Effect of Putrescine, GA3, 2, 4-D, and Calcium on Extending Harvest Season of Navel Orange

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ABSTRACT

The present study was conducted in 2007/2008 and 2008/2009 seasons in order to extend harvest season and maintain fruit quality for better marketability of Washington navel oranges growing in clay soil by preharvest foliar sprays (at color break stage) with GA3, 2,4-D, putrescine and calcium either alone or in combinations. Trees were harvested at mid- February (late in the season). All sprayed substances delayed fruit softening, peel ageing and fruit color break and decreased creasing and preharvest fruit drop. Also, fruit TSS, sugars and V.C. contents were increased. The treatments had positive influence on extending harvest season without any deterioration in fruit characteristics. In general, spraying the different substances in combinations gave better results, especially for putrescine and 2,4-D.

INTRODUCTION

Oranges are of the worldwide popular fruits mostly consumed fresh. Orange fruit faces pre- or post-harvest a number of rind disorders such as creasing, splitting, puffing, peel pitting and senescence. Delaying peel senescence prolongs the fruits life, improves fruit quality and extends its marketing season, which enhances the crop value and contributes to growers returns. Thus, any manipulation of the final stage of fruit development in order to delay rind senescence improves fruit quality and extends its exporting season. Egypt's navel orange export season starts from mid-November to mid- February and navel oranges growers tend to expand harvest period by keeping the fruit for longer time on the trees in order to extend marketing season, for a high economic return. This procedure leads to the appearance of the previous mentioned disorders, and thus shortens its shelf life and marketing ability. Controlling of rind aging mainly rind softening in navel oranges is important to prolong the life of quality fresh orange fruit as long as possible after harvest.

Growth regulators application is one of several tools which when properly used enables citrus growers to extend marketing period with no loss of fruit quality (Ismail 1997). Gibberellic acid when applied pre-harvest retarded rind softening and fruit maturation (Coggins 1981, Ismail 1997). Creasing, splitting, puffing and peel pitting can be reduced in intensity or minimized using gibberellic acid and synthetic auxins or a mixture of both (Agusti et al. 2002). According to Chapman (1983), exogenous applications of GA3 at the citrus fruit color change stage will maintain the peel quality of late-harvested fruit and will reduce mesocarp cracking. In addition pre-harvest sprays of 2,4-D alone or with GA3 is proven effective in better peel quality of on tree-stored fruit, and reducing late season fruit drop therefore, extending the harvest season, as well as retarding rind senescence and lowering fruit decay (Golddchmidt and Eilati 1970, Ismail1997). Moreover, Tumminelli et al. (2005) noticed an increase in ethylene production in the albedo tissues of Satsuma mandarin with the ripening stage and it increases with peel aging. In the meantime, Zheng and Zhang (2004) reported a gradual decline in the concentrations of free polyamines in mandarin fruits after harvest that was parallel to peel senescence. Polyamines are group of natural compounds that are believed to have anti-senescence function by inhibition of the formation of enzymes essential to the synthesis of ethylene (Ke and Romani 1988) thus, retard ripening and extend fruit shelf life. They also improve fruit quality by reducing mechanical damage and increasing fruit firmness (Valero et al 1998a and b, Perez-Vicente et al. 2002). In addition, many researches focused on extending citrus fruit life by pre- or post-harvest treatments with calcium (Valero et al. 1998b, El-Hilali et al. 2004).

The present study was conducted to evaluate the effect of pre-harvest foliar sprays of GA3, 2, 4-D, putrescine and calcium on extending harvest season and maintain fruit quality for better marketability of Washington navel oranges without economical loss.

MATERIALS AND METHODS

Plant material and treatment

Thirty-five years old Washington navel orange trees (*Citrus sinensis*, L.), budded on sour orange rootstock were planted at 4.5x4.5 m apart in a private citrus orchard at El-Tarh region, EL- Behera Governorate. The soil was clay, well-drained with water table about 110 cm and pH 8. Trees were subjected to the same cultural practices usually done in the orchard. In January of both seasons, calcium superphosphate (15.5% P_2O_5) was added at the rate of 250 kg per feddan. Ammonium nitrate (33% N) was applied at the rate of 250 kg in

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March, 250 kg in May and 200 kg in August of both seasons per feddan. In August of both seasons, 100 kg per feddan potassium sulfate (48% K_2O_5) was added. Trees were irrigated with Nile water every 15-20 days.

Fifty five trees were selected as uniform as possible and were sprayed once at the beginning of fruit color break (about month and half before commercial harvest date) with 10 ppm GA3, 10 ppm 2, 4-D, 5 mmol PUT (Putrescine) and 1500 ppm chelated calcium either alone or in combinations. Treatments were arranged in a complete randomized design with five replicates (each replicate consisting of one tree) and the trees were treated with eleven foliage treatments (5 x 11 = 55 tree), water only, PUT, 2, 4-D, GA3, Ca, PUT + 2,4-D, PUT + GA3, PUT + Ca, 2,4-D + GA3, 2,4-D + Ca and GA3 + Ca. The surfactant Nourfilm (produced by Alam Chemca) at the rate of 40 cm^3/100 L water was added to all sprayed chemicals in order to obtain best results. Trees were harvested late in the harvest season (mid-February) at the end of export period.

Determination of fruit physical parameters

The percentage of pre-harvest fruit drop, rind ageing, softening, creasing and fruits unfit for export and peel thickness (mm) and fruit color were recorded at harvest date (mid- February). Peel softening and fruits unfit for export were measured depending on the scale of export specifications. Rind ageing was estimated as the percent of fruit with peel pitting. Fruit color was estimated by giving five degrees of color stages as follow; 1= 100 % green, 2= 25% green, 3= 50% orange, 4= 75% orange and 5=100% orange.

Determination of fruit chemical parameters

A sample of ten fruits was taken from each replicate at harvest date in both growing seasons, in order to estimate, electrolyte leakage (EL), fruit carotenoids, total soluble solids (TSS), acidity, TSS/acidity ratio, sugars, and vitamin C (VC) contents.

Acidity (%) and VC (mg/100 ml juice) were determined by titration according to AOAC (1980). Electrolyte leakage (EL) was estimated in fruit peel by taking six 12-mm-diameter discs were prepared from each orange peel (total weight 0.6 g) and incubated in 10 ml 0.3 M mannitol (five samples per treatment). Electrolyte leakage was measured after 3 h of incubation in a shaking water bath at 25°C. The electrical conductivity was measured at room temperature with a microprocessor conductivity meter (WTW, Weilheim, Germany). The tissue and the mannitol solution then were frozen at -20°C, thawed, and boiled for 10 min. Total conductivity was then measured. Rates of electrolyte leakage are expressed as changes in the percentage of the total conductivity per hour (Lafuente et al., 1991). Carotenoids (mg/100 g peel fresh weight) were measured according to the method of Wensttein (1957). Sugar content (%) was determined according to the method of Malik and Singh (1980).

Statistical analysis

All data were tested for treatments effects on analyzed parameters by one-way analysis of variance (ANOVA) technique. Means for comparison were separated using least significant difference (LSD $P < 0.05$) according to Snedecor and Cochran (1980). The Statistical analysis was performed with the help of SAS Institute, 1989.

RESULTS AND DISCUSSION

Fruit physical parameters

The effects of the different treatments on fruit physical parameters at harvest date are presented in Table (1). The data of both seasons indicated that spraying calcium alone increased peel thickness in comparison with the control. In addition, peel thickness increased by spraying GA3, PUT + Ca, 2,4-D + Ca (in the first season) and PUT + 2,4-D, Put + GA3, 2,4-D + GA3 and GA3 + Ca (in the second season) compared to the control with no significant differences among the mentioned treatments (except GA3 + Ca which indicated the highest increase in fruit peel thickness in the second season only). However, fruit color break was not significantly affected by any of the sprayed substances when compared with the control in both seasons. A significant decrease in rind ageing percent was obtained by most foliar sprays in the first season. Moreover, fruit softening and creasing percentages were significantly decreased by all treatments in both seasons compared to the control. In general, the combinations of the used substances resulted in the highest decrease as compared with spraying each substance alone especially for 2, 4-D and putrescine. Similarly, fruit drop and number of fruits unfit for exportation were significantly decreased by all treatments in both seasons as compared to the control.

The data of the present study showed that in general all sprayed growth regulators had positive influences in decreasing fruit external characteristics disorder and delaying fruit senescence at harvest date. Senescence is the final stage of fruit growth and characterized by breakdown of cell wall components leading to loss of tissue structure (Buchanan-Wollaston 1997), which all contribute to the weakening of peel structure and leads to the final demise of the fruit. Previous studies have suggested the promotive role of ethylene on the process of fruit ripening and senescence (Lelievre et al. 1997). In non-climacteric fruit, such as citrus, ethylene is not required for the coordination of ripening of the fruit (Giovannoni 2001), however, it plays an important role

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Table 1. Effect of PUT, 2, 4-D, GA3 and Ca foliar sprays on fruit physical characteristics at harvest during 2007/2008 and 2008/2009 seasons

Treatments	Parameters						
	Peel thickness (mm)	Color	Rind ageing (%)	Softening (%)	Creasing (%)	Fruit drop (%)	Fruit unfit for export (%)
			Season 2007/2008				
water	30.4	5.00	8.66	31.5	28.6	18.1	57.4
PUT	32.7	4.94	5.26	18.5	14.7	10.4	30.5
2,4-D	31.8	5.00	4.21	15.9	15.3	5.37	25.7
GA3	34.2	4.86	5.26	14.9	15.8	6. 39	23.5
Ca	35.3	5.00	3.45	19.6	17.8	5.45	21.7
PUT +2,4-D	32.6	4.96	4.78	6.28	9.76	7.68	27.5
PUT + GA3	33.3	4.83	3.16	8.64	10.9	10.6	28.9
PUT + Ca	34.8	4.94	5.54	7.87	9.87	9.03	26.3
2,4-D +GA3	32.7	4.98	2.28	7.75	8.87	4.57	23.5
2,4-D + Ca	35.4	5.00	4.60	6.87	10.8	8.64	36.9
GA3 + Ca	33.2	5.00	4.23	6.07	9.87	4.46	24.7
$L.S.D._{0.05}$	3.0	NS	2.68	3.84	4.76	4.69	6.89
			Season 2008/2009				
water	31.8	5.00	6.89	34.8	37.6	20.5	54.4
PUT	33.8	5.00	4.86	15.6	20.3	16.2	34.3
2,4-D	33.6	5.00	6.57	17.1	21.1	9.57	28.3
GA3	34.6	4.87	4.60	12.9	15.4	11.0	24.3
Ca	34.8	4.98	4.26	17.6	20.2	10.6	30.3
PUT +2,4-D	35.4	4.98	3.98	12.0	16.5	6.63	23.5
PUT + GA3	34.8	4.86	3.74	10.9	10.6	8.85	20.3
PUT + Ca	33.6	5.00	6.21	9.76	9.08	10.5	32.7
2,4-D +GA3	34.8	5.00	4.68	11.9	13.3	6.74	24.7
2,4-D + Ca	34.4	4.96	6.64	14.7	13.7	4.58	28.5
GA3 + Ca	43.8	5.00	7.42	10.0	16.1	7.67	35.9
$L.S.D._{0.05}$	2.9	NS	4.07	3.75	2.86	4.06	7.46

in the senescence process. Association between the sprayed substances specially the polyamines (for example putrescine in our study) and ethylene has been recently reviewed and investigated (Pandey et al 2000, Giovannoni 2001, and Valero et al. 2002). In this sense, there is evidence of an interrelationship between ethylene and polyamines during fruit ripening and senescence (Pandey et al. 2000). They play an inhibitory role on ethylene production through inhibition of ACC synthesase and ACC oxidase (Apelbaum et al. 1981, Lee et al 1997), thus delaying ethylene emission. Polyamines have been reported to reduce softening, delay senescence and reduce decay in several fruits (Saftner and Baldi 1990, Kramer et al. 1991). Other beneficial effects of polyamines application on fruit are retarding color changes and increasing fruit firmness (Valero et al. 1999, Valero et al. 2002). Putrescine application leads to changes in cell wall stability (Messiaen et al. 1997) by inhibition of the action of polygalacturonase and pectin methyl esterase involved in softening, and also cross-link pectic substances in the cell wall, producing rigidification and increasing fruit firmness (Martinez-Romero et al. 2002, Perez-Vicente et al. 2002). This might explain the decrease in fruit softening by putrescine application reported for lemon (Valero et al. 1998b) and obtained for navel orange in our study.

Moreover, GA3 sprays in the present study increased peel thickness and decreased rind ageing, softening, creasing, color change and the number of unfit export fruits. Similar results were obtained for Hamlin, Valencia, navels and blood oranges and mandarins (Coggins 1973, Davies et al. 1997, Davies et al. 1999, Pozo et al. 2000). GA3 is known to delay and retard chlorophyll degradation in citrus (El-Otmani and Coggins 1991). Its role is not limited only to the regulation of rind color, but also in delaying the more general process of peel ageing (Baez-Sanudo et al. 1992). This might be due to the association of GA3 with the reduction of fruit peel growth as has been reported for mandarins (Pozo et al. 2000). In addition, improvement in fruit physical characters by calcium sprays in the present study was measured specially an increase in peel thickness and decrease in rind ageing, softening and creasing as obtained by Sayed et al (2004) working on grapefruit and El-Hilali et al (2004) working on mandarin. Also, Storey et al (2005) reported rind fruit disorders as a result of calcium deficiency. Calcium role in the physiological disorder related to ripening, fruit quality and shelf life is well established (Wimwright and Burbage, 1989). Calcium is involved in cell wall membrane metabolism and it contributes to the maintenance of configuration of specific enzymes (Jones and Lunt 1967). Addition of calcium improves rigidity of cell walls and obstructs enzymes such as polygalacturonase from reaching their active sites (John 1987), thereby retarding tissue softening and delaying ripening. Repeated sprays of calcium solutions increased the proportion of unaffected navel orange fruit with albedo breakdown (Treeby and Storey 2002).

The positive influence on decreasing fruit drop by the sprayed substances in our study is obvious. It is well established that plant growth regulators are involved in control of abscission (Sexton and Robersts 1982), Ethylene accelerates mature citrus fruit abscission, and as previously mentioned that putrescine inhibits ethylene production, this might explain its effect on decreasing fruit drop. In addition, 2, 4-D is widely used in citrus in order to reduce the incidence of mature fruit drop and its primary action is to delay the development of the abscission layer (Coggins 1973). GA3 and Ca sprays influence might be due to the increase in the thickness of both juncture zone and the pedical as well as increasing the connections of vascular system and cell adhesion in union zone as reported for grapefruit by Sayed et al. (2004).

Fruit chemical parameters

The data in Table (2) indicated that all foliar sprays increased fruit TSS content in both seasons as compared with the control. A significant increase in fruit acidity was obtained by spraying GA3 alone, Ca alone, PUT + GA3 and GA3 + Ca in the first season, whereas, fruit acidity content was not significantly affected by any of the treatments in the second season. The TSS/acidity ratio increased significantly by spraying 2, 4-D alone, Put + Ca and 2,4-D + Ca in the first season, whereas, in the second season all foliar sprays increased TSS/Acidity ratio compared to the control. Fruit vitamin C content increased significantly by all treatments (except 2, 4-D + GA3 in the first season and PUT + 2, 4-D in both seasons). However, spraying 2, 4-D alone resulted in lower VC content than the control in the second season only. Electrolyte leakage was significantly decreased in the first season by spraying PUT alone, 2,4-D alone, PUT + 2, 4-D, and PUT + GA3, whereas, in the second season it was decreased by all foliar sprays except spraying 2,4-D alone. In both seasons, fruit content of reduced sugars was significantly increased by all sprays (except PUT + 2, 4-D in the first season, PUT alone in the second season and 2, 4-D alone in both seasons). In addition, only foliar sprays of PUT + 2,4-D and 2,4-D + Ca resulted in higher non reduced sugars content than the control in the first season, whereas, in the second season all foliar sprays resulted in higher non reduced sugars content than the control. In addition, total sugars content increased significantly by all treatments in both seasons. Moreover, spraying 2, 4-D + GA3 and 2,4-D + Ca obtained the highest total sugars values when compared with all other treatments in both seasons. The data of both seasons indicated that all foliar sprays (except 2,4-D alone in both seasons, Put + 2,4-D and 2,4-D + Ca in the first season and 2, 4-D + GA3 and 2,4-D + Ca in the second season) decreased fruit carotenoids content as compared with the control. Spraying GA3 + Ca indicated the lowest value of carotenoids content in both seasons.

The improved fruit appearance obtained for navel orange fruits in our study by the sprayed substances reflected better fruit internal characters at harvest date. TSS and sugars contents were increased. It is reported that at the late stages of citrus fruit development, soluble solids accumulate in the juice sacs (Coggins 1981). Similar increases in TSS, VC and sugars contents were reported by EL-Hilali et al. (2004) on Fortune mandarin, El-Otmani et al. (2004) on Clementine mandarin, and Saleem et al. (2008) on Blood Red orange. The decrease in carotenoids by spraying PUT and GA3 might be attributed to that the same substances led to a decrease in fruit color as obtained for the first harvest date in this study. Ethylene induces fruit coloration and carotenoids content in navel oranges (Rodrigo and Zacarias 2007), that might be due to inhibition of ethylene production by putrescine and GA3 sprays which decreased carotenoids content and lowered fruit coloration.

Table 2. Effect of PUT, 2, 4-D, GA3, and Ca foliar sprays on fruit chemical characteristics at harvest during2007/2008and 2008/2009 seasons

Treatment	Parameters								
	TSS (%)	Acidity (%)	TSS/ Acidity	VC[1] (mg/ 100ml)	EL[2]	Sugars (%)			Carotenoids (mg/100g)
						Red.	Non-red.	Total	
Season 2007/2008									
water	10.7	0. 94	11.47	56	304	3.26	4.39	7.65	8.34
PUT	11.6	1.08	10.8	66	262	3.76	4.67	8.43	6.54
2,4-D	10.9	0.86	12.7	59	248	3.60	4.80	8.40	8.78
GA3	12.0	1.18	10.2	68	278	3.74	5.04	8.78	5.68
Ca	11.8	1.16	10.2	65	284	3.78	5.14	8.92	6.12
PUT +2,4-D	11.5	0.96	12.3	54	268	3.68	5.26	8.94	7.12
PUT + GA3	12.3	1.12	11.0	68	253	3.76	4.78	8.54	5.65
PUT + Ca	12.2	0.96	12.7	73	277	3.94	4.86	8.80	6.12
2,4-D +GA3	11.2	0.92	12.2	58	287	4.66	4.96	9.62	6.56
2,4-D + Ca	12.1	0.96	12.6	59	289	4.68	5.25	9.93	7.48
GA3 + Ca	12.3	1.14	10.8	64	289	4.46	4.98	9.44	4.89
L.S.D. 0.05	0.24	0.18	1.08	2.6	29	0.46	0.78	0.65	1.56
Season 2008/2009									
water	11.1	1.02	10.9	52	282	3.67	4.21	7.88	8.97
PUT	12.1	0.93	13. 0	59	260	3.88	4.68	8.56	7.65
2,4-D	12.7	0.98	12.9	49	294	3.89	4.86	8.75	8.65
GA3	11.9	0.89	13.3	67	257	4.02	4.78	8.80	6.13
Ca	12.3	0.84	13.6	64	273	4.45	4.88	9.33	6.43
PUT +2,4-D	12.1	0.90	13.5	50	248	4.47	5.00	9.47	6.64
PUT + GA3	12.0	0.94	12.7	60	260	4.36	4.94	9.30	6.76
PUT + Ca	12.4	0.86	14.4	64	265	4.28	4.87	9.15	6.87
2,4-D +GA3	11.8	0.80	14.8	55	270	4.66	5.08	9.74	7.87
2,4-D + Ca	12.2	1.00	12.2	56	261	4.75	5.17	9.92	8.01
GA3 + Ca	12.2	0.89	13.7	65	274	4.66	5.06	9.72	5.98
L.S.D. 0.05	0.67	NS	1.15	2.3	16	0.27	0.46	0.22	1.18

[1] Vitamin C, [2] Electrolyte leakages

The improvement measured in the external and internal fruit characteristics in the present study indicates that GA3, PUT, Ca and 2, 4-D sprays might enable on-tree storage and later harvest. The combined effect of these substances on delaying fruit senescence, permits longer harvest season with only modest losses from fruit drop and without risks of fruit quality loss as similarly was obtained by Greenberg et al.(1992) on mandarins.

CONCLUSION

Finally, it might be concluded that spraying the different used substances or their combinations will allow growers to have longer on- tree storage for navel oranges, thus, expand harvest period and export season of fruit with a good quality.

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الملخص العربي

تأثير الرش بالبيتروسين وحامض الجيبريلليك و2,4-D والكالسيوم على إطالة موسم جمع البرتقال أبو سرة

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أجريت هذه الدراسة على ثمار البرتقال ابو سرة المنزرع في أرض طينية خلال موسمي 2008/2007 ، 2009/2008 بغرض إطالة موسم جمع وتصدير الثمار في مصر مع الاحتفاظ بصفات جودة الثمار الخارجية لزيادة قدرتها التسويقية وذلك عن طريق الرش قبل الحصاد بكل من البيتروسين وحمض الجيبيريلليك والكالسيوم و2,4-D منفردة أو في التوليفات المختلفة وذلك عند بداية انكسار اللون الاخضرللثمار وبداية تكوين اللون الاصفر.جمعت الثمار فى ميعاد متأخر وهو نهاية موسم تصدير البرتقال بسرة (منتصف فبراير) عن ميعاد الجمع التجارى (منتصف نوفمبر– بداية موسم التصدير).

وقد أظهرت النتائج أن جميع المواد المستخدمة أدت الى تأخير حدوث ليونة الثمار (الطراوة) وانكسار اللون الاخضر مع أنخفاض نسبة تدهورالقشرة والتبحير، كما أدت الى تقليل نسبة تساقط الثمار قبل الجمع. أدت المعاملات المختلفة أيضا الى زيادة محتوى الثمار من المواد الصلبة الذائبة الكلية والسكريات وفيتامين ج. كان لجميع المعاملات تأثير ايجابى على إطالة موسم جمع الثمار بدون حدوث تدهور لصفات الثمار الخارجية والداخلية مما يتيح الفرصة لتأخير موسم التصدير دون حدوث تدهور فى صفات جودة الثمار بنسبة عالية، بصفة عامة أوضحت النتائج أن رش المواد المستخدمة فى توليفات أعطت نتائج أفضل عن رشها منفردة وخاصة بالنسبة للبتروسين و2,4-D.