Influences of some chemical substances used to induce early harvest of 'Canino' apricot trees

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Abstract: Brassinolide and hydrogen cynamide were sprayed to accelerate yield harvesting on 8 years old 'Canino' apricot (*Prunus persica* L. Batch). Trees were grown in Elbostan region, where the soil is sandy and drip irrigated at 2009 & 2010 years. All treatments were positively affected shoot growth more than control. However, progressive increment of leaf growth parameters (leaf area, dry weight and SLW) and leaf chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll content) were parallel to the brassinolide concentrations on spraying solution. Trees sprayed with dormex + 50 ppm of brassinolide (DM 50) recorded the biggest yield (weight and number per tree) and crop monetary value, while there was positive influences of both substances on hastening harvest dates of 'Canino' fruits. Sprayed trees of brassinolide and hydrogen cynamide (D, M100, DM25 & DM50) started harvest and reached peak of harvest earlier than control trees. Progressive increment of fruit weight, length, diameter and size values were linearly related to the brassinolide concentrations on spraying solution, while not affected fruit firmness. On the other hand, 'Canino' apricot trees which applied with hydrogen cynamide only (D) harvested the firmest fruit. All investigated treatments increased soluble solids content (SSC) and decreased acidity percentage of fruit juice. M 100, DM 25 and DM 50 treatments improved 'Canino' apricot fruit color (H*), while there was no clear trend of treatments on records of fruit color lightness (L*).
[Gabr, M. A.; Fathi M. A.; Azza I. Mohamed and Mekhaeil G. B. **Influences of some chemical substances used to induce early harvest of 'Canino' apricot trees.** Nature and Science 2011;9(8):59-65]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: 'Canino' apricot tree, Brassinolide , hydrogen cynamide

1-Introduction

Brassinosteroids (BRs) are a class of poly hydroxyl steroids which have been recognized as a class of plant hormones. These were first explored when Mitchell *et al.* (1970) reported that cell division and elongation were promoted by the treatment of organic extracts of rape (*Brassica napus* L.) pollen. Brassinolide (BL) was the first isolated brassinosteroid when Michael *et al.* (1979) isolated the biologically active molecule.

Montoya *et al*. (2005) showed that brassino steroids are essential for many physiological functions in plants, however little is known concerning where and when they are synthesized. In young tomato seedlings BR synthesis activity was observed mainly in apical and root tissues undergoing expansion. In flowers, synthesis activity was observed in the pedicel joints and ovaries, whereas in fruits it was strongest during early seed development and was associated with the locular jelly and seeds. Quantitative measurements of endogenous BR indicated intense biosynthesis in developing tomato fruits, which were also found to contain high amounts of brassinolide. Moreover, brassino steroids stimulate cell elongation and cell division (Kauschmann *et al.*, 1996), and BR has specific effect of differentiation. Underling physiological pathways include modification of cell wall properties, effects on carbohydrate assimilation , allocation, and control of aquaporin activities. Brassinosteroids apparently coordinates and integrates diverse processes required for growth, partly via interactions with phytohormones setting the frame for BR responses (Müssig, 2005).

The growth induced by brassinosteroid has been related to increase in RNA and DNA content, polymerase activity, protein synthesis (Kalinich *et al.*1985) carbohydrate fraction, reducing sugars, non reducing sugars and starch (Vardhini and Rao, 1998). The yield increase in fruit trees may be related to improvement in the assimilation efficiency of photosynthetic carbon of the sprayed trees. The brassinosteroid application in wheat and mustard plants stimulated photosynthetic activity expressed by acceleration in CO_2 fixing, increase protein biosynthesis (Braun and Wild, 1984) and in mustard, increased photosynthetic rates that were directly related to growth and seed production (Hayat *et al.* 2000).

Kauschmann *et al.* (1996) reported that brassinosteroids which show high structural similarity to animal steroid hormones elicit a variety of growth responses when exogeneously applied to plant tissues. Moreover, Clouse (1996) explained that BRs have been shown to enhance tracheary element differentiation, stimulate membrane hyperpolarization and ATPase activity, promote ethylene biothynsesis, control microtubule orientation and alter mechanical properties of cell walls. Krizek and Mandava (1983) recorded that growth response of bean plants treated with BR produced a sequential increase in elongation, curvature and swelling. In addition, brassinosteroid treatment greatly stimulated accumulation of photosynthates in the treated internode. This suggests a possible mobilization role for BR in the intact plant. While, Amzallag (2002) verified that BR increased leaf lenhening in Sorghum.

Fruit set of navel orange trees treated with BL was increased while there were no differences in quality between treated and untreated fruits (Sugiyama and Kuraishi, 1989). As well as, in persimmon, grapevine and citrus, Watanabe *et al.* (1997) reported that BR compound showed the practical effects for fruit setting. While, Wang *et al.* (2004) reported that brassinolide increased fruit weight and sugar content of orange. In passion fruit orchards, brassinosteroid increased fruit number per plant and in turn yield per hectare and soluble solids content was 1° Brix greater than the control (Gomes *et al.*, 2006). Symons *et al.* (2006) showed that increases in endogenous BR levels are associated with ripening of nonclimacteric fruits such as grape. Also, they verified that application of BRs to grape berries promoted ripening, while brassinazole, an inhibitor of BR biosynthesis, delayed fruit ripening.

In pecan, Youssef *et al*. (1994) and El-Sharkawy and Osman (2009); Kuden and Son (2005) in apricot; Bahlool *et al*. (1994) and Stino and Rashed (2002) in pear and El-Sayed *et al*. (2002) in vines, reported that hydrogen cynamide (Dormex) induced bud opening and advanced flowering date. Additionally, Attala and Stino (1999) revealed that H_2CN_2 at different concentrations and time of application were improved bud burst and accelerate vegetative growth.

In Spain, Brunton *et al*. (1994) showed that hydrogen cynamide (Dormex®) application is used in all apricot fresh market cultivars to advance and uniformize the ripening. In their study, they concluded that hydrogen cynamide treatments advanced fruit weight, firmness, color, SSC and titrable acidity. On the way, Son and Kuden (2005) in apricot and plum; and El-Sharkawy and Osman (2009) in pecan, cleared that hydrogen cynamide treatments hastened flowering and harvesting time. They added that hydrogen cynamide treatments improved fruit set and increased yield as weight and number of fruit per tree, while enhanced fruit physical and chemical properties. In addition, Bahlool *et al*. (1994) reported that fruit set percentage was realized, yield was increased, fruit diameter and firmness slightly decreased and TSS was increased, while acidity was not affected as a result of spraying Dormex.

The aims of present investigation were, therefore, to induce trees to yield earlier, increase yield and improve fruit quality of 'Canino' apricot.

2. Materials and methods

This investigation was carried out during years 2009 & 2010 on eight years old 'Canino' apricot (*Prunus armeniaca* L. Batch) trees budded on apricot seedling rootstocks. Trees were grown at Elbehira governorate, Elbostan region where the soil is sandy and drip irrigation is practiced. Trees were blocked on the basis of trunk circumference at the beginning of the experiment to minimize within-treatments variation due to differences in tree size. Treatments were arranged as a random complete blocks design with a single tree plot replicated three times for each treatment. Obtained data were statistically analyzed by a computer based data analysis software package "MSTAT".

Trees of treatments (M50) and (M100) were sprayed with 50 and 100 ppm brassinolide, respectively. At the same time, trees of treatments (D), (DM25) & (DM50) were subjected to spray with 2 % hydrogen cynamide (Dormex®) + 3 % mineral oil (Caple 2®) and then sprayed with 0, 25 &50 ppm brassinolide (Milagrow®), respectively. While, control trees (C) were sprayed with tap water only.

Three branches, five years old, in different directions on each tree were selected and labeled for measuring all growth parameters. All current shoots developed on these branches were counted to get shoot number and measured to get shoot length (cm). All leaves on fifteen shoots (five shoots per branch) were measured by using Li-Core-3100 Areameter to get leaf area (cm^2). Leaves were dried at 70° C and weighted to get leaf dry weight (g.) and then specific leaf weight (SLW) was calculated as ($mg.cm^{-2}$).

Chlorophyll a and chlorophyll b were extracted from fresh leaves with DMF and were determined using a spectrophotometer as reported by Rami and Porath (1980). The concentration of chlorophyll a and chlorophyll b and its total value were calculated by Rami's formulas as (µg / ml) (Rami 1982), while presented in this study as ($mg.cm^{-2}$).

At picking dates, fruit number per tree was counted, fruit yield per tree was weighted and average fruit weight was calculated. These data were used in estimating crop monetary value considering a farm-gate price of Egyptian pounds for every harvest date separately, and then calculated for total yield per tree.

Fruit firmness was recorded by Magness – Taylor type pressure tester with a standard 7/16 $inch^2$ plunger, to determinate fruit firmness and recorded in $Lb/inch^2$. Fruit skin color measurements (a*, b*, L*

& H°) were determined using tri-stimulus colorimeter (Hunter – type DP 9000). The instrument estimated skin color of fruits with color metric CIE Lab method where L* measure lightness scale readings and the two coordinates a * and b* included. Positive values of a* is a measure of redness and becomes greenish measure when values changed into negative, while b* of yellowness and blueness (- b*) on the Hue circle. The Hue angle [H° = arc tan (b*/a*)] describe the relative amounts of redness and yellowness where point at 0°/360° is defined for red/magenta, 90° yellow, 180° for green and 270° for blue color (McGuire, 1992 and Voss, 1992). Total soluble solids (TSS) were determined by using a hand refractometer and total acidity percentage was estimated according to (A.O.A.C., 1990).

3. Results and discussion

3.1. Response of shoot growth with Hydrogen cynamide and Brassinolide spraying treatment

Response of shoot growth as shoot length and diameter, and leaf growth and efficiency as leaf area, dry weight and specific leaf weight (SLW) of 'Canino' apricot trees to brassinolide (Milagrow®) and hydrogen cynamide (Dormex®) treatments is shown in table (1). Data illustrated that all treatments were positively affected shoot growth than control, significantly. At the same time, trees of M100 & DM50 treatments recorded the highest values of shoot length and diameter. In concerning with leaf growth parameters, data illustrated that there was no significant difference between control trees and trees of D treatment which sprayed with hydrogen cynamide and mineral oil only, and both recorded the lowest leaf area, dry weight and specific leaf weight (SLW) records, significantly.

However, progressive increment of these parameters was parallel to the brassinolide concentrations on spraying solution, in both seasons.

These results are in harmony with Attala and Stino (1999) who revealed that H_2CN_2 at different concentrations and time of application were improved bud burst and accelerate vegetative growth. While, Amzallag (2002) verified that BR increased leaf lenhening in Sorghum. While, Kauschmann *et al.* (1996) reported that brassinosteroids elicit a variety of growth responses when exogeneously applied to plant tissues. They indicated that BR are essential for proper plant development and play an important role in the control of cell division and elongation. It could be explained in light of Müssig (2005) investigation which discussed that brassinosteroids apparently coordinates and integrates diverse processes required for growth, partly via interactions with phytohormones setting the frame for brassinosteroid responses

Table (1): Effect of Hydrogen cynamide and Brassinolide spraying on vegetative growth of 'Canino' apricot trees.

Treatments	Shoot length (cm)	Shoot diameter (cm)	Leaf dry weight (g)	Leaf area (cm^2)	SLW ($mg.cm^{-2}$)
2009					
C	68.43	0.88	0.225	26.20	8.58
M50	79.17	1.00	0.276	27.70	9.99
M100	95.10	1.18	0.335	30.83	10.90
D	77.00	0.96	0.229	26.13	8.76
DM25	85.20	1.15	0.255	27.77	9.18
DM50	95.07	1.34	0.304	29.60	10.27
LSD at 5 %	5.792	0.08136	0.01522	1.144	0.9856
2010					
C	66.78	0.82	0.265	24.09	10.98
M50	91.30	1.19	0.293	28.01	10.49
M100	88.26	1.30	0.348	31.80	10.96
D	77.89	0.96	0.229	24.30	9.44
DM25	85.78	1.06	0.281	28.67	9.82
DM50	89.22	1.20	0.306	33.66	9.10
LSD at 5 %	6.957	0.09096	0.05753	1.146	1.461

3.2. Response on chlorophyll content with Hydrogen cynamide and Brassinolide spraying treatment

Data of brassinolide and hydrogen cynamide treatments on chlorophyll content of 'Canino' apricot leaf were arranged in table (2). Statistical analysis showed that there was no significant difference between control trees and D treatment trees and both recorded the lowest chlorophyll a, chlorophyll b and total chlorophyll content,

However, progressive increment of these parameters was parallel to the brassinolide concentrations on sprayed solution, in both seasons.

These results are in line with Müssig (2005) who reported that underling physiological pathways of brassinosteroids include effects on carbohydrate assimilation and allocation. Additionally, Krizek and Mandava (1983) recorded that brassinosteroid treatment greatly stimulated accumulation of photosynthates in the treated inter-node of bean plants. While, Montoya *et al*. (2005) showed that brassino steroids are essential for many physiological functions in plants.

Table (2): Effect of Hydrogen cynamide and Brassinolide spraying on chlorophyll content of 'Canino' apricot leaf.

Treatments	Chlorophyll a ($mg.cm^{-2}$)	Chlorophyll b ($mg.cm^{-2}$)	Total chlorophyll ($mg.cm^{-2}$)
2009			
C	3.32	2.18	5.50
M50	3.80	2.49	6.30
M100	4.37	2.83	8.20
D	3.37	2.15	5.52
DM25	3.74	2.35	6.10
DM50	3.85	2.71	6.57
LSD at 5 %	0.1819	0.1076	1.642
2010			
C	3.33	2.19	5.46
M50	3.73	2.48	6.21
M100	4.15	2.80	6.95
D	3.32	2.23	5.54
DM25	3.75	2.54	6.32
DM50	4.19	2.83	6.40
LSD at 5 %	0.2034	0.1151	0.5032

3.3. yield and crop monetary value of 'Canino' apricot tree with response of Hydrogen cynamide and Brassinolide spraying

In concerning with influences of bud break substance and brassinolide spraying on yield and crop monetary value of 'Canino' apricot trees. Data in table (3) cleared that control trees recorded the lowest significant fruit weight and number and crop monetary value per tree than other treatments. In most cases, trees sprayed with 50 ppm of brassinolide (M 50) had no significant difference than control trees. On the other hand, trees sprayed with dormex + 50 ppm of brassinolide (DM 50) recorded the biggest yield (weight and number per tree) and crop monetary value of 'Canino' apricots.

These results found support in results of Son and Kuden (2005) and El-Sharkawy and Osman (2009) who showed that hydrogen cynamide treatments improved fruit set and increased yield as weight and number of fruit per tree. In addition, Bahlool et al. (1994) reported that fruit set percentage was realized and yield was increased as a result of spraying Dormex. In concerning of brassinolide, in navel orange, Sugiyama and Kuraishi (1989) reported that applications increased fruit set, as in japanese persimmon, grapevine and citrus (Watanabe et al., 1997) which resulted in increased fruit number per tree and in turn yield per hectare in passion fruit orchards (Wang *et al*., 2004).

In tomato, Montoya *et al.* (2005) discussed that brassinosteroids synthesis activity was observed mainly in tissues undergoing expansion. In flowers, synthesis activity was observed in the pedicel joints and ovaries, whereas, in fruits it was strongest during early seed development and was associated with the locular jelly and seeds. Quantitative measurements of endogenous brassinosteroids indicated intense biosynthesis in developing fruits, which were also found to contain high amounts of brassinolide. In addition, brassinolide positively affected fruit cell division and elongation (Krizek and Mandava, 1983; Clouse, 1996; Müssig, 2005 and Montoya *et al.*, 2005) and stimulated accumulation of photosynthates (Krizek and Mandava, 1983 and Müssig, 2005) through increase carbohydrate assimilation (Müssig, 2005). By the same way brassinolide enhance tracheary element differentiation and control microtubule orientation (Clouse, 1996) which suggests a possible mobilization role for brassinosteroid (Krizek and Mandava, 1983) and in turn promote photosynthates accumulation in fruits. Braun and Wild (1984); (Kalinich *et al.*1985); Vardhini and Rao (1998) and Hayat *et al.* (2000) supported these findings and explanation which reported that yield increase in fruit trees may be related to improvement in the assimilation efficiency of photosynthetic carbon and protein biosynthesisof the sprayed trees.

3.4.The influences of Hydrogen cynamide and Brassinolide spraying on harvest dates of 'Canino' apricot fruits

At the same time, Diagrams (1) & (2) illustrated the positive influences of both substances on advancing harvest dates of 'Canino' apricot fruits. Both diagrams showed that sprayed trees of brassinolide and hydrogen cynamide (D, M100, DM25 & DM50) started harvest and reached peak of harvest earlier than control trees, in both seasons. Advanced dates of starting picking and heavy quantity of fruits which harvested of treated trees led to significant increase in crop monetary value per tree which reflected on grower income.

In apricot, Brunton *et al*. (1994) showed that hydrogen cynamide (Dormex®) application is used to advance and uniformize the ripening while, Son and Kuden (2005) and El-Sharkawy and Osman (2009) cleared that hydrogen cynamide treatments hastened flowering and harvesting time.

Table (3): Effect of Hydrogen cynamide and Brassinolide spraying on yield and crop monetary value of 'Canino' apricot trees.

Treatments	Fruit yield / tree (Kg)	Fruit number / tree	Crop monetary value (£E)
		2009	
C	45.6	828.2	83.95
M50	51.3	776.2	86.70
M100	58.7	952.2	146.8
D	52.6	940.9	133.7
DM25	57.9	941.5	161.0
DM50	63.5	955.2	177.1
LSD at 5 %	11.72	83.88	17.86
		2010	
C	32.8	605.5	66.25
M50	37.1	572.6	79.95
M100	46.2	675.0	108.0
D	39.3	667.2	96.52
DM25	44.0	708.6	110.9
DM50	46.2	740.6	111.6
LSD at 5 %	4.834	80.54	17.96

Besides, brassinosteroids altered mechanical properties of cell walls, stimulated membrane hyperpolarization and ATPase activity and promote ethylene biothynsesis (Clouse, 1996) which may be advanced early maturity of treated 'Canino' apricot fruits. While, Symons et al. (2006) reported that application of brassinosteroids on grape berries promoted ripening.

3.5.Response of hydrogen cynamide and brassinolide spraying on Physical characteristics of 'Canino' apricot fruits

Data of physical characteristics of 'Canino' apricot fruits as affected by hydrogen cynamide and brassinolide spraying were arranged in Table (4).

Data inTable (4) showed that control trees recorded the lowest fruit weight, length, diameter and size significantly, during investigated seasons. 'Canino' apricot trees which applied with hydrogen cynamide only (D) did not differentiate than control trees while harvested the firmest fruit, significantly. However, progressive increment of fruit weight, length, diameter and size values was linearly related to the brassinolide concentrations on spraying solution, in both seasons. On the other hand, brassinolide treatments decreased fruit firmness records in 2009, while there was no difference among treatments in second season.

Obtained results are in line with those of Brunton *et al*. (1994) who showed that hydrogen cynamide treatments advanced fruit weight and firmness. Son and Kuden (2005) in apricot and plum; and El-Sharkawy and Osman (2009) in pecan, cleared that hydrogen cynamide treatments enhanced fruit physical properties. While, Bahlool *et al*. (1994) reported that fruit diameter and firmness slightly decreased as a result of spraying Dormex. In accordance, Wang *et al.* (2004) reported that brassinolide increased fruit weight of orange. Besides, Symons *et al*. (2006) showed that increases in endogenous brassinosteroids levels, but not indole-3-acetic acid (IAA) or GA levels, are associated with ripening of nonclimacteric fruits. Also, they verified that application of brassinosteroids on grape berries promoted ripening.

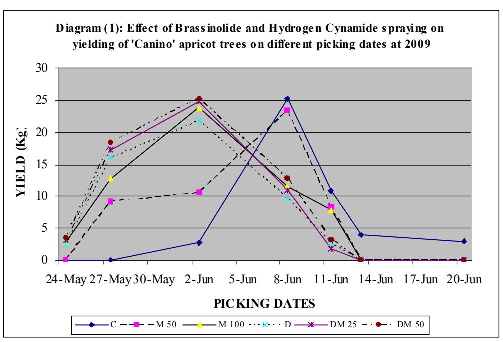

Diagram (1): Effect of Brassinolide and Hydrogen Cynamide spraying on yielding of 'Canino' apricot trees on different picking dates at 2009

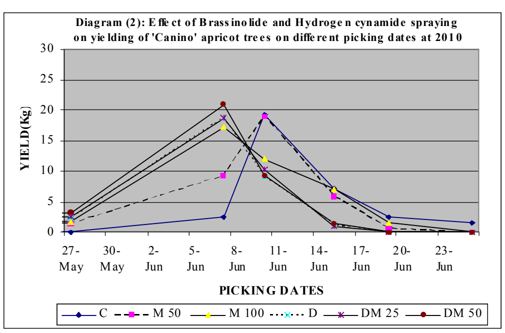

Diagram (2): Effect of Brassinolide and Hydrogen cynamide spraying on yielding of 'Canino' apricot trees on different picking dates at 2010

Table (4): Effect of Hydrogen cynamide and Brassinolide spraying on physical characteristics of 'Canino' apricot fruits.

Treatments	Fruit weight (g)	Fruit length (cm)	Fruit diameter (cm)	Fruit size (cm^3)	Fruit firmness ($Lb/inch^2$)
			2009		
C	55.07	4.73	4.33	51.25	8.54
M50	66.10	4.90	4.67	49.17	8.07
M100	61.65	5.03	4.87	58.33	8.25
D	55.95	4.53	4.57	49.17	9.13
DM25	61.53	5.30	5.05	64.58	8.77
DM50	66.49	5.10	5.02	60.83	8.50
LSD at 5 %	5.349	0.2228	0.1819	4.694	0.5146
			2010		
C	54.23	4.60	4.30	48.92	9.19
M50	64.86	4.83	4.30	51.97	8.73
M100	68.54	4.83	4.63	53.67	8.35
D	58.92	4.67	4.42	50.09	9.40
DM25	62.14	5.13	4.80	52.73	9.20
DM50	62.43	4.97	4.77	54.18	8.27
LSD at 5 %	2.446	0.1726	0.2074	2.238	0.7209

3.6. Effect of Hydrogen cynamide and Brassinolide spraying on chemical characteristics and color of 'Canino' apricot fruits.

Response of chemical characteristics and color of 'Canino' apricot fruits on to hydrogen cynamide and brassinolide applications is shown in table (5). Data showed that all investigated treatments increased soluble solids content (SSC) and decreased acidity percentage of fruit juice than control trees in a significant manner in both seasons. At the same time, both sprayed trees of M 100 and DM 50 treatments recorded the highest SSC and lowest acidity %, while other treatments recorded intermediate values.

Table (5): Effect of Hydrogen cynamide and Brassinolide spraying on chemical characteristics and color of 'Canino' apricot fruits.

Treatments	SSC %	Acidity %	Fruit color (L*)	Fruit color (H*)
		2009		
C	8.17	0.97	39.60	48.89
M50	9.00	0.82	41.57	49.87
M100	10.97	0.72	41.06	54.52
D	9.17	0.81	34.94	55.59
DM25	10.70	0.78	42.91	60.01
DM50	11.33	0.71	44.30	62.87
LSD at 5 %	0.6419	0.04068	2.786	3.902
		2010		
C	8.57	0.98	37.03	50.03
M50	9.43	0.87	34.91	49.56
M100	12.49	0.69	41.73	55.08
D	9.59	0.85	36.90	49.16
DM25	10.57	0.78	31.23	60.19
DM50	11.06	0.71	35.00	61.54
LSD at 5 %	0.4865	0.06559	1.966	3.117

Moreover, M 100, DM 25 and DM 50 treatments significantly improved 'Canino' apricot fruit color (H*), while there was no clear trend of treatments on records of fruit lightness (L*).

These results could be explained in light of Brunton *et al*. (1994) showed that hydrogen cynamide treatments advanced fruit color, SSC and titrable acidity. While, Bahlool *et al*. (1994) reported that TSS was increased and acidity was not affected as a result of spraying Dormex. On the way, Son and Kuden (2005) and El-Sharkawy and Osman (2009) showed that hydrogen cynamide treatments enhanced fruit chemical properties. In concerning with influences of brassinolide, Symons *et al*. (2006) showed that increases in endogenous brassinosteroid levels are associated with ripening of nonclimacteric fruits. They verified that application of brassinosteroid on grape berries promoted ripening. Moreover, Wang *et al.* (2004) reported that brassinolide increased sugar content of orange. While in passion fruit orchards, Gomes *et al.* (2006) showed that brassinosteroid increased soluble solids content (SSC) with about 1° Brix greater than the control which passion fruit pulp is very acid and any increase in the Brix value can contribute to juice quality.

Results of this investigation concluded that hydrogen cynamide and brassinolide applications improve vegetative growth; enhance physiological status and directing apricot trees to harvest earlier and improve fruit yield and quality. It could be said that the treatment of spraying trees 2% hydrogen cynamide + 3% mineral oil + 50 ppm of brassinolide (DM50) was a recommended treatment to apply on 'Canino' apricot trees.

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6/25/2011