Morphological changes in the apex of *Prunus persica* L. during floral transition and effects of gibberellin on flower bud differentiation

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Abstract

The aim of the research was to study the morphological and histochemical evolution of the bud meristems of 'Lavinia' nectarine cultivar. Moreover, the effectiveness of Release LC (a gibberellin chemical compound) in controlling the rate of flower bud differentiation was also evaluated. During a two-year period, the Release LC was applied in postharvest to avoid problems of possible chemical residues on marketable fruits. To determine the effect of treatment, several biological parameters such as initial flower and vegetative bud number, flower bud drop, evolution of the flower bud phenological stages, rate of bloom and fruit set were recorded. To establish the floral differentiation stage, the meristematic apices were collected before and after treatment and microscopically observed. The thin sections were analysed using histological (apex size, developmental stages of meristematic apex, co-axial stage), and histochemical (RNA fluorescent staining) techniques. In 'Lavinia' cv., the critical phase of the meristematic apex evolution occurred from May to June (60 and 90 days after full bloom): the presence of triple apices increased rapidly, the co-axial phase was achieved, the width and height of the meristematic dome increased markedly and the RNA appeared by a weak staining. As regards the flower bud differentiation control by exogenous treatments with Release LC, the different results obtained in our experiments indicate that the efficacy of treatment strictly depends on the growth stage of a meristematic apex.

Key words: Chemical thinning, flower bud differentiation, gibberellins, meristematic apex morphology, nectarine

Introduction

In many fruit species, the control of the crop load is an unavoidable operation in a modern orchard management system, to optimise the size and quality of fruits, prevents alternate bearing and balances the fruit-to-shoot ratio (Costa and Vizzotto, 2000). Thinning must be often performed every year and the hand thinning of fruitlets is the technique that ensures the best results, although the costs are very high and it takes between 100 to 500 h ha^{-1} depending on vigour and flower production, thinning intensity and season (Jimenez and Diaz, 2002). Consequently, more cheaper alternative means are still under evaluation. Chemical and bioregulator substances (Carbaryl, NAA, GAs and 6-BAP) are effective as fruit thinners in apple (Dennis, 2000; Fallahi and Willemsen, 2002), while no satisfactory results have been achieved in peach and nectarine (Southwick *et al.,* 1996; Costa and Vizzotto, 2000; Garcia-Pallas *et al.*, 2001). Chemical fruit thinning could create an uneven distribution of fruits in trees or preferentially remove large fruit at the expense of smaller fruit (Southwick *et al.*, 1996). Indirect thinning methods were applied for inhibiting flowering intensity by chemical applications during flower bud induction and, thus, reducing costs of hand thinning. Gibberellin seems able to reduce the number of potential floral buds, inhibiting the transition of meristematic apices from the vegetative to floral stage, when applied before floral differentiation (Oliveira and Browning, 1993; Gonzales-Rossia *et al.,* 2006). Investigations on *Prunus persica* [L.] Batsch., have indicated that GA treatments from 0 to 47 days after full bloom (Byers *et al.*, 1990) and from mid-June to early July (Southwick *et al.*, 1996) inhibit flower

bud differentiation, reducing the subsequent crop load. However, conflicting results didn't allow generalising on application time and chemical concentration for the same cultivar (Taylor and Geisler-Taylor, 1998, Garcia-Pallas *et al.*, 2001). In addition, fruit load regulation through the chemical control of flower bud differentiation may increase the risk of total crop loss caused by accidental events (*i.e.* spring frost injury), which can occur during the ontogenetic cycle. Favourable responses have been shown in areas generally without adverse winter and spring weather stress (Southwick *et al.*, 1995).

Better understanding of chemical thinning action mechanism could be helpful to explore the evolution of a meristematic apex under morphological and physiological aspects. These aspects may be studied using classical histological techniques and by advanced cytochemical methods. In particular, cytochemical studies showed a relationship between the floral transition process (evocation) and RNA synthesis. During the evocation phase, responding to the floral stimulus, flowering genes are de-repressed and alternatively genes responsible for vegetative patterns of morphogenesis are eliminated (Evans, 1971). The new genetic order in the apical meristem leads to an increase in the RNA content, which is considered one of the earliest indicators of the evocation process (Bernier *et al.*, 1981; Buban and Faust, 1982) and involved in the transition from vegetative to reproductive phase (Wada *et al.*, 2002).

The aim of our research was to study the morphological and histochemical evolution of the meristematic apices and the effectiveness of Release LC (a gibberellin chemical compound)

in controlling the rate of flower bud differentiation on a nectarine cultivar. We chose a postharvest treatment to avoid problems of possible chemical residues on marketable fruits, considering the currently increasing pressure from consumers for fruits and vegetables cultivated according to low environmental impact systems.

Materials and methods

Plant material and experimental site: The experimental trials were carried out over two consecutive growing seasons (2002 and 2003) on mature (ten-years-old) 'Lavinia' nectarine peach trees located in a germplasm collection at the Department of Cultivation and Protection of Woody Species of Pisa-University (Italy; altitude 6 m, lat. 43.02 °N, long. 10.36 °E). The trees were grafted onto GF677, shaped as central leader and subjected to the usual cultural practices of the area (pruning, irrigation, and fertilisation). During the experimental trials minimum and maximum daily temperature and rainfall (Fig. 1) were acquired from the Department of Agronomy and Environmental Management (Pisa, University) and from the weather station of the 'Agenzia Regionale per lo Sviluppo e l'Innovazione nel settore Agricolo-forestale' (ARSIA-Florence).

Chemical Treatments: Release LC, a liquid formulation of gibberellic acid (4% w/w) was used in postharvest at the concentration of 80 mg L^{-1} resulted effective for peach (Costa and Vizzotto, 2000; Bartolini *et al.,* 2002). The chemical was sprayed to dripping point on the trees using a high pressure handgun, and Tween 20 (polyoxyethylene-20 sorbitan monolaurate, Sigma Chem. Co.) surfactant was added at 0.02% (v/v). The same trees were used every year and were treated the day after the fruit harvest: July 26th the first year and July 21st the second year. A non-sprayed control was included in the experiment. A randomised complete-block design with one-tree plots of four replications each was performed.

Biological and morphological observations under field conditions: One-year-old fruiting shoots (10/tree) of about 50 cm in length were tagged before treatment. Subsequently they were analysed from summer to spring, to evaluate the treatment effects in the current season and flowering and fruiting in the next season. The following biological and morphological parameters were recorded: a) initial flower and vegetative bud number; b) evolution of the flower bud phenological stages (Baggiolini, 1952); c) monthly count of the persisting flower buds for the evaluation of bud drop; d) rate of bloom and e) fruit set.

Histological and histochemical analysis: To establish the floral differentiation stage, the meristematic apices (25 for each sampling time) were periodically collected from the median portion of one-year-old fruiting shoots and microscopically observed. During the first year the apex collection covered a period of 40 days, from about 10 days before harvest to one month later (end of August). During the second year, the sampling of apices covered a longer period of 60 days from initial fruit-set to harvest time.

The meristematic apices were fixed in Carnoy (ethanol and glacial acetic acid 3: 1 v/v) and subsequently prepared for the anatomical and histochemical observations. The apices, after dehydration in a graded ethanol series, were embedded in histoplast and longitudinally sectioned (10 μm) with the cut parallel to the axis, using a Shandon microtome. The slides were then dewaxed, hydrated in an ethanol-water graded series and stained with Acridine Orange solution for 15 minutes (stock acridine orange 0.1% in the ratio 1/9 in Walpole's buffer at pH 4.2) for RNA localisation according to Bitonti *et al.* (2002). The slides were mounted with a synthetic mountant (Shandon) and examined using a Nikon epifluorescent microscope equipped with a 100W mercury lamp plus an excitation filter (B, type IF 420-490).

The width and height of the apical dome (Fig. 2a) were measured using a graduate ocular, on the same slides used for the histochemical analysis, and the pertinent structural features described. In particular, the co-axial stage was identified within the node, which consists of the achievement of lateral apices at the same level as the central one (Fig. 2b), as described by Giannino *et al.* (2003).

Representative selected sections was photographed with a digital camera (Olympus C-2000 z) equipped to the microscope.

Data analysis: Statistical analysis were conducted on treatment means using Student's *t*- test procedure, and analysis of variance (ANOVA) adopting the LSD multirange test for means separation (Statgraphics *Plus* software ver. 5; Manugistics, Inc., USA).

Results

First Year (summer 2002 – spring 2003)

Biological observations related to Release LC treatment: After the treatment with Release LC, applied the day after fruit harvest (July 26th), a particularly wet period occurred and in the following two months about 200 mm of rain were recorded (Fig. 1a). The treatment showed its efficacy in controlling the number of differentiated flower buds (Fig. 3a). In October, the treated trees had a number of flower buds significantly lower than untreated ones. From winter to spring, the differences were reduced due to a light increase of the flower bud drop recorded in the control trees. However, the flower bud drop was similar among the treatments and the highest percentages (about 27%) were found just before full blooming (March, 25th). After about 15 days from full bloom, a severe spring frost (-4°C) occurred (Fig. 1b), when the young fruits were at the phenological stage I (Baggiolini, 1952). After frost, it was possible to detect different fruit retention: while the fruits persisted on treated trees, on untreated trees all fruits dropped (Fig. 3b).

Morphological and histochemical observations: **Preharvest time (-10 days)**: At this time, in the first fifteen days of July, all sampled nodes were constituted by triple apices. The co-axial stage (lateral and central apices at the same level) were reached in about 50% of the examined samples. The meristematic dome measured about 133 μm in width and 77 μm in height (Table 1).

The histological analysis made it possible to distinguish different stages of floral differentiation (Fig. 4), according to Legave (1975). The buds vegetatively differentiated were about 30% while in 25% of the apices the receptacle appeared (stage B) and only the external layer of the tunica was noticed, because the other two layers were confused with the underlying cells. The remaining apices showed morphological features of undifferentiated buds (stage A) characterized by a conical shape, with three-layered tunica and recent foliar primordium according to the 'tunica-
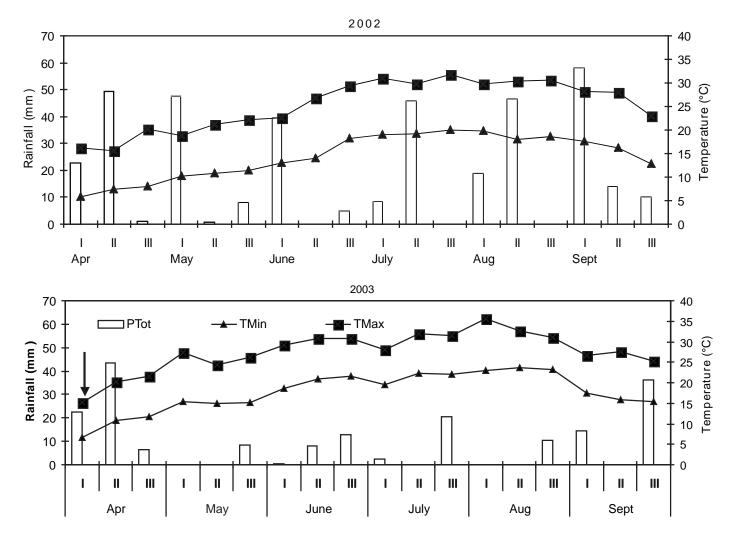
Fig. 1. Minimum and maximum temperatures (lines) and total rainfall (mm) (bars) recorded during the spring-summer season in 2002 (a) and 2003 (b) years.

corpus' model (Fig. 4). The external cellular layers of the tunica were characterised by very close cells with a dense cytoplasm and a scarcely visible nucleus.

Harvest time (July, 25th) the day before Release LC treatment: During few days (10) elapsed from the previous sampling time, the size of the meristematic dome increased both in width and height. The growth in height was particularly significant with values (about 140 μm), 80% greater compared to the previous value. Moreover, all the apices examined were at the coaxial phase. As

regards the floral differentiation, the apices analyzed showed, in similar percentage (about 30%), vegetative, undifferentiated or differentiated (stage B) features (Table 1).

Postharvest (30 days after Release LC treatment): One month after fruit yield and Release LC treatment, a different evolution of apices between treated and untreated trees was observed (Table 1). In treated trees, 24% of the apices showed the anatomical characteristics of an undifferentiated stage (stage A), while 35% clearly showed morphological features of vegetative buds.
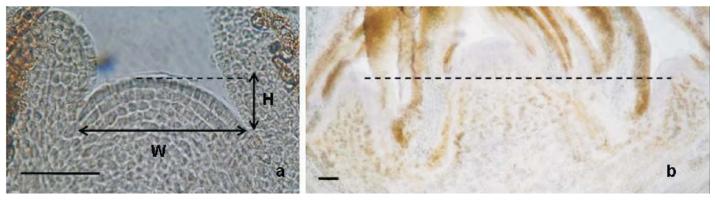

Fig. 2. Representation of measurements performed on longitudinal median sections of meristematic apices of 'Lavinia' peach cultivar. a) Height (H) and width (W) of meristematic dome (x 400; scale bar: 50 μm); b) Coaxial stage: achievement of lateral apices at same level as the central one (x 160; scale bar: 200 μm).

The remaining samples (41%) were at stages B and D-E of the flower differentiation. The untreated samples didn't show undifferentiated apices (stage A); most flower buds were at stage D-E, corresponding to the appearance of sepal, petal and stamen primordial; the remaining buds (36%) showed clear vegetative characteristics (Table 1).

Second Year (Summer 2003 – spring 2004)

Biological observation related to Release LC treatments: In this year, from July to September about 85 mm of rain was recorded

Table 1. Morphological characteristics of meristematic dome (Year 2002) and stages of floral differentiation in 'Lavinia' peach cultivar. The Release LC treatment was applied on the day after fruit harvest

Characterstic	- 10 days	Harvest time (July, 25th)	+ 30 days	
			Control	Release
(a) Meristematic dome				
Width (μm)	133.3 (±10.3)	166.7 (±15.3)*		
Height (μm)	76.7 (±16.3)	136.6 (±15.3)*		
Coaxial phase (%)	50	100		
(b) Differentiation stage (%)				
Vegetative buds (%)	30	32	36	35
Stage A	45	35	0	24
Stage B	25	33	0	25
Stage C	0	0	25	0
Stage D-E	0	0	39	16

Data represent the means values of 10 replicates (±SD). Between columns * denote significant difference by Student's *t* test ($P \leq 0.05$).
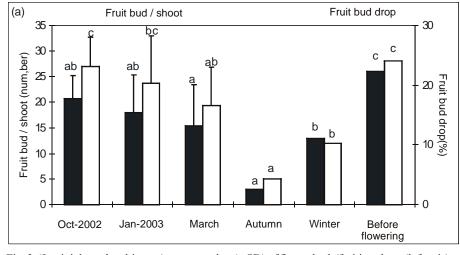
and temperatures were particularly elevated mainly in the minimum daily values which never gone down below 20°C (Fig. 2b).

The Release LC treatment wasn't able to affect the differentiation process. From autumn to spring, in treated trees the number of differentiated flower buds per shoots was significantly higher

Table 2. Morphological characteristics of meristematic dome (Year 2003) (a): stages of floral differentiation (b) and type of node (c) in 'Lavinia' peach cultivar, recorded at May, June and July. (a) Width, height (μm) and coaxial phase (%) reached inside the triad apices. (b) Most advanced differentiation stage: undifferentiated apex (stage A), differentiated apex (stages B and C). (c) Type of node: percentage distribution of single, double and triple node related to the number of meristematic apices (Year 2002)

Characterstic	May, 20th	June, 10th	July, 20th
(a) Meristematic dome			
Width (μm)	84.0 (±11.4)b	116.7 (±5.8)a	122.67 (±21.5)a
Height (μm)	37.0 (±11.0)b	35.2 (±1.1)b	60.0 (±24.0)a
Coaxial phase (%)	27	86	100
(b) Differentiation Stage	A	B	B and C
(c) Type of node (%)			
Single	25	30	0
Double	65	30	20
Triple	10	40	80

Data represent the means values of 10 replicates (± SD). Between columns, values with different letters are significantly different at $P \leq 0.05$ (LSD test).
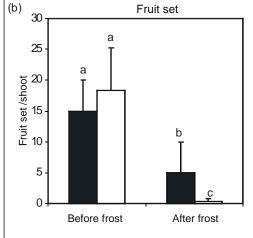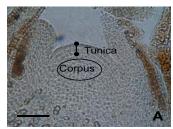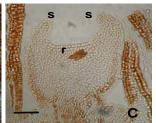

Fig. 3. 'Lavinia' peach cultivar: a) mean number (± SD) of flower buds/fruiting shoot (left axis) and percentage of flower buds drop (right axis) recorded from October 2002 to March 2003, in untreated (□) and treated (■) trees; b) mean number (± SD) of fruit set/fruiting shoot recorded before and after a spring frost (April) in untreated (□) and treated (■) trees. Means followed by different letters differ significantly at $P \leq 0.05$ (LSD test).
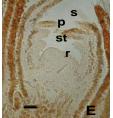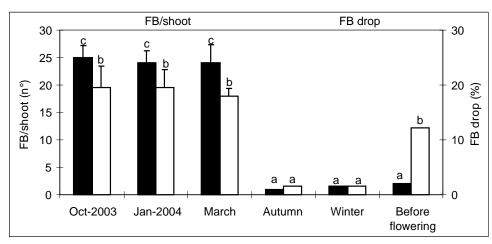

Fig. 4. Developmental stages observed in meristematic apex of 'Lavinia' peach cultivar. Stage A (x 400; scale bar: 50 μm): undifferentiated meristematic apex constituted by 'tunica' (external zone of the meristematic apex constituted by three layers of cells) and 'corpus' (under the tunica constituted by mother cells). Stages B – E: evolution of floral differentiation; Stage B (x 400; scale bar 50 μm): receptacle primordium arrangement; Stage C (x 200; scale bar: 200 μm): sepal primordia; Stage D (x 100; scale bar: 200 μm): petal primordia; Stage E (x 100; scale bar: 200 μm) stamen primordia (r: receptacle; s: sepal; p: petal; st: stamen).
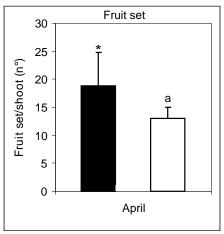
Fig. 5. 'Lavinia' peach cultivar: a) mean number (± SD) of flower buds/fruiting shoot (left axis) and percentage of flower buds drop (right axis) recorded from October 2003 to April 2004 in untreated (□) and treated (■) trees. Means followed by different letters differ significantly at $P ≤ 0.05$ (LSD test); b) mean number (± SD) of fruit set/fruiting shoot recorded in April in untreated (□) and treated (■) trees. Asterisk (*) denote significant difference by Student's t test ($P ≤ 0.05$).

than untreated trees (Fig. 5). This trend was also confirmed in terms of fruit set and treated trees had a highest number of fruits per shoot. In the treated trees, a negligible flower bud drop from autumn to spring was recorded and there was a larger tendency to retain fruits. On the other hand, in untreated trees a later flower bud drop occurred, just before flowering. We must remark that, on treated trees, a high percentage (more than 30%) of flowers showed double and triple pistils, abnormality observed in a very low rate on untreated trees (Fig. 6).

Morphological and histochemical observations

May, 20th (60 days after full bloom): At this time, corresponding to fruit enlargement, the meristematic dome measured 84 and 37 μm in width and height, respectively. The sampled nodes were mainly constituted by double apices (65%) and the co-axial phase was observed only in 27% of the apices. In meristematic apices, the cellular layers of the tunica were well visible, denoting an undifferentiated status (stage A) (Table 2). The histochemical procedure for RNA detection didn't give a positive reaction and no fluorescent signal was observed in the meristematic apices (Fig. 7a).

June, 10th (80 days after full bloom): The morphology of nodes showed several modifications in comparison to the observations of the previous month (Table 2). A significant increase in

meristematic dome width was noticed, while the height dimension was unchanged. In particular, an increase of 30% in the number of triple apices was observed and the coaxial phase was achieved in most samples. The histological observations showed the loss of the tunica layers of the meristematic apices, denoting a prevalently presence of the differentiation stage B.

At meristematic apex level, it was possible to notice the appearance of a uniformly diffused fluorescent reaction related to the RNA localisation (Fig. 7b).

July, 20th (1 day before harvest time): The growth of the apices was underlined by a substantial increase in meristematic dome height, reaching about 2-fold higher values with respect to the previous records, while the width remained substantially unchanged (Table 2). Moreover, the sampled nodes showed triple apices which were mostly differentiated (stages B and C) and the coaxial phase was reached. The intensity of RNA staining fluorescent signal was very strong compared to the reactions observed on the previous dates. The localisation of the RNA
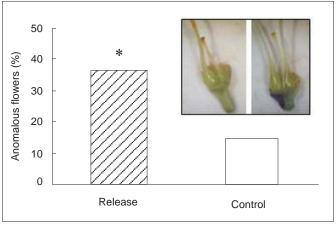

Fig 6. 'Lavinia' peach cultivar: percentage of anomalous flowers carrying double and triple pistils, recorded at blooming time (March, 20th 2003) on untreated (□) and treated (■) trees. Asterisk (*) denote significant difference by Student's t test ($P ≤ 0.05$).
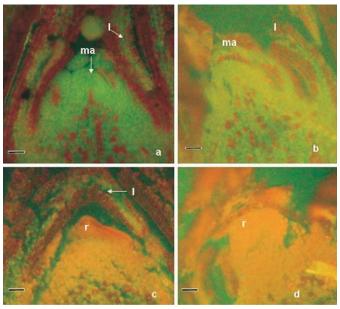

Fig. 7. 'Lavinia' peach cultivar: Fluorescence degree of RNA signal detected by acridine orange staining on meristematic lateral apices (x 200), sampled on May (a), June (b), July 3th, (c) and July 20th (d). (ma) meristematic apex; (r) receptacle primordium; (l) leaf primordium (Scale bars: 50 μm).

reaction was diffused on the apex and particularly concentrated on floral developing primordium (Fig. 7 c, d).

Discussion

The results obtained during a two year period permitted to identify the critical phase of the meristematic apex evolution in Lavinia cv., and to clarify the different results in the control of flower bud differentiation rate obtained by a postharvest application of Release LC.

The meristematic apex showed significant morphological and anatomical changes from May to June, 60 and 90 days after full bloom. In this period, the presence of triple apices increased rapidly, the co-axial phase was achieved, the meristematic dome growth markedly in width and height and the apex lost the tunica layering. This latter change has long been considered one of the classical anatomical parameters for defining flower apex induction (Tombesi, 1965; Martinez-Tellez et al., 1982; Buban and Faust, 1982). The histochemical analysis carried out for the RNA localisation seems to confirm June as the month most involved in the differentiation process. In this period, we observed the first RNA appearance by a weak staining and the strongest signal was related to the morphological apex evolution, denoted by an increase in the meristematic dome dimensions and the attainment of the co-axial phase. These changes occurred in concomitance with the final peach fruit growth (data not shown). The increase in RNA detection in the meristematic apex is considered to be the first indicator of a transition period called 'floral evocation', responding to the floral stimulus (Evans, 1971; Bernier et al., 1981; Pinney and Polito, 1990). In this phase, RNA and proteins are synthesised and mitotic activity increases when the stimulus triggering flower initiation reaches the apical meristem (Buban and Faust, 1982). In apple, during the transition period, a gene involved in floral differentiation began clearly to express itself (Wada et al., 2002).

As regards the different effect of Release LC on the flower bud differentiation control, the results were supported by the morphological and histochemical analysis. In the first year, this chemical compound was able to reduce the initial number of differentiated flower buds: at the time of treatment, the apices were mostly still undifferentiated and, later, a slower evolution of floral differentiation was observed. A similar retardative effect, on overall flower bud development, was reported in peach after exogenous GA_3 applications (Corgan and Widmoyer, 1971; Basconsuelo et al., 1995). It should be pointed out that following a late spring frost, the fruit yield of untreated trees was all injured, whereas some fruits persisted on plants treated the previous summer. The greater increase in cold hardiness wasn't due to a delay in bloom, as reported by Corgan and Widmoyer (1971) and Basconsuelo et al. (1995), but it could be related to a different physiological or biological status of the young fruits, hypothesis which need further and appropriate analysis. This late spring frost should affected the differentiation process of the current year which was faster. Indeed, at the same treatment time as the previous year, the apices were mainly at an irreversible stage, and thus the Release LC was ineffective in controlling the flower bud differentiation rate. Clanet and Borsani (1972) showed that GA treatments didn't induce any trouble in the differentiation process when chemical was sprayed at more advanced stage of

bud differentiation (stage C). In addition, the drought conditions occurred during the summer season could have interacted with the chemical treatment, also inducing the appearance of anomalous flowers. In treated trees, high percentages of double and triple pistils were observed at blooming time in accordance with Garcia-Pallas et al.(2001) and Reinoso et al. (2002) which found a relationship between exogenous gibberellin and high floral anomalies. As consequence of strong competitions, these anomalies produce non-marketable fruits involving high costs to reduce the fruitlets to increase the final fruit size at maturity.

From a practical point of view, the postharvest application of the Release LC in 'Lavinia' cultivar not appeared an useful technique to control the flower bud differentiation rate. The different results of our experiments confirm that the response to chemical treatments may be mediated by numerous endogenous and exogenous factors. The efficacy of Release LC seems strictly depending on the growth stage of a meristematic apex. The sensitivity to this treatment proved to be during the 'transition phase' which is influenced by several physiological and environmental factors (Tromp, 2000). The high variability of the climatic conditions occurring between different years didn't allow to predict the efficacy of a chemical treatment, also within the same cultivar. The strategy of postharvest applications to avoid chemical contamination of marketable fruits fails, so as to use this approach like an indirect technique of fruit thinning in order to provide a reduction in costs.

Acknowledgements

References

Baggiolini, M. 1952. Stades repères de l'abricotier. *Rev. Romande Agric. Vitic. Arboric.*, 8: 28-29.

Bartolini, S., L. Andreini and C. Vitagliano, 2002. Il controllo della carica delle gemme a fiore in alternativa al diradamento dei frutti di pesco. *Proceedings of VI Giornate Scientifiche SOI (Italian Horticultural Society)* held at Spoleto from 23-25th April, 2002, pp. 199-200.

Basconsuelo, S., H. Reinoso, E. Lorenzo and R. Bottini, 1995. Dormancy in peach (*Prunus persica* L.) flower buds. IV. Morphogenesis of excised buds as influenced by chilling and gibberellin A_3. *Plant Growth Reg.*, 16: 113-119.

Bernier, G., J.M. Kinet and R.M. Sachs, 1981. *The physiology of flowering. Vol. II Transition to reproductive growth.* CRC Press, Boca Raton.

Bitonti, M.B., R. Cozza, A. Chiappetta, D. Giannino, M. Ruffini Castiglione, W. Dewitte, D. Mariotti, H. Van Onckelen and A.M. Innocenti, 2002. Distinct nuclear organization, DNA methylation pattern and cytokinin distribution mark juvenile, juvenile-like and adult vegetative apical meristems in peach (*Prunus persica* [L.] Batsch). *J. Exp. Bot.*, 53(371): 1047-1054.

Buban, T. and M. Faust, 1982. Flower bud induction in apple trees. Internal control and differentiation. *Hort. Rev.*, 4: 174-203.

Byers, R.E., D.H. Carbaugh and C.N. Presley, 1990. The influence of bloom thinning and GA_3 sprays of flower bud numbers and distribution in peach trees. *J. Hort. Sci.*, 65: 143-150.

Clanet, H. and O. Borsani, 1972. Contribution a l'etude de l'action de l'acide gibberellique sur la croissance des rameaux et l'evolution des bourgeons a fleur du pecher, consequences pratiques. *Ann. Amelior. Plantes*, 22(1): 61-79.

Corgan, J.N. and F.B. Widmoyer, 1971. The effects of gibberellic acid on flower differentiation, date of bloom and cold hardiness of peach. *Proc. Am. Soc. Hort. Sci.*, 96: 54-57.

Costa, G. and G. Vizzotto, 2000. Fruit thinning of peach trees. *Plant Growth Reg.*, 31: 113-119.

Dennis, F.G. Jr, 2000. The history of thinning. *Plant Growth Reg.*, 31: 1-16.

Evans, L.T. 1971. Flower induction and the florigen concept. *Ann. Rev. Plant Physiol.*, 22: 365-394.

Fallahi, E. and K.M. Willemsen, 2002. Blossom thinning of pome and stone fruit. *HortScience*, 37(3): 474-477.

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. *Scientia Hortic.*, 90: 265-278.

Giannino, D., G. Mele, R. Cozza, L. Bruno, G. Testone, C. Ticconi, G. Frugis, M.B. Bisonti, A.M. Innocenti and D. Mariotti, 2003. Isolation and characterization of a maintenance DNA-methyltransferase gene from peach (*Prunus persica* [L.] Batsch): transcript localization in vegetative and reproductive meristems of triple buds. *J. Exp. Bot.*, 54(393): 2623-2633.

Gonzalez-Rossia, D., M. Juan, C. Reig and M. Agustì, 2006. The inhibition of flowering by means of gibberellic acid application reduces the cost of hand thinning in Japanese plums (*Prunus salicina* Lindl.). *Scientia Hortic.*, 110(4): 319-323.

Jimenez, C.M. and J.B.R. Diaz, 2002. Fruit distribution and early thinning intensity influence fruit quality and productivity of peach and nectarine trees. *J. Am. Soc. Hort. Sci.*, 127(6): 892-900.

Legave, J.M. 1975. La différentiation du bourgeon a fleur et le repos hivernal chez l'abricotier *Prunus armeniaca* Vulgaris. *La Pomologie Francaise*, 17(9): 150-168.

Martinez-Tellez, J.J., R. Monet and P. Crossa-Raynaud, 1982. Contribution a une meilleure connaissance de la biologie florale et de la fécondation chez le pêcher. *Arboriculture Fruitiere,* 338: 39-45.

Oliveira, C.M. and G. Browning, 1993. Gibberellin structure-activity effects on flower initiation in mature trees and on shoot growth in mature and juvenile *Prunus avium*. *Plant Growth Regul.*, 13(1): 55-63.

Pinney, K. and V.S. Polito, 1990. Flower initiation in "Manzanillo" olive. *Acta Hortic.*, 286: 203-205.

Reinoso, H., V. Luna, C. Daurìa, R.P. Pharis and R. Bottini, 2002. Dormancy in peach (*Prunus persica*) flower buds. VI. Effects of gibberellins and an acyclohexanedione (Trinexapac-ethyl) on bud morphogenesis in field experiments with orchard trees and on cuttings. *Can. J. Bot.*, 80: 664-674.

Southwick, S.M., K.G. Weis, J.T. Yeager and H. Zhou, 1995. Controlling cropping in 'Loadel' cling peach using gibberellin: Effects on flower density, fruit distribution, fruit firmness, fruit thinning and yield. *J. Am. Soc. Hort. Sci.*, 120: 1087-1095.

Southwick, S.M., K.G. Weis and J.T. Yeager, 1996. Chemical thinning of stone fruits in California. *Good Fruit Grower*, 1: 34-52.

Taylor, B.H. and D. Geisler-Taylor, 1998. Flower bud thinning and winter survival of "Redhaven" and "Cresthaven" peach in response to GA_3 sprays. *J. Am. Soc. Hort. Sci.*, 123(4): 500-508.

Tombesi, A. 1965. Tipiche variazioni della struttura nell'apice di pesco dallo stadio indifferenziato a quello riproduttivo. *Riv. Ortoflorofrutt. It.*, 49: 401-429.

Tromp, J. 2000. Flower-bud formation in pome fruits as affected by fruit thinning. *Plant Growth Reg.*, 31: 27-34.

Wada, M., Q. Cao, N. Kotoda, J. Soejima and T. Masuda, 2002. Apple has two orthologues of FLORICAULA/LEAFY involved in flowering. *Plant Mol. Biol.*, 49: 567-577.