Europ.J.Hort.Sci., **68** (3). S. 123–128, 2003, ISSN 1611-4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Prohexadione-Ca Positively Affects Gas Exchanges and Chlorophyll Content of Apple and Pear Trees

Sabatini, E., M. Noferini, G. Fiori, L. Corelli Grappadelli and G. Costa
(Dipartimento di Colture Arboree, University of Bologna, Bologna, Italy)

Summary

Prohexadione-Ca is a new generation growth retardant which inhibits dioxygenase enzymes activity mimicking 2-oxoglutarate and affecting both gibberellin and phenolic compounds metabolism. Several reports regarding the use of this growth retardant in apple and pear show the positive effects of prohexadione-Ca on yield and average fruit weight. On the contrary only a few trials have been performed regarding the effect of prohexadione-Ca on tree photosynthesis. This paper reports studies carried out over four-years on apple and pear to evaluate the photosynthetic efficiency of trees treated with prohexadione-Ca: whole-canopy experiments were performed on apple field trees, and leaf measurements on potted apple scions and field pear trees. Prohexadione-Ca positively affected leaf mass area and clorophyll content; net photosynthesis was often increased after application with the exception of a late spring application on vigorously growing trees.

From these studies the positive influence of prohexadione-Ca on fruit weight and yield might be attributed to improved ecophysiological efficiency.

Zusammenfassung

Positiver Einfluss von Prohexadione-Ca auf Gaswechsel und Chlorophyll-Gehalt bei Apfel- und Birnenbäumen. Prohexadion-Ca ist ein Wachstumsregulator der neuen Generation, der die Enzymaktivität von Dioxygenasen durch Imitieren von 2-Oxoglutarat hemmt und sowohl den Gibberellin- als auch den Phenol-Stoffwechsel beeinflusst. Zahlreiche Untersuchungen zur Anwendung des Wachstumhemmers zeigten bei Äpfeln und Birnen einen positiven Einfluss von Prohexadion-Ca auf Erntemenge und durchschnittliches Fruchtgewicht. Nur wenige Versuche befassten sich mit der Wirkung von Prohexadion-Ca auf die Photosynthese der Bäume. Die hier vorgestellte Arbeit umfasst einen Zeitraum von vier Jahren, in dem mittels Kronen-Untersuchungen an ausgepflanzten Apfelbäumen sowie Blattmessungen an getopften Apfelklonen und ausgepflanzten Birnenbäumen, die Photosyntheseleistung von mit Prohexadion-Ca behandelten Apfel- und Birnbäumen untersucht wurde. Dabei zeigte sich, dass Prohexadion-Ca eine positive Wirkung auf die Größe der Blattfläche und den Chlorophyll-Gehalt hat. Die Nettophotosyntheserate steigerte sich häufig nach der Anwendung; eine Ausnahme stellte hierbei die Anwendung im späten Frühjahr an starkwüchsigen Bäumen dar.

Unsere Untersuchungen zeigen, dass die positive Wirkung von Prohexadion-Ca auf Fruchtgewicht und Erntemenge auf eine verbesserte ökophysiologische Leistungsfähigkeit zurückzuführen sein dürfte.

Key words. whole canopy photosynthesis – dioxygenase inhibitors – prohexadione-Ca – chlorophyll – NIR – SPAD

Introduction

Crop yield depends on many factors, including photosynthetic efficiency and carbohydrate partitioning within the plant. The effects of growth regulators on such parameters have been studied since the first molecules became available on the market, as commercially available tools to manipulate both growth and photosynthate partitioning.

Since the '80s, several contributions have shown growth regulator effects on photosynthetic activity, carbon partitioning in the different plant organs, dry weight accumulation and leaf area development (DE-JONG and DOYLE 1984). For example, one of the most popular plant growth regulators (PGR) at that time (paclobutrazol) was shown to influence the gas exchanges and carbohydrate partitioning. In apple, it was found that paclobutrazol increases water use efficiency, via an enhancement of net photosynthesis (Pn) without changes in stomatal opening or transpiration. This response can be explained only by higher mesophyll conductance to CO_2 (CATSKY and TICHA 1982).

Prohexadione-Ca is a new generation growth retardant, which inhibits dioxygenase enzymes activity, as a mimic of 2-oxoglutarate (WINKLER 1997; RADEMACH-

ER 2000), thus affecting both gibberellin and phenolic compounds metabolism (ROEMMELT et al. 1999). This includes a defence mechanism against several fruit pests, including fire blight and scab (RADEMACHER et al. 1999; COSTA et al. 2001a, b). Several reports for apple and pear, (EVANS et al. 1997; BOMBEN et al. 1998; COSTA et al. 2001a, b), show the positive effects of prohexadione-Ca on yield and average fruit weight. Prohexadione-Ca has also been reported as capable of increasing total non-structural carbohydrates (TNC), nitrogen accumulation and stimulating stomatal opening. This latter parameter is normally associated with large carbon assimilation (GUAK et al. 2001).

This paper reports four-year studies, on apple and pear effects of prohexadione-Ca on single leaf and whole tree gas exchanges, and on chlorophyll and dry matter content of the leaves. Our goal was to evaluate, if the positive influence of prohexadione-Ca on fruit weight and yield (EVANS et al. 1997; BOMBEN et al. 1998; COSTA et al. 2001a, b) could be attributed to improved ecophysiological efficiency.

Materials and Methods

Leaf photosynthesis and chlorophyll content

Leaf photosynthesis was measured in 1999 with a portable porometer type CIRAS-1 (PP Systems, Hitchin, UK) on fully-grown 'Abbé Fétel'/'Beurré Hardy'/'Quince C' trees. The experimental design was 60 completely randomised trees, replicated 6 times with ten trees per treatment. Trees were treated with concentrations of prohexadione-Ca ranging from 200 to 375 ppm, distributed in several applications as reported in Table 1.

- J_{max} (μmol e^- m^{-2} s^{-1}): maximum activity of the electron transport chain;
- Vc_{max} (μmol CO_2 m^{-2} s^{-1}): *in vivo* Rubisco activity;
- Leaf mass per area;
- Chlorophyll content.

Whole canopy photosynthesis

Except for 2000, when a simpler, less automated system was used, whole canopy determinations were carried out using a system capable of simultaneously and automatically monitoring up to six trees over extended periods of time. Both gas exchange systems, however, operated as open systems, following the general outlay described by CORELLI GRAPPADELLI and MAGNANINI (1993, 1997).

In 2000, the gas exchanges of six two-year-old, potted 'Golden Delicious'/M9 apple scions were recorded for two days before the prohexadione-Ca application. Subsequently, four apple trees were treated with either 250 ppm or 500 ppm prohexadione-Ca. Two apple trees served as controls. Data were collected four times per day, during the central hours (from 10:00 to 16:00). The experiment was terminated 15 days after the prohexadione-Ca application.

In 2001, a late season application (15 September 2001) of 250 ppm prohexadione-Ca was made on two-year-old apple trees, 'Mondial Gala'. Before the prohexadione-Ca application, tree gas exchanges were monitored for one week. The measurements were continued up to 45 days after the spray. At the end of the experiment, chlorophyll and dry matter content of the leaves were determined.

In 2002, a similar study was initiated on six apple trees, monitoring the trees' gas exchanges for three days prior to the prohexadione-Ca treatment. Three days later, the trees were sprayed with 250 ppm; a second application at the same concentration was repeated after 10 more days. Four unsprayed trees served as controls. At the end of the experiment, ten leaves per tree were collected from current growth shoots, 6–7 nodes below the shoot tip for chlorophyll and dry matter determinations. The tree leaf area was estimated by means of allometric relationships between shoot diameter and its leaf area.

Table 1. Prohexadione-Ca treatments performed in 1999 on 'Abbé Fétel' pear trees.

Treatments in 1999	1st application	2nd application	3rd application	4th application
ProCa 50 ppm x 4	28/4	6/5	18/5	31/5
ProCa 125 ppm x 3	30/3	23/4	13/5	n. a.

n. a. – not applied

In early July and mid-August, leaf samples were collected for chlorophyll determination; the second sample was collected after a new flush of growth occurred. At the same time, shoot length, number of nodes, number of leaves per node, leaf area were measured. Chlorophyll content was assessed either destructively, via a spectrophotometric approach (PORRA et al. 1989), and non-destructively, by a Near Infrared Spectrometer (NIRs) and by a SPAD-502 Clorophyll Meter (Minolta) (PORRO et al. 2001).

A/C_i curves

Ten leaves/tree were selected from current growth shoots, 6–7 nodes below the shoot tip on three-year-old apple trees, which had been sprayed with 250 ppm prohexadione-Ca, and from untreated trees. On these leaves, A/C_i curves were calculated according to Farquar et al. (1980), yielding the following parameters:

Results

Leaf photosynthesis and chlorophyll

Prohexadione-Ca increased chlorophyll content in leaves of current growth shoots on a fresh mass basis (Table 2) in both July and August, irrespective of the dose-applied, except for a higher content in the

Table 2. Chlorophyll content of pear leaves in control and prohexadione-Ca treated leaves, as determined by three different methods.

Treatment in 1999	Chlorophyll *a* and *b* (µg/g)		Relative SPAD units		NIRs (µg/g)	
	July	August	July	August	July	August
Prohexadione-Ca 200 ppm	260 a	491 a	45 a	52 a	260 a	409 a
Prohexadione-Ca 375 ppm	180 b	494 a	43 a	51 a	218 b	412 a
Control	127 c	335 b	36 b	49 a	140 c	312 b

Means with the same letter are not statistically different (Duncan's test, p= 0,05); statistical comparison is performed for each single measurements date.

200 ppm treatment in July. While the trends are the same irrespective of the analytical methods, the SPAD seems to be the least sensitive, as it did not distinguish between the two treatments in July, and between the treatments and the control in August, while other the chemical analysis and the NIRs measurements were in very good agreement .

Net photosynthesis was larger in the prohexadione-Ca treated apple leaves: on both date, CO_2 uptake was 50 % larger than in the control irrespective of the concentration of prohexadione-Ca applied (Fig. 1).

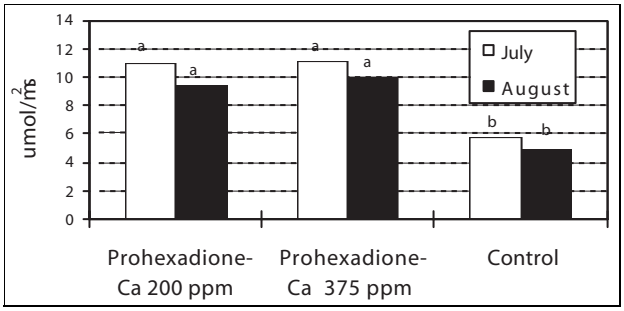


Fig. 1 Photosynthesis measured on control and prohexadione-Ca treated 'Abbé Fétel' trees, in 1999. (Means with the same letter are not statistically different (Duncan's test, p= 0,05)

A/C$_i$ curves

Ten days after the prohexadione-Ca treatment, treated apple leaves of current shoots contained more chlorophyll per leaf area than control ones. Leaf mass per area and J_{max} were higher in the treated leaves. J_{max} was, as above, significantly higher even when expressed per unit of leaf mass per area. This indicates that this increase is not only a consequence of a morphological changes, (i.e., thicker leaves, but of a physiological one as well (Table 3). Vc_{max} was higher in the prohexadione-Ca-treated leaves, but this difference disappeared, when expressed on a leaf mass per area (LMA) basis (Table 3).

Whole Canopy CO_2 Assimilation Measurements

Both applications of prohexadione-Ca, 250 and 500 ppm, to potted apple trees (2000) increased net canopy carbon exchange rate. The control tree's gas exchange remained constant over the time of the experiment: 250 ppm prohexadione-Ca doubled canopy photosynthesis, while 500 ppm prohexadione-Ca increased it by about 40 % (Fig. 2).

Similar data were obtained in the late application trial in September 2001, when the daily canopy net carbon exchange was also increased after prohexadione-Ca application. Interestingly, this increase was recorded mainly in the hours following peak photosynthesis, when the rates of the treated tree remained higher than those of the control tree (Fig. 3). In our experience, during the afternoon, stomatal conductance of untreated trees tends to decrease and this might be an indication that prohexadione-Ca cause maintenance of larger stomatal opening.

In the early application trial (2002), the results were quite different from the 2 years before (Table 4). Whole canopy net carbon exchange rates were higher in treated than in control trees before prohexadione-Ca application, and the difference remained after the treatment, therefore no influence of prohexadione-Ca can be assumed. Water use efficiency (WUE) was positively affected by prohexadione-Ca, with higher values ten days after treatment, whereas all control trees showed an opposite tendency. This effect is due to increased transpiration in the controls 10 days after treatment (DAT), whereas the treated trees maintained constant transpiration, or even reduced it.

The lack of prohexadione-Ca effects on net photosynthesis can also be observed in Fig. 4, which clearly shows that the treated tree reached higher rates than the

Table 3. Biochemical parameters described in control and treated leaves.

Treatment	µg chl cm^{-2}	LMA	J_{max} µmol CO_2 m^{-2} s^{-1}	J_{max}/LMA µmol CO_2 g^{-1} s^{-1}	Vc_m µmol CO_2 m^{-2} s^{-1}	Vc_{max}/LMA µmol CO_2 g^{-1} s^{-1}
Control	46.8	14.0	221	15.9	113	8.1
Prohexadione-Ca	56.8	16.5	299	18.2	132	8.0
Statistics	**	**	**	*	*	NS

**: significantly different for p<0.01; *: significantly different for p< 0.05; NS: not statistically different.

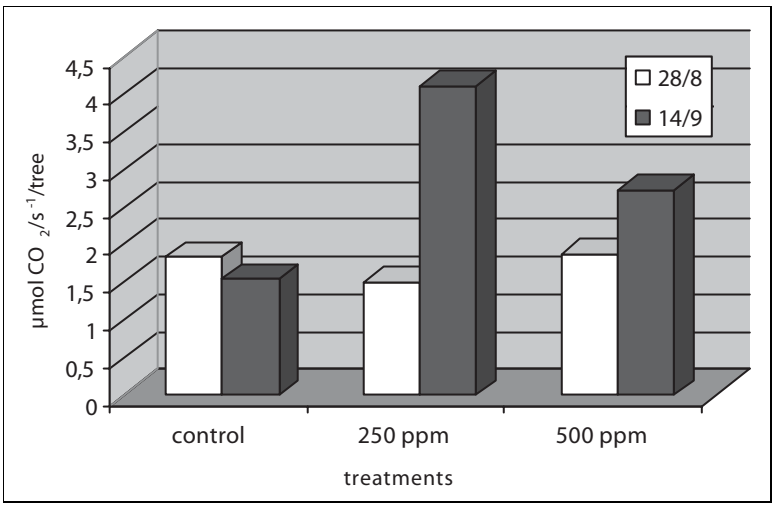


Fig. 2 Daily averages of net photosynthesis in treated and untreated apple scions immediately before prohexadione-Ca application (28/8/00) and 15 days after (14/9/00).

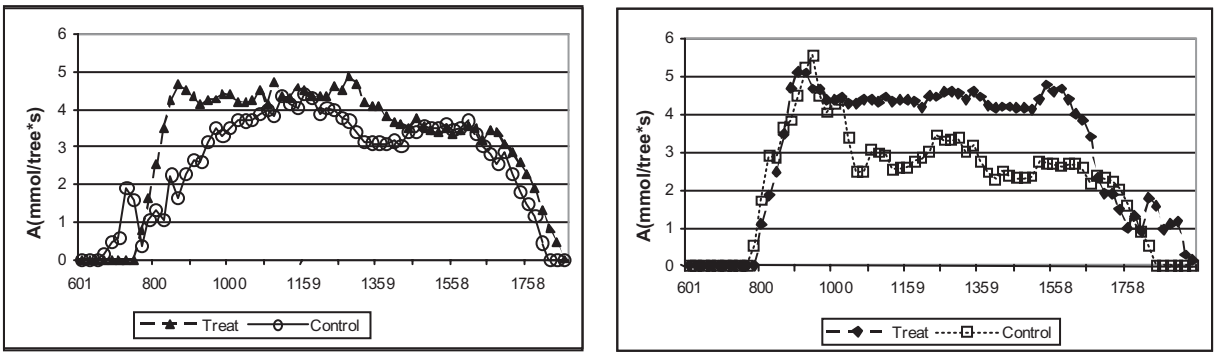


Fig. 3 The daily response of photosynthesis to a late application of prohexadione-Ca.

control before the prohexadione-Ca application. The same difference was recorded 20 days after the application, which suggests that this difference is probably due to a random variation between the days reported.

Discussion

The 2000 whole canopy experiment showed a positive influence of the growth regulator on the gas exchange. Photosynthesis was more than doubled in the 250 ppm treatment and the effect was long-lasting, as it remained apparent at least until the end of the experiments, i.e. 15 days after prohexadione-Ca application. These results were contradicted, at least in part, by those obtained in 2002, at the whole canopy level: here, no differences appeared. This lack of response in 2002 might be due to active growth of the young trees during this part of the season: this growth, witnessed by the similar number of nodes in both control and treated trees, may have masked the positive effect of the growth regulator in 2002. In response, the control trees may have been photosynthesising at high rates to meet the internal growth demand for carbon. Transpiration rate was also

Table 4. Data measured at 11 a.m. with the whole canopy gas exchange system (2002), before and 10 days after prohexadione-Ca treatment, in the six trees used.

DAT	Chamber/ Treatment	PAR* µmol m^{-2} s^{-1}	T °C (outside)	D (Kpa)	A µmol CO_2 m^{-2} s^{-1}	E mmol H_2O m^{-2} s^{-1}	WUE	G_{CO2} mmol m^{-2} s^{-1}	A acc (g)
0	1/contr.	1285	30.48	0.98	4.28	0.49	9.54	226.5	2.66
	2/contr				5.79	0.58	9.99	375	3.3
	3/ProCa				8.18	1.51	5.42	457	4.86
	4/ProCa				8.44	1.61	5.23	571	5.76
+ 10	1/contr.	1024	30.2	1.15	7.66	1.18	6.46	524.5	4.33
	2/contr				6.7	0.88	7.63	527.5	3.68
	3/ProCa				9.26	0.95	9.72	424	4.49
	4/ProCa				11.1	1.67	6.62	510.2	5.64

PAR*: photosynthetic active radiation (reduced considering the attenuation of sunlight due to the plastic chamber); D: vapour pressure deficit in the atmosphere; A: CO_2 net assimilation; E: transpiration; G_{CO2}: total conductance to CO_2 exchange; A acc: organic carbon accumulated.

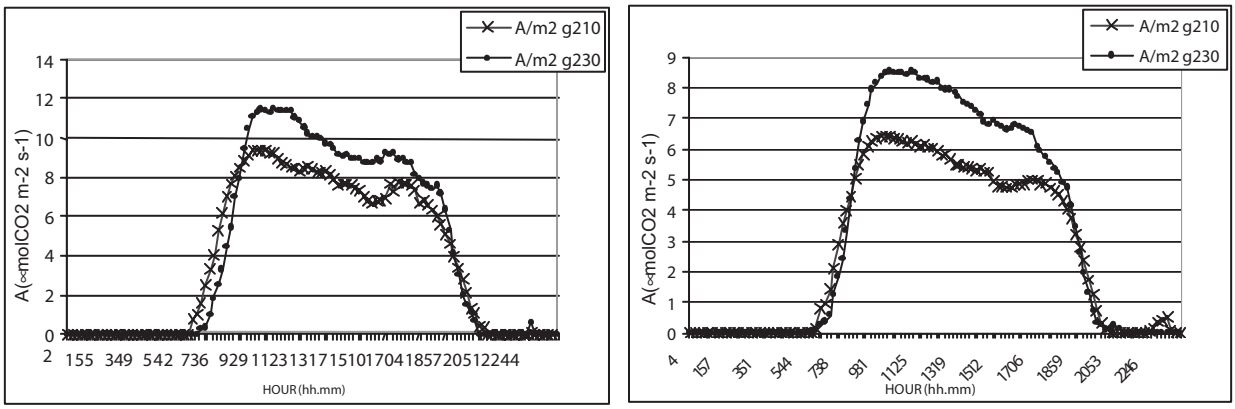


Fig. 4 Net photosynthesis during the whole day before treatment (0 DAT=DOY 210) and 20 DAT (DOY 230) in a prohexadione-Ca treated plant (left) and a control plant (right).

affected, in agreement with the results of GUAK et al. (2001), who measured a decrease in stomatal conductance in treated plants in the central hours of the day.

Moreover, the A/C_i curves showed that both J_{max} and V_{max} were positively affected, an indication that prohexadione-Ca may influence both electron transport and carboxylation efficiency, although in a different way. The higher V_{max} values might in fact simply be due to a greater number of chloroplasts, since the difference disappeared, if expressed on LMA basis. The J_{max} value, on the other hand, remained statistically significant even if expressed in these terms. LMA and total chlorophyll content per leaf area of the treated leaves were significantly higher. This difference however, disappeared when chlorophyll content was expressed in terms of fresh and dry weight, thus showing that the higher chlorophyll content is most probably a consequence of an increase in palisade parenchyma and leaf thickness.

Conclusions

In conclusion, positive results on photosynthetic properties of the leaves have been recorded in most of our studies, with the exception of the 2002 whole canopy trial. Prohexadione-Ca appears in general as capable of promoting leaf maturation and the attainment of greater photosynthetic capacity. When this does not occur, as may have been the case in the 2002 whole canopy experiment, these positive effects may not appear. In the 2002 study, prohexadione-Ca, in fact, reduced internode length, but it did not stop shoot growth, in comparison with the control (data not shown). Typically, under our conditions, the spring growth flush lasts well into July.

Further work is needed to investigate a putative detrimental effect of prohexadione-Ca on respiration. This hypothesis is supported by the findings of HECKMAN et al. (2002), who showed that 2-oxoglutarate analogues are able to block the respiratory electron transport chain, by binding to the ubiquinone active site. Moreover, 2-oxoglutaric acid is involved in the tricarboxylic acid cycle, where it is dehydrogenated and decarboxylated to succinyl-CoA, with the formation of NADH. MILLAR et al. (1999) purified and characterised an enzymatic complex responsible for these reactions in *Solanum tuberosum* mitochondria. Furthermore, MAJAMAA et al. (1985) measured an inhibition of this enzyme using 2-oxoglutarate analogues; therefore higher CO_2 assimilation measured after prohexadione-Ca treatment could possibly be also related to a decrease in respiratory losses, which would result in higher net photosynthesis.

Acknowledgements

These investigations were supported by the European Commission (QLK5-CT-1999-01583).

References

BOMBEN, C., G. VIZZOTTO and G. COSTA 1997: Controllo della crescita vegetativa nel melo: una nuova molecola, il Prohexadione-Calcio. IV Giornate Scientifiche SOI, 1–3 Aprile, San Remo, 189–190.

CATSKY, J. and I. TICHA 1982: Photosynthetic characteristic during ontogenesis of leaves. Intracellular conductance and its components. Photosynthetica **16**, 253–284.

CORELLI GRAPPADELLI, L. and E. MAGNANINI 1993: A whole-tree sytem for gas-exchange studies. HortScience **28**, 41–45.

CORELLI GRAPPADELLI, L. and E. MAGNANINI 1997: A whole-tree gas-exchanges: can we do cheaper? Acta Hort. **451**, 279–285.

COSTA, G., C. ANDREOTTI, F. BUCCHI, E. SABATINI, C. BAZZI and S. MALAGUTI 2001a: Prohexadione-Ca (Apogee): growth regulation and reduced fire blight incidence in pear. HortScience **36**, 931–933.

COSTA, G., F. SPINELLI, E. SABATINI and W. RADEMACHER 2001b: Incidence of Scab (*Venturia inaequalis*) in apple as affected by different plant bioregulators. 9th International Symposium on Bioregulators in Fruit Production , August 19–22, 2001. Seoul, Korea.

DE JONG, T. M. and J. F. DOYLE 1984: Leaf gas exchange and growth responses of mature 'Fantasia' nectarine trees to paclobutrazol. J. Amer. Soc. Hort. Sci. **109**, 878–882.

EVANS, R. R., J. EVANS and W. RADEMACHER 1997: Prohexadione-Calcium for suppression of vegetative

growth in eastern apples. Acta Horticulturae **451**, 663–666.

FARQUAR, G. D., S. Von CAEMMERER and J. A. BERRY 1980: A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149**, 78–90.

GUAK, S., D. NEILSEN and N. E. LOONEY 2001a: Growth, allocation of N and carbohydrates, and stomatal conductance of greenhouse grown apple treated with prohexadione-Ca and gibberellins. Journal of Horticultural Science and Biotechnology **76**, 746–752.

HECKMAN, N. L., T. E. ELTHON, G. L. HORST and R. E. GAUSSOIN 2002: Influence of trinexapac-ethyl on respiration of isolated wheat mitochondria. Crop Science **42**, 423–427.

MAJAMAA, K., T. M. TURPEENNIEMI-HUJANEN, P. LATIPAA, V. GUNZLER, H. M. HANAUSKE-ABEL, I. E. HASSINEN and K.I. KIVIRIKKO 1985: Differences between collagen hydroxylases and 2-oxoglutarate dehydrogenase in their inhibition by structural analogues of 2-oxoglutarate. Biochem. J. **229**, 127–133.

MILLAR, A. H., S. A. HILL and C. J. LEAVER 1999: Plant mithocondrial 2-oxoglutarate dehydrogenase complex: purification and characterisation in potato. Biochem. J. **343,** 327–334.

PORRA, R. J., W. A. THOMPSON and P. E. KRIEDEMANN 1989: Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochem. Biophys. Acta **975**, 384–394.

PORRO, D., C. DORIGATTI, M. STEFANINI and A. CESCHINI 2001: Use of SPAD Meter in diagnosis of nutritional status in apple and grapevine. Acta Hort. **564**, 243–252.

RADEMACHER, W., G. E. STAMMLER and P. CREEMERS 1999: Prohexadione-Ca: effects against apple scab (*Venturia inaequalis*). 96th Int. Conference of ASHS, Minneapolis, 28–31 July, Abstract **223**, 480.

RADEMACHER, W. 2000: Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. Annual Review of Plant Molecular Biology Vol.**51**.

ROEMMELT, S., D. TREUTTER, J. B. SPEAKMAN and W. RADEMACHER 1999: Effects of prohexadione-Ca on the flavonoid metabolism of apple with respect to plant resistance against fire blight. Acta Hort. **489**, 359–363.

WINKLER, V. W. 1997: Reduced risk concept for prohexadione calcium, a vegetative growth control plant growth regulator in apples. Acta Horticulturae **451**, 667–671.

Received May 19, 2003 / Accepted June 13, 2003

Addresses of authors: Sabatini E., M. Noferini, G. Fiori, L. Corelli Grappadelli and G. Costa, Dipartimento di Colture Arboree, University of Bologna, 40126 Bologna, Italy, e-mail: gcosta@agrsci.unibo.it.