

The effect of growth regulators on fruit shape and inorganic nutrient concentration in leaves and fruit of 'Red Delicious' apples

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In this work, the effects of gibberellins, cytokinins and some of their commercial mixtures, on fruit shape of 'Red Delicious' clones ('Royal Red Delicious', 'Red Chief' and 'Imperial Double Red Delicious') were studied. Quantitative determinations of nutrient elements in leaves and fruit, and measurements of the rates of photosynthesis and transpiration, water use efficiency, stomatal conductance and internal CO₂ concentration were additionally made. Promalin, Perlan, Novagib+kinetin, benzyladenine and kinetin improved apple shape by increasing the ratio of fruit height/diameter and fruit fresh weight in some cases, whereas GA₃ had no effect. The effects on inorganic nutrient content of growth regulators used varied between leaves and fruits, among cultivars tested, among treatments and year to year. GA₃ and Perlan, but not Promalin, increased photosynthesis and transpiration rates and stomatal conductance. Flowering of the next two years was negatively affected by GA₃ and Promalin, less so by Perlan, but not by kinetin and benzyladenine.

Key words: Apple, fruit quality, phyto regulators, inorganic elements.

INTRODUCTION

Fruit quality in apple is determined by taste, aroma, colour and shape, which characterize each particular cultivar. The elongated fruit shape and the presence of five well-developed calyx lobes characterize cultivars belonging to the 'Red Delicious' group of clones. Fruits with these characteristics are considered as 'typical' for their cultivar. According to Marcelle (1995), the ratio fruit height/diameter (H/D) constitutes a factor of fruit quality, the value of which must be >0.95 for 'Golden Delicious' apples in order to be commercially acceptable. The lack of 'typical' shape has been considered as a disadvantage in marketing. Various pre-harvest manipulations and treatments may remedy such defects. Promalin (GA₄/GA₇ + BA) improved fruit shape, as it did

chemical thinning at the end of the flowering period (Williams, 1978, Burak & Buyukyilmaz, 1997). The cytokinin BA (benzyladenine) and the cytokinin-like CPPU [N-(2-chloro-4-pyridyl) N-phenylurea] caused fruit thinning and affected the vegetative growth and the nutrient content of leaves and fruit (Miller 1988, Elfving & Cline, 1993).

Low temperatures before or after flowering and high temperatures after flowering are the usual reasons for the non-typical shape of apples (Chandler, 1965, Stylianidis *et al.*, 2000). Nitrogen, K, Ca and B may also affect fruit shape. Typical fruit shape in 'Golden Delicious' was achieved when nitrogen content in leaves was 2.6% (on a dry weight basis), but fruits were oblate (flat) when nitrogen content dropped to 1.01% and spherical when it was 3.35% (Bergmann, 1992). The depth of calyx cavity was affected positively by potassium (Marcelle, 1995), while oblate apples had a high calcium content

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(Politis, 1996). According to Shear & Faust (1980), boron deficiency may cause the formation of oblate fruits with an open calyx cavity. Stylianidis *et al.* (2000) recognized that boron deficiency could play a role in the appearance of oblate apples in the apple-producing provinces of West and Central Macedonia.

In the present work, the effects of various gibberellins, cytokinins and some of their commercial mixtures on fruit shape, and on nutrient content of leaves and fruit of apple cultivars belonging to the 'Red Delicious' group of clones were investigated. In order to check whether the overall physiological status of the trees has been affected by the various treatments, certain photosynthetic parameters (photosynthetic and transpirational rates, stomatal conductance, water use efficiency and internal CO₂ concentration) were also recorded in the experimental trees.

MATERIALS AND METHODS

The experiments were carried out on 'Royal Red Delicious', 'Imperial Double Red Delicious' and 'Red Chief' clones of the 'Red Delicious' group, in apple orchards of Northern Greece.

The treatments were the following:

1. Control
2. Spray with GA₃ (10 mg/l)
3. Spray with benzyladenine (BA) (50-300 mg/l)
4. Spray with kinetin (6-furfurylamino-purine) (50, 100 and 150 mg/l)
5. Spray with Promalin (GA₄/GA₇ 1.8% w/w + BA 1.8% w/w) (0.5, 1.0 and 1.5 ml/l)
6. Spray with Perlan (GA₄/GA₇ 19.2 g/l + BA 19.2 g/l) (0.5, 1.0 and 1.5 ml/l)
7. Spray with Novagib (GA₄/GA₇ 19.2 g/l) (0.5 ml/l) + kinetin (150 mg/l)
8. Spray with Promalin (0.5 ml/l) + GA₃ (10 mg/l)
9. Spray with Perlan (0.5 mg/l) + GA₃ (10 mg/l)

The sprays were done once at petal fall, in four consecutive years (1999, 2000, 2001 and 2002). The fruits were harvested according to the acceptable commercial harvesting criteria and the fresh weight and the height and diameter of mature fruit were determined. Quantitative determinations of nutrient elements in fruit and leaves were also made, the latter giving a reliable picture of the nutrient status of the whole tree (Faust, 1989). In 2000, measurements of photosynthetic and transpiration rates, water use efficiency, stomatal conductance and internal concentration of CO₂ were done in 40 leaves per treat-

ment from the median region of current growth shoots, three months after the treatment, with a portable Li-6200 instrument (Li-Cor, Lincoln, NE, USA).

Determinations of nutrient elements

Samples of leaves and fruits were washed first with tap and then with distilled water. They were then dried in an oven (Heraeus) at 68°C for 72 h and grounded to a fine powder. The Kjeldahl method was used for the determination of nitrogen, the phosphovanadomolybdenic ammonium method for phosphorus and the azomethine-H method for boron. The K, Ca, Mg, Fe, Mn and Zn contents were determined by means of spectrophotometric atomic absorption.

Statistical analysis

All experiments were arranged according to a randomized complete-block design with five replicates to each treatment. The replicates represented individual branches ('Royal Red Delicious' and 'Imperial Double Red Delicious') or the entire tree ('Red Chief'). Experimental data were subjected to ANOVA, and statistical differences were determined with the LSD test. The standard error of the means was calculated where appropriate. For the statistical analysis, the software StatView for Macintosh was used.

RESULTS

Benzyladenine (BA) resulted in an increase in the H/D ratio of 'Royal Red Delicious' fruit, which reached a value of 1.19 compared to 0.94 of the control (Table 1). Fruit shape of 'Imperial Double Red Delicious' was also improved by Promalin, and BA and less so by kinetin (Table 2). Perlan, Promalin, and kinetin alone or with Novagib, improved fruit

TABLE 1. The effect of gibberellin and benzyladenine on the ratio height/diameter (H/D) of apple fruits cv. Royal Red Delicious. Sprays were done at petal fall in 1999

Treatment	H/D
Control	0.94b
Gibberellin A ₃ 10 ppm	1.00ab
Benzyladenine 75 ppm	1.19a
Benzyladenine 150 ppm	1.12ab
Benzyladenine 300 ppm	1.07ab

TABLE 2. The effect of growth regulators on height/diameter (H/D) ratio and fresh weight of apples, cv. Imperial Double Red Delicious. Sprays were done at petal fall in 2000

Treatment	H/D	Fruit fresh weight (g)
Control	0.92cd	235ab
Gibberellin A ₃ 10 ppm	0.94bcd	223bc
Kinetin 50 ppm	0.92cd	193e
Kinetin 100 ppm	0.90d	213cd
Kinetin 150 ppm	0.97abcd	201de
Benzyladenine 50 ppm	0.96bcd	219bc
Benzyladenine 100 ppm	1.04ab	224bc
Benzyladenine 150 ppm	1.02abc	225bc
Promalin 0.5‰	0.98abcd	221bc
Promalin 1.0‰	1.00abcd	245a
Promalin 1.5‰	1.08a	214cd

TABLE 3. The effect of various growth regulators on the ratio height/diameter (H/D) and the fresh weight of apple fruits cv. Red Chief. The sprays were done at petal fall in 2001

Treatment	H/D	Fruit fresh weight (g)
Control	0.91b	202de
Gibberellin A ₃ 10 ppm	0.93b	220bc
Kinetin 150 ppm	0.97ab	228bc
Kinetin 200 ppm	0.99ab	245a
Perlan 0.5‰	1.07a	199e
Perlan 1.0‰	1.07a	227bc
Perlan 1.5‰	1.02ab	218bc
Novagib 0.5‰ + Kinetin 150 ppm	1.01ab	215cd
Promalin 0.5‰	1.02ab	233ab
Promalin 1.0‰	1.00ab	213cde
Promalin 1.5‰	1.05a	217cd

TABLE 4. The effect of growth regulators on photosynthetic parameters of apple trees cv. Red Chief. Sprays were done at petal fall in 2000

Treatment	Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2}\text{s}^{-1}$)	Transpiration rate ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$)	Water use efficiency ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}^{-1}$)	Stomatal conductance ($\text{mol m}^{-2}\text{s}^{-1}$)	Internal CO ₂ concentration ($\mu\text{L.L}^{-1}$)
Control	6.82c	2.56d	2.66a	85b	203a
GA ₃ 10 ppm	9.31a	3.79a	2.46b	130a	204a
Promalin 1.5‰	6.53d	2.99c	2.18c	96b	215a
Perlan 1.2‰	8.88b	3.41b	2.60a	122a	200a

shape in 'Red Chief' (Table 3). GA₃, kinetin, Perlan and Promalin caused an increase in fruit fresh weight. GA₃ and Perlan increased the photosynthetic and transpiration rates and stomatal conductance, but they did not affect the internal CO₂ concentration. On the other hand, GA₃ and Promalin decreased the water use efficiency (Table 4).

The effect of the growth regulators on inorganic nutrient content varied between leaves and fruit and among treatments in the same clone and year to year. Perlan, Promalin and kinetin, which improved fruit shape of 'Red Chief', also caused an increase in the K, and only kinetin, the Ca content of fruit in 2002. GA₃, which did not improve fruit shape, caused an increase in the K content (Table 5, data for 2000 and 2001 were similar and therefore not shown). The Ca content of leaves was increased by

some treatments and decreased by others. A decrease in leaf K content of 'Red Chief' was observed in all treatments in 2000, in some of them in 2002, while it remained unaffected in 2001. Boron was significantly increased by GA₃ and not significantly by all other chemicals (Table 6, data for 2000 and 2001 are not shown).

All treatments raised the level of Ca and decreased K, Fe, B in the leaves of 'Imperial Double Red Delicious' in 2000. The N and Mn contents were also decreased in most treatments (Table 7). The Fe and, in most cases, the Zn content of fruit increased, while the content of B decreased (Table 8, data for 2001 and 2002 were similar and not shown).

GA₃, Promalin and to a lesser extend Perlan, exerted a negative effect on next year's flowering, while kinetin had no effect.

TABLE 5. The effect of growth regulators on inorganic element content in apple fruit, cv. Red Chief. Sprays were done at petal fall in 2002

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	B
	% d.wt.					ppm			
Control	0.17ab	0.07a	0.49c	0.05ab	0.04a	11a	1b	1b	15b
Gibberellin A ₃ 10 ppm	0.20a	0.05a	0.61a	0.06ab	0.04a	10ab	1b	2a	23a
Perlan 0,5‰	0.20a	0.05a	0.59ab	0.05ab	0.04a	8abc	2a	2a	16b
Perlan + GA ₃	0.15b	0.05a	0.53bc	0.05ab	0.04a	10ab	1b	1b	18ab
Promalin 0,5‰	0.19ab	0.08a	0.58ab	0.05ab	0.03a	7bc	1b	1b	18ab
Promalin + GA ₃	0.20a	0.05a	0.57ab	0.04b	0.04a	6c	2a	1b	18ab
Kinetin 200 ppm	0.19ab	0.06a	0.59ab	0.07a	0.05a	11a	2a	1b	19ab
Kinetin + GA ₃	0.19ab	0.05a	0.53bc	0.04b	0.04a	8abc	1b	1b	19ab

TABLE 6. The effect of growth regulators on inorganic element content in leaves of apple trees, cv. Red Chief. Sprays were done at petal fall in 2002

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	B
	% d.wt.					ppm			
Control	2.22ab	0.25a	1.07a	1.39cd	0.18a	107b	51bc	13a	66ab
Gibberellin A ₃ 10 ppm	2.31ab	0.24a	1.11a	1.29de	0.17a	123ab	68a	14a	78a
Perlan 0,5‰	2.35a	0.27a	0.97b	1.24e	0.18a	120b	48c	13a	67ab
Perlan + GA ₃	2.22ab	0.28a	0.85c	1.38cd	0.19a	115b	53βc	14a	67ab
Promalin 0,5‰	2.29ab	0.28a	1.13a	1.46bc	0.18a	114b	63ab	15a	75ab
Promalin + GA ₃	2.34a	0.26a	0.86c	1.84a	0.22a	137a	69a	14a	64b
Kinetin 200 ppm	2.31ab	0.24a	0.84c	1.53b	0.19a	111b	55bc	12a	68ab
Kinetin + GA ₃	2.18b	0.28a	0.96b	1.46bc	0.18a	113b	57abc	13a	74ab

TABLE 7. The effect of growth regulators on inorganic element content in leaves of apple trees, cv. Imperial Double Red Delicious. Sprays were done at petal fall in 2000

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	B
	% d.wt.					ppm			
Control	2.40bc	0.25a	1.12a	1.72e	0.30a	150a	29b	25ab	50a
Gibberellin A ₃ 10 ppm	2.30cd	0.19b	0.97cd	2.55a	0.40a	109cd	22de	27a	38cd
Gibberellin A ₃ 50 ppm	2.40bc	0.21ab	1.17a	2.10bcd	0.33a	112c	26bcd	22bc	36de
Kinetin 100 ppm	2.32bcd	0.22ab	1.02bc	2.17bc	0.34a	111c	27bc	21bc	38cd
Kinetin 150 ppm	2.09e	0.22ab	0.67f	2.13bcd	0.34a	101de	40a	21bc	32e
Benzyladenine 50 ppm	2.23d	0.23ab	0.87de	2.22b	0.36a	113c	23cde	23abc	36de
Benzyladenine 100 ppm	2.11e	0.25a	0.78e	2.00d	0.37a	124b	26bcd	22bc	44b
Benzyladenine 150 ppm	2.33bcd	0.23ab	0.91d	2.05cd	0.35a	127b	23cde	23abc	49a
Promalin 0,5‰	2.38bc	0.23ab	1.08ab	2.24b	0.39a	98ef	26bcd	25ab	44b
Promalin 1,0‰	2.42ab	0.21ab	0.97cd	1.99d	0.33a	91f	21e	22bc	42bc
Promalin 1,5‰	2.51a	0.19b	0.97cd	1.83a	0.32a	96ef	19e	19c	38cd

TABLE 8. The effect of growth regulators on inorganic element content in apple fruit, cv. Imperial Double Red Delicious. Sprays were done at petal fall in 2000

Treatment	N	P	K	Ca	Mg	Fe	Mn	Zn	B
Control	0.24a	0.09a	0.61ab	0.06a	0.04a	18fg	2b	4ef	18ab
Gibberellin A ₃ 10 ppm	0.21a	0.08a	0.58ab	0.06a	0.04a	15gh	2b	3f	21a
Gibberellin A ₃ 50 ppm	0.24a	0.08a	0.63a	0.07a	0.05a	30c	3b	5de	14bcde
Kinetin 100 ppm	0.21a	0.08a	0.58ab	0.04a	0.05a	23de	2b	6cd	13cde
Kinetin 150 ppm	0.23a	0.08a	0.58ab	0.05a	0.04a	20ef	2b	7bc	14bcde
Benzyladenine 50 ppm	0.25a	0.06a	0.55ab	0.05a	0.04a	26d	2b	9a	12de
Benzyladenine 100 ppm	0.21a	0.08a	0.56ab	0.06a	0.04a	41a	1b	8ab	11e
Benzyladenine 150 ppm	0.24a	0.08a	0.51b	0.07a	0.05a	35b	2b	9a	14bcde
Promalin 0,5‰	0.24a	0.09a	0.61ab	0.06a	0.04a	7j	2b	5de	16bcd
Promalin 1,0‰	0.20a	0.09a	0.57ab	0.06a	0.04a	13hi	2b	4ef	17abc
Promalin 1,5‰	0.27a	0.09a	0.51b	0.07a	0.04a	10ij	1c	5de	14bcde

DISCUSSION

Promalin, Perlant, and kinetin alone and in combination with Novagib, increased the fresh weight of apples and improved their shape by increasing the H/D ratio in 'Red Chief'. An increase in the H/D ratio has also been observed in 'Golden Delicious' and 'Jonagold' by a combination of GA₃ and the synthetic cytokinin CPPU (Bangerth, 1994). Kinetin alone gave similar results in the first year of its application (Stylianidis *et al.*, 2000). On the contrary, GA₃ alone had no effect on fruit shape. Promalin and BA caused a significant improvement in apple shape of 'Imperial Double Red Delicious', while kinetin did the same but to a lesser extent. A similar increase in H/D ratio, caused by Promalin has also been observed in 'Starking Delicious' (Unrath, 1974, Curray & Williams, 1983, Burak & Buyukyilmaz, 1997). BA had also a beneficial effect on 'Royal Red Delicious'. Generally, BA was more effective than kinetin in improving fruit shape in all cultivars tested.

According to Faust (1989), the nutrient status of leaves affects the levels of nutrients in fruits and the opposite. In our experiments, the effects of various chemicals on inorganic nutrient content varied between leaves and fruits in the same cultivar, although great differences between treatments and controls were not observed. Wherever fluctuations in the nutrient content were detected, these concerned mainly changes in K, Ca and B. Although it has been reported that elements like N, Ca, K and B affect fruit shape (Bergmann, 1992), no correlation could be established between fruit shape and fruit nutrient

status. This observation was corroborated by the fact that although GA₃ increased Ca, K and B contents in fruit, it did not affect their shape. An increase in Ca, caused by GA₃ has been also reported by Sass (1993). This has been further supported by Elfving & Cline (1993) who observed variations in the nutrient content of 'Empire' apples from year to year, (after treatment with benzyladenine), but no correlation between fruit size and nutrient status have been established.

Reduced flower differentiation and fruit set the year after GA₃ application have been observed in 'Starking' and 'Golden Delicious' (Buban *et al.*, 1993). In our experiments, GA₃, Promalin, and Perlant, negatively affected next year's flowering, the latter two probably due to the GA they contained. On the other hand, kinetin did not exert any detrimental effect on flowering. CPPU, with a cytokinin-like action, also did not affect flowering in certain apple cultivars (Basak, 1997). From this point of view, whenever the need of chemically regulating apple fruit shape arises, synthetic cytokinins are to be preferred, since they lack any adverse side effect on return bloom.

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