourth or fewer of the flowers had petal primordia in the control and 25 mg L^{-1} applications while the higher concentrations did not have any. Stamen primordia were not visible either in the GA_3 treatments. As the time advanced until August, 28th in 2009 gibberellin treatments other than 25 mg L^{-1} did not contain any pistil primordia. Towards the end of August, there were some acceleration in the progression and 2–8 % of the flowers exposed to higher GA_3 concentrations (50 and 100 mgL^{-1}) started to develop pistils. It was not until the mid-September that the highest dose of GA_3 had flowers with pistil primordia formed. There were clear and significant differences between the lower half of the GA_3 doses and the higher doses at the end of the sampling date. The highest dose of GA_3 caused a remarkable delay in the pistil formation compared to the control and other treatments. By the time the other applications had their pistils almost fully developed in the flower buds, the highest dose resulted in little more than 50 % pistil formation.

Effect of gibberellic acid applications on the flower number per bud was examined in the dormant buds the following dormant seasons of 2008 and 2009 (Table 3). Statistical analysis showed that flower number per bud was under the influence of both season and the treatment. In both seasons, the increasing dose of GA_3 caused the number of flowers per bud to decrease compared with the control buds, except for the 25 mg L^{-1} in the first season. Reduction in the number was 8–9 % in the 25–50 mg L^{-1} and 40–53 % in the 100–250 mg L^{-1}, respectively compared to the control. In the second year, there were differences between the control and 25 mg L^{-1}. The 50–100 mgL^{-1} applications resulted in the similar decreases. The reduction compared to the control was at a similar range (29–48 %). Differences between the seasons showed that the control trees had 8.9 % less flowers per bud while the greatest decrease was observed in the trees treated with 50 mg L^{-1} GA_3. The trees that received 100 mg L^{-1} GA_3 had 7.2 % fewer flowers in the buds. Applying 250 mg L^{-1} GA_3 did not resulted in fewer buds between the seasons, but very slight increase occurred.

Discussion

In this study, flower buds induction and initiation started in early-July and was completed around mid-September. Flower induction, initiation and differentiation in sweet cherries starts, under growing conditions of Turkey, from the end of June to mid-September depending upon the

Table 1. Effect of GA_3 applications on the progression of flower bud formation in '0900 Ziraat' sweet cherry cultivar (2008).

Sampling date	GA_3 treatments (mg L^{-1}).	Flowers (%) differentiating into				
		Flower primordia	Sepal primordia	Petal primordia	Stamen primordia	Pistil primordia
July 24	Control	1.8 a	26.9 a	61.8 a	9.5 a	–
	25	11.7 a	24.9 a	50.3 a	13.1 a	–
	50	7.3 a	32.4 a	47.3 a	13.0 a	–
	100	11.4 a	35.8 a	37.7 a	15.1 a	–
	250	15.2 a	33.2 a	51.6 a	–	–
August 4	Control	–	2.7 b	44.4 a	50.1 a	2.8
	25	10.8 a	19.9 ab	49.3 a	20.0 b	–
	50	8.9 a	19.5 ab	52.3 a	19.3 b	–
	100	13.6 a	39.7 a	26.6 a	20.1 b	–
	250	10.3 a	39.8 a	49.9 a	–	–
August 14	Control	–	–	24.3 ab	61.2 a	14.5 a
	25	–	17.6 b	18.6 b	61.5 a	2.3 a
	50	3.3 a	40.2 ab	36.5 ab	20.0 b	–
	100	5.7 a	34.9 ab	36.2 ab	23.2 b	–
	250	10.1 a	43.2 a	46.7 a	–	–
August 24	Control	–	–	8.5 b	34.2 b c	57.3 a
	25	–	–	4.2 b	77.3 a	18.5 b
	50	–	7.9 b	47.8 a	37.4 b	6.9 b
	100	–	35.1 a	52.6 a	12.3 c	–
	250	–	47.2 a	52.8 a	–	–
September 4	Control	–	–	–	2.3 c	97.7 a
	25	–	–	–	39.4 b	60.6 b
	50	–	–	–	45.2 b	54.8 b
	100	–	7.5 a	11.1 a	74.9 a	6.5 c
	250	–	12.3 a	22.8 a	64.9 ab	–
September 14	Control	–	–	–	–	100.0 a
	25	–	–	–	3.3 b	96.7 ab
	50	–	–	–	7.4 ab	92.6 ab
	100	–	–	4.1 a	14.1 ab	81.8 bc
	250	–	–	7.6 a	23.7 a	68.7 c

varieties (Engin and Ünal 2007). Sweet cherry flower initiation was reported to occur at the end of June in Japan (Watanabe 1983), mid-late July in central Washington (Guimond et al. 1998). Orchard management and cultural practices at this critical time can be optimized with the acknowledgement in order to favor floral development.

Our results clearly showed that GA_3 application markedly affected the progression of floral differentiation in the '0900 Ziraat' sweet cherry cultivar. Applications of GA_3 at 100 and 250 mg L^{-1} concentrations slowed down the development of flower buds. In addition, when GA_3 was re-applied at same dose the following year, flower bud initiation also was delayed. Inhibitory effects of applied gibberellins on floral initiation were reported for sweet cherry (Lenahan et al. 2006) and peach (Garcia-Pallas et al. 2001). The action of gibberellins in reducing floral bud potential is because transition into a floral state was obstructed before the inductive period (Bernier 1988). In grapes, gibberellins favor inflorescence axis formation and promote flowering. However, later on, it inhibits flowering because it leads to form tendrils (Vasconcelos et al. 2009). Increased shoot growth was also reported as the reason for inhibition of floral induction following GA_3 application (Wilkie et al. 2008).

Table 2. Effect of GA_3 applications on the progression of flower bud formation in '0900 Ziraat' sweet cherry cultivar (2009).

Sampling date	GA_3 treatments (mg L^{-1}).	Flowers (%) differentiating into				
		flower primordia	sepal primordia	petal primordia	stamen primordia	pistil primordia
July 29	Control	–	67.7 a	23.2 a	9.1	–
	25	9.8 bc	69.9 a	20.3 a	–	–
	50	25.6 b	57.8 a	16.6 a	–	–
	100	72.4 a	27.6 b	–	–	–
	250	81.9 a	18.1 b	–	–	–
August 8	Control	–	8.8 b	31.3 a	47.4 a	12.5
	25	–	39.4 a	41.5 a	19.1 b	–
	50	10.9 a	48.8 a	40.3 a	–	–
	100	12.7 a	42.2 a	45.1 a	–	–
	250	13.4 a	55.7 a	30.9 a	–	–
August 18	Control	–	–	20.1 b	64.3 a	15.6 a
	25	–	15.9 c	19.8 b	60.1 a	4.2 a
	50	4.5 a	47.1 ab	48.4 a	–	–
	100	10.5 a	71.2 a	18.3 b	–	–
	250	10.0 a	33.1 b	56.9 a	–	–
August 28	Control	–	–	2.5 c	14.3 b	83.2 a
	25	–	–	19.9 b	69.3 a	10.8 b
	50	–	23.4 a	37.8 ab	31.1 b	7.7 b
	100	–	38.1 a	48.9 a	11.3 b	1.7 b
	250	–	36.5 a	46.3 a	17.2 b	–
September 7	Control	–	–	–	8.5 c	91.5 a
	25	–	–	–	28.8 bc	71.2 ab
	50	–	–	5.5 a	37.2 b	57.3 b
	100	–	–	21.6 a	75.2 a	3.2 c
	250	–	4.7	21.1 a	74.2 a	–
September 17	Control	–	–	–	–	100.0 a
	25	–	–	–	2.2 b	97.8 a
	50	–	–	–	9.5 ab	90.5 ab
	100	–	–	8.6 a	16.7 ab	74.7 bc
	250	–	–	17.1 a	29.3 a	53.6 c

GA_3 application also resulted in a lower number of flowers per bud in the following dormant season. Similar to our results, Southwick et al. (1997) showed that flowering was reduced by 50 % in the 'Paterson' apricot. Facteau et al. (1989) reported that increasing dose of GA_3 (0–150 mg L^{-1}) diminished flowering in the 'Bing' and 'Lambert' sweet cherry cultivars. Webster and Goldwin (1981) indicated that GA_3 sprays often caused a reduction in the number of floral buds in the subsequent spring in the plum cv. 'Victoria'. However, Lenahan et al. (2006) reported no adverse effects of GA_3 on the flower number per reproductive bud in the 'Bing' cultivar. The overall reason the researchers stated for GA_3 reducing flowers per bud was that floral buds were impaired. In the present study we concluded that the action of GA_3 in impairing floral buds might be due to the facts that i) the floral organs involved were replaced by leaf-like appendages, to the some extent that none of flower buds contained single normally-developed flower primordia, ii) some of the flowers were still at stage of sepal-petal differentiation and the other floral organs had not been formed, and iii) flower primordia were unequally developed and some of them were much smaller than the other.

Table 3. Effect of GA_3 application in the dormant seasons of 2008 and 2009 on the number of flowers (flowers/bud) in '0900 Ziraat' sweet cherry.

Treatments (mg L^{-1})	2008		2009		Differences between the seasons (%)
	Flower/bud	Reduction[y] (%)	Flower/bud	Reduction (%)	
Control	3.77 Aa*		3.43 Aa		– 9
25	3.37 Aa	– 8,9	2.43 Bb	–29,2	–28
50	3.10 Ba	– 7,8	1.97 Cb	–42,6	–36
100	2.23 Ca	–40,9	2.07 Cb	–39,7	– 7
250	1.77 Da	–53,1	1.80 Da	–47,6	+ 2

* Means followed by the same capital letter within a column, and means followed by the same small letter in a row are not significantly different (n = 20, $P < 0.05$). [y] compared to the control.

Conclusion

Timing of GA_3 application is critical in relation to floral bud induction. Detailed information of the floral bud induction and initiation of the cultivars should enable GA_3 applications to be more precise and the effects to be within the expectations. However, since all the empirical evaluations are within the limitations of cultivar, site, rootstock and climate, the end results might not be applicable to other conditions. In order for gibberellin applications to be used in a crop load management in sweet cherry, more studies should be conducted.

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References

Bernier, G. 1988: The control of floral evocation and morphogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. **39**, 175–219.

Buban, T., C.R. Hampson, R.L. Anderson, R.L. Perry and A.D. Webster 1993: Using plant growth regulators to increase fruit set in sour cherry trees. Acta Hort. (ISHS) **410**, 307–310.

Cline, J.A. and M. Trought 2007: Effect of gibberellic acid on fruit cracking and quality of Bing and Sam sweet cherries. Can. J. Plant Sci. **87**, 545–550.

Engin, H., F. Şen, G. Pamuk and Z. Gökbayrak 2009: Investigation of physiological disorders and fruit quality of sweet cherry. Europ. J. Hort. Sci. **74**, 118–123.

Engin, H. and A. Ünal 2007: Examination of flower bud initiation and differentiation in sweet cherry and peach by using scanning electron microscope. Turk. J. Agric. For. **31**, 373–379.

Facteau, T.J., K.E. Rowe and N.E. Chestnut 1989: Flowering in sweet cherry in response to application of gibberellic acid. Sci. Hort. **38**, 239–245.

Garcia-Pallas, I., J. Val and A. Blanco 2001: The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA_3 as an alternative to hand thinning. Sci. Hort. **90**, 265–278.

Gonzalez-Rossia, D., C. Reig, M. Juan and M. Agusti 2007: Horticultural factors regulating effectiveness of GA_3 inhibiting flowering in peaches and nectarines (*Prunus persica* L. Batsch). Sci. Hort. **111**, 352–357.

Guimond, M.C., P.K. Andrews and G.A. Lang 1998: Scanning electron microscopy of floral initiation in sweet cherry. Amer. Soc. Hort. Sci. **123**, 509–512.

Horvitz, S., A.F. López Camelo, A. Yommi and C. Godoy 2003: Application of gibberellic acid to 'Sweetheart' sweet cherries: effects on fruit quality at harvest and during cold storage. Acta Hort. (ISHS) **628**, 311–316.

Lenahan, O.M., M.D. Whiting and D.C. Elfving 2006: Gibberellic acid inhibits floral bud induction and improves 'Bing' sweet cherry fruit quality. HortSci **41**, 654–659.

Looney, E.N. 1983: Growth regulator use in the production of *Prunus* species. In: Nickell, L.G. (ed.): Plant Growth Regulation Chemicals. CRC Press Inc. Boca Raton, Florida, 27–41.

McLaughlin, J.M. and D.W. Greene 1991: Fruit and hormones influence flowering of apple. I. Effect of cultivar. J. Amer. Soc. Hort. Sci. **116**, 446–449.

Southwick, S.M. and J.T. Yeager 1991: Effects of postharvest gibberellic acid sprays return bloom of 'Patterson' apricot. Acta Hort. (ISHS) **293**, 459–466.

Southwick, S.M. and K. Glozer 2000: Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. HortTech. **10**, 744–751.

Southwick, S.M., J.T. Yeager and K.G. Weis 1997: Use of gibberellins on 'Patterson' apricot (*Prunus armeniaca*) to reduce hand-thinning and improve fruit size and firmness: Effects over three seasons. J. Hort. Sci. **72**, 645–652.

Vasconcelos, M.C., M. Greven, C.S. Winefield, M.C.T. Trought and V. Raw 2009: The flowering process of *Vitis vinifera*: A review. Amer. J. Enol. Vitic. **60**, 411–434.

Watanabe, S. 1983: Scanning electron microscope observations of flower bud differentiation in sweet cherry (*Prunus avium* L.). J. Yamagata Agr. For. Soc. **77**, 15–18.

Webster, A.D. and G.K. Goldwin 1981: The hormonal requirements for improved fruit setting of plum, *Prunus domestica* L. cv. 'Victoria'. J. Hort. Sci. **56**, 27–40.

Wilkie, J.D., M. Sedgley and T. Olesen 2008: Regulation of floral initiation in horticultural trees. J. Exp. Bot. **59**, 3215–3228.

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