UNIVERSIDAD POLITÉCNICA DE VALENCIA

DEPARTAMENTO DE PRODUCCIÓN VEGETAL

CONTROL DE LA FLORACIÓN EN EL GÉNERO *PRUNUS*. FACTORES CLIMÁTICOS Y NUTRICIONALES

TESIS DOCTORAL

Presentada por:
D. Diego Esteban González Rossia

Dirigida por:
Dr. D. Manuel Agustí Fonfría

Valencia, Junio de 2006

SONETO

Si para recobrar lo recobrado
debí perder primero lo perdido,
si para conseguir lo conseguido
tuve que soportar lo soportado,

si para estar ahora enamorado
fue menester haber estado herido,
tengo por bien sufrido lo sufrido,
tengo por bien llorado lo llorado.

Porque después de todo he comprobado
que no se goza bien de lo gozado
sino después de haberlo padecido.

Porque después de todo he comprendido
que lo que el árbol tiene de florido
vive de lo que tiene sepultado.

Francisco L. Bernardez

"No debe haber barreras para la libertad de preguntar. No hay sitio para el dogma en la ciencia. El científico es libre y debe ser libre para hacer cualquier pregunta, para dudar de cualquier aseveración, para buscar cualquier evidencia, para corregir cualquier error"

J. Robert Oppenheimer

"Trabajar, trabajar, no estar nunca contento del resultado,
pero no tener que arrepentirse de la intención…"

Jorge B. Mullor

AGRADECIMIENTOS

Constantemente el destino va intercalando pruebas en el camino de nuestras vidas que cada uno resuelve de manera única y personal. El haber venido a España a realizar esta tesis doctoral fue para mí una de esas pruebas, que he podido sortear favorablemente gracias al apoyo de muchas personas. Por ello, quiero expresar mi más sincero agradecimiento a todos aquellos que han estado a mi lado durante todo este tiempo brindándome su cariño y a los que, directa o indirectamente, me han acompañado en la ardua, paciente y generosa labor de investigar, en especial:

A los técnicos de las Cooperativa de Alginet, Carlet y de la Estación Experimental de Carlet (Consellería de Agricultura, Pesca y Alimentación de Valencia), por su inestimable colaboración.

A los compañeros, que en una larga sucesión itinerante, han pasado por el Laboratorio de Citricultura de la UPV, por tantos buenos momentos vividos juntos.

A los amigos, argentinos, uruguayos, chilenos, holandeses y españoles por brindarme su cariño, consejo y tolerancia, en los buenos y en los malos momentos.

A Norberto Gariglio y Rubén Pilatti, por su permanente apoyo, consejo y guía desde mis primeros pasos en investigación en la Facultad de Agronomía de Esperanza (UNL - Argentina) hasta la fecha.

A Manolo Agustí, mi director, por confiar en mí y darme la posibilidad, los medios y el apoyo necesario para desarrollar este trabajo, y lo que es más importante, por ofrecerme su amistad.

A Rai e Isabel, por estar siempre allí cuando los necesité y por su cariño.

A Vicente Clavijo, por ser como un segundo padre para mí aquí en España.

A Pedro, mi hermano, por haberme escuchado y aconsejado siempre y por ser además, mi mejor amigo.

A mi papá, por guiarme en el aprendizaje de la ciencia y la investigación, y por ser mi referente como hombre y como profesional.

A mi mamá, por su amor de madre y por haber dado siempre todo, en pos de la felicidad y bienestar de sus hijos.

Y finalmente a Laura, por su amor, paciencia, dulzura, confianza…. en definitiva, por ‘TODO’.

A todos, muchísimas gracias

General index

Tables index

Figures index

Page

RESUME

Most *Prunus* species have the tendency to set a large number of fruits, but it reduces the possibility of attaining commercial fruit size and quality at harvest. As a derived effect, alternate bearing cycles can be initiated since gibberellins produced in the seeds are the main cause of inhibition of floral bud initiation. To increase individual fruit size, growers thin fruit manually, but it represents a high cost that reduces growers' returns. Paradoxically, the effect of gibberellins can be used agronomically to overcome these problems.

This PhD Thesis develops an easy agronomical technique to reduce flowering, and consequently the cost of hand-thinning of *Prunus* cultivars, by means of gibberellic acid application, exploring the implications in the floral process of fruit and inhibitors of gibberellins biosynthesis (paclobutrazol, prohexadione-Ca), through carbohydrates and nitrogen fractions content. We also studied the temperature requirements to sprout or bloom and their dependence on sugar's content, using the 'one node cutting' technique.

Experiments were carried out in three commercial orchards in the area of Valencia, Spain, and one in Esperanza, Argentina. The application of gibberellic acid during the floral induction period reduced flowering by 40%-50% in peaches and plums, being 50 mg l^{-1} of GA_3 the most appropriate concentration. Gibberellic acid application also modified floral distribution along the shoot and some aspects of PSII efficiency. Our results evidenced an inhibitory effect of fruit on the flowering process, which acts directly, by reducing the number of floral buds, and indirectly, by reducing the number of developing shoots, their length, number of nodes and number of buds. We then hypothesize that flowering is not an inductive process but an inhibitory process. Neither soluble sugars in phloem sap of shoots nor carbohydrate reserves in fibrous roots, have been linked to the floral initiation process, but with energy supplied to sprout. However, we found evidences of some disturbances of the nitrate reduction process in trees tending to flower scarcely.

The experiments giving artificial chilling showed that chilling enhances leafing and blooming but with larger effect over the former. Excessive chilling diminished the percentage of flower budbreak in low chilling cultivars. Moreover, the mean time to budbreak (MTB) of leaf buds decreased faster with chilling, compared with flower buds. Experiments demonstrated a negative correlation between the number of

chill hours accumulated till budbreak and the number of heat units accumulated from then to full bloom, and that chilling induces changes in carbohydrates and nitrogen metabolism in the adjacent tissues of buds. Finally, the results proved that budbreak starts when bud weight increases significantly with respect to its initial weight at leaf fall, in both floral and vegetative buds. Thus, results confirm the utility of the excised shoots method to complement field experiments in the search for a unique model of dormancy process.

RESUMEN

La mayoría de las especies de *Prunus* tienen tendencia a cuajar un gran número de frutos, lo que reduce las posibilidades de obtener frutos con buen tamaño y calidad. Pero, además, esto puede derivar en ciclos de alternancia, ya que las giberelinas producidas en las semillas son la principal causa de inhibición de la iniciación floral. Por ello, los agricultores recurren al aclareo manual para incrementar el tamaño individual de los frutos, aunque ello represente un alto costo que repercute grandemente sobre sus beneficios. Paradójicamente, el efecto inhibidor de las giberelinas puede ser usado como alternativa agronómica para la solución de estos problemas.

Esta tesis doctoral describe una técnica agronómica sencilla basada en la aplicación de ácido giberélico (GA_3), para reducir la floración y consecuentemente los costos de aclareo manual en cultivares de *Prunus*. Además, se abordó el estudio de las implicaciones en el proceso de la floración de los frutos y los inhibidores de la síntesis de giberelinas (paclobutrazol, prohexadione-Ca) a través de los carbohidratos y las fracciones nitrogenadas. Complementariamente, se estudiaron los requerimientos de temperatura para la brotación y floración, y su dependencia de los contenidos en carbohidratos, mediante el cultivo de varetas.

Los experimentos se llevaron a cabo en tres huertos comerciales de la zona de Valencia, España, y en un huerto situado en Esperanza, Argentina. La aplicación de GA_3 durante el período de inducción floral redujo la floración en unos 40%-50% en melocotones, nectarinas y ciruelos, siendo 50 mg l^{-1} la dosis más adecuada de GA_3. La aplicación de GA_3 también afectó la distribución de las flores a lo largo del ramo y algunos aspectos de la eficiencia del PSII. Los resultados evidenciaron un efecto inhibidor del fruto sobre el proceso de floración, que actúa directamente mediante la reducción del número de yemas florales, e indirectamente reduciendo el número de brotes en desarrollo, su longitud, el número de nudos y el número de yemas. De ahí que se proponga la hipótesis de que la floración no es un proceso inductivo, sino más bien un proceso inhibitorio. Tanto el contenido de azúcares solubles del floema de brotes, como los carbohidratos presentes en las raíces fibrosas, no han podido ser relacionados con el proceso de iniciación floral, aunque sí con los requerimientos energéticos para brotar. Sin embargo, se han encontrado evidencias de algunas perturbaciones en el proceso de reducción del ión nitrato en árboles con escasa tendencia a florecer.

Los experimentos en los que se aplicó frío artificial a varetas, demostraron que el frío promueve la brotación de yemas vegetativas y florales, siendo mayor el efecto sobre las primeras. Por otro lado, se observó que una gran acumulación de frío disminuye el porcentaje de brotación de las yemas de flor en cultivares con bajos requerimientos de frío. Además, el tiempo medio de brotación (MTB) de yemas vegetativas disminuyó más rápidamente con el frío que el de yemas florales. En esta tesis se demuestra que existe una correlación negativa entre el número de horas acumuladas hasta la brotación y el número de unidades de calor acumuladas desde la brotación hasta la floración, y que el frío induce cambios en el metabolismo de los carbohidratos y el nitrógeno de los tejidos adyacentes a las yemas. Finalmente, se comprueba que la brotación comienza cuando el peso de las yemas aumenta significativamente respecto de su peso inicial al inicio de la latencia, tanto en yemas de flor como en yemas vegetativas. De este modo, los resultados confirman la utilidad del método de varetas como complemento de los experimentos de campo en la búsqueda de un modelo único que explique el proceso de la dormancia.

RESUM

La majoria de les espècies *Prunus* tenen tendència a fer un gran nombre de fruits, però això redueix la possibilitat d'aconseguir una grandària i qualitat del fruit comercial en la collita. Com a efecte derivat, alterna cicles productius que poden ser iniciats des de gibberel·lines produïdes a les llavors, que són la causa principal d'inhibició del procés d'iniciació de la inducció floral. Per a augmentar la grandària individual del fruit, els cultivadors aclareixen manualment els fruits, però això significa un cost alt que en redueix els ingressos. No obstant això, l'efecte de les gibberel·lines pot ser usat agronòmicament per a vèncer aquests problemes.

Aquesta tesi PhD desenvolupa una tècnica agronòmica senzilla per a reduir la floració i consegüentment el cost de l'aclarida manual de cultivars de *Prunus*, per mitjà de l'aplicació d'àcid gibberèl·lic, i estudia les conseqüències del fruit, dels inhibidors de la síntesi de gibberel·lines (paclobutrazol, prohexadiona de calci) i de les fraccions de carbohidrats i nitrogen en el procés floral. També se estudien les necessitats de temperatura per a brotar o florir, i la seua dependència en el contingut de sucres, mitjansant la tècnica de *one node cutting*.

Els experiments es van dur a terme en tres horts comercials a la zona de València, Espanya, i un a Esperanza, l'Argentina. L'aplicació d'àcid gibberèl·lic durant el període d'inducció floral redueix la floració entre 40%-50% en bresquilleres i pruneres, en què 50 mg l^{-1} de GA_3 és la concentració més apropiada. L'aplicació d'àcid giberèl·lic també afecta la distribució floral al llarg del rebrot i alguns aspectes de l'eficiència del PSII. Els resultats també evidencien un efecte inhibitori del fruit en el procés de floriment, el qual actua directament, per reducció del nombre de gemmes florals, i indirectament, per reducció del nombre de rebrots desenvolupats, la seua llargada, nombre de nodes i nombre de gemmes. Aleshores admetem com a hipòtesi que la florida no és un procés inductiu sinó un procés inhibitori. Ni sucres solubles en el floema de la saba dels brotons ni reserves de carbohidrats en arrels fibroses s'han vinculat al procés d'iniciació floral, sinó amb l'energia subministrada per a brotar. De totes maneres, trobem evidències d'algunes pertorbacions dels processos de reducció del nitrat en els arbres que tendeixen a penes a florir.

Els experiments realitzats amb refrigeració artificial mostren que una refrigeració excessiva minva el percentatge de brotada floral en menys cultivars refrigerats i minva el percentatge de brotada floral. D'altra banda, el temps mitjà de brotació (MTB) de gemmes vegetatives va disminuir mes

rapidament per l'efecte del fred que el de les gemmes de flor. El treball també demostra una correlació negativa entre el nombre d'hores de fred acumulat fins a la brotada i el nombre d'unitats de calor acumulada des de l'aclarida fins a la plena floració, i aquesta refrigeració indueix a canvis metabòlics en carbohidrats i nitrogen en el teixit adjacent de les gemmes. Finalment, demostra que la brotada comença quan el pes de la gemma augmenta significativament respecte al seu pes en la caiguda de les fulles, tant en gemmes florals com vegetatives. Així, els resultats confirmen la utilitat del mètode de conreu de varetas per a complementar els experiments al camp en la recerca d'un model únic del procés de la laténcia.

PRESENTATION

The Memory I present here as a PhD Thesis, has been organized as a summary of several chapters, each one with own entity. Each chapter has been introduced, presented and discussed as a scientist article, although a general introduction and a final general discussion are also provided.

The general objective of this PhD Thesis is to contribute to the knowledge of flowering process on the *Prunus* genus. To achieve it, we studied the effect of fruit on floral bud initiation and development and the effect of gibberellic acid spray on the inhibition of the process, since there are a lot of reports indicating the effect of gibberellins controlling flowering. This effect has been used, in our study, to develop an easy agronomical technique to reduce flowering and, thus, to reduce the cost of hand-thinning the fruit. On the other hand, the study tries to enhance flowering production by inhibiting the inhibitor, that is, by applying some inhibitors of gibberellins' synthesis, such as paclobutrazol and prohexadione-Ca. The effect of chilling on vegetative bud and floral bud differentiation has also been studied, in order to state the temperature requirements to sprout or bloom, respectively, and their dependence on sugar's content. Nitrogen requirements have been also studied.

The order of the chapters follows a logical sequence, presenting the results chronologically, that is, the effect of fruit and hormonal control followed by the effect of chilling.

CHAPTER 1

General introduction

1- General introduction

In annual plants, the transcription from vegetative to reproductive growth is a unique change in the plant life, affecting the identity of the meristematic apex to make it develop flowers instead of leaves. The occurrence of this transitional process is a mayor adaptative feature to overcome adverse environmental conditions. However, most fruit trees are policarpic plants and need to save some vegetative buds for insuring their future growth (Lang, 1965).

In most annual plants, sexual reproduction (flowering) begins with a change in the photoperiod (photoperiodic sensitivity). Some plants remain vegetative till they sense a shortening in day length to a certain amount of hours (short day plants), while other plants only flower when day length overcomes a specific number of light hours (long day plants). Nevertheless, photoperiodic requirements are not strict and plants growing under long day conditions would flower earlier and with fewer leaves than those growing under short day conditions. With the use of genetic molecular models, genes involved in the temporary control of flowering were identified on the plant model *Arabidopsis thaliana* (Araki, 2001; Mouradov *et al.*, 2002; Samach and Coupland, 2000). Initially, these genes elucidate only individual steps from the flowering process, depending on phenotypic and environmental conditions. But recently, they have been identified converging in time to unchain flowering: the transcription of the meristematic tissues into reproductive growth begins when the flowering promoter proteins reach a threshold concentration. Two genes are known to encode this sort of proteins (Kobayashi *et al.*, 1999; Kardailsky *et al.*, 1999; Samach *et al.*, 2000; Lee *et al.*, 2000). Genetics, plant age, endogenous hormones levels and environmental conditions converge to control the levels of transcription of both genes in relation to flowering (Kobayashi *et al.*, 1999; Kardailsky *et al*., 1999; Samach *et al*., 2000).

Although there is enough evidence of the influence of photoperiod on the regulation of dormancy in temperate species, in most fruit trees the influence of photoperiod seams not to have a direct involvement in the control of flower induction. Low temperatures and drought are both signalling that, when interrupted, provoke a flush of flowering, which was demonstrated in *Citrus* (Spiegel-Roy and Goldschmidt, 1996) and mango (Davenport and Nuñez-Elisea, 1997). Both type of stress lead to a decrease or virtually stop root growth, suggesting a common regulatory process in both species.

But the recurrent production of flowers, year after year, in fruit tree species puts some doubt about the need of a positive annual induction. Thus, it is possible to argue that after juvenility trees are constantly induced to flower, but the bud developmental process is under one or various inhibitors control (Goldschmidt and Samach, 2004; Martinez-Fuentes *et al*., 2004), which would act in a quantitative manner. The fruit appears to be the main source of these inhibitors since causes flower inhibition on the following season. Moreover, the number of fruits in the tree is directly related with the intensity of flower inhibition.

Among the currently known plant hormones, gibberellins are the strongest related with flowering (Pharis and King, 1985). Its application has repeatedly demonstrated to inhibit flower bud differentiation in a number of fruit tree species (Goldschmidt *et al.*, 1997). It seems that gibberellins interfere in the crossing points of different metabolic pathways involved in the control of the plant flowering process, thus playing a crucial role in ensuring the biodiversity derived from the sexual reproduction of plants.

This way, the roll of the fruit in the flowering process becomes crucial, but it is still unknown when and how it intervenes with the process. Luckwill (1970) anticipated that the gibberellins synthesised in the seeds are the ones responsible for flower inhibition. Nevertheless, it has been proved that other tissues, like the ovary walls, can exert this action particularly in parthenocarpic fruits (Monselise, 1978) and even the active growing roots could be the source of gibberellins that would interfere with the flowering process (Carr *et al.*, 1964). Moreover, it was also suggested an effect of gibberellins reducing budbreak as an indirect effect of gibberellins reducing flower intensity (García-Luis *et al*., 1995; Syvertsen *et al*., 2003).

Thus, it becomes clear that in woody species many aspects of the flowering process are still unclear, especially those related to the influence of the fruits on the inhibition of flower differentiation in lateral meristems and to the role of low temperatures in the ripening of differentiated buds. The knowledge about these mechanisms could derivate in a new technique, which would allow improving the fruit quality and profitability of fruit tree orchards.

In this work, we outline the hypothesis that every bud from a fruit tree has all the information required for being inducted to flowering, but inhibitor factors from the fruit block this process by a hormonal pathway, particularly dominated by endogenous gibberellins. We also propose that

the low autumn-winter temperatures are capable of modulate the availability of reserves, which guaranty an adequate budbreak. Accordingly, nutritional aspects may become important, but are irrelevant for the induction and differentiation process.

References

Araki, T. 2001. Transition from vegetative to reproductive phase. Current Opinion in Plant Biology 4: 63-68.

Carr, D.J.; Reid, D.M. and Skeene, K.G.M. 1964. The supply of gibberellins from the root to the shoot. Plant 63: 382-392.

Davenport, T.L. and Nuñez-Elisea, R. 1997. Reproductive physiology. In: Litz, R.E. (Ed.). *The Mango: Botany*. Production and Uses, CAB Intl.

García-Luis, A.; Kanduser, M. and Guardiola, J.L. 1995. The influence of fruiting on the bud sprouting and flower induction responses to chilling in *Citrus*. Journal of the American Society of Horticultural Science 70: 817-825.

Goldschmidt, E.E.; Tamin, M. and Goren, R. 1997. Gibberellins and flowering in *Citrus* and other fruit trees: a critical analysis. Acta Horticulturae 463: 201-207.

Goldschmidt, E.E. and Samach, A. 2004. Aspects of flowering in fruit trees. Acta Horiculturae 653: 23-27.

Kardailsky, I.; Shukla, V.K.; Ahn, J.H.; Dagenais, N.; Christensen, S.K.; Nguyen, J.T. ; Chory, J.; Harrison, M.J. and Weigel, D. 1999. Activation tagging of the floral inducer FT. Science 286: 1962-1965.

Kobayashi, Y.; Kaya, H.; Goto, K.; Iwabuchi, M. and Araki, T. 1999. A pair of related genes with antagonistic roles in mediating flowering signals. Science 286: 1960-1962.

Lang, A. 1965. Physiology of flower initiation. In: Lang, A. (Ed), *Encyclopedia of Plant Physiology*. XV/1: 1380-1536.

Lee, H.; Suh, S.S.; Park, E.; Cho, E.; Ahn, J.H.; Kim, S.G.; Lee, J.S.; Kwon, Y.M. and Lee, I. 2000. The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis.* Genes & Development 14: 2366-2376.

Luckwill, L.C. 1970. The control of growth and fruitfulness of apple trees. In: Luckvill, L.C. and Cutting, C.V. (Eds.). *Physiology of tree crops*. Academic Press, New York, EEUU, pp. 237-254.

Martinez-Fuentes, A.; Mesejo, C.; Juan, M.; Almela, V. and Agustí, M. 2004. Restrictions on the exogenous control of flowering in *Citrus*. Acta Horticulturae 632: 91-98.

Monselise, S.P. 1978. Understanding of plant processes as a basis for successful growth regulation in citrus. Proc. International Society of Citriculture 1: 250-255.

Mouradov, A.; Cremer, F. and Coupland, G. 2002. Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14: 111-130.

Pharis, R.P. and King, R.W. 1985. Gibberellins and reproductive development in seed plants. Annual Review in Plant Physiololy 36: 517-568.

Samach, A. and Coupland, G. 2000. Time measurement and the control of flowering in plants. Bioessays 22: 38-47.

Samach, A.; Onouchi, H.; Gold, S.E.; Ditta, G.S.; Schwarz-Sommer, Z.; Yanofsky, M.F. and Couplant, G. 2000. Distinct roles of *CONSTANS* target genes in reproductive development of *Arabidopsis*. Science 288: 1613-1616.

Spiegel-Roy, P. and Goldschmidt, E.E. 1996. Biology of Citrus. Cambridge University Press.

Syvertsen, S.P.; Goni, C. and Otero, A. 2003. Fruit load canopy shading affect leaf characteristics and net gas exchange of 'Spring' navel orange trees. Tree Physiology 23 (13): 899-906.

CHAPTER 2

Objectives

2- Objectives

In accordance with the hypothesis proposed, the objectives of this work are:

2.1- Physiological objectives

2.1.1- Influence of fruit on flowering in *Prunus* species. Nutritional aspects.

2.1.2- Influence of gibberellins on flowering in *Prunus* species. Vegetative and reproductive relationships. Photosynthetic disorders. Nutritional aspects.

2.1.3- Temperature influence on the breaking of dormancy of flower buds in *Prunus* species. Nutritional aspects: carbohydrates and nitrogen metabolism.

2.2- Agronomical objectives

2.2.1- Flower bud inhibition by means of gibberellic acid sprays as an indirect technique of fruit thinning. Influence on yield and fruit quality.

2.2.2- Adjustment of a technique for quantifying the cold requirements of different varieties of *Prunus persica* L. Batsch.

CHAPTER 3

The effect of fruit on flower bud initiation and development in peach

3- The effect of fruit on flower bud initiation and development in peach

Abstract

The objectives of this study were to quantify the effects of crop load on flowering and the relationships between flowering and phloem sap carbohydrates and nitrogen fractions content from buds burst to dormancy in 'Zincal 5' nectarine. Fruit reduced significantly the number of flowers per tree both indirectly, by reducing the number of shoots per tree and the number of nodes per shoot, and directly, by reducing the number of floral buds per node. The magnitude of the response depended on the number of fruits developed per tree, trees keeping all fruits up to senescence flowered 35% less than trees thinned by hand to 40% of fruits at pit hardening, and 55% less than trees completely thinned in bloom by hand. Trees keeping all fruits had significant lower content of glucose and sorbitol in phloem sap of mixed branches up to harvest date and full vegetative growth, respectively, but not significant relationships were found between their concentration and flowering intensity following spring. Sucrose only differed at full vegetative growth, while fructose did not show any significant difference due to crop load. In fibrous roots, trees completely thinned in bloom showed lower starch content just prior to bud burst, indicating that its content is not associated to flower bud induction and differentiation but with building materials and energy supply to bud burst. Nitrate-nitrogen fraction was significantly higher and ammonia-nitrogen fraction significantly lower in trees tending to flower scarcely, indicating some disturbance of the nitrate reduction process in these trees.

Introduction

Fruit is the most important inhibitory factor of flowering of most polycarpic (perennial) plants, both in evergreen and deciduous species. In fruit trees, this effect has been amply demonstrated in pome fruit trees, mainly in apple and pears (Jonkers, 1979), and *Citrus* trees (Moss, 1971). In stone fruit trees, a negative correlation between percentage of set and floral bud developed also has been found (Couranjou, 1970) and partial flower thinning increased the number of subsequent developed floral buds (Myers *et al*., 2002).

Gibberellins have been reported to inhibit the initiation of flower buds in apple (Luckwill, 1970), pears (Griggs and Iwakiri, 1961), *Prunus* (Bradley and Crane, 1960) and *Citrus* (Monselise and Halevy, 1964). Although other kind of endogenous phytohormones, such as cytokinins, auxins and ethylene, may possibly play an indirect role influencing

budbreak, no direct regulatory role in flowering has been shown. Thus, gibberellins are apparently positioned in the intersection between flowering plant programs, and they interfere with flower formation (Golsdchmidt *et al*., 1997). Actively growing rootlets may be the source of the inhibitory endogenous gibberellins (Carr *et al*., 1964), but seeds and fruit tissues are also important source of gibberellins (Chan and Cain, 1967; Monselise, 1978; Marino and Greene, 1981). In apples, flower bud formation coincides with embryo growth in developing fruitlets and it allows Luckwill (1970) to hypothesize that gibberellins produced in seed inhibit flower formation. Nowadays, a vast amount of experiments support this hypothesis, since for many polycarpic plants, both evergreen and deciduous species, gibberellic acid sprayed when natural flower induction takes place, prevents flower bud formation or decreases flowering intensity. This is the case of *Citrus* (El-Otmani *et al*., 2000), *Pome* fruit trees (Jonkers, 1979) and stone fruit trees (Painter and Stembridge, 1972).

Despite of it, the depressed vegetative growth due to fruit, concomitant to the flowering inhibitory effect, does not agree with the high levels of gibberellins produced by fruit. García-Luis *et al*. (1995) showed a significant reduction of bud sprouting due to fruit in *Citrus*, and Syvertsen *et al*. (2003) showed that total fruit removal early in the season favours tree vegetative growth also in *Citrus*. This demonstrates that fruit diminishes the proportion of buds that are able to sprout, thus reducing the number of buds ready to flower, and also proves the competition between reproductive and vegetative growth. The population of developing fruits creates a cumulative sink of both mineral and organic nutrients, and the depletion of reserves as a result of it has been suggested as one of the reasons of the reduction of vegetative growth (Monselise and Goldschmidt, 1982). Although these conditions would depress root growth and shift nutritional balance toward flower initiation (Monselise and Goren, 1969), the effect of fruit on flowering is unrelated to carbohydrate accumulation in the roots or the leaves (García-Luis *et al*., 1995) and no consistent correlation between carbohydrate levels and flowering has been found (Goldschmidt *et al*., 1985; Lovatt *et al*., 1988). However, some disturbance on the nitrate reduction process has been shown in *Citrus* with regard to flowering process (Monselise *et al*, 1981). A similar study has not been done in *Prunus* species.

In this work we study the effect of fruit on flower bud formation and vegetative growth in peach, with special attention to carbohydrates concentration and nitrogen fractions in shoots' phloem sap and reserves in roots, in relation to flowering.

Materials and methods

Experiments were conducted during four consecutive years (2000-2003) in a commercial orchard of nectarine trees, cv. 'Zincal 5' (*Prunus persica* L. Batsch), located in Alginet, Spain (latitude 39.27º N – longitude 0.47º W – altitude 30 metres), using seven-year-old trees, grafted onto 'GF677' rootstock, planted 5x5 m apart, grown in a loamy clay soil with drip irrigation. Trials were conducted as randomized complete blocks design with one-tree plots and ten replicates. Same trees were used every year.

Every year, just after budbreak, all flowers of a set of ten trees were removed (NF), whereas all flowers of a second set of ten trees left up to fruit senescence (AF); a third set of ten trees were hand thinned at pit hardening to 40% of fruits per tree according to commercial criteria (TF). In spring, number of mixed shoots, fruiting shoots, spurs, premature shoots and water sprouts per tree were counted. Ten apical and ten lateral shoots, mixed and fruiting shoots, ten spurs and ten premature shoots per tree, all around the tree and at 1.5-2.0 m height, were also labelled. From them, length, number of flowers and distribution to flower shoots type, number of nodes, average internode's length and fresh weight and dry weight of wood was recorded.

Flowering intensity was evaluated by both number of flowers per m of shoot length and total number of flowers per tree. The former was calculated by counting the flowers and measuring the length of labelled terminal mixed and fruiting shoots. Total number of flowers per tree was evaluated by multiplying average number of flowers per shoot of mixed shoots, fruiting branches, spurs and premature shoots, by total number of each type of shoots per tree. Average number of flowers per node was also calculated.

At pit hardening (PH), harvesting date (HD), full vegetative growth (VG), leaf abscission (LA), dormancy (DM) and bud bursting (BB) stages, two mixed shoots per tree were sampled for concentrations of sugars and starch. Shoots were transported to the lab at low temperature (≤5ºC), cut in segments and mixed those of every two trees, in the same order for all sample dates. Bark of shoots was removed at low temperature (≤5ºC), lyophilized and stored at -40ºC until sugar analysis. At bud bursting stage, fibrous roots were also sampled, transported, mixed, lyophilized and stored as for mixed shoots.

Powdered samples (100 mg dry wt) were extracted with 1.0 ml of 80% ethanol. Known amounts of mannitol (Sigma Chemical, Madrid, Spain), a sugar absent in the extracts, were added to the extracts as internal standard. The extracts were then boiled for 5 min and centrifuged at low speed (10000 rpm) for 10 min. The supernatant was removed and the pellet extracted twice as above. The combined supernatant was evaporated in vacuum at 45ºC to 0.5 ml and purified sequentially by cation exchange Dowex 50X8-100 (Sigma Química, Madrid, Spain) and anion exchange Dowex 1X4-400 (Sigma Química, Madrid, Spain) chromatography. The columns (1.0 ml volume) were pre-equilibrated with 2M HCl and 1M Na_2CO_3, respectively, and washed with water before sample application. The elutes (1.0 ml extract and 2 ml water washing) were then passed through a C18 Sep-Pak cartridge (Waters-Millipor, Barcelona, Spain). Sugars were analysed using a Spectra System HPLC equipped with a P2000 gradient Pump, a RI-150 differential refractometer and a ChromQuest Chromatography Data System for Windows NT. Twenty microlitre aliquots of the filtered extracts were injected onto a Waters Sugar-PAK^{TM} I Chromatography column (Waters; USA). Column temperature was kept at 85ºC and mQ water was used as solvent at a flow rate of 0.5 ml min^{-1}. Fructose, glucose, sorbitol, and sucrose were identified by their retention times. Sugar quantification was performed using peak area calculation related to regression curves for known concentrations of sugars (Sigma Química, Madrid, Spain), taking into account the recovery of each sample.

Starch was determined in the remaining pellets after extraction of soluble sugars. The residues were dissolved in a small volume of water, the pH was adjusted to 6.0 and the starch was gelatinized by autoclaving the extracts for 2h at 130ºC. A 2.5 ml aliquot of 2M Na-acetate buffer (pH 4.8) and 5 ml amyloglucosidase (650 units, from Rhizopus mold, Sigma Química, Madrid, Spain) were added to the extracts. These were adjusted with water to a final volume of 20 ml and enzymatic digestions were performed for 2h at 55ºC under continuous shaking. After filtration, the released glucose was determined by HPLC as above.

Total and soluble fractions of nitrogen were also analyzed. Proteic nitrogen (N-proteic), ammonia nitrogen (N-NH_4^+) and nitrate nitrogen (N-NO_3^-) (measured as the combined pool of NO_3^-+NO_2^-) content were determined according to AOAC (1984), Raigón et al. (1992) and Beljaars et al. (1994). Powdered bark samples (500 mg dry wt) were homogenised in 10 ml 5 % cold (4ºC) trichloroacetic acid (TCA) using a magnetic shaker (RO5 IKA-WERKE, Staufen, Germany) for 15 min. The probe was rinsed with 30 ml 5% cold TCA, which was added to the homogenate. The

homogenate was stored at 4ºC for 15 min and then filtered through 90 mm Schleider & Shvell filter paper. The residuum, containing N-Prot, was rinsed three times with 10 ml 5% cold TCA, which was added to the filtered solution. The filtered solution was completed up to 100 ml with mQ water and stored at 4ºC until $N\text{-}NH_4^+$ analysis. For N-proteic analysis, the solid residue and the filter paper were digested, by the micro-Kjeldahl method, with 10 ml 96% concentrated sulphuric acid, 10 ml H_2O_2, and 3 g catalyst mixture K_2SO_4:$CuSO_4$:Se (10:1:0.1), at 450ºC for 30 min; then, digested sample was distilled in a Foss Kjeltec 2200 Auto Distillation® with 40% w/v NaOH and 2% boric acid solution, and titrated with 0.1 N HCl. Results are expressed as mg N-Prot per g dried wt. $N\text{-}NH_4^+$ was determined by means of a FIAstar 5000 Analyzer® (Flow Injection System, Höganäs, Sweden) equipped with an ammonia cassette including a gas diffusion membrane and a 5027 Auto-Sampler. mQ water was used as a carrier, 0.5 M NaOH as a Reagent 1, and the acid –base indicator solution as a Reagent 2. Forty µl of the filtered solution containing $N\text{-}NH_4^+$ were injected into the carrier stream merged with the NaOH stream. The colour shift was measured at 590 and 720 nm. Results are expressed as µg $N\text{-}NH_4^+$ per g dry wt.

For $N\text{-}NO_3^-$ fraction analysis, powdered bark samples (500 mg dry wt) were homogenized in 50 ml mQ water, using a magnetic shaker for 30 min. The homogenized was filtered through 90 mm Schleider & Shvell filter paper. Two hundred µl of the filtered solution were injected in the FIAstar 5000 Analyzer® equipped with a nitrate-nitrite cassette, dialysis membrane and cadmium reducing column. The colour shift was measured at 540 and 720 nm. Results are expressed as µg $N\text{-}NO_3^-$ per g dry wt.

Analysis of variance was performed on the data, using Newman-Keuls multiple range test for means separation.

Results

In peaches, flowering is inversely related to the number of preceding fruits. In our experiments under Mediterranean climatic conditions, a growing adult tree of 'Zincal 5' nectarine thinned up to 40% of developing fruit at pit hardening produced 55 flowers m^{-1} shoot on average. In contrast, removal of all flowers at anthesis increased the average number of flowers in next season up to 63-66 flowers m^{-1}, whereas the presence of total flowers and fruits up to senescence stage reduced it down to 45-49 flowers m^{-1} on average. The results were steady during the four years of experiments (Figure 3.1). In terms of number of flowers per tree, all flowers removal increased number of flowers in the next spring from

$32.9x10^3$ flowers per tree in the fruit thinned-control-trees to $45.9x10^3$ flowers per tree, whereas keeping all fruits on the tree up to senescence reduced it down to $20.8x10^3$ flowers per tree. Distribution of flowers according to the shoot's type is shown in Figure 3.2.

Fruit also affected vegetative growth. Fifty days after flower removal, terminal shoots of deflowered trees had more nodes (14.7 on average) than those of not thinned trees (11.5 nodes). Furthermore, shoot length and leaf dry weight was significantly larger in deflowered trees compared to not thinned trees (data not shown). At winter rest period, terminal shoots had grown 60% more in deflowered and thinned trees than in not thinned trees (Table 3.1). Besides, number of nodes per terminal shoot was significantly larger in deflowered trees. Also number of flowers per node was significantly higher in shoots that have not fruits or had few fruits (thinned trees) compared to shoots that had all fruits up to senescence (Table 3.1). On the other hand, trees having all fruits developed significant lower number of shoots (6500, aprox.) than hand-thinned trees at pit hardening (8500) and it, in turn, lower than deflowered trees (12000), the premature shoots (Figure 3.3) and water sprouts being the most affected shoots. Among bearing shoots, fruiting shoot was the most affected shoot decreasing down to 50% due to the presence of fruit, followed by mixed shoot (25%); not significant differences were observed for spurs (Figure 3.3). In lateral shoots, not significant differences due to fruit were found in any of parameters measured (data not shown).

The time-course of concentration of the sugar translocating sorbitol in the bark tissues of mixed shoots, showed significantly higher values for deflowered trees up to full vegetative growth stage, at which presented a peak of concentration (Figure 3.4A). From then to bud burst stage concentration declined continuously and not significant differences due to fruit were observed. Sucrose content was markedly lower than sorbitol content and also presented a peak of concentration during vegetative growth; but it exhibited a second peak, larger than the first one, at dormancy, followed by a sharp decline down to bud burst stage (Figure 3.4B). Only during full vegetative growth sucrose concentration differed significantly between treatments. The hexose glucose showed significant differences in concentration due to fruit up to harvest date, the concentration being higher for deflowered trees, whereas hexose fructose does not show significant differences due to fruit. From then, not significant differences due to fruit were observed for both glucose and fructose. Their concentration declined down to dormancy and remained almost constant until bud burst (Figures 3.4C and 3.4D).

Starch accumulation in the fibrous roots depended significantly on the crop load. At bud burst, deflowered trees presented significant lower starch concentration in the roots (94.5±1.1) than not thinned trees (114.3±1.9) or hand thinned trees at pit hardening (112.9±4.3). These lower value in the fibrous roots of trees tending to flower more profusely (see Figures 3.1 and 3.2) corresponded with higher concentration of hexoses in their fibrous roots (data not shown) which correlated positively with number of flowers per m of mixed shoot, both for glucose (r^2= 0.501; ns) and fructose (r^2= 0.897; $P \leq 0.05$). Not significant differences in starch content were observed in the bark tissues of mixed shoots due to fruit (data not shown); it remained almost constant (60-64 mg g^{-1} dw) from leaves abscission to bud burst.

Ammonia-nitrogen fraction in bark tissues of mixed shoots decreased from harvest date (110-120 µg g^{-1} dw) down to leaf abscission (70-80 µg g^{-1} dw) and remained almost constant up to bud burst. Nitrate-nitrogen fraction decreased from harvest date (30-37 µg g^{-1} dw) down to dormancy (12-15 µg g^{-1} dw) in bark tissues of mixed shoots from deflowered trees or hand-thinned trees, remaining almost constant up to bud burst; but trees that had all fruits up to senescence showed a peak of nitrate-nitrogen fraction at dormancy (Table 3.2) followed by a sharp decrease down to bud burst, reaching same values that deflowered and thinned trees. Not significant differences due to fruit were observed during the period of study, except for dormancy. At dormancy, trees that support all developed fruits had the highest concentration of nitrate mentioned above and the lowest of ammonia-nitrogen fraction in the bark tissues of mixed shoots (Table 3.2). At this stage, nitrate-nitrogen correlated negatively (r^2= 0.878; $P \leq 0.05$) and ammonia-nitrogen positively (r^2= 0.653; $P \leq 0.05$) with number of flowers m^{-1} of mixed shoot burst following spring. Proteic-nitrogen fraction and total nitrogen did not show significant differences due to fruit during the period of this study (data not shown). On the contrary, fibrous roots of deflowered trees had, prior to bud burst, significant higher concentration of proteic-nitrogen (11.7±0.3 mg g^{-1} dw) than those that support all fruits (8.9±0.3) or thinned trees (8.8±0.1). Concentration of this proteic- nitrogen fraction of fibrous roots correlated positively with number of flowers m^{-1} of mixed shoot (r^2= 0.701; $P \leq 0.05$) burst following spring.

Discussion

In deciduous trees flower initiation occurs during the first stages of fruit development, and a strong effect of developing fruit inhibiting bud initiation is found. Fruit tree species such as pear, apple, plum, apricot and even evergreen fruit tree species, such as mango, olive and citrus suffer this

effect and reach to alternate the crop. A biennial cycle is very usual, so that an "on-year" (large yields) is followed by an "off-year" (little or no yield) and so on for a sequence of several years (Monselise and Goldschmidt, 1982).

Our results show evidences that, in peach, this inhibitory effect of fruit on flowering takes place through both indirect and direct mechanism. Indirectly, by reducing the number of shoots and the number of nodes per shoot, that is, by reducing the number of buds that, potentially, can develop into flower, directly by reducing the number of flower buds per node. In accordance with our results, Syvertsen *et al* (2003) showed that, in *Citrus*, fruit removal early in the season favour tree vegetative growth, which increase crop potential for the following year, and Byers *et al* (1990) reported an increase of 103% in the number of flower buds per limb by means of bloom thinning mature peach trees. However, no effect on the total number of shoots per tree was reported in these studies. On the other hand, Myers *et al* (2002), demonstrated that partial flower thinning increased the number of flower buds per node. Our results agree with those of these authors, but improve them since give information for the entire tree. Furthermore, in our experiments partial thinning of fruit at pit hardening and total thinning of flower during bloom increased in a same proportion the number of flower buds per node compared to not thinned trees, indicating that there must be a threshold value of fruit number above which inhibition of flowering takes place, and that fruit interferes with flowering induction process during the cell division fruit growth stage, that is, up to pit hardening, in agreement with the results for pome fruits (Chan and Cain, 1967). The combination of the reduction in the number of shoots in the tree, the number of nodes per shoot and the number of flower bud per node, give rise to the reduction of number of flowers per tree due to fruit.

The effect of fruit inhibiting flowering of woody plants is under hormonal control (Goldschmidt *et al*., 1997). In fruit tree species the annual production of flowers is understood, nowadays, as a process constantly induced, but with their flower buds development under the control of an inhibitor that acts in a quantitative manner (Goldschmidt and Samach, 2004; Martínez-Fuentes *et al*., 2004). Gibberellins are, among the currently known plant hormones, the most strongly associated with flowering (Pharis and King, 1985; Goldschmidt *et al*., 1997) also in peaches, as it is proved by the inhibition obtained applying gibberellic acid during flower bud induction (Painter and Stembridge, 1972). Seeds, particularly in stone fruits, are an important source of gibberellins which lead to inhibit flowering (Hoad, 1978).

With regard to carbohydrate contents, the high amounts of energy required for fruit development is the reason of lower sorbitol and glucose concentration in the phloem sap of our not deflowered trees compared to deflowered or fruit thinned trees, rather than a reason related with flowering, a process which in itself does not appear to require much energy. However, the fact that fruit development, shoot elongation and flower induction take place at the same time, make difficult to explain the role of carbohydrates in these processes. In fruit trees, it seems to be an antagonism between flowering and shoot elongation, mediated by fruit that reduces number of developing shoots and number of nodes per shoot. Since there are conditions when a new vegetative flush may become a better sink than fruits (Quinlan and Preston, 1971), some kind of competition between vegetative and reproductive sinks exists. According to our results, in peaches competition favours to fruit, due to its large seed, and carbohydrates are transported to and consumed by fruit, preferably. Furthermore, this effect restarts every year and, thus, differences in flowering of our trees, according to treatments, persist along the years.

Another aspect of interest is that when fruit completes the development, its power sink stops, and from then no differences on hexoses content in the phloem sap were found due to fruit. At dormancy, and prior to bud burst, the higher content of glucose, compared to fructose, agrees with the key role in triggering the onset of budbreak referred to glucose (Maurel *et al*., 2004).

Carbohydrate reserves in fibrous roots were not related with fruit load up to dormancy, indicating that its content is not associated to the process of flower bud induction and differentiation. However, prior to budbreak trees which were more prone to flower had lower starch concentration in fibrous roots, which corresponded with higher concentration of sorbitol and glucose, proving that bud needs a supply of buildings materials and energy during the early events of budbreak.

Higher levels of $N\text{-}NO_3^-$ on trees that support all fruits up to senescence corresponded with lower values of $N\text{-}NH_4^+$, suggesting some disturbance of the nitrate reduction process in trees with lower number of flower buds. This result agrees with that of Monselise *et al* (1981) for *Citrus*. Lovatt *et al*. (1988) hypothesized that the accumulation of ammonia, which increases with severity or duration of low temperature and water-deficit stress, is an important factor in the regulation of flower initiation in *Citrus* and other tropical and subtropical evergreen tree crops, as showed by Nevin and Lovatt (1987) in mango. Furthermore, urea or ammonium fertilization enhanced flower production in *Citrus* (Lovatt *et*

al., 1988) and apple (Edwards, 1986), respectively. Our results in nectarine agree with this ammonium content and flowering relationship.

In conclusion, in peaches flower intensity depends on the number of developed fruit, both indirectly, because fruit reduces the number of developing shoots and the number of nodes per shoot, and directly, because reduces the number of flower buds. Neither soluble sugars in phloem sap of shoots nor the carbohydrate reserves in fibrous roots have been associated to the flower bud initiation. Evidences of some disturbance of the nitrate reduction process in trees tending to flower scarcely are shown.

References

Beljaars, P.R.; Van Dijk, R. and Van der Horst, G.M.J. 1994. Determination of Nitrate in Vegetables by Continuous Flow: Interlaboratory Study. Journal of AOAC International 77: 1522-1529.

Bradley, M.V. and Crane, J.C. 1960. Gibberellin-induced inhibition of bud development in some species of *Prunus.* Science 13: 825-826.

Byers, R.E.; Carbaugh, D.H. and Presley, C.N. 1990. The influence of bloom thinning and GA_3 sprays on flower bud numbers and distribution in peach trees. Journal of the American Society of Horticultural Science 65: 143-150.

Carr, D.J.; Reid, D.M. and Skeene, K.G.M. 1964. The supply of gibberellins from the root to the shoot. Planta 63: 382-392.

Chan, B.C. and Cain, J.C. 1967. The effect of seed formation on subsequent flowering in apple. Proceedings of the American Society of Horticultural Science 91: 63-67.

Couranjou, J. 1970. Recherches sur les causes génétiques de l'alternance du prunier domestique (*Prunus domestica* L.) I. Mise en evidence de deux charactères de productivité comme facteurs d'alternance. Incidence d'un élément du milieu sur l'expression de l'alternance et sa modification. Nature Biotechnology 20: 297-318.

El-Otmani, M.; Coggins, C.; Agustí, M. and Lovatt, C.J. 2000. Plant Growth Regulators in Citriculture: World current uses. Critical Review in Plant Sciences 19: 395-447.

Edwards, G.R. 1986. Ammonia, arginine, polyamines and flower initiation in apple. Acta Horticulturae 179: 363.

García-Luis, A.; Kanduser, M. and Guardiola, J.L. 1995. The influence of fruiting on the bud sprouting and flower induction responses to chilling in *Citrus*. Journal of the American Society of Horticultural Science 70: 817-825.

Goldschmidt, E.E.; Aschkenazi, N.; Herzano, Y.; Schaffer, A.A. and Monselise, S.P. 1985. A role for carbohydrate levels in the control of flowering in *Citrus*. Scientia Horticulturae 26: 159-166.

Goldschmidt, E.E.; Tamin, M. and Goren, R. 1997. Gibberellins and flowering in citrus and other fruit trees: A critical analysis. Acta Horticulturae 463: 201-207.

Goldschmidt, E.E. and Samach, A. 2004. Aspects of flowering in fruit trees. Acta Horticulturae 653: 23-27.

Griggs, W.H. and Iwakiri, B.T. 1961. Effects of gibberellin and 2,4,5-trichlorophenoxypropionic acid sprays on Barlett pear trees. Proceedings of the American Society of Horticultural Science 77: 73-89.

Hoad, G.V. 1978. The role of seed derived hormones in the control of flowering in apple. Acta Horticulturae 80: 93-103.

Jonkers, H. 1979. Biennial bearing in apple and pear: a literature survey. Scientia Horticulturae 11: 303-317.

Lovatt, C.J.; Zheng, Y. and Hake, K.D. 1988. A new look at the Kraus-Kraybill hypothesis and flowering in Citrus. Proceedings of 6th International Citrus Congress 1: 475-483.

Lovatt, C.J.; Zheng, Y. and Hake, K.D. 1988. Demonstration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. Israel Journal of Botany 37: 181-188.

Luckwill, L.C. 1970. The control of growth and fruitfulness of apple trees. In: Luckvill, L.C. and Cutting, C.V. (Eds.). *Physiology of tree crops*. Academic Press, New York, EEUU, pp. 237-254.

Marino, F. and Greene, W.D. 1981. Involvement of gibberellins in the biennial bearing of ''Early McIntosh'' apples. Journal of the American Society of Horticultural Science 106: 593-596.

Martínez-Fuentes, A.; Mesejo, C.; Juan, M.; Almela, V. and Agustí, M. 2004. Restrictions on the exogenous control of flowering in Citrus. Acta Horticulturae 632: 91-98.

Maurel, K.; Leite, G.B.; Bonhomme, M.; Guilliot, A.; Ragau, R.; Petel, G. and Sakr, S. 2004. Trophic control of bud break in peach (*Prunus persica*) trees: a possible role of hexoses. Tree Physiology 24: 579-588.

Monselise, S.P. 1978. Understanding of plant processes as a basis for successful growth regulation in citrus. International Society of Citriculture 1: 250-255.

Monselise, S.P.; Goldschmidt, E.E. and Golomb, A. 1981. Alternate bearing in citrus and ways of control. Proceedings of the International Society for Citriculture 1: 239-242.

Monselise, S.P. and Goldschmidt, E.E. 1982. Alternate bearing in fruit trees. Horticultural Review 4: 128-173.

Monselise, S.P. and Goren, R. 1969. Flowering and fruiting-interactions of exogenous and internal factors. Proceedings First International Citrus Symposium 3: 1105-1112.

Monselise, S.P. and Halevy, A.H. 1964. Chemical inhibition and promotion of citrus bud induction. Proceedings of the American Society of Horticultural Science 84: 141-146.

Moss, G.I. 1971. The effect of fruit on flowering in relation to biennial bearing in sweet orange (*Citrus sinensis*). Journal of Horticultural Science 46: 177-184.

Myers, S.C.; Savelle, A.T.; Tustin, D.S and Byers, R.E. 2002. Partial flower thinning increases shoot growth, fruit size, and subsequent flower formation of peach. Journal of the American Society of Horticultural Science 37: 647-650.

Nevin, J.M and Lovatt, C.J. 1987. Demonstration of ammonia accumulation and toxicity in avocado leaves during water-deficit stress. South-African-Avocado-Growers-Association 10: 51-54.

Painter, J.W and Stembridge, G.E. 1972. Peach flowering response as related to time of gibberellin application. HortScience 7: 389-390.

Pharis, R.P. and King, R.W. 1985. Gibberellins and reproductive development in seed plants. Annual Review of Plant Physiology 36: 517-568.

Quinlan, J.D and Preston, A.P. 1971. The influence of shoot competition on fruit retention and cropping of apple trees. Journal of Horticultural Science 46: 525-534.

Raigón, M.D.; Pérez-García, M.; Maqueira, A. and Puchades, R. 1992. Determination of available nitrogen (nitric and ammoniacal) in soils by flow injection analysis. Analisis 20: 483-487.

Syvertsen, S.P.; Goni, C. and Otero, A. 2003. Fruit load canopy shading affect leaf characteristics and net gas exchange of ‘Spring’ navel orange trees. Tree Physiology 23 (13): 899-906.

Table 3.1: Influence of fruit on the terminal shoots (mixed and fruiting shoots) characteristics from mixed branches of nectarine 'Zincal 5' trees completely thinned in bloom (NF) by hand, thinned to 40% of fruits by hand at pit hardening (TF) and not thinned (AF). Values corresponding to dormancy stage. Each value is the mean ± SE of 100 branches from 10 trees.

	Shoot length	Shoot weight ($g\ m^{-1}$)	Internode's length	Node Nº	Nº flower buds per node
NF	38.8 ± 0.8 a	42.3 ± 1.6 a	1.5 ± 0.1 a	25.6 ± 0.4 a	0.84 ± 0.03 a
TF	35.9 ± 0.7 a	27.3 ± 1.3 b	1.7 ± 0.1 a	21.1 ± 0.5 b	0.88 ± 0.04 a
AF	24.1 ± 0.5 b	30.7 ± 0.7 b	1.2 ± 0.1 b	19.8 ± 0.4 b	0.49 ± 0.01 b

Different letters in the same column indicate significant differences ($P \leq 0.05$).

Table 3.2: Nitrate-nitrogen and ammonia-nitrogen content in 'Zincal 5' nectarine phloem sap on fully thinned in bloom by hand (NF), thinned to 40% fruit by hand at pit hardening (TF) and not thinned (AF) trees. Values corresponding to dormancy stage. Each value is the mean ± SE of 10 trees.

	$N\text{-}NO_3^-+NO_2^-$ ($\mu g\ N\ g^{-1}$ dw)	$N\text{-}NH_4^+$ ($\mu g\ N\ g^{-1}$ dw)
NF	11.3 ± 0.5 b	63.8 ± 2.1 a
TF	13.5 ± 1.3 b	63.0 ± 2.4 a
AF	38.2 ± 2.5 a	53.3 ± 1.6 b

Different letters in the same column indicate significant differences ($P \leq 0.05$).

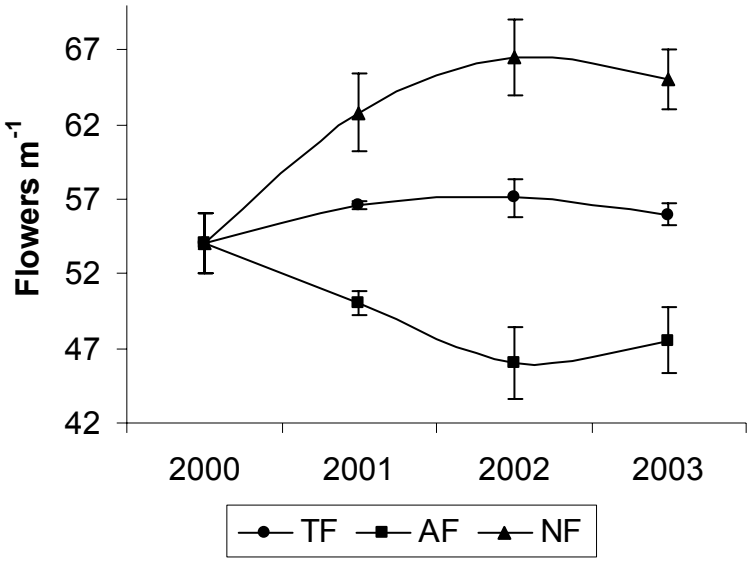

Figure 3.1: Yearly effect of fruit on average number of flowers located in mixed and fruiting terminal shoots from mixed branches of nectarine 'Zincal 5'. Values for trees completely thinned in bloom (NF), hand-thinned to 40% of fruits at pit hardening (TF) and not thinned (AF). Each value is the average of 10 trees. Standard errors are given as vertical bars. Except for year 2000, differences among trees are significantly different ($P \leq 0.05$).

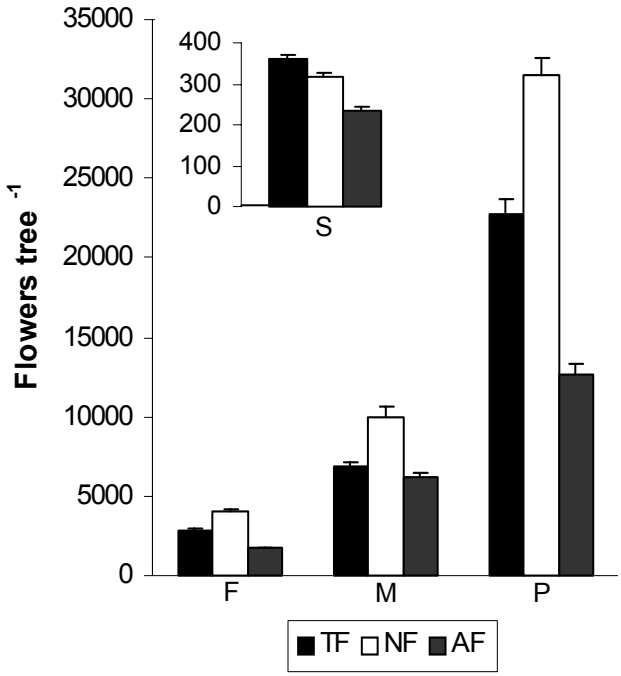

Figure 3.2: Effect of fruit on the pattern of flower distribution according to shoot types in nectarine 'Zincal 5'. Values expressed as total flowers per tree located in spurs (S), fruiting branches (F), mixed shoots (M) and premature shoots (P) for trees completely thinned in bloom (NF), hand-thinned to 40% of fruits at pit hardening (TF) and not thinned (AF). Each value is the average of 10 trees. Standard errors are given as vertical bars.

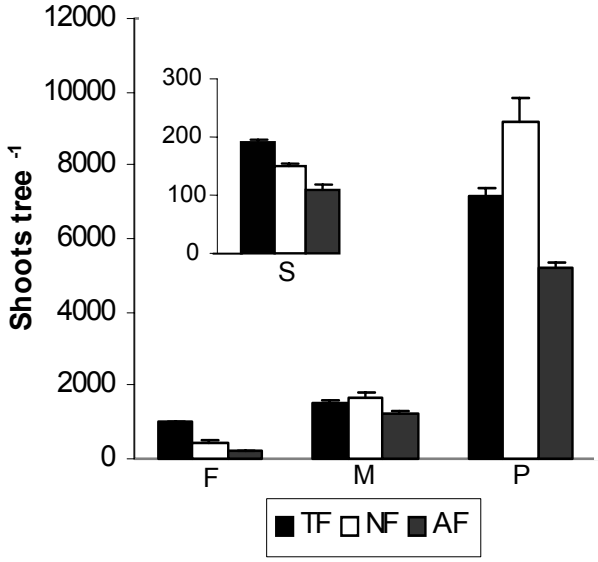

Figure 3.3: Effect of fruit on the number of shoots developed per tree of nectarine 'Zincal 5'. Values expressed as total number of spurs (S), fruiting branches (F), mixed shoots (M) and premature shoots (P) per tree completely thinned in bloom (NF), hand-thinned to 40% of fruits at pit hardening (TF) and not thinned (AF). Each value is the average of 10 trees. Standard errors are given as vertical bars.

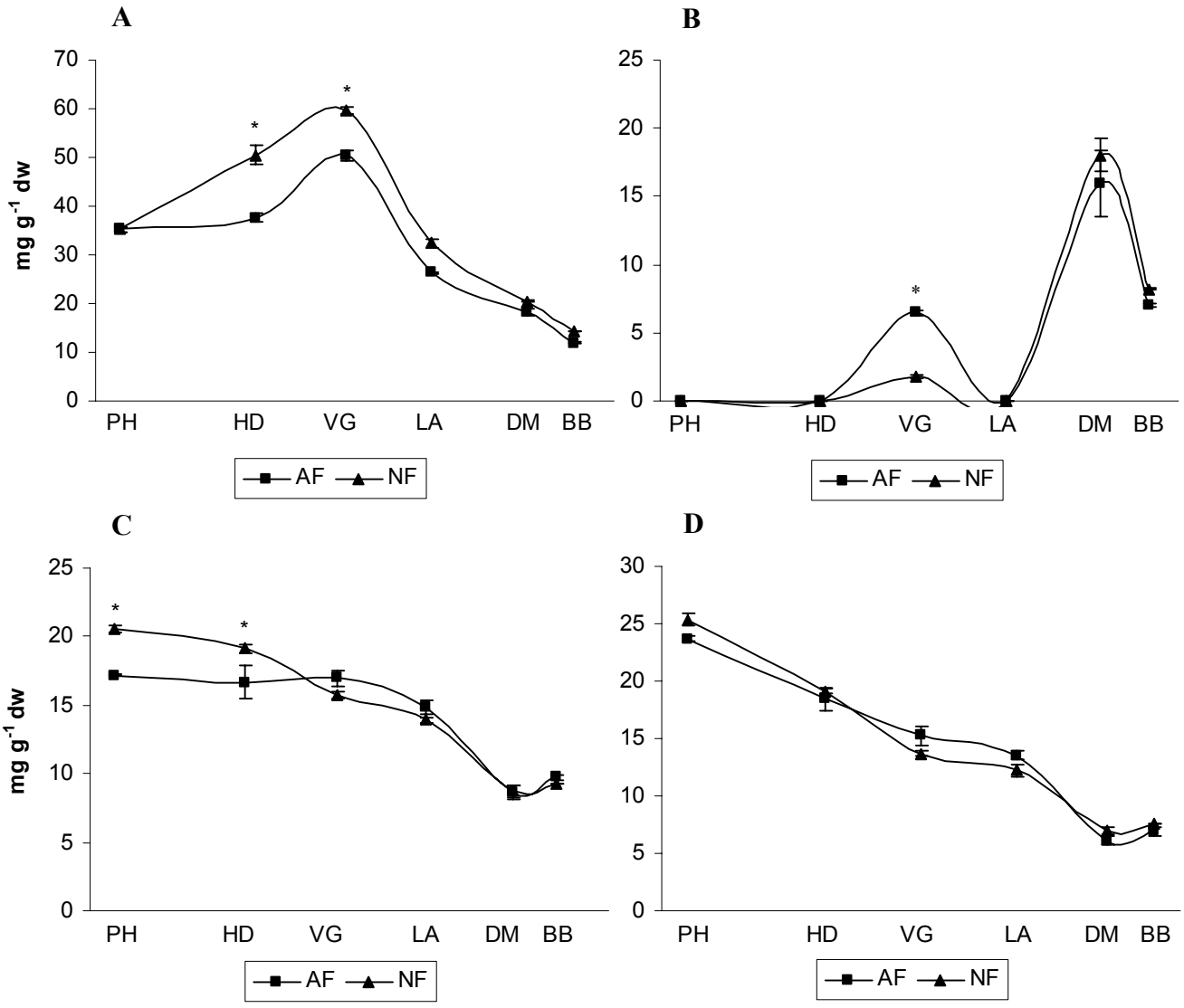

Figure 3.4: Effect of fruit on the time-course of sorbitol (A), sucrose (B), glucose (C) and fructose (D) concentration in phloem sap of nectarine 'Zincal 5' trees completely thinned in bloom (NF) by hand and not thinned (AF). Values are the average of 10 trees. Standard errors are given as vertical bars. (*) Significant at $P \leq 0.05$.

CHAPTER 4

Factors regulating effectiveness of GA_3 inhibiting flowering in peaches and nectarines (*Prunus persica* L. Batsch)

4- Factors regulating effectiveness of GA_3 inhibiting flowering in peaches and nectarines (*Prunus persica* L. Batsch)

Abstract

The application of gibberellic acid during the flower bud induction period reduced significantly flowering in peaches and nectarines. The magnitude of the response significantly depended on the total amount of active material applied per tree. Results show, for cultivars tested, a higher sensitivity of peach to GA_3 in comparison with nectarine. Concentrations of 0.5 or 1.0 g $tree^{-1}$ of gibberellic acid reduced flowering by about 50% in both peaches and nectarines and gave rise to a reduction in the cost of hand thinning of approximately 50%, without affecting yield and fruit characteristics. The effect was higher in the basal part of the shoots and reduced from the base to the apical part. This is of crucial importance for winter pruning, since a reduction of shoot length in gibberellic acid-treated trees, could lead to an excessive reduction in the number of flowers at bloom and even to a reduction in yield.

Introduction

Peaches, in optimum growing conditions, set a large number of fruits, which reduces the possibility of attaining commercial fruit size and quality at harvest (Faust, 1989; Costa and Vizzotto, 2000; Southwick and Glozer, 2000). Eighty percent of the variance found in the final fruit size can be explained by variations in the number of fruits initially set in Mediterranean climatic conditions (Agustí *et al*., 1997). Accordingly, hand thinning has proved to be a reliable way of improving fruit size (Costa and Vizzotto, 2000; Bergh, 1992; Havis, 1962; Knight, 1980; Lilleland, 1965), but this spends between 100 and 500 h ha^{-1} depending on tree vigour and flower production, thinning intensity and season (Clanet *et al*., 1987; Hilaire and Guiauque, 1994; Southwick and Weis, 1996, Southwick *et al*., 1995), and is therefore an expensive preharvest cultural practice (Mizelle and Westberry, 1989).

Alternative thinning techniques have been tested as substitutes for hand thinning to improve fruit size, such as girdling branches (Agustí *et al*., 1998), flower thinning by hand, or by means of mechanical methods or chemical treatments (Byers *et al.*, 1990; Moran and Southwick, 2000; Myers *et al*., 1993; Weinberger, 1941), and chemical fruit thinning using caustic materials (Byers and Lyons, 1985; Zilkah *et al.*, 1988), growth regulators (Byers *et al*., 1990; Stembridge and Gambrell, 1971; Tromp, 2000) and photosynthetic inhibitors (Byers *et al*., 1984; Del Valle *et al*.,

1985). However, effective chemical fruit thinning, as used in apples (Fallahi and Willemsen, 2002), has had very limited success in stone fruit trees (Southwick *et al*., 1995).

Gibberellins (GAs) produced in the seeds are the main cause for the negative effect of fruits on flower bud formation in many species (Faust, 1989); consequently, the application of these hormones shows a significant effect reducing the number of flowers per tree. This has been confirmed in citrus, apple and stone fruit trees by means of gibberellic acid (GA_3) applied at flower bud induction, which reduced flowering in the following season (Monselise and Halevy, 1964; Bradley and Crane, 1960; Luckwill and Silva, 1979; Painter and Stembridge, 1972; Southwick and Yeager, 1991). Thus, the inhibition of flower bud induction by applying GA_3 during the flower bud induction period appears to be a reliable method for reducing the number of flowers developing the following spring (El-Otmani *et al.*, 2000; Southwick *et al.*, 1995; Southwick and Yeager, 1991) and, thus, as an indirect method of chemical fruit thinning (García-Pallas *et al*., 2001; Agustí *et al*., 2001).

This paper presents the results obtained during a four-year experimentation period in an attempt to find the appropriate GA_3 concentration, spray timing and spraying method for adequate flower reduction in the peach and nectarine cultivars commercially grown in Valencia (Spain). We also measured the effect of GA_3 treatments on fruit quality, yield and vegetative growth the following season and quantified the cost of hand thinning of treated trees and any residual effects on flowering two years after treatment.

Materials and methods

Experiments were carried out over four consecutive years (1999-2002) on adult trees (8-10 years old) of 'Springlady' peach and 'Zincal 5' nectarine (*Prunus persica* L. Batsch), grafted onto 'GF677' rootstock, planted 5x5 m apart, grown in a sandy-loamy soil, with drip irrigation in the area of Valencia, Spain (latitude 39.28º N – longitude 0.22º W – altitude 30). Fertilization, pest management and pruning were in accordance with normal commercial practice. At pit hardening, all trees were hand-thinned to the same fruit spacing (15 to 20 fruit cm^{-1}). Time spent per tree on hand thinning was recorded.

The GA_3 (Arabelex-L; 1.6% w/v; Aragro; Madrid - Spain) was applied as a foliar spray to the whole tree at concentrations of 0, 25, 50, 75, 100, 150 and 200 mg l^{-1}, depending on the experiment, using a pressurised

handgun (30 atm) and turbo-sprayer (60 atm), spraying 3200-6000 l ha^{-1} and 1400–1600 l ha^{-1}, respectively, depending on the tree size. A surfactant (alkyl polyglycol ether, 20% p/v) was added to the spray solution at a concentration of 0.05%. In all the experiments, a concentration of 0 mg l^{-1} was considered as control. In the fourth year, and to generalize the response, trees of four peach varieties, 'Springlady', 'Candor', 'Sherman' and 'Maycrest', and of five nectarine varieties 'Zincal 5', 'Alginet', 'Valencia', '866' and 'Sunrise', were sprayed with 50 mg l^{-1} at pit hardening. Flower density at full bloom was measured and the percentage of inhibition with regard to control trees was recorded.

Trees were selected for their uniformity in size, vigour and crop load. A randomised complete block design was used in all the experiments, with 5-8 plots of one replication each.

In early winter, 10 homogeneous current season shoots per tree were randomly selected and their length measured. Each shoot was arranged longitudinally from base to apex into three segments, termed *proximal*, *middle* and *distal*, respectively, to record the location of flower distribution on the shoots. At full bloom, the number of flowers developed on each segment was recorded. Data are expressed as the mean number of flowers per m of shoot length.

Fruits were harvested according to appropriate commercial size and colour standards. Yield, average fruit weight and diameter and number of fruits per tree were recorded at each harvest date. Fruit colour was measured by determining the 'a' and 'b' Hunter coordinates of 20-fruit samples randomly picked from each replicate at the first harvest date. Three measurements per fruit were made around the equator circumference, using a Minolta chromameter CR-300. Flesh firmness was assessed using a fruit pressure tester FT-011 (Facchini, Italy) with a 4-mm-diameter flat cylinder probe. Total soluble solids (TSS) concentration of juice (ºBrix) was assessed by digital refractometer (Atago, Japan).

Ten lateral and 10 apical homogeneous current season shoots per tree were collected at harvest time, and their total length, length of internodes and number of nodes was determined in order to verify the effect of treatments on vegetative growth.

Analysis of regression and variance were performed on the data using the Duncan's multirange test for means separation. Percentages were analysed after arc sine transformation.

Results

The application of 50 mg l^{-1} of GA_3 significantly reduced flowering in 'Springlady' peach compared to controls when applied during the flowering induction period. The magnitude of the response depended on the date of treatment, ranging from 40%-50%, for treatments carried out 85-100 days after anthesis (DAA), to 20% for treatments applied 120-130 DAA (Figure 4.1). This inhibitory effect was always higher in the proximal shoot segment, followed by the middle segment and by the distal segment, the latter being not significantly different from that of control trees for all spraying dates (Figure 4.2). The effect also depended on the concentration applied. The higher the GA_3 concentration applied, the lower the percentage of shoots that presented flowers in their proximal and middle segments, both in peach cv. 'Springlady' and nectarine cv. 'Zincal 5' (Figure 4.3). Regarding the effect of date of treatment, no significant differences with untreated trees were found for the distal segments due to treatment either in peach or nectarine.

Gibberellic acid sprays during the period of highest sensitivity (85-100 DAA), showed a significant concentration effect on flower inhibition (Table 4.1). In peach cv. 'Springlady' and nectarine cv. 'Zincal 5', 25 mg l^{-1} GA_3 spraying with a pressurized handgun significantly reduced the number of flowers per m of shoot length. The effect became saturated at 50 mg l^{-1} for peach, whereas for nectarine the effect increased up to 100 mg l^{-1} GA_3. Applications with turbo sprayer evolved similarly to the pressurized handgun, but the effect saturated at 150 mg l^{-1} GA_3 (Table 4.1).

However, when the results are expressed according to the amount (g) of the active material applied per tree, it (x) correlates linearly and significantly with the percent of flowers inhibited (y), both for peaches ($y=0.06x+19.34$; $r^2=0.900$, $P\leq0.05$) and nectarines ($y=0.04x+9.08$; $r^2=0.85$, $P\leq0.05$). These equations differ significantly ($P\leq0.05$) both in the slope and in the ordinate, indicating a higher sensitivity of peaches trees to GA_3 than nectarine trees. Thus, 0.5 g and 1.0 g of GA_3 per tree are needed to inhibit 50% of flowers in peaches and nectarines, respectively.

This response to GA_3 treatments was tested and confirmed in other nectarine and peach cultivars commercially grown in Spain (Table 4.2). Spraying procedure significantly affected the magnitude of the response because of the differences in volume of solution applied per tree. Thus, the percentage of flower inhibition was nearly 50% when a pressurized handgun was used, but only 20% with a turbo-sprayer (Table 4.2).

Furthermore, the results were confirmed and peaches proved to be more sensitive to GA_3 sprays than nectarines, irrespective of spraying method.

As a consequence of the reduction in flowering intensity by GA_3 sprays, there was also a reduction in the time spent on hand thinning (Table 4.1). This time was reduced by 35-40% and 45-55% for concentrations of 50 mg l^{-1} and 75 mg l^{-1}, respectively, both for peach and nectarine, when pressurised handgun was used, and by 55% and 60% for 100 mg l^{-1} and 150 mg l^{-1} of GA_3, respectively, when turbo sprayer was used in nectarine. For pressurised handgun, higher concentrations of GA_3 (75-100 mg l^{-1}) did not reduce the time spent on hand thinning. For turbo sprayer, 200 mg l^{-1} GA_3 reduced it by more than 90%, but the drastic reduction in fruit number per tree due to this concentration makes it inapplicable.

As it is shown in Table 4.1, only GA_3 concentrations higher than 100 mg l^{-1} significantly reduced the number of fruits harvested per tree and yield (kg $tree^{-1}$), both in peach and nectarine trees, and irrespective to the spraying procedure. This is because the number of fruits left on the shoots by hand thinning was selected according to commercial practices. In peach cv. 'Springlady', control trees and GA_3 treated trees up to 75 mg l^{-1} had 5.0-6.0 fruits per m of shoot length after hand thinning on an average, whereas trees treated with 100 mg l^{-1} GA_3 had 4.4 fruits per m. Similarly, in nectarine cv. 'Zincal 5' control and 25 mg l^{-1} GA_3 treated trees had 8.8 fruits per m of shoot length after hand thinning, whereas trees treated at 50 mg l^{-1} GA_3 or higher concentrations had 6.0-7.0 fruits per m.

At pit hardening, fruit weight of 'Springlady' peach was positively and linearly correlated with the number of flowers per m of shoot length (r^2 = 0.74; $P \leq 0.5$) and with the GA_3 concentration applied (r^2 = 0.90; $P \leq 0.05$). Fruit size of both 'Springlady' and 'Zincal 5' cultivars was larger compared with the untreated control trees when 75 mg l^{-1} GA_3 was applied. Besides, treatment increased fruit weight of peach and nectarine by 27% and 16%, respectively. Differences in fruit weight were maintained, and even increased, during linear development fruit stage. In 'Springlady' peach, fruits of control trees reached a diameter of 59.4 mm at harvest, which was significantly lower than that of fruits from trees treated at 50 mg l^{-1} GA_3 or higher concentrations (63-64 mm) (Table 4.1). Similarly, fruit diameter of nectarine was increased for 25 mg l^{-1} GA_3 or higher concentrations.

The accelerated fruit development by cause of flowering reduction led to an advanced fruit maturity, which is shown in Table 3 for nectarine 'Zincal 5'. At harvest, fruit colour and total soluble solids were significantly higher for fruits treated at 25 mg l^{-1} GA_3 and higher

concentrations, with regard to untreated fruits. Accordingly, the number of fruits harvested at the first picking date increased up to applications of 100 mg l^{-1} GA_3 (Table 4.1). However, even when the process of fruit maturity involves a natural softening, fruit firmness showed a significant increase with increasing GA_3 concentration applied (Table 4.3).

The reduction in the initial number of reproductive developing sinks, also affected vegetative growth at harvest. Internode length of lateral shoots was significantly increased in nectarine cv. 'Zincal 5' when trees were treated with 50 mg l^{-1} GA_3 or higher concentrations, with regard to control; internode length of apical shoots was significantly increased only at 150 mg l^{-1} GA_3 concentrations or higher (data not shown).

The residual effects of different GA_3 concentrations applied to peach cv. 'Springlady' were evaluated at bloom in the second spring after treatment. There were not significant differences in the number of flowers per m of shoot length between control and treated trees (data not shown). The small differences found between treatments could not be attributed to the treatments themselves. Indeed, flower density in control trees did not vary significantly the two following years after treatments (51.1±6.1 and 59.4±3.9 flowers per m, respectively).

Discussion

Gibberellins diffusing from the developing fruits are the major cause of alternate bearing in apples (Williams and Edgerton, 1981). This effect of gibberellins takes place very soon, within 3 to 8 weeks after full bloom (Chan and Cain, 1967; Faust, 1989). To demonstrate the flower bud induction period in peach under Mediterranean climatic conditions, Jourdain and Clanet (1987) used GA_3 sprays which had previously proved as flower inhibitors in *Citrus* (Monselise and Halevy, 1964), resulting that it takes place between late April and late July. Nowadays, the use of gibberellic acid to reducing flowering is a common practice in *Citrus* (El-Otmani *et al.*, 2000), avocado (Salazar-García and Lovatt, 2000), apricot (Southwick and Weis, 1996), mango (Turnbull *et al.*, 1996), *Pomes* (McArtney y Li, 1998) and even in peach (Painter and Stembridge, 1972; Southwick and Glozer, 2000).

In peaches, the application of GA_3 causes asymmetric flower distribution along the shoot. In treated shoots, the lowest density of flower buds is located in the proximal segment of the shoot, whereas the higher one is found in the distal segment, increasing gradually from the former to the latter, which is in agreement with results by Byers *et al.* (1990). It has

been suggested that GA_3 persistence in the external surface of treated organs is very limited (Monselise *et al.*, 1976), thus GA_3 action on buds developing later in the season (August and September) is reduced, explaining the higher flower density on the distal shoot segment in the following spring after treatment. This is a critical point for planning winter pruning, since the removal of apical segments in GA_3 treated trees could lead to an excessive reduction in the number of flowers at bloom and even reduce the yield.

In our experiments, only concentrations of 100 mg l^{-1} GA_3 or higher concentrations significantly affected fruit yield, irrespective of the spraying procedure. However, for concentrations between 0 and 100 mg l^{-1} GA_3, the reduction in the number of harvested fruits was related not only to the GA_3 treatment itself but also to the number of fruits left by hand thinning. Workers have a tendency to leave fruits uniformly distributed along the shoot, without compensating for the lower number of fruits developed on the proximal segment of the shoot. However, the reduction in the number of fruits was compensated by an increase in fruit weight, and thus there were no significant differences in yield for 0-100 mg l^{-1} GA_3. Hansen and Christensen (1974) did not observe any differences in the number of fruits and fruit size at harvest in GA_3 treated trees, compared to control trees. In previous experiments, we observed similar results by hand thinning of fruits (unpublished data), which are in accordance with the results found by Marini (2002). However, it is important to note that fruit from treated trees grew faster and matured earlier than fruit from untreated trees, and it can be explained by the reduction in the number of competing flowers. This effect is similar to that obtained with the application of synthetic auxins (Agustí *et al*., 1993; 1999) and scoring branches (Agustí *et al*., 1998), both performed at the onset of cell enlargement fruit stage. The response is associated with the increase in sink strength by auxins and the increase in carbohydrate supply to the fruits by scoring branches, that is, with higher nutrients supply. Accordingly, it is not surprising that the reduction in the number of flowers by GA_3, which reduces competition for nutrients among developing fruitlets, accelerates fruit development and anticipates the harvest date.

Despite of it, fruit firmness increased in GA_3 treated trees. This response is similar to that found by Southwick *et al.* (1995), and can be explained by the reduction of competition between developing fruit on GA_3 treated trees. Under these conditions, the number of cells in fruit tissues increases (Ho, 1988) and also its resistance, similarly to what occurs in apples fruits after flower thinning (Westwood *et al.*, 1967).

In conclusion, GA_3 applied at the flower induction period reduces significantly flowering in peaches and nectarines, the response depending more on the total amount of active material applied per tree rather than on the concentration used. This reduction gives rise to a reduction of hand-thinning costs by 50%, approximately, without affecting yield and fruits characteristics, but increasing returns to the growers. Our experiments also demonstrates that, for the cultivars tested, peach sensitivity to GA_3 treatment almost double that of nectarine and, thus, 0.5-1.0 g $tree^{-1}$ of GA_3 reduces their flowering by about 50%, respectively. The effect appears mainly in the basal part of the shoots, and it warns of winter pruning procedure.

References

Agustí, M.; Almela, V.; Andreu, I.; Juan, M. and Zacarias; L. 1999. Synthetic auxin 3,5,6-TPA promotes fruit development and climacteric in *Prunus persica* L. Batsch. Journal of Horticultural Science and Biotechnology 74: 556-560.

Agustí, M.; Andreu, I.; Juan, M.; Almela, V. and Zacarias, L. 1998. Effects of ringing branches on fruit size and maturity of peach and nectarine cultivars. Journal of Horticultural Science and Biotechnology 73: 537-540.

Agustí, M.; Juan, M.; Almela, V.; Andreu, I. and Speroni, C. 1997. *Estímulo del desarrollo de los frutos de hueso*. Consellería de Agricultura, Pesca y Alimentación. Valencia, Spain.

Agustí, M.; Juan, M.; Almela, V. and Speroni, C. 1993. The effect of 2,4-DP on fruit development in apricots (*Prunus armeniaca* L.). Scientia Horticulturae 57: 51-57.

Agustí, M.; Juan, M.; Gariglio, N.; González-Primo, D. and Almela, V. 2001. Inhibición de la floración en el melocotonero. Su utilidad como técnica del aclareo de frutos. IV Congreso Ibérico Ciencias Hortícolas. Resúmenes 215.

Bergh, O. 1992. Cumulative effect of time of hand thinning on fruit size of Golden Delicious and Granny Smith apples. South African Journal of Plant Soil 9: 64-67.

Bradley, M.V. and Crane, J.C. 1960. Gibberellin-induced inhibition of bud development in some species of *Prunus*. Science 131: 825-826.

Byers, R.E.; Carbaugh, D.H. and Presley, C.N. 1990. The influence of bloom thinning and GA_3 sprays on flower bud numbers and distribution in peach trees. Journal of the American Society of Horticultural Science 65: 143-150.

Byers, R.E. and Lyons Jr., C.G. 1985. Peach flower thinning and possible sites of action of desiccating chemicals. Journal of the American Society of Horticultural Science 100: 662-667.

Byers, R.E.; Lyons Jr., C.G.; Del Valle, T.B.; Barden, J.A. and Young, R.W. 1984. Peach fruit abscission by shading and photosynthetic inhibition. HortScience 19: 649-651.

Chan, B.G. and Cain, J.C. 1967. The effect of seed formation on subsequent flowering in apple. Proceedings of the American Society of Horticultural Science 91: 63-67.

Clanet, H.; Salles, J.C. and Pinet, C. 1987. L`eclairssage des fleurs ou des jeunes fruit du pecher. Arboricult. Fruitere 395: 53-60.

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

Del Valle, T.B.; Barden, J.A. and Byers, R.E. 1985. Thinning of peaches by temporary inhibition of photosynthesis with terbacil. Journal of the American Society of Horticultural Science 110: 804-807.

El-Otmani, M.; Coggins, C.W.; Agustí, M. and Lovatt, C. J. 2000. Plant Growth Regulators in Citriculture: World current uses. Critical Review in Plant Sciences 19: 395-447.

Fallahi, E. and Willemsen, K.M. 2002. Blossom thinning of Pome and Stone fruit. Horticultural Science 37: 474-477.

Faust, M. 1989. *Physiology of temperature zone fruit trees*. John Wiley & Sons, New York, USA.

García-Pallas, I.; Val, I. and Blanco, A. 2001. The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA_3 as an alternative to hand thinning. Scientia Horticulturae 90 (3/4): 265-278.

Hansen, P. and Christensen, J.V. 1974. Fruit thinning. III. Translocation of 14C assimilates to fruit from near and distant leaves in the apple 'Golden Delicious'. Horticultural Research 14: 41-45.

Havis, A.L. 1962. Effect of fruit thinning of 'Redhaven' peach. Proceedings of the American Society of Horticultural Science 80: 172-176.

Hilaire, C. and Guiauque, P. 1994. In: *Pêcher: Les variétés et leur conduite*. Brochure. Centre Techique Interprofessionel des Fruits et Légumes. Paris, France.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. Annual Review of Plant Physiology and Plant Molecular Biology 39: 355-378.

Jourdain, J.M. and Clanet, H. 1987. L'eclaircissage. Fruit et Legumes 41: 16-20.

Knight, J.N. 1980. Fruit thinning of the apple cultivar Cox´s Orange Pippin. Journal of Horticultural Science 55: 267-273.

Lilleland, O. 1965. Growth and thinning of the peach fruit. Proceedings Peach Congress, Verona, Italy. 1-10.

Luckwill, L.C. and Silva, J.M. 1979. The effect of daminozide and gibberellic acid on flower initiation, growth and fruiting of apple cv. Golden Delicious. Journal of Horticultural Science 54: 217-223.

Marini, R.P. 2002. Pruning peach trees. Virginia Cooperative Extension. Virginia Technical Horticulture Publication 422 (020): 1-10.

McArtney, S.J. and Li, S.H. 1998. Selective inhibition of flowering on "Braebun" apple trees with gibberellins. Journal of Horticultural Science 33: 699-700.

Mizelle Jr., W.O. and Westberry, G.O. 1989. Cost analysis. In: Alan Crozier (Ed.). *The biochemistry and physiology of gibberellins. Vol. 2*. Praeger, New York. USA, pp. 6-14.

Monselise, S. P. and Halevy, A. H. 1964. Chemical inhibition and promotion of citrus bud induction. Proceedings of the American Society of Horticultural Science 84: 141-146.

Monselise, S.P.; Weiser, M.; Shafir, N.; Goren, R.; Goldschmidt, E. 1976. Creasing of orange peel: physiology and control. Journal of Horticultural Science 51: 341-351.

Moran, R.E. and Southwick, S.M. 2000. Chemical bloom thinning of pome and stone fruits. In: Basra, A.S. (Ed.). *Plant growth regulators in agriculture and horticulture: Role and commercial uses*. Food Products Press, Binghamton, New York, USA.

Myers, S.C.; King, A.; Savelle, A.T. 1993. Bloom thinning of 'Winblo' peach and 'Fantasia' nectarine with monocarbamide dihydrogensultate. HortScience 28: 616-617.

Painter, J.W. and Stembridge, G.E. 1972. Peach flowering response as related to time of gibberellin application. HortScience 7: 389-390.

Salazar-García, S. and Lovatt, C.J. 2000. Use of GA_3 to manipulate flowering and yield of "Hass" avocado. Journal of the American Society of Horticultural Science 125: 25-30.

Southwick, S.M. and Glozer K. 2000. Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. HortTechnology 10 (4): 744-751.

Southwick, S.M. and Weis, K.G. 1996. Bloom thinning 'Loadel' cling peach with a surfactant. Journal of the American Society of Horticultural Science 121: 334-338.

Southwick, S.M. and Yeager, J.T. 1991. Effects of postharvest gibberellic acid application on return bloom of 'Patterson' apricot. Acta Horticulturae 293: 459-466.

Southwick, S.M.; Weis, K.G. and Yeager, J.T. and Zhou 1995. Controlling cropping in 'Loadel' Cling Peach using Gibberellin: effect on flower

density, fruit distribution, fruit firmness, fruit thinning and yield. Journal of the American Society of Horticultural Science 120 (6): 1087-1095.

Stembridge, G.E. and Gambrell, C.E. 1971. Thinning peaches with bloom and post-bloom applications of 2-chloroethyl phosphonic acid. Journal of the American Society of Horticultural Science 96: 7-10.

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

Turnbull, C.G.; Anderson, K.L. and Winston, E.C. 1996. Influence of gibberellin treatment on flowering and fruiting patterns in mango. Australian Journal of Experimental Agriculture 36: 603-611.

Weinberger, J.H. 1941. Studies on time for peach thinning from blossoming to maturity. Proceedings of the American Society of Horticultural Science 38: 137-140.

Westwood, M.N.; Batjer, L.P. and Billingsley, H.D. 1967. Cell size, cell number, and fruit density of apples as related to fruit size, position in cluster, and thinning method. Proceedings of the American Society of Horticultural Science 91: 51-62.

Williams, M. W. and Edgerton, L.J. 1981. Fruit thinning of apples and pears with chemicals. U.S. Department of Agriculture. Information Bulletin 289.

Zilkah, S.; Klein, I. and David, I. 1988. Thinning peaches and nectarines with urea. Journal of Horticultural Science 63: 209-216.

Table 4.1: Effect of gibberellic acid (GA_3) applied during the period of flower bud induction on flowering density, time spend on hand-thinning, average fruit diameter, total number of fruit at harvest, percent of fruit harvested at first picking date and yield per tree in peach cultivar 'Springlady' and nectarine cultivar 'Zincal 5'. Trees were hand-thinned to the same fruit spacing at 45 days after anthesis (DAA). Each value is the mean of at least 6 trees.

Spraying Method	GA_3 (mg l^{-1})	Nº flowers m^{-1} shoot length	Thinning (min $tree^{-1}$)	Fruit diameter (mm)	Yield		
					Nº fruits $tree^{-1}$	1st picking (%)	kg $tree^{-1}$
'Springlady' peach							
Pressurised handgun (*)	0	51.1 a	52.6 a	59.4 b	333 a	48.0a	36.0a
	25	38.4 b	43.9 ab	63.1 ab	339 a	50.2a	39.2a
	50	25.5 c	32.7 bc	62.8 ab	282 ab	56.3b	32.1a
	75	24.6 c	28.5 c	64.3 a	288 ab	57.4bc	33.9a
	100	18.8 c	24 c	64.3 a	248 b	61.1c	28.7b
'Zincal 5' nectarine							
Pressurised handgun (*)	0	79.6 a	54.0 a	51.3 b	749 a	83.0a	44.2a
	25	57.0 b	46.2 a	54.0 a	682 a	88.5b	49.1a
	50	39.5 c	36.0 b	54.8 a	607 ab	92.4bc	46.0a
	75	27.5 c	24.0 c	54.8 a	597 ab	93.2bc	46.4a
	100	18.0 d	18.0 c	55.0 a	414 b	95.0c	33.2b
Turbo-sprayer (**)	0	55.7 a	30.2 a	50.3 b	519 a	nd	44.5a
	50	51.6 b	24.4 b	53.1 a	458 ab	nd	41.2a
	100	44.8 c	14.4 c	53.7 a	432 b	nd	38.7ab
	150	31.1 d	10.6 c	52.3 ab	349 c	nd	29.9b
	200	30.1 d	2.0 d	52.7 ab	233 d	nd	20.4c

Mean separation within a column by Duncan's multiple range test ($P \leq 0.05$).
(*) 2nd year of research. Sprays were performed 101 DAA.
(**) 3rd year of research. Sprays were performed 106 days DAA. ND: Not determined

Table 4.2: Influence of spraying procedure on the effect of gibberellic acid on flower bud inhibition in peaches and nectarines. Values expressed as percentage of flowers with regard to the untreated controls. Date of treatment: 101-105 days after anthesis. Concentration applied: 50 or 75 mg l^{-1}. Data for the fourth year of research. Trees were hand-thinned to the same fruit spacing at 45 days after anthesis.

	Pressurised handgun		Turbo-sprayer		Average
Peach	'Springlady' I	50,1	'Candor'	22,1	40,0 a
	'Springlady' II	58,1	'Sherman'	20,5	
	'Springlady' III[z]	55,0	'Maycrest'	34,3	
Nectarine	'Zincal 5'	50,4	'Valencia'	15,4	25,6 b
	'Alginet'[z]	32,1	'866'	17,1	
			'Sunrise'	12,9	
Average		49,1 a		20,4 b	

Mean separation within columns and lines by Duncan's multiple range test ($P \leq 0.05$).
[z]: 75 mg l^{-1}.

Table 4.3: Effect of gibberellic acid (GA_3) concentration applied on 'Zincal 5' nectarine fruit characteristics at harvest. Trees were hand-thinned to the same fruit spacing at 45 days after anthesis. Values corresponding to the second year of research. Sprays were performed 101 days DAA with a pressurized handgun. Each value is the mean of 25 fruits per tree, from at least 6 trees per treatment.

GA ($mg\ l^{-1}$)	Fruit colour		Fruit firmness (N)	TSS (º Brix)
	a	a/b		
0	16.9 b	0.9 b	8.0 d	8.7 c
25	18.0 a	1.1 a	8.4 c	9.1 bc
50	17.4 ab	1.1 a	8.8 b	9.5 ab
75	18.3 a	1.1 a	9.2 a	9.7 ab
100	18.8 a	1.2 a	9.3 a	10.0 a

Mean separation within a column by Duncan's multiple range test ($P \leq 0.05$).

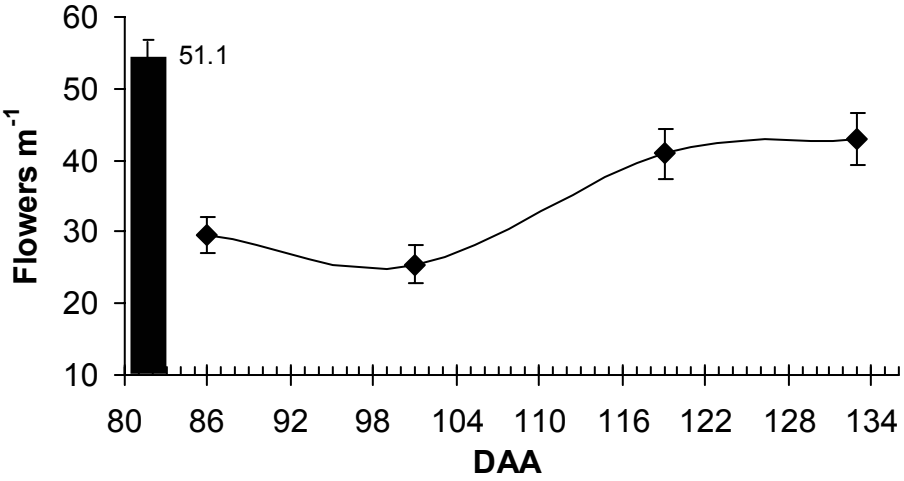

Figure 4.1: Influence of gibberellic acid (50 mg l^{-1}) on flowering intensity of cv. 'Springlady' peach tree. Date of treatment effect. Untreated control value is given as a solid bar. Standard errors are given as vertical bars. Values expressed as flowers per m of shoot length. Each value is the average of 8 trees.

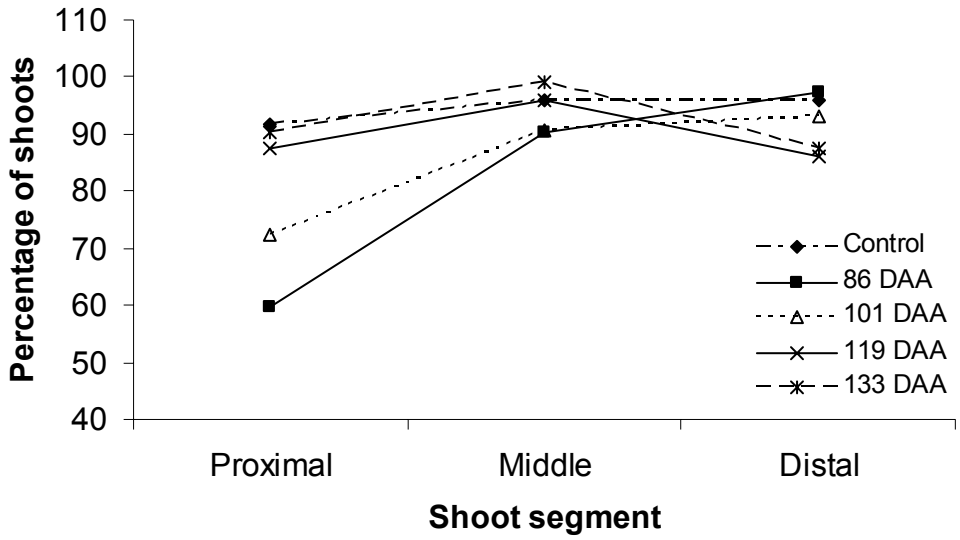

Figure 4.2: Effect of treatment date of gibberellic acid (50 mg l^{-1}) on the percentage of shoots of cv. 'Springlady' peach trees flowered in their proximal, middle and distal segments. Each value is the average of 10 shoots per tree from 8 trees.

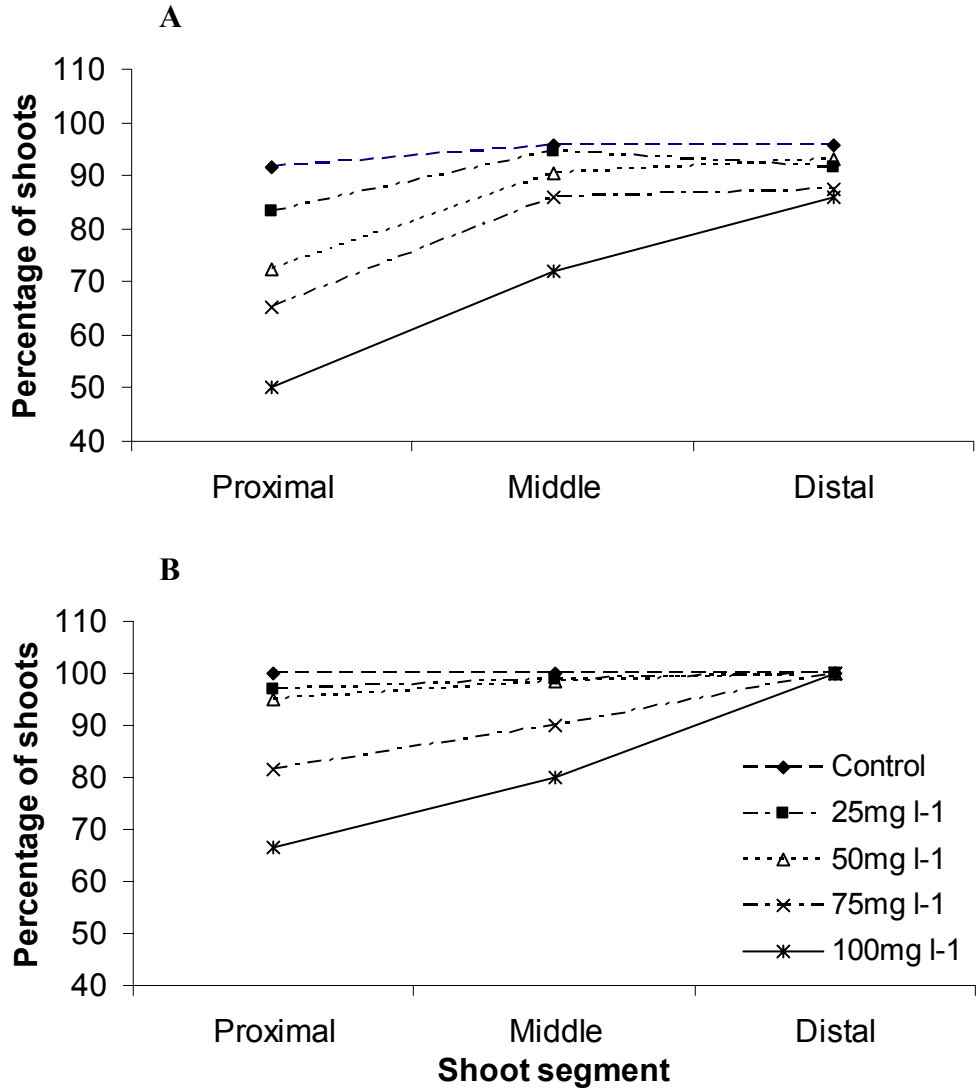

Figure 4.3: Effect of gibberellic acid concentration applied on the percentage of shoots of peach 'Springlady' (A) and nectarine 'Zincal 5' (B) flowered in their proximal, middle and dista*l* segments. Each value is the average of 10 shoots per tree from 8 trees.

CHAPTER 5

Hormonal regulation of reproductive and vegetative growth in *Prunus persica* L. Batsch

5- Hormonal regulation of reproductive and vegetative growth in *Prunus persica* L. Batsch

Abstract

In order to investigate the effect of different growth regulators such as gibberellic acid, paclobutrazol and prohexadione-Ca during flower bud induction, two experiments were carried out in two consecutive years and followed during 3 years, on adult trees of nectarine 'Zincal 5'. The application of PCB had no effect on flowering the following season but increased flowering and yield the second and third season. PCB significantly reduced shoot and internodes length during the second and third season. The soil application of 2 g $tree^{-1}$ of PCB increased fruit quality and the number of fruits collected at first harvest. Trees were more sensitive to soil than to leaf applied PCB, and its effect lasted longer in apical than lateral shoots. PHD had no effect on vegetative or reproductive growth in none of the years. Sucrose significantly decreased in the bark tissue of shoots from trees with a low tendency to flowering (GA_3 treated) and increased in trees with a high tendency to flowering (PCB treated). Total sugar concentration was not affect in the bark and increased in the roots of PCB treated trees. Starch decreased in the bark of PCB treated trees. These evidences suggest a possible effect of PCB treatment on carbohydrate metabolism. Also, GA_3 and PCB seam to affect nitrogen metabolism in the roots, in relation to flowering.

Introduction

Gibberellins (GAs) are tetracyclic diterpenoid compounds that play an important role in many aspects of plant growth and development (Ben-Tal, 2000). One of these is the inhibitory effect of GAs cause on bud induction (Faust, 1989; Luckvill, 1970). The exact means by which gibberellins affect this process is still not known, but application during maximum flower bud induction reduces flowering in *Prunus*, *Pome* and *Citrus* species (Moran and Southwick, 2000; Tromp, 2000; Southwick and Davenport, 1987).

Contrary to GAs, a number of applied chemicals that inhibit growth also stimulate flowering in a variety of broad-leaved trees (Kozlowski and Pallardy, 1997). This is the case of paclobutrazol (PCB), a substance derivated from triazole, which acts as a growth retardant in many plants (Hamid and Williams, 1997; Okuda *et al.*, 1994; Vu and Yelonosky, 1992; Fletcher *et al.*, 2000), and has been found to specifically inhibit the three oxidative steps of the gibberellins (GA) precursor *ent*-kaurenoic acid

(Hedden and Graebe, 1985). There are many reports of enhanced flowering following application of triazole derivates in temperate and tropical fruit trees (Kozlowski and Pallardy, 1997), but a few studies gave reliable results on the enhancement of flowering after treatment with paclobutrazol (Delgado *et al.*, 1986; Iwahori and Tominaga, 1986; Ogata *et al.*, 1995). Prohexadione-Ca (PHD) is another growth retardant that, unlike PCB, interferes in the latter stages of GA biosynthesis, affecting the 3β hydroxylation pathway (Nakayma *et al.*, 1990; Brown *et al.*, 1997). This substance belongs to a new group of GA inhibitors, the acylcyclohexanodiones, and is considered a safer compound than PCB because it metabolizes quickly, is used in a much lower concentration and once applied it degrades in just a few weeks (Evans *et al.*, 1999). Up to now, it has been mainly used for controlling apple growth (Owens and Stover, 1999; Unrath, 1999; Medjdoub *et al.*, 2004; Medjdoub *et al.*, 2005).

To our knowledge, the appropriate time for the application of a GA biosynthesis inhibitor has always been at the beginning of the growing season or mid autumn (Okuda *et al.*, 1996; Mehouachi *et al.*, 1996; Kozlowski and Pallardy, 1997) but never during full flower bud induction, when GA_3 is mostly used for reducing flowering in the following season (Southwick and Glozer, 2000). It is still unclear whether the effect of growth regulators on flower bud induction is a direct effect on the process, or if they act indirectly through their effect on growth rate. Many authors give the period of maximum flower bud induction, in the northern hemisphere as between June and early July (Southwick and Glozer, 2000; Marini, 2002).

It has been suggested that the accumulation of NH_4^+ concentration in citrus and avocado is related to flower bud formation (Lovatt *et al.*, 1988; Nevin and Lovatt, 1987). Also, an increase in wood nitrogen was reported in peach due to paclobutrazol application (Szewczuk, 1994), and the amount of stored N was strongly correlated to those of non-structural carbohydrates (Jordan *et al.*, 2001). Applications of paclobutrazol on mango trees were also effective in suppressing vegetative growth, as compared to the control, affecting the total non-structural carbohydrate content in shoots before flowering, which led to higher rates of flowering and better yield and fruit quality (Yeshitela, 2004). The possible involvement of carbohydrates in flowering has also been suggested in *Citrus* by Goldschmidt *et al.* (1985).

The objective of this research was to investigate the effect of growth retardants such as PCB and PHD on flower bud induction and vegetative growth, compared with GA_3, an effective flower bud inhibitor, applied

during maximum flower bud induction. Residual effects on vegetative growth and flower density were evaluated throughout a period of three consecutive years. Carbohydrates and nitrogen concentrations in the bark tissue of shoots and roots was also investigated at budbreak in relation to flowering.

Materials and methods

Plant material

Two experiments were carried out on two consecutive years on adult plants (8-10 years old) of early maturing nectarine (*Prunus persica* L. Batsch, *leavis*) cv. 'Zincal 5' grafted onto 'GF677' rootstock, from a commercial orchard situated in Alginet, Spain (latitude 39.27º N – longitude 0.47º W – altitude 30 metres). The trees were planted at 5x5 m apart (400 trees/ha) in sandy-loamy soil and drip irrigated. Fertilization, pesticide applications and pruning were in accordance with normal commercial practice. All trees were consistently hand-thinned to the same fruit spacing (15 to 20 cm), 45-49 days after anthesis (DAA) during hardening.

Experimental design and spray treatments

Trees were selected for their uniformity of size, vigour and crop load, arranged into eight blocks and randomly assigned to the treatments. The experiments were all randomized complete blocks with one repeat measure.

The GA_3 treatments were delivered as dilute, full coverage foliar sprays applied to wetness with pressurised hand-gun (30 atm; 3200 l ha^{-1}). A surfactant (alquil poliglicol eter, 20% w/v) was added to each spray treatment at 0.05% (v/v). Paclobutrazol [(2RS, 3RS)-1-(4-chlorophen-yl)-4,4-dimethyl-2-(1,2,4-triazol-lyl)penta-3-ol; 25% w/v] (PCB) and prohexadione-Ca (3-oxido-4propionyl-5-oxo-cyclohexene-carboxylate) (PHD) preparation and spray were equal to GA_3 when applied to the leaves, and dissolved in 2 l of distilled water when applied to the soil.

Experiment 1 (2001-2002). The experiment was designed to comparatively measure the effect of GA_3 and high concentration PCB on flower density, crop load, fruit quality and vegetative growth. For these purposes, trees were treated in early June 2001 with 600 mg $tree^{-1}$ GA_3 (Arabelex-L; 1.6% w/v; Aragro; Madrid – Spain) on 16 g $tree^{-1}$ PCB (Cultar; 25% w/v; Syngenta; Madrid – Spain), as a foliar spray, and 16 g $tree^{-1}$ PCB applied to soil. Two GA_3 + PCB combine treatments were also

applied as a foliar spray and to soil and a group of 8 trees were water sprayed only, and left as control trees. Late in winter, 10 homogeneous current season shoots per tree were randomly selected and their length measured. Each shoot was arranged longitudinally from the base to the apex into three segments termed *proximal*, *middle* or *distal*, respectively, for a clear distinction of the flower distribution on the shoots. At full bloom, the number of flowers developed on each shoot was recorded. Data are expressed as the mean number of flowers per m of shoot length. At pit hardening, time employed to thinning and fruit size-weight was measured. Fruits were harvested according to appropriate commercial size and colour standards. The average fruit weight and diameter and number of fruits per tree were recorded at each harvest date. Fruit colour was measured by determining the 'a' and 'b' Hunter coordinates on 20-fruit samples randomly picked from each replicate at first harvest date. Three measurements were made per fruit around the equator, using a Minolta Chroma Meter CR-300. Flesh resistance was determined by a fruit pressure tester FT-011 (Facchini, Italy) using a 4-mm-diameter flat cylinder. Total soluble solids (TSS) were measured refractometrically using a digital refractometer (Atago, Japan) and expressed as ºBrix. Ten lateral and ten apical homogeneous current season shoots were collected from each tree at the end of the growing season (November) and at first harvest, and their total length, length of internodes and number of nodes were determined in order to verify the effect of treatments on vegetative growth. Residual effects were evaluated during a 3–year period.

Experiment 2 (2002-2003). The experiment was designed to measure the effect of PHD with respect to GA_3 and PCB in a lower dose. Trees were treated in early June 2002 with 400 mg tree^{-1} GA_3 or 2 g tree^{-1} PCB, as a foliar spray, 2 g tree^{-1} PCB applied to soil, 2 g tree^{-1} PHD (Regalis; 10% w/w; BASF; Ludwigshafen – Germany) as foliar spray, 2 g tree^{-1} PHD applied to soil and two combined treatments, one GA_3 + PCB and one GA_3 + PHD applied to soil. A group of 8 trees were only water sprayed and left as control trees. Flower density, thinning time, fruit quality, crop load, and vegetative growth were evaluated as described for experiment 1.

Measurements of carbohydrates

In late January (28 Jan 2002) of the current season, at budbreak, five annual shoots and roots (<3 mm wide, 15-20 g per tree) were collected from trees of experiment 1, and their carbohydrate and nitrogen contents determined. The bark tissue was cold extracted from the shoots with a sharp scalpel. After taking fresh weights, the tissues were frozen, lyophilized, grounded and stored at -40º C. The carbohydrate determination

procedures were base on the Moing *et al*. (1992) and Mehouachi *et al.* (1995) methodology, as follows: Soluble sugar was extracted three times from 100 mg ground samples, using 80% hot ethanol. The residue was washed with mQ water three times. The supernatant and rinse-water collected were filtered and the filtrate evaporated to dryness, then dissolved in mQ water and subjected to HPLC. Sugars were analyzed in a Spectra System® HPLC with a P2000 gradient Pump, a RI-150 differential refractometer, a ChromQuest Chromatography Data System for Windows NT and a Sugar-Pack I 300 x 6.5 mm column. Column temperature was kept at 85°C (Croco-Cil oven) and pure mQ water was used as solvent at a flow rate of 0.5 ml min^{-1}. Mannitol, fructose, glucose, sucrose, sorbitol, raffinose and stachyose were identified by their retention time. Sugar content was determined using peak area calculation related to regression curves for known concentrations of sugar (Sigma Química, Madrid, Spain). Total sugar concentration was calculated by adding all sugar concentrations.

The starch was gelatinized by autoclaving the remaining insoluble pellets at 130 ºC for 2 h in 6 ml of mQ water. Sodium-acetate buffer and amyloglucosidase were added to the extracts and enzymatic digestions were performed at 55ºC for 2 h. After filtration, the amount of released glucose in the supernatant was measured by HPLC, as described above. Each determination was repeated three times.

Nitrogen fraction analysis

Protein nitrogen (N-prot), ammonium nitrogen ($N\text{-}NH_4^+$) and nitric nitrogen ($N\text{-}NO_3^-+NO_2^-$) analysis were based on the techniques described by AOAC (1984), Raigón *et al*. (1992), Levey *et al*. (2000) and Beljaars *et al.* (1994), with some modifications described below.

Protein nitrogen (N-prot). To determine the amount of N-prot, 0.5 g sub-sample were dissolved with 10 ml of cold (4ºC) trichloroacetic acid 5% (TCA 5%), which precipitates protein, and homogenized in a magnetic stirring (AGIMATIC-E) for 15 minutes. Thereafter, 30 ml of cold TCA 5% were added and the final solution was stored at 4ºC for 15 minutes. The sample was then filtered with a 90 mm Schleicher and Shvell filter paper and the residue, containing the N-prot, washed three times with 10 ml of cold TCA 5%. The filtered solution was completed to 100 ml with mQ water and stored at 4ºC for further $N\text{-}NH_4^+$ analysis with a Fiastar 5000 Analyser® (Flow injection system-FIA). The solid residue with the filter paper was digested by micro-Kjeldahl method in order to obtain the N-prot fraction. Samples (two to four replicates per sample) were digested with 10

ml of concentrated sulphuric acid, 10 ml of H_2O_2 and a 3 g of a mixed of K_2SO_4:$CuSO_4$:Se (10:1:0.1) as catalysers, at 450ºC for 30 minutes; distilled in a Foss Kjeltec 2200 Auto Destillation® with 40% w/v sodium hydroxide and a 2% boric acid solution (Laboratorios MALAB), and finally titrated with 0.1 N HCl factor 1. The percentage of N-prot was calculated according to the following equation:

$$\%\ \text{N - prot} = \frac{\text{ml HCl} * \text{HCl factor} * 0.1 * 100 * 14}{1000 * [\text{sample weight (g)}]}$$

Final results were expressed as mg of nitrogen per g of dried sample (mg N g^{-1} dw).

FIA method for N-NH_4^+ determination. The determination of N-NH_4^+ was carried out in a FIAstar 5000 Analyzer equipped with an ammonia cassette including a gas diffusion membrane, and a 5027 Auto-Sampler, using mQ water as carrier, sodium hydroxide 0.5 M as Reagent 1, and the acid-base indicator solution as Reagent 2. Then, 40 µl of the eluted solution from the N-prot extraction were injected into a carrier stream merged with a sodium hydroxide stream, and the colour shift measured photometrically at 590 nm. Values were expressed as µg of nitrogen per g of dried sample (mg N g^{-1} dw).

FIA method for N-NO_3^-+NO_2^- determination. The N-NO_3^-+NO_2^- were extracted from 0.5 g of dry pounded bark from annual shoots, with 50 ml of mQ water shacken for 30 minutes and filtered with a 90 mm Schleicher and Shvell paper. Two hundred µl of the extracted solution were injected into the FIAstar 5000 Analyser equipped with a nitrite cassette, dialysis membrane and cadmium reduction column. The colour shift was measured at 540 nm. Values were expressed as µg of nitrogen per g of dried sample (mg N g^{-1} dw).

Statistical analysis

Linear regressions and analysis of variance were performed on the data, and means separated using the Student-Newman-Keuls's multiple range tests. Percentages were analysed after arc sine transformation.

Results

Application of GA_3 significantly reduced and PCB soil applied slightly increased flower per m of shoot length in the following season, in both experiments 1 and 2 (Table 5.1). Flower distribution among shoots segments, also varied with treatment. The percentage of flowers increased

in the distal segments for 600 mg tree^{-1} GA_3 in 2001-2002, whereas PCB did not modify flower distribution on the shoot (Table 5.2). Variations in the flower density highly affected the time required for thinning (Table 5.3). The time required for thinning was significantly reduced in GA_3 and slightly increased in PCB treated trees, with respect to control trees (Table 5.3). Fruit weight and diameter at pit hardening significantly increased in trees treated with soil applied PCB, whereas no effects were shown for the other treatments (Table 5.3). In experiment 1, total soluble solids decreased and a/b colour relationship increased in fruits from PCB soil treated trees, while GA_3 did not affect fruit quality, with respect to controls (Table 5.4). In experiment 2, fruit diameter and fresh weight increased in 2 g tree^{-1} soil applied PCB with respect to control (Table 5.1 and 5.4). It is interesting to note that PCB applied to soil at 2 g a.i. tree^{-1} increased by 16 % the percentage of fruits collected at first harvest date with respect to the controls, and a significant fruit abscission was observed in PCB treated trees (data not shown).

In both experiments, GA_3 and PCB treatments had no effect on the number of fruits per tree at harvest, compared with the controls. Nevertheless, soil applied PCB significantly increased yield in both experiments (Table 5.1). At the end of the growing season (November 2001), 600 mg tree^{-1} GA_3 treated trees significantly increased final shoot length respect to controls, while no effect was shown in the rest of the treatments (Table 5.5). In all cases, combined treatments with GA_3+GA_3inhibitors PCB and PHD had an intermediate effect between GA_3 and GA_3inhibitors applied alone. Independently of the application method, PHD had no significant effect on flower density, flower distribution, yield and fruit quality when applied in mid June (Table 5.1, 5.2, 5.4 and 5.5).

During the second season after spraying, trees treated with 16 g trees^{-1} PCB significantly increased flowers per m of shoot length and fruit diameter at pit hardening, whereas no residual effects were evident in GA_3 treated trees, as it is shown in Table 5.6. PCB also improved fruit weight and anticipated fruit maturity the second season after treatment, with respect to control (Table 5.7). In the third season after treatment, the effect of PCB at higher doses on flower density was still significantly higher than controls, but to a much lesser degree with respect to the second season (Table 5.8).

Although PCB had no effect in the first season, a significant decrease in shoot growth was found during two periods of the second and third seasons after treatment, with respect to control (Table 5.9). A sharp decrease in length of shoots and internodes and also in the number of

leaves was shown in PCB treated trees in both lateral and apical shoots, with higher differences with respect to controls in the 2002 season than in the 2003 season. The effects of PCB lasted longer in apical shoots than in lateral shoots.

At budbreak in the second season after treatment, when major changes in vegetative and reproductive growth were evident, analysis of carbohydrate, starch and nitrogen contents were performed on bark tissue of annual shoots and roots. Raffinose decreased and fructose increased significantly in the bark tissue of annual shoots from PCB treated trees, while glucose increased and sucrose decreased significantly in GA_3 treated trees in relation to controls (Figure 5.1). Significant increases were shown in fructose, sorbitol, raffinose and total sugar concentration while sucrose significantly decreased in roots from PCB treated trees (Figure 5.2). On the other hand, roots from GA_3 treated trees only showed a significant increase in raffinose concentration (Figure 5.2). Starch content decreased in the bark of annual shoots from PCB treated trees, while no effect was shown in GA_3 treated trees or in starch concentration in the roots for either treatment, with respect to control (Figure 5.3).

Ammonium-nitrogen ($N\text{-}NH_4^+$) concentration increased in the bark of PCB treated trees while $N\text{-}NO_3^-+NO_2^-$ concentration increased in GA_3 treated trees (Figure 5.4). $N\text{-}NH_4^+$ also increased in the roots of PCB and GA_3 treated trees but not significantly (Figure 5.5). Neither PCB nor GA_3 showed any effect on N-prot concentration in the bark and roots, with respect to controls (Figure 5.4 and 5.5).

Discussion

Gibberellic acid (GA_3), at 400 and 600 mg a.i. per tree, significantly reduced the number of flowers per m of shoot length the following season, with no negative effect on yield or fruit quality. These result agree with those of Southwick and Glozer (2000) and González Rossia *et al.* (data unpublished) in other *Prunus* species. García-Pallas *et al.* (2001) found no secondary effects on vegetative growth following GA_3 spray in nectarine, which apparently contradicts our results in experiment 1, but not those in experiment 2, which suggests there is a threshold in the GA_3 concentration effect on vegetative growth. The GA_3 sprays even reduced flower bud density when the tendency for nectarine shoots to develop flower buds was intensified with paclobutrazol, in line with what Casper and Taylor (1989) had earlier found in peach.

Flore (1994) found a negative correlation between growth vigour and the readiness to lay down flower buds, and since young leaves are sources of GAs, as long as shoot growth continues and young leaves spands, GAs will inhibit bud formation (Tromp, 2000). Several authors reported a promotion of flower bud differentiation and a decrease in vegetative growth in peach and nectarine by means of PCB application (Huett *et al.*, 1997; Kozlowski and Pallardy, 1997). In our experiments, soil applied paclobutrazol in mid June slightly increased flowering the following season by 4.1 % and 2.5% for 2 g a.i. tree^{-1} and 16 g a.i. tree^{-1}, respectively, and had no effect on flower distribution or shoot length. The absence of differences in annual shoot length and flower density with respect to control during the first season could be explained by the lateness of the treatment period. Significant differences in flowering and shoot growth the following season were reported in PCB treatments performed from the beginning of petal-fall to as late as mid May in peach (Blanco *et al.*, 2002; Chen *et al.*, 1997). At this time much of the vegetative growth has already occurred due to the early budbreak of this early-maturing variety, and it is possible to assume that treatments with GA biosynthesis inhibitors do not directly affect flower induction. The decrease in vigour, found in the second and third season after treatment, and the increased effect on flowering promotion, suggest an indirect effect of PCB on flower bud induction rather than a direct effect. These findings are in opposition to those of Browning *et al* (1992), who suggested that PCB had a direct effect on flower promotion.

Foliar applications of PCB were ineffective in inducing flowering of ‘Zincal 5’ nectarine, which may have been due to the treatment period, dosages used or its limited mobility in the plant, as had been found by Okuda *et al.* (1996) in satsuma mandarin.

According to Evans *et al.* (1999), prohexadione-Ca is quickly absorbed by the leaves, does not persist in the plant or in the environment more than a few weeks and has no residual effects the following year. It was also reported to inhibit apple, but not peach, tree growth (Byers and Yoder, 1999). These results were confirmed in our research when applied in mid June.

PCB applied to soil increased fruit size at pit hardening by 11%-12% and yield by 10%-36% in the second season. It denotes the increasing effect of the growth regulator, starting at the beginning of the second growth season. These results agree with those of Szewczuk (1994) and Chen *et al.* (1997) and could be explained by the decrease in the demand for resources for vegetative growth as a consequence of reduced vigour,

which can be mainly redirected to fruit growth. Soil application of PCB also increased yield at third year, in agreement with Zha (1994).

In experiment 2 we measured an advance in the harvest date, which coincides with previous results by George and Nissen (1993), but no previous reports were found in the literature about the PCB effect increasing fruit abscission in the season following harvest.

Sorbitol concentration in the bark appeared to play a negligible role in the budbreak process, which agrees with previous results by Maurel *et al*. (2004). The increase of sorbitol concentration in the roots of PCB treated trees, together with the increase in fructose, correlate with the increase in total sugar concentration, suggesting a an alteration of the carbohydrates metabolism in the roots.

The results obtained with sucrose are interesting, as it significantly decreased in trees with a low tendency to flowering (GA_3 treated), with respect to controls. And even though no differences were found in sucrose concentration in the bark of trees with a high tendency to flowering (PCB treated), with respect to the controls, there was a reduction in the level of sucrose in their roots, suggesting a possible increase in the upward movement of sucrose at the beginning of the second growing season. It has recently been demonstrated that sucrose moving in the phloem is the shoot-to-root signal essential for flowering and that sucrose stimulates the root-to-shoot movement of cytokinin in the xylem sap in *Sinapis* (Havelange *et al.*, 2000). It had earlier been suggested that the balance between GAs produced in the seeds of young fruits and cytokinins coming from the roots controls flower formation in apple (Luckwill, 1970).

In relation to this hypothesis, Buban *et al.* (1978) demonstrated that the cytokinin export from roots to shoots was greater in apple rootstocks fertilized with ammonium than it was for rootstocks fertilized with nitrate. In our work, the bark tissue of annual shoots of PCB treated trees showed increased $N\text{-}NH_4^+$ concentration, while GA_3 treated trees increased $N\text{-}NO_3^- + NO_2^-$ concentration at budbreak. Previous results by González Rossia *et al.* (data unpublished) in nectarine 'Zincal 5' showed that $N\text{-}NH_4^+$ significantly increased before budbreak in trees completely defruited the year before, while trees completely loaded till fruit senescence had a significant increase in the concentration of $N\text{-}NO_3^- + NO_2^-$ in the bark tissue of annual shoots before budbreak. Nevin and Lovat (1987) also suggested a relationship between increasing NH_4^+ concentration in mango and citrus leaves and flower induction.

Jordan *et al.* (2001) found an increased amount of stored N, which strongly correlated with those of non-structural carbohydrates in peach. In our work, the increase in NH_4^+ in the roots of PCB treated trees was also related to the increase in total soluble carbohydrates.

Carbohydrate distribution in a low-vigour tree is such that flower bud formation may not have enough carbohydrates to proceed (Faust, 1989). This statement agrees with the significant decrease in starch concentration in the bark of annual shoots of PCB treated trees. However, total sugar concentration in the bark of annual shoots was not affected and even increased in the roots, as Okuda *et al.* (1996) had found in satsuma mandarin.

Growth regulators that decrease shoot growth generally increase root growth (Faust, 1989). In our experiment, the decrease in starch concentration in the roots of PCB treated trees, suggests an increase of metabolic activity in these organs, which increases growth and reduces the accumulation of reserves. The high levels of glucose and fructose, together with the low levels of sucrose in the roots of PCB treated plants, suggest a higher catabolism of sucrose in these organs (Quick and Schaffer, 1996). In conclusion, the application of GA biosynthesis inhibitors during the maximum bud induction period produced no significant increase in flowering the following season. PHD had no effect on vegetative growth or flowering. PCB increased yield the second and third season after spraying with respect to controls. Flower induction seems to be indirectly influenced by PCB through its inhibitory effect on vegetative growth. PCB affects carbohydrate metabolism, and both GA_3 and PCB appear to affect nitrogen metabolism in shoots, which is related to flowering. Further research is needed to clarify the possible relationship between root-to-shoot sucrose transport, and also the effect of cytokinins from the roots on flower promotion.

References

AOAC. 1984. In: Williams, S. (Ed.). *Official Methods of Analysis of the Association of Official Analytical Chemists*. Association of Official Analytical Chemists, Arlington, Virginia.

Beljaars, P.R.; Van Dijk, R. and Van der Horst, G.M.J. 1994. Determination of Nitrate in Vegetables by Continuous Flow: Interlaboratory Study. Journal of AOAC International 77: 1522-1529.

Ben-Tal, J. 2000. Are gibberellins capable of replacing the environmental flowering signal? Israel Journal of Plant Sciences 48: 205-215.

Blanco, A.; Monge, E. and Val, J. 2002. Effects of paclobutrazol and crop-load on mineral element concentration in different organs of 'Catherine' peach trees. Journal of Plant nutrition 25(8): 1667-1683.

Brown, R.G.S.; Kawaide, H.; Yang, Y.Y.; Rademarcher, W. and Kamiya, Y. 1997. Daminozide and prohexadione-Ca have similar modes of action as inhibitors of the late stages of gibberellin metabolism. Physiologia Plantarum 101: 309-313.

Browning, G.; Kuden, A. and Blake, P. 1992. Site of (2RS, 3RS)-paclobutrazol promotion of axilliary flower initiation in pear cv. Doyenne du Comece. Journal of Horticultural Science 67: 121-128.

Buban, T.; Varga, A.; Tromp, J.; Knegt, E. and Bruinsma, J. 1978. Effects of ammonium and nitrate nutrition on the level of zeatin and amino nitrogen in xylem sap of apple rootstocks. Z. Pflanzenphysiol 89: 289-295.

Byers, R. E. and K. S. Yoder. 1999. Prohexadione-calcium inhibits apple, but not peach, tree growth, but has little influence on apple fruit thinning or quality. HortScience 34: 1205-1209.

Casper, J.A. and Taylor, B.H. 1989. Growth and development of young 'Loring' peach trees after foliar sprays of paclobutrazol and GA_3. HortScience 24: 240-242.

Chen-JianYa; Wu-XiaoMei; Chen-MaoQuan. 1997. The restraint effect of PP333 on the growth of peach top. Journal of Zhejiang-Forestry-Science and Technology 17 (1): 30-32.

Delgado, R.; Casamayor, R.; Rodriguez, J.L.; Cruz, P. and Fajardo, R. 1986. Paclobutrazol effects on oranges under tropical conditions. Acta Horticulturae 179: 537-544.

Evans, J.R.; Evans, R.R.; Regusci, C.L. 1999. Mode of action, metabolism, and uptake of BAS 125W, prohexadione-Ca. HortScience 34: 1194-1135.

Faust, M. 1989. *Physiology of temperature zone fruit trees*. John Wiley & Sons, New York, USA.

Fletcher, R.A.; Giller, A.; Sankhla, N.; Davis, T.D. 2000. Triazoles as plant growth regulators and stress protectants. Horticultural Review 24: 55-108.

Flore, J.A. 1994. Stone fruit. In: Schaffer, B. and Anderson, P.C. (Eds.). *Handbook of environmental physiology of fruit crops, Vol. 1, Temperate crops*. CRC Press, Inc., Boca Raton, Florida, pp. 233-270.

García-Pallas, I.; Val, J. and Blanco, A. 2001. The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA_3 as an alternative to hand thinning. Scientia Horticulturae 90 (3/4): 265-278.

George, A.P. and Nissen, R.J., 1993. Effects of growth regulants on defoliation, flowering, and fruit quality of the low chill peach cultivar

Flordaprice in subtropical Australia. Australian Journal of Experimental Agriculture 33 (6): 787-795.

Goldschmidt, E.E.; Aschkenazi, N.; Herzano, Y.; Schffer, A.A. and Monselise, S.P. 1985. A role for carbohydrate levels in the control of flowering in *Citrus*. Scientia Horticulturae 26: 159-166.

Hamid, M.M. and Williams, R.R. 1997. Translocation of paclobutrazol and gibberellic acid in Sturt's Desert Pea (*Swainsona formosa*). Plant Growth Regulation 23: 167-171.

Havelange, A.; Lejeune, P.; Bernier, G. 2000. Sucrose/cytokinin interaction in *Sinapis alba* at floral induction: a shoot-to-root-to-shoot physiological loop. Physiologia Plantarum 109 (3): 343-350.

Hedden, P. and Graebe, J.E. 1985. Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. Journal of Plant Growth Regulation 4: 111-122.

Huett, D.O.; George, A.P.; Slack, J.M. and Morris, S.C. 1997. Diagnostic leaf nutrient standards for low-chill peaches in subtropical Australia. Australian Journal of Experimental Agriculture 37(1): 119-126.

Iwahori, S. and Tominaga, S. 1986. Increase in first-flush flowering of the Meiwa kumquat, *Fortunella crassifolia* Swingle, trees by paclobutrazol. Scientia Horticulturae 28: 347-353.

Jordan, M.O.; Gomez, L.; Mediene, S.; Neilsen, D.; Fallahi, B.; Nielsen, G.; Peryea, F. 2001. Regulation of N uptake in young peach trees in relation to the management of carbon and nitrogen stores. Acta-Horticulturae 564: 63-70.

Kozlowski, T.T. and Pallardy, S.G. 1997. Physiological regulation of vegetative growth. In: Kozlowski, T.T. and Pallardy, S.G. (Eds.). *Growth control in woody plants*. Academic Press, pp. 73-165.

Levey, D.J.; Bissell, H.A. and O´Keefe S.F. 2000. Conversion of nitrogen to protein and amino acids in wild fruits. Journal of Chemical Ecology 26 (7): 1749-1763.

Lovatt, C.L.; Zheng, Y. and Hake, K.D. 1988. Demonstration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. Israel Journal of Botany 37: 181-188.

Luckvill, L.C. 1970. The control of growth and fruitfulness of apple trees. In: Luckvill, L.C. and Cutting, C.V. (Eds.). *Physiology of tree crops*. London, New York: Academic Press, pp. 237-254.

Marini, R.P. 2002. Pruning peach trees. Virginia Cooperative Extension. Virginia Technical Horticulture Publication 422 (020): 1-10.

Maurel, K.; Leite, G.B.; Bonhomme, M.; Guilliot, A.; Ragau, R.; Petel, G. and Sakr, S. 2004. Trophic control of bud break in peach (*Prunus persica*) trees: a possible role of hexoses. Tree Physiology 24: 579-588.

Medjdoub, R.; Val, J. and Blanco, A. 2004. Prohexadione-Ca inhibits vegetative growth of 'Smoothee Golden Delicious' apple trees. Scientia Horticulturae 1001: 243-253.

Medjdoub, R.; Val, J. and Blanco, A. 2005. Inhibition of vegetative growth in red apple cultivars using prohexadione-calcium. Journal of Horticultural Science and Biotechnology 80 (2): 263-271.

Mehouachi, J.; Serna, D.; Zaragoza, S; Agustí, M.; Talon, M. and Primo-Milo, E. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. Plant Science 107: 189-197.

Mehouachi, J.; Tadeo, F.R.; Zaragoza, S.; Primo-Millo, E. and Talon, M. 1996. Effects of gibberellic acid and paclobutrazol on growth and carbohydrate accumulation in shoots and roots of citrus rootstock seedlings. Journal of Horticultural Science 71 (5): 747-754.

Moing, A.; Carbonne, F.; Rashad, M.H.; Gaudillère, J.P. 1992. Carbon fluxes in mature peach leaves. Plant Physiology 100: 1878-1884.

Moran, R.E. and Southwick, S.M. 2000. Chemical bloom thinning of pome and stone fruits. In: Basra, A.S. (Ed.). *Plant growth regulators in agriculture and horticulture: Role and commercial uses*. Food Products Press, Binghamton, New York, USA.

Nakayama, I.; Miyazawa, T.; Kobayashi, M.; Kamiya, Y.; Abe, H. and Sakurai, A. 1990. Effects of a new plant growth regulator, prohexadione-calcium (BX-112), on shoot elongation caused by exogenous applied gibberellins in rice (*Oryza sative* L.) seedlings. Plant Cell Physiology 31: 195-200.

Nevin, J.M and Lovatt, C.J. 1987. Demonstration of ammonia accumulation and toxicity in avocado leaves during water-deficit stress. South-African-Avocado-Growers-Association 10: 51-54.

Ogata, T.; Ueda, Y.; Shiozaki, S.; Horiuchi, S. and Kawase, K. 1995. Effects of gibberellin synthesis inhibitors on flower setting of Satsuma mandarin. Journal of the Japanese Society for Horticultural Science 64: 251-259.

Okuda, H.; Kihara, T. and Iwagaki, I. 1994. Effects of annual foliar application of paclobutrazol for 9 years on the growth, yield and fruit quality of Satsuma mandarin trees. Bulletin of the Fruit Tree Research Station 26: 61-69.

Okuda, H.; Kihara, T. and Iwagaki, I. 1996. Effects of paclobutrazol application to soil at the beginning of maturation on sprouting, shoot growth, flowering and carbohydrate contents in roots and leaves of satsuma mandarin. Journal of Horticultural Science 71 (5) 785-789.

Owens, C.L.; Stover, E. 1999. Vegetative growth and flowering of young apple trees in response to prohexadione-calcium. HortScience 34: 1194-1196.

Quick, W.P. and Schaffer, A.A. 1996. Sucrose metabolism in sources and sinks. In: Zamski, E. and Schffer, A.A. (Eds.). *Photoassimilate distribution in Plants and Crops*. Source-Sink relationships. Marcel Dekker, Inc.

Raigón, MD; Pérez-García, M; Maqueira, A; Puchades, R. 1992. Determination of available nitrogen (nitric and ammoniacal) in soils by flow injection analysis. Analisis 20: 483-487.

Southwick, S.M. and Davenport, T.L. 1987. Modification of the water stress-induced floral response in Tahiti lime. Journal of the American Society of Horticultural Science 112: 231-236.

Southwick, S.M. and Glozer K. 2000. Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. HortTechnology 10 (4): 744-751.

Szewczuk, A. 1994. Effect of irrigation and paclobutrazol on the growth, flowering and fruiting of peach. Journal of Fruit and Ornamental Plant Research 2 (2): 37-47.

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

Unrath, C.R. 1999. Prohexadione-Ca: a promising chemical for controlling vegetative growth of apples. HortScience 34: 1197-1200.

Vu, J.C.V. and Yelenosky, G. 1992. Growth and photosynthesis of sweet orange plants treated with paclobutrazol. Journal of Plant Growth Regulation 11: 85-89.

Yeshitela, T.B. 2004. Effect of cultural practices and selected chemicals on flowering and fruit production in some mango (*Mangifera indica* L.) cultivars. PhD Thesis. Department of Plant Production and Soil Science. Faculty of Natural and Agricultural Sciences. University of Pretoria. USA.

Zha-DouLu. 1994. The study of the effect of PP333 (paclobutrazol) on the early bearing and high yield of densely planted peach trees. Journal of Beijing Forestry 16 (3): 31-37.

Table 5.1: Paclobutrazol (PCB), prohexadione-Ca (PHD) and gibberellic acid (GA_3) effect on flower density, fruit diameter at harvest and yield parameters the season following application in nectarine cultivar 'Zincal 5'. All trees were consistently hand-thinned to the same fruit spacing at 45 days after anthesis (DAA). Each value is the mean of 8 trees. Treatments were performed on June 2001 and June 2002, respectively.

Treatments	Nº flowers m^{-1} shoot length	Fruit diameter at harvest (mm)	Nº fruits $tree^{-1}$ at harvest	Harvest (kg $tree^{-1}$)
2001-2002				
Control	57.1 a	51.7 abc	461 ab	38.0 ab
GA_3 600 mg $tree^{-1}$	40.9 b	52.4 ab	380 ab	33.9 ab
PCB leaf 16 g $tree^{-1}$	59.8 a	52.2 abc	494 a	35.4 ab
PCB soil 16 g $tree^{-1}$	58.5 a	50.7 bc	455 ab	41.7 a
GA_3 + PCB leaf	45.8 b	50.4 c	387 b	31 0 b
GA_3 + PCB soil	42.7 b	53.2 a	436 ab	36.0 ab
2002-2003				
Control	56.3 ab	51.5 b	322 a	26.0 b
GA_3 400 mg $tree^{-1}$	43.5 d	53.5 ab	328 a	29.0 ab
PCB leaf 2 g $tree^{-1}$	49.4 bcd	54.4 a	303 a	28.2 b
PCB soil 2 g $tree^{-1}$	58.6 a	56.1 a	353 a	35.6 a
PHD leaf 2 g $tree^{-1}$	51.3 bc	53.5 ab	348 a	30.7 ab
PHD soil 2 g $tree^{-1}$	52.2 bc	53.9 ab	351 a	32.6 ab
GA_3 + PCB soil	44.0 d	53.6 ab	398 a	31.6 ab
GA_3 + PHD soil	46.8 cd	54.4 a	328 a	35.9 a

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.2: Paclobutrazol (PCB), prohexadione-Ca (PHD) and gibberellic acid (GA_3) effect on flower distribution on a mixed shoot the following spring after treatment in nectarine cultivar 'Zincal 5'. Each value is the mean of 10 shoots per tree and 8 trees per treatment.

Treatments	Flower distribution a mixed shoot (%)		
	Proximal	Middle	Distal
	2001-2002		
Control	32 a	34 a	34 b
GA_3 600 mg tree^{-1}	24 b	32 a	44 a
PCB leaf 16 g tree^{-1}	30 ab	34 a	36 b
PCB soil 16 g tree^{-1}	31 a	32 a	37 b
GA_3 + PCB leaf	26 ab	36 a	38 b
GA_3 + PCB soil	28 ab	35 a	37 b
	2002-2003		
Control	26 a	31 a	43 a
GA_3 400 mg tree^{-1}	24 a	31 a	46 a
PCB leaf 2 g tree^{-1}	25 a	32 a	43 a
PCB soil 2 g tree^{-1}	24 a	30 a	46 a
PHD leaf 2 g tree^{-1}	23 a	33 a	44 a
PHD soil 2 g tree^{-1}	22 a	35 a	43 a
GA_3 + PCB soil	24 a	32 a	44 a
GA_3 + PHD soil	20 a	32 a	48 a

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.3: Paclobutrazol (PCB) and gibberellic acid (GA_3) effect on hand thinning time (min $tree^{-1}$ $worker^{-1}$), fruit diameter and fresh weight at harvest the season following application in nectarine cultivar 'Zincal 5'. All trees were consistently hand-thinned to the same fruit spacing at 45 days after anthesis (DAA). Each value is the mean of 8 trees. Treatments were performed on June 2001.

Treatments	Thinning (min $tree^{-1}$ $worker^{-1}$)	Fruit diameter at pit hardening (mm)	Fresh fruit weight at pit hardening (g)
Control	20.3 ab	20.5 b	5.2 b
GA_3 600 mg $tree^{-1}$	10.7 d	20.0 b	4.9 b
PCB leaf 16 g $tree^{-1}$	18.5 bc	21.0 ab	5.3 ab
PCB soil 16 g $tree^{-1}$	23.5 a	22.9 a	6.2 a
GA_3 + PCB leaf	14.5 cd	21.3 ab	5.46 ab
GA_3 + PCB soil	18.0 bc	20.8 ab	5.0 b

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.4: Paclobutrazol (PCB), prohexadione-Ca (PHD) and gibberellic acid (GA_3) effect on fruit fresh weight, fruit diameter at harvest and yield parameters the season following application in nectarine cultivar 'Zincal 5'. All trees were consistently hand-thinned to the same fruit spacing at 45 days after anthesis (DAA). Each value is the mean of 30 fruits per tree and 8 trees per treatment.

Treatments	Fruit fresh weight (g)	TSS (ºBrix)	Fruit firmness (N)	Skin fruit colour (Hunter)			
				L	a	b	a/b
2001-2002							
Control	83.0 ab	10.1 a	19.6 a	43.9 abc	15.2 b	21.0 a	0.9 bc
GA_3 600 mg tree^{-1}	89.0 a	10.2 a	17.6 a	46.6 a	14.6 b	20.9 a	0.8 c
PCB leaf 16 g tree^{-1}	84.0 ab	9.3 b	17.6 a	40.2 c	17.7 ab	17.1 b	1.1 b
PCB soil 16 g tree^{-1}	77.9 b	9.3 b	16.7 a	36.1 d	19.7 a	14.2 ab	1.6 a
GA_3 + PCB leaf	79.2 b	9.7 ab	18.6 a	45.0 ab	14.8 b	19.8 a	0.8 c
GA_3 + PCB soil	82.8 ab	9.4 ab	18.6 a	41.3 bc	15.8 b	17.3 ab	1.01 bc
2002-2003							
Control	81.2 b	8.1 a	16.7 ab	37.4 a	18.8 a	13.8 a	1.4 a
GA_3 400 mg tree^{-1}	89.3 ab	9.2 a	18.6 ab	36.1 a	18.9 a	12.6 a	1.5 a
PCB leaf 2 g tree^{-1}	93.4 ab	8.8 a	15.7 b	35.3 a	19.0 a	11.8 a	1.6 a
PCB soil 2 g tree^{-1}	100.9 a	8.6 a	15.7 b	36.0 a	19.0 a	12.9 a	1.5 a
PHD leaf 2 g tree^{-1}	88.1 ab	8.4 a	20.6 a	34.2 a	19.5 a	11.5 a	1.7 a
PHD soil 2 g tree^{-1}	92.7 ab	8.7 a	18.6 ab	34.8 a	18.4 a	11.6 a	1.6 a
GA_3 + PCB soil	90.4 ab	8.6 a	16.7 ab	34.8 a	19.7 a	11.8 a	1.7 a
GA_3 + PHD soil	91.7 ab	8.7 a	20.6 a	36.6 a	19.4 a	13.2 a	1.5 a

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.5: Paclobutrazol (PCB), prohexadione-Ca (PHD) and gibberellic acid (GA_3) effect on annual shoot length at the end of vegetative growth (November 10) on the current year of treatment, in nectarine cultivar 'Zincal 5'. Each value is the mean of 80 shoots per treatment.

Treatments	Annual shoot length (cm)
2001-2002	
Control	31.6 b
GA_3 600 mg tree^{-1}	35.2 a
PCB leaf 16 g tree^{-1}	33.0 ab
PCB soil 16 g tree^{-1}	32.8 ab
GA_3 + PCB leaf	31.5 b
GA_3 + PCB soil	32.4 b
2002-2003	
Control	32.4 a
GA_3 400 mg tree^{-1}	33.5 a
PCB leaf 2 g tree^{-1}	30.18 a
PCB soil 2 g tree^{-1}	32.2 a
PHD leaf 2 g tree^{-1}	35.2 a
PHD soil 2 g tree^{-1}	31.5 a

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.6: Residual effect of paclobutrazol (PCB) and gibberellic acid (GA_3) on flower density and fruit diameter at pit hardening during the second season (2002-2003) after application, in nectarine cultivar 'Zincal 5'. Each value is the mean of at least 8 trees. Treatments were performed on June 2001.

Treatments	Flowers m^{-1} (12/02/2003)	Fruit diameter (mm) at pit hardening (17/04/2003)
Control	55.8 b	30.1 b
GA_3 600 mg $tree^{-1}$	54.2 b	30.9 b
PCB leaf 16 g $tree^{-1}$	60.1 b	32.7 b
PCB soil 16 g $tree^{-1}$	132.7 a	38.1 a

Mean separation within a column b y Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.7: Residual effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) on fruit quality aspects and yield at harvest (May 2003) during the second season after application, in nectarine cultivar 'Zincal 5'. Each value is the mean of 30 fruits per tree and 8 trees per treatment. Treatments were performed on June 2001.

Treatments	Fruit fresh weight (g)	TSS (ºBrix)	Fruit firmness (N)	Skin colour relation a/b	Nº fruits tree^{-1}	% of first collected fruits
Control	78.1 b	8.9 b	20.6 a	1.4 b	400 a	27.5 b
PCB soil 16 g tree^{-1}	119.3 a	10.1 a	8.8 b	2.0 a	403 a	67.3 a

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

Table 5.8: Residual effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) on flower density (flowers m^{-1}) during the second (2002-2003) and third (2003-2004) season after application, in nectarine cultivar 'Zincal 5'. Each value is the mean of 80 annual shoots per treatment. Treatments were performed on June 2001.

Crop year	Control	PCB soil
2002-2003	55.8 aB	132.7 aA
2003-2004	63.4 aA	67.5 bA

Means followed by different lower-case letters in the same column and by different capital letters in the same line differ significantly ($P \leq 0.05$).

Table 5.9: Residual effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) on vegetative growth during the second (2002-2003) and third (2003-2004) season after application, in nectarine cultivar 'Zincal 5'. Each value is the mean of 80 annual shoots per treatment. Treatments were performed on June 2001.

Treatments	Date	Apical shoot			Lateral shoot		
		Shoot length (cm)	Internodes length (cm)	Nº leaves m^{-1}	Shoot length (cm)	Internodes length (cm)	Nº leaves m^{-1}
Control	20/05/2002	26.8 b	1.6 a	83.6 b	18.1 b	1.27 b	91.7 c
	11/11/2002	35.9 a	1.7 a	-	34.4 a	1.9 a	-
PCB soil	20/05/2002	2.0 e	0.2 c	840.0 a	1.3 d	0.1 d	930.1 a
	11/11/2002	2.6 e	0.2 c	-	2.8 d	0.2 d	-
Control	26/05/2003	22.6 c	1.6 a	101.4 b	25.0 ab	1.7 a	77.7 c
	12/11/2003	29.7 ab	1.6 a	-	31.9 a	1.9 a	-
PCB soil	26/05/2003	2.3 e	0.2 c	958.3 a	7.3 c	0.5 c	260.7 b
	12/11/2003	12.8 d	0.8 b	-	19.8 b	1.1 b	-

Mean separation within a column by Student-Newman-Keuls' multiple range test ($P \leq 0.05$).

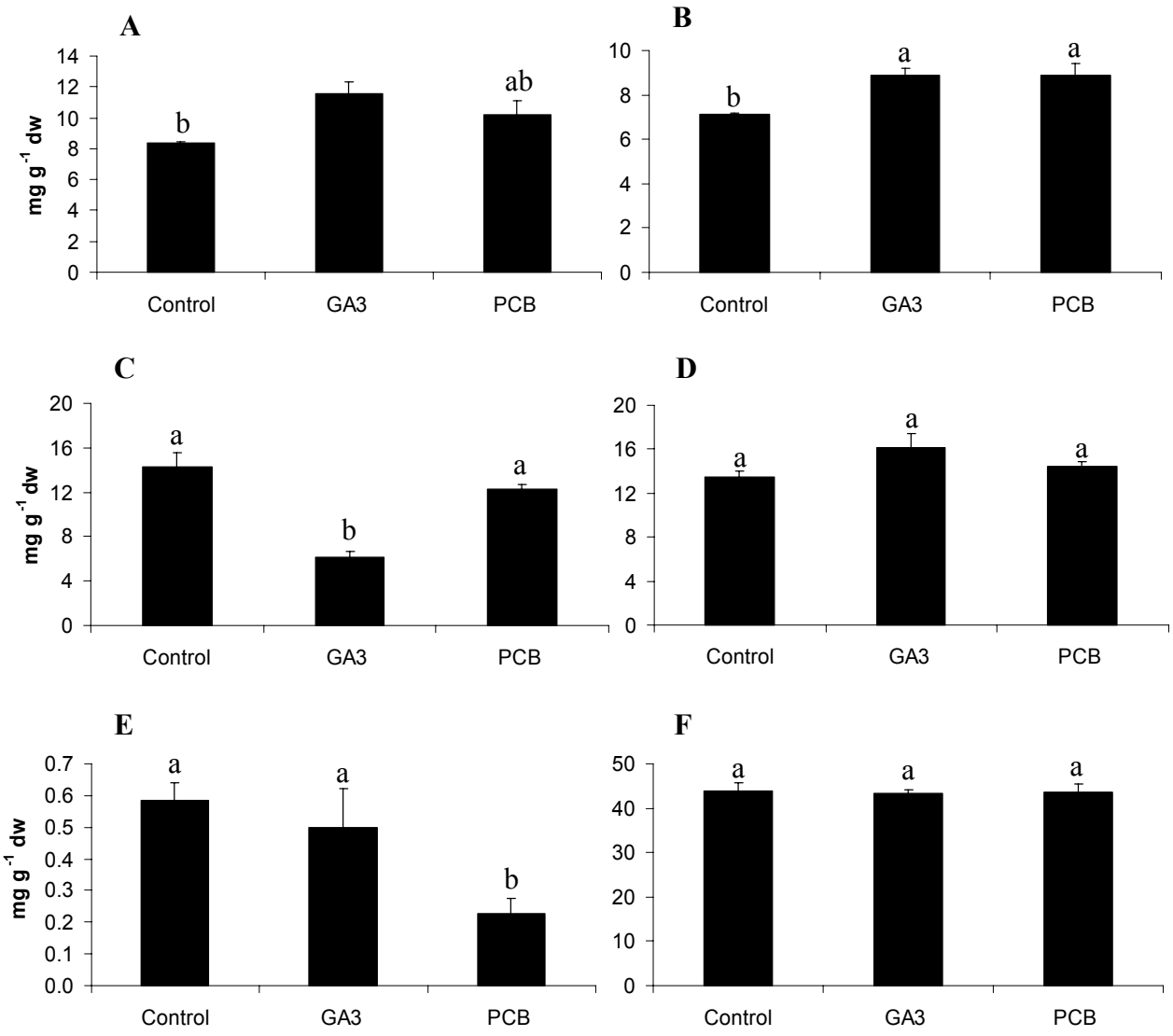

Figure 5.1: Effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) and 600 mg tree^{-1} leaf sprayed gibberellic acid (GA_3) on glucose (A), fructose (B), sucrose (C), sorbitol (D), raffinose (E) and total sugar (F) concentration in the bark tissue of annual shoots of nectarine cv 'Zincal 5', at budbreak. Standard errors are given as vertical bar.

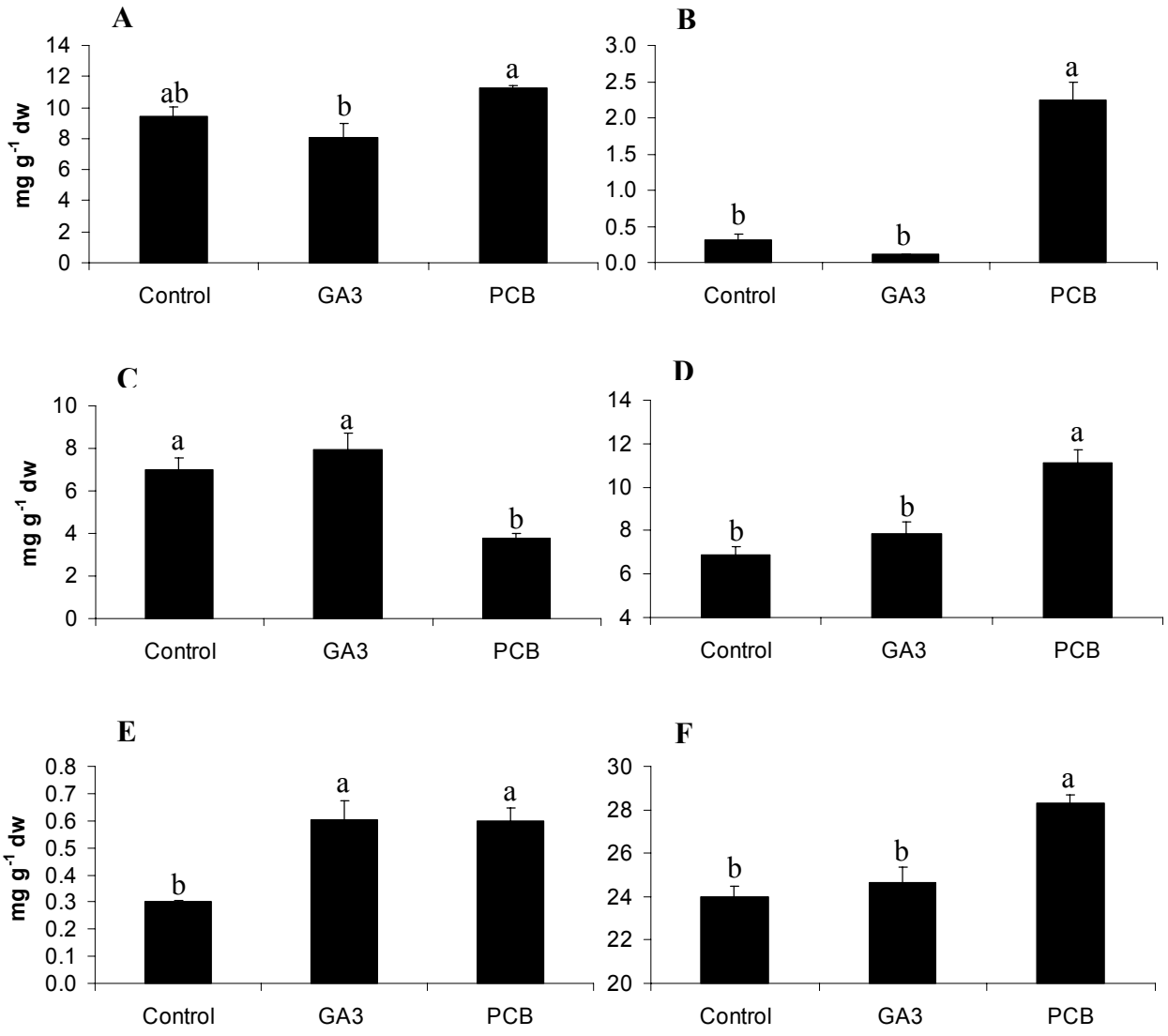

Figure 5.2: Effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) and 600 mg tree^{-1} leaf sprayed gibberellic acid (GA_3) on glucose (A), fructose (B), sucrose (C), sorbitol (D), raffinose (E) and total sugar (F) concentration in annual roots of nectarine cv 'Zincal 5', at budbreak. Standard errors are given as vertical bar.

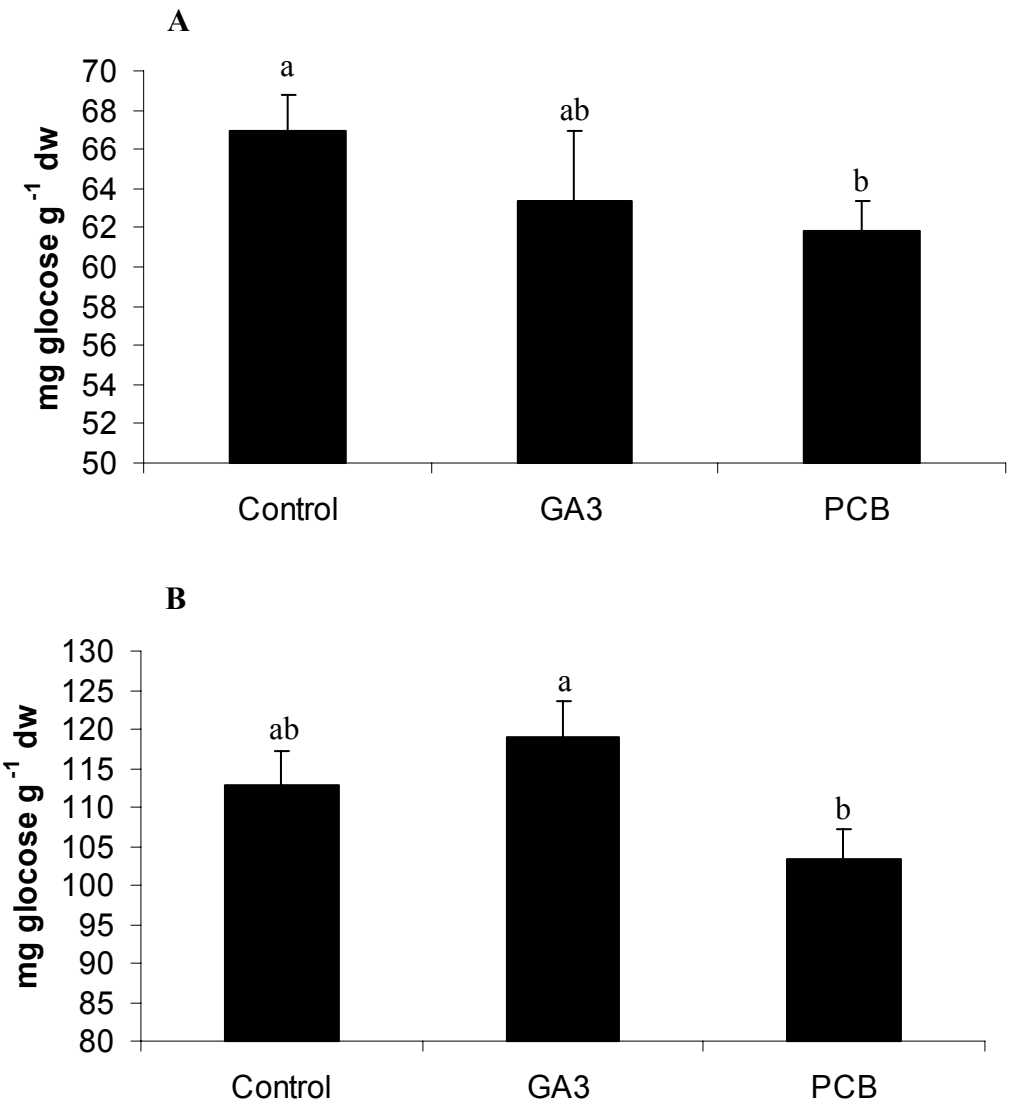

Figure 5.3: Effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) and 600 mg tree^{-1} leaf sprayed gibberellic acid (GA_3) on starch (mg glucose g^{-1} dw) concentration in the bark tissue of annual shoots (A) and in annual roots (B) of nectarine cv 'Zincal 5', at budbreak. Standard errors are given as vertical bar.

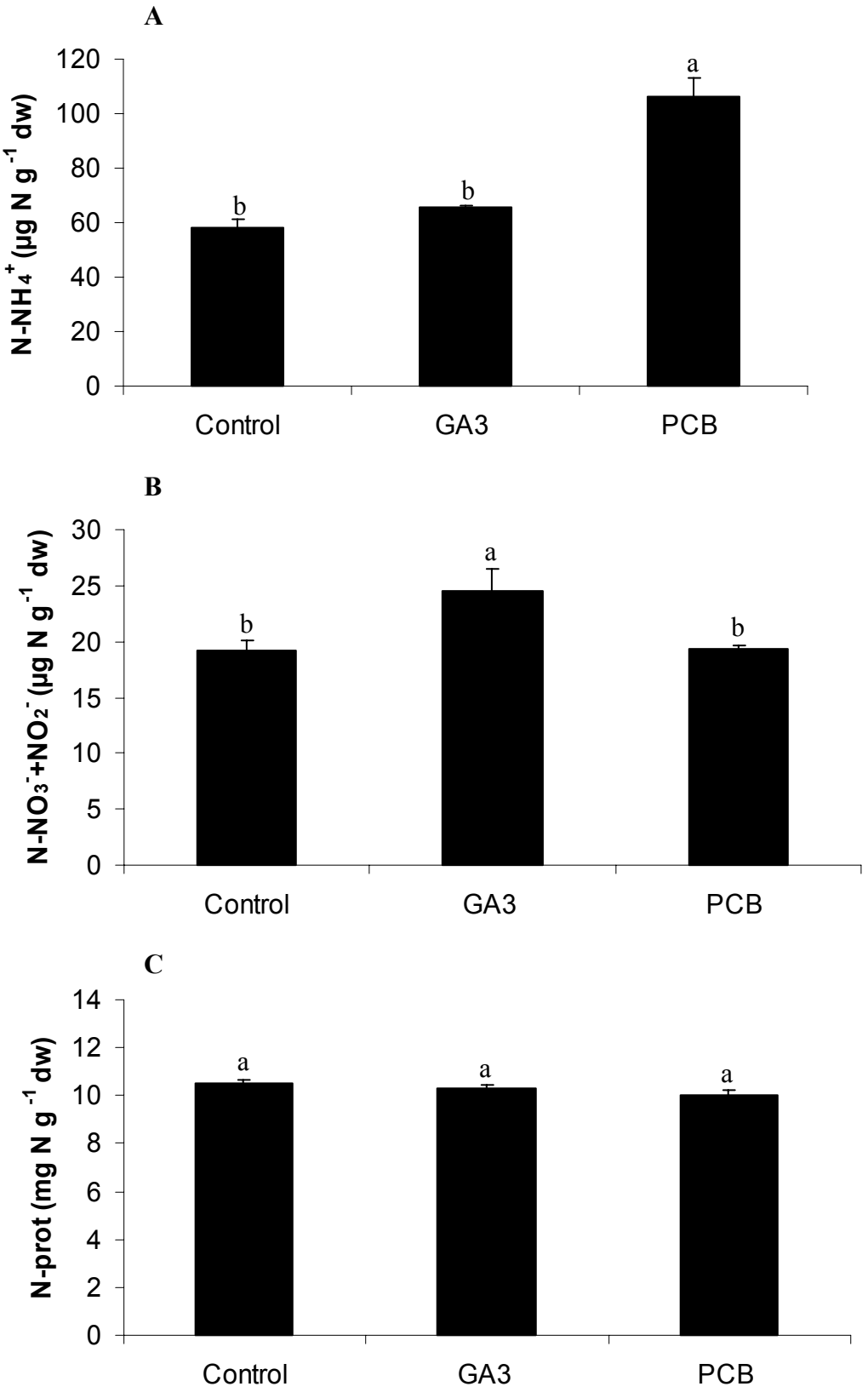

Figure 5.4: Effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) and 600 mg tree^{-1} leaf sprayed gibberellic acid (GA_3) on N-NH_4^+ (A), NO_3^-+NO_2^- (B) and N-protein (C) concentration in the bark tissue of annual shoots of nectarine cv 'Zincal 5', at budbreak. Standard errors are given as vertical bar.

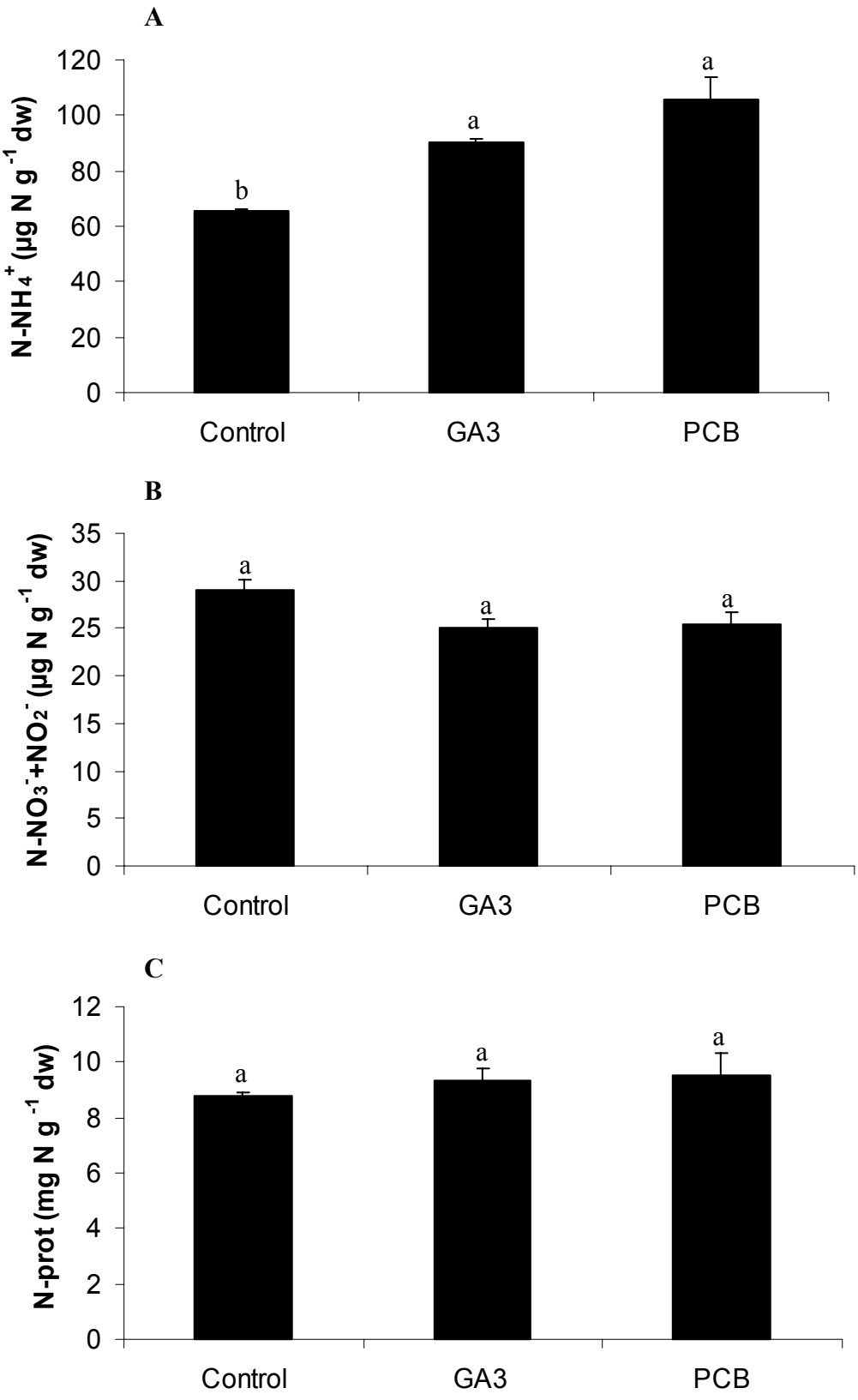

Figure 5.5: Effect of 16 g tree^{-1} soil applied paclobutrazol (PCB) and 600 mg tree^{-1} leaf sprayed gibberellic acid (GA_3) on $N\text{-}NH_4^+$ (A), $NO_3^-+NO_2^-$ (B) and N-protein (C) concentration in annual roots of nectarine cv 'Zincal 5', at budbreak. Standard errors are given as vertical bar.

CHAPTER 6

The inhibition of flowering by means of gibberellic acid application reduces the cost of manual thinning in Japanese plums (*Prunus salicina* Lindl.)

6- The inhibition of flowering by means of gibberellic acid application reduces the cost of manual thinning in Japanese plums (*Prunus salicina* Lindl.)

Abstract

The application of gibberellic acid during flower bud induction significantly reduced flowering of 'Black Diamond' and 'Black Gold' Japanese plums. The response depended on the concentration applied and on the type of shoot. Mixed shoots had a similar response in both varieties, flowering being reduced by 40% for GA_3 50 mg l^{-1} and by 75%-90% for GA_3 75 mg l^{-1} or higher concentration. With regard to spurs, GA_3 50 mg l^{-1} reduced flowering intensity by 40% and 25% in 'Black Gold' and 'Black Diamond', respectively, and GA_3 75 mg l^{-1} or higher concentration reduced flowering by 70% and 50%, respectively. This partial inhibition of flowering significantly reduced the cost of manual thinning. The best GA_3 concentration was found to be 50 mg l^{-1}, since it reduced the cost of thinning by 45%-47% and increased final fruit weight by 7%-33% for 'Black Diamond' and 'Black Gold', respectively. Not significant differences in yield and in mature fruit characteristics of treated trees were found compared to untreated trees.

Introduction

In *Prunus* species, final fruit size depends to a great extent on the total number of fruits per tree (Costa and Vizzotto, 2000; Webster and Spencer, 2000). In these species, percentage of fruit set is unaffected for a wide range of flower bud production, hence an early reduction of competition among developing fruits is, of importance for increasing final fruit size at maturity. However, reduction of developing fruitlets results in an abundant flowering the following spring. For this reason manual thinning is generally performed every year in most stone fruits species, but it involves high costs.

Reduction of flowering in fruit trees is due to the inhibitory effect of gibberellins produced by seeds of developing fruits (Chan and Cain, 1967; Hoad, 1984). Gibberellins are thought to move from the fruit to the nearby nodes, where they inhibit the initiation of new floral primordia which usually initiated about six to eight weeks after fruit set (Webster and Spencer, 2000). Based on this inhibitory effect, the application of gibberellic acid (GA_3) during flower bud induction interrupts the floral process and partially reduces flowering. This technique has been successfully applied in apple trees (Luckwill, 1970; McArtney and Li,

1998; Tromp, 2000), avocados (Salazar-García and Lovatt, 2000) and mangos (Turnbull *et al*., 1996). In citrus, GA_3 treatment during autumn rest period reduced flowering by up to 50% the following season, depending on the concentration used (Monselise and Halevy, 1964; El-Otmani *et al*., 2000). Applied to apricots between May and July considerably reduced the subsequent flowering, also depending on the concentration applied (Southwick *et al*., 1995b). In peach, similar results have been achieved, and it has also been shown that the response depends both on date of treatment and on the concentration and volume of the hormone applied per tree (Painter and Stembridge, 1972; Taylor and Geisler-Taylor, 1998). This partial inhibition of flowering results in a partial reduction of the number of developing fruits and, thus, can be used as an indirect thinning method, providing a marked reduction in thinning costs. A similar study, however, has not been carried out in plums.

The aim of this work was to set a technique for inhibiting flowering intensity in Japanese plums (*Prunus salicina* L.) by means of application of GA_3 during flower bud induction and, thus, reducing costs of hand thinning. We also studied the effect of GA_3 treatment on fruit quality and on developing shoots.

Materials and methods

The experiments were carried out with same results in two consecutive developmental periods (2001/02 - 2002/03) in commercial orchards of adult trees of Japanese plum (*Prunus salicina* L.), cvs. 'Black Gold' and 'Black Diamond', grafted onto 'Marianna GF-81' rootstock, and located in Lliria, Spain (39º 39' latitude - 00º 38' longitude; 180 metre altitude). Trees were pruned to the classic vase shape, planted 6x4 m apart, grown in a loamy-clay soil, with drip irrigation. In both orchards the plum cv. "Fortune" was used as pollinator.

To determine the optimum concentration, GA_3 (Arabelex-L; 1.6% w/v; Aragro; Madrid - Spain) was applied at concentrations of 0, 25, 50, 75, 100, 125 and 150 mg l^{-1} 106 days after anthesis (DAA). A non-ionic wetting agent (nonylphenylpolyethyleneglycol ether, 20 % w/w) was added at a rate of 0.05 %. Treatments were sprayed by hand-gun at a pressure of 25-30 atm, wetting the trees to the point of runoff. Each tree received on average 10 litres of liquid spray. Different trees were used every year. A randomised complete-block design with one-tree plots of eight replications each was performed.

Eight mixed shoots, 8 spur shoots and 8 premature shoots were labelled per tree all around the tree. At flowering, length, number of flowers and percentage of fruit set were recorded.

At pit hardening, manual thinning was carried out according to traditional method (15-20 cm of separation between fruits). The time spent for thinning was measured per tree and per worker and the number of fruits remaining on the labelled shoots was recorded.

At colour break (June 21, 2002), 16 fruits per tree and repetition were sampled from all around the tree at a height of 1.5-2.0 m for colour analysis, flesh firmness and concentration of total soluble solids. Fruit colour was established determining the a and b Hunter co-ordinates; three measurements per fruit were made around the equator, using a Minolta CR-300 chroma-meter (Tokyo, Japan). Flesh firmness was assessed using a fruit pressure tester FT-011 (Facchini, Italy) using a 3 mm diameter flat cylinder probe. Total soluble solids (TSS) concentration of juice (ºBrix) was assessed with a digital refractometer (Atago, Tokyo, Japan).

At harvest (July 5-10, 2002), fruits picked were counted and weighed per tree, those affected by cracking were also counted, and fruit characteristics were evaluated as above.

Regression analysis or analysis of variance was performed on the data using the Student-Newman-Keuls' multirange test for means separation. Percentages were analysed following arc sine transformation.

Results

The effect of GA_3 reducing flowering in Japanese plum depended on the type of shoot and on the concentration applied. Mixed shoots showed similar response for both cultivars, reducing flowering by 40%-50% for 25-50 mg l^{-1}, and 75%-90% for 75 mg l^{-1} or higher concentration (Figure 6.1A). For spur shoots, in the cv. 'Black Gold' GA_3 50 mg l^{-1} reduced flowering by 40%, whereas 75 mg l^{-1} or higher concentration reduced flowering by 50%-80% (Figure 6.1B). In the cv. 'Black Diamond' GA_3 50 mg l^{1} reduced flowering only by 25%; not higher reduction than 50% were found for the GA_3 highest concentration (100 mg l^{-1}) (Figure 6.1B). A significant relationship between flowers cm^{-1} and the GA_3 concentration applied were found for both 'Black Gold' ($r^2 = 0.96$; $P \leq 0.05$) and 'Black Diamond' Japanese plums ($r^2 = 0.90$; $P \leq 0.05$).

GA_3 also affected flowering distribution shoot types. In Japanese plums, spurs per tree are higher in number (65%) than mixed shoots (15%); the remainder 20% are premature shoots and water shoots. The weighted mean adjustment for each type of shoot indicates a saturating effect of GA_3 for 75 mg l^{-1} in mixed shoots and for 100 mg l^{-1} in spur shoots in both cultivars (Table 6.1). Flowering reduction also increased fruit weight at pit hardening, both in 'Black Gold' and in 'Black Diamond' (Figure 6.2).

As a result of the reduction in flowering, time spent on hand thinning in the cv. 'Black Diamond' was reduced from 42 min per worker in control trees, to 23 and 15 min per worker in trees treated with GA_3 50 mg l^{-1} and 75 mg l^{-1}, respectively (Table 6.2). In the cv. 'Black Gold', time spent on thinning was reduced from 65 min per worker in control trees, to 34 and 16 min per worker in trees treated with GA_3 50 mg l^{-1} and 75 mg l^{-1} (Table 6.2). Only GA_3 150 mg l^{-1} significantly improved the response in comparison to 75 mg l^{-1} in both cultivars.

Since thinning was carried out under commercial criteria that is, leaving the fruits regularly distributed every 15-20 cm along the branche, fewer fruits were harvested from treated trees than from untreated trees. The response reached statistical significance for GA_3 50 mg l^{-1} or higher concentration treated trees in the cv. 'Black Diamond', or GA_3 25 mg l^{-1} or higher concentration treated trees in cv. 'Black Gold' (Table 6.3). The effect of thinning revealed at colour break, when a close inverse relationship between the number of fruits per tree and fruit weight (g), were found both for 'Black Diamond' ($r = -0.917$; $P \leq 0.05$) and for 'Black Gold' ($r = -0.970$; $P \leq 0.05$). The increased fruit weight partially compensated the reduction in the total number of fruits, and yield was only reduced for GA_3 75 mg l^{-1} or higher concentration in both varieties (Table 6.3).

Fruit from GA_3 treated trees grew faster and advanced fruit colour break. This effect was most noticeable in the cv. 'Black Gold', for which GA_3 50 mg l^{-1} treated fruit showed at colour break, significant differences in weight, diameter, TSS and fruit colour, compared to control (Table 6.4). In cv. 'Black Gold' there were not significant differences in flesh firmness of treated fruit compared to control fruit, however in cv. 'Black Diamond' flesh firmness was significantly reduced by treatment (Table 6.4). Not significant differences were observed in the number of fruits affected by cracking at maturity in treated fruit compared to control fruit (data not shown). Same results on fruit characteristics were found for 2002/03 developmental period (data not shown).

Discussion

Japanese plum (*Prunus salicina* Lindl), like other stone fruit species such as European plum (*Prunus domestica* L.), apricot (*Prunus armeniaca* L.) or peach (*P. persica* L. Batsch), tends to produce a great number of blossoms (Southwick and Glozer, 2000) and, also a great number of fruits. This makes it necessary to thin fruit manually so that the fruits remaining on the tree reach a commercially appropriate size. The thinning of fruits affects carbohydrates partitioning and promotes vegetative growth and affects induction and differentiation of floral buds (Byers *et al.*, 1990).

Polar gibberellins, such as GA_3, are known to inhibit flower bud induction in many angiosperm trees, including temperate fruit crops, citrus and mango (Sedgley and Griffin, 1989). Accordingly, its use in commercial peach orchards for reducing the number of flower, and, consequently, the number of developing fruitlets significantly reduces hand-thinning costs (Painter and Stembridge, 1972). Japanese plums, however, had not been studied for this purpose.

In our experiments, pre-harvest applications of GA_3 during flower bud induction, significantly reduced the number of flowers developed in the subsequent spring in two Japanese plum cultivars, 'Black Diamond' and 'Black Gold'. The effect depended on the concentration applied and on the type of shoot. Our experiments also show that 'Black Gold' proved to be more sensitive to GA_3 than 'Black Diamond'. The greater sensitivity to GA_3 observed in mixed shoots with respect to spurs is due to the difference in vigour between these types of shoot. Since there is an inverse relationship between vigour and the number of floral buds produced (Tromp, 2000), it is thought that GA_3 affects more acutely to the more vigorous shoots than the less vigorous, such as spurs. These results agree with those found by Southwick and Fritts (1995) for different Japanese plum cultivars, such as 'Black Amber' and 'Friar', but these authors did not study differential sensitivity of shoots or the effect of GA_3 on yield and fruit quality.

With regard to the commercial effect of the treatments, the best results were obtained for GA_3 50 mg l^{-1} which reduced the number of flowers per cm^{-1} by 31%-43%. Although at this concentration there was a reduction in the number of fruits harvested per tree compared to the control, the increase in fruit weight compensated for this effect and yield per tree (Kg $tree^{-1}$) was not significantly affected. The early reduction in the number of floral buds allows the tree to maximize its capacity to increase fruit size (Weinberger, 1941). The increase in fruit size and the advance fruit ripening obtained in

the Japanese plums studied are in agreement with results found by Garcia-Pallas *et al.* (2001) in 'Crimson Gold' nectarine.

Pre-harvest application of GA_3 50 mg l^{-1} had no detrimental effect on fruit quality at harvest. Moreover, in the cv. 'Black Gold' it increased fruit firmness and also improved fruit colour. Southwick and Glozer (2000) obtained similar results in *Prunus domestica* L. for concentrations of between 31 and 62 mg l^{-1} of GA_3.

According to our results, the optimum concentration of GA_3 can be taken as 50 mg l^{-1}. At this concentration, the mean saving in thinning time was 45% for the cv. 'Black Diamond' and up to 47% for the cv. 'Black Gold'. These saving costs, although they do not include the cost of the product (GA_3) and the application, are greater than those estimated by Southwick *et al.* (1995a) for the 'Loadel' peach.

In conclusion, the use of GA_3 to inhibit flowering appears as an indirect technique of fruit thinning and as a useful method for controlling the cost of hand thinning in Japanese plum cultivars. In this work, the optimum effect was found for GA_3 50 mg l^{-1} applied 106 DAA, but it must be subjected to testing for specific cultivars and climatic conditions and for pruning in order to avoid excessive depletion of the number of developing fruits and, consequently, of final yield.

References

Byers, R.E.; Carbaugh, D.H. and Presley, C.N. 1990. The influence of bloom thinning and GA_3 sprays on flower bud numbers and distribution in peach trees. Journal of the American Society of Horticultural Science 65: 143-150.

Chan, B.G. and Cain, J.C. 1967. The effect of seed formation on subsequent flowering in apple. Proceedings of the American Society of Horticultural Science 91: 63-67.

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

El-Otmani, M.; Coggins, C.; Agustí, M. and Lovatt, C.J. 2000. Plant Growth Regulators in Citriculture: World current uses. Critical Review in Plant Sciences 19: 395-447.

García-Pallas, I.; Val, I. and Blanco, A. 2001. The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA_3 as an alternative to hand thinning. Scientia Horticulturae 90 (3/4): 265-278.

Hoad, G.V. 1984. Hormonal regulation of fruit bud formation in fruit trees. Acta Horticulturae 149: 13-23.

Luckwill, L.C. 1970. The control of growth and fruitfulness of apple trees. In: Luckvill, L.C. and Cutting, C.V. (Eds.). *Physiology of tree crops*. Academic Press, New York, EEUU, pp. 237-254.

McArtney, S.J. and Li, S.H. 1998. Selective inhibition of flowering on "Braebun" apple trees with gibberellins. Journal of Horticultural Science 33: 699-700.

Monselise, S. P. and Halevy, A. H. 1964. Chemical inhibition and promotion of citrus bud induction. Proceedings of the American Society of Horticultural Science 84: 141-146.

Painter, J.W. and Stembridge, G.E. 1972. Peach flowering response as related to time of gibberellin application. HortScience 7: 389-390.

Salazar-García, S. and Lovatt, C.J. 2000. Use of GA_3 to manipulate flowering and yield of "Hass" avocado. Journal of the American Society of Horticultural Science 125: 25-30.

Sedgley, M. and Griffin, A.R. 1989. Sexual reproduction of tree crops. Academic Press, London and San Diego.

Southwick, S.M. and Glozer K. 2000. Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. HortTechnology 10 (4): 744-751.

Southwick, S.M. and Fritts, R. 1995. Commercial chemical thinning of stone fruit in California by gibberellins to reduce flowering. Acta Horticulturae 394: 135-147.

Southwick, S.M.; Weis, K.G. and Yeager, J.T. and Zhou 1995a. Controlling cropping in 'Loadel' Cling Peach using Gibberellin: effect on flower density, fruit distribution, fruit firmness, fruit thinning and yield. Journal of the American Society of Horticultural Science 120 (6): 1087-1095.

Southwick, S.M; Yeager, J.T. and Zhou, H. 1995b. Flowering and fruiting in 'Patterson' apricot (*Prunus armeniaca*) in response to postharvest application of gibberellic acid. Journal of the American Society of Horticultural Science 94: 492-495.

Taylor, B.H. and Geisler-Taylor, D. 1998. Flower bud thinning and winter survival of 'Redhaven' and 'Cresthaven' peach in response to GA_3 sprays. Journal of the American Society of Horticultural Science 123 (4): 500-508.

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

Turnbull, C.G.; Anderson, K.L. and Winston, E.C. 1996, Influence of gibberellin treatment on flowering and fruiting patterns in mango. Australian Journal of Experimental Agriculture 36: 603-611.

Webster, A.D. and Spencer, J.E. 2000. Fruit thinning plums and apricots. Plant Growth Regulation 31: 101-112.

Weinberger, J.H. 1941. Studies on time for peach thinning from blossoming to maturity. Proceedings of the American Society of Horticultural Science 38: 137-140.

Table 6.1: Effect of gibberellic acid concentration applied on flowers cm^{-1} as weighted average values for spurs and mixed shoots of 'Black Diamond' and 'Black Gold' plums. Treatments applied at flower bud induction (106 DAA) on June 2001, values for spring 2002. Each value is the average of 8 trees.

GA_3 concentration (mg l^{-1})		0	25	50	75	100	125	150
'Black Diamond'	Mixed Shoots	0.38 aC	0.21 bC	0.22 bC	0.11 cC	0.07 cB	0.06 cB	0.04 cC
	Spurs	3.61 aB	3.10 abB	2.50 bB	2.20 bc B	1.70 cA	1.50 cA	1.80 cA
	Weighted Mean	3.99 a	3.29 ab	2.75 b	2.33 bc	1.79 c	1.53 c	1.82 c
'Black Gold'	Mixed Shorts	0.54 aC	0.34 bC	0.26 bC	0.06 cC	0.03 cB	0.05 cB	0.05 cC
	Spurs	7.12 aA	6.10 abA	4.10 b AC	3.30 bcA	1.70 cA	1.30 cA	1.40 cA
	Weighted Mean	7.66 a	6.42 ab	4.38 b	3.41 bc	1.92 c	1.33 c	1.41 c

Means followed by different lower-case letters in the same line and by different capital letters in the same column differ significantly ($P \leq 0.05$).

Table 6.2: Effect of GA_3 concentration on time spent per tree and worker on hand thinning (min) at pit hardening in plums cv 'Black Diamond' and cv. 'Black Gold'. Treatments applied at flower bud induction (106 DAA) on June 2001. Date of thinning April 2002. Each value is the average of 8 trees.

GA_3 concentration (mg l^{-1})	0	25	50	75	100	125	150
cv. 'Black Diamond'	41.5 a	23.7 b	22.7 b	15.1 c	9.5 cd	9.4 cd	6.8 d
cv. 'Black Gold'	64.5 a	42.3 b	34.0 c	15.8 d	11.1 de	10.5 de	8.9 e

Means followed by different letters in the same line differ significantly ($P \leq 0.05$).

Tabla 6.3: Effect of GA_3 concentration on the number of fruits harvested per tree and yield (Kg tree^{-1}) in plums cv 'Black Diamond' and cv. 'Black Gold'. Treatments applied during floral bud induction (mide June) on 2001. Date of harvest 5-10 July 2002. Each value is the average of 8 trees.

GA_3 concentration (mg l^{-1})		0	25	50	75	100	125	150
cv. 'Black Diamond'	Number of fruits per tree	427.5 a	352.4 ab	323.8 b	165.5 c	119.5 c	136 c	68.6 d
	Yield (Kg tree^{-1})	41.5 a	36.6 a	33.5 a	18.2 b	11.9 b	15.7 b	8.1 b
cv. 'Black Gold'	Number of fruits per tree	451.3 a	318.3 b	280.9 b	109.8 c	90.1 c	94.6 c	68.5 c
	Yield (Kg tree^{-1})	40.6 a	38.7 a	33.6 a	15.5 b	12.5 b	13.1 b	9.7 b

Means followed by different letters in the same line differ significantly ($P \leq 0.05$).

Tabla 6.4: Effect of GA_3 concentration on fruit characteristics at colour break the season following treatment, in cv. 'Black Diamond' and cv. 'Black Gold' plums. Values for 2001 treatments applied at flower bud induction (106 DAA). Date of analysis June 21, 2002. Each value is the average of 8 trees.

	cv. 'Black Diamond'			cv. 'Black Gold'		
GA_3 concentration (mg l^{-1})	0	50	100	0	50	100
Fruit weight (g)	75.9 b	82.6 b	93.3 a	73.9 c	100.8 b	114.1 a
Fruit diameter (mm)	51.8 b	52.7 b	56.9 a	50.5 c	56.9 b	59.6 a
TSS (ºBrix)	10.7 b	10.4 b	11.5 a	11.5 b	13.2 a	12.8 a
Flesh firmness (N)	14.7 a	12.7 b	12.6 b	16.5 a	15.9 a	15.7 a
Skin colour 'a'	-13.6 b	-3.4 a	-5.3 a	-8.7 b	2.9 a	3.9 a
Skin colour 'a/b'	-0.5 b	0.0 a	0.0 a	-0.3 b	0.3 a	0.4 a

Means followed by different letters in the same line for each cultivar differ significantly ($P \leq 0.05$).

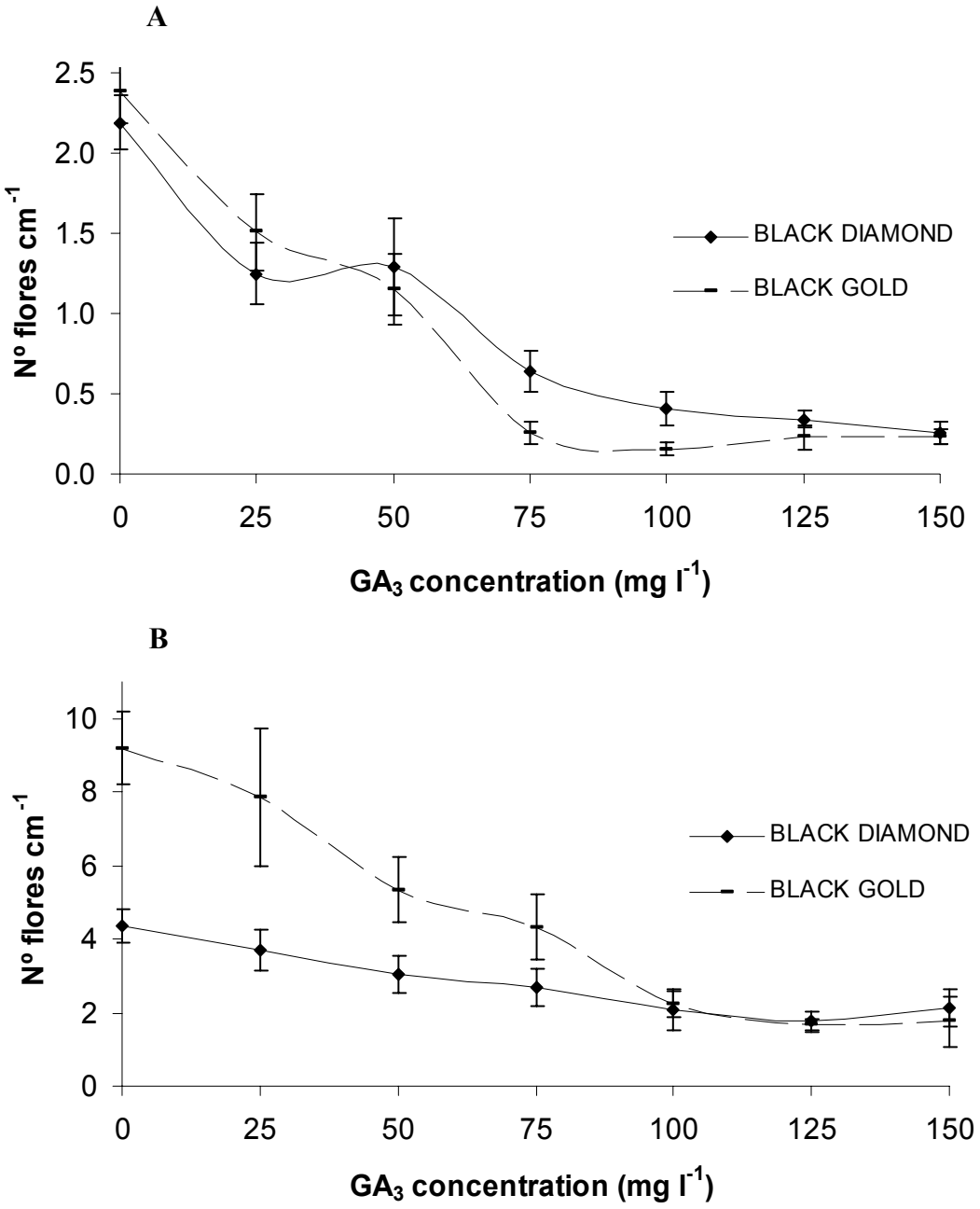

Figure 6.1: Effect of gibberellic acid (GA_3) on the number of flowers per cm of mixed (A) and spur shoots (B), in cv. 'Black Diamond' and cv. 'Black Gold' plums. SE are given as vertical bars. Treatments applied during floral bud induction (mide June 2002). Values for the spring of 2003. Each value is the average of 8 trees. SE are given as vertical bars.

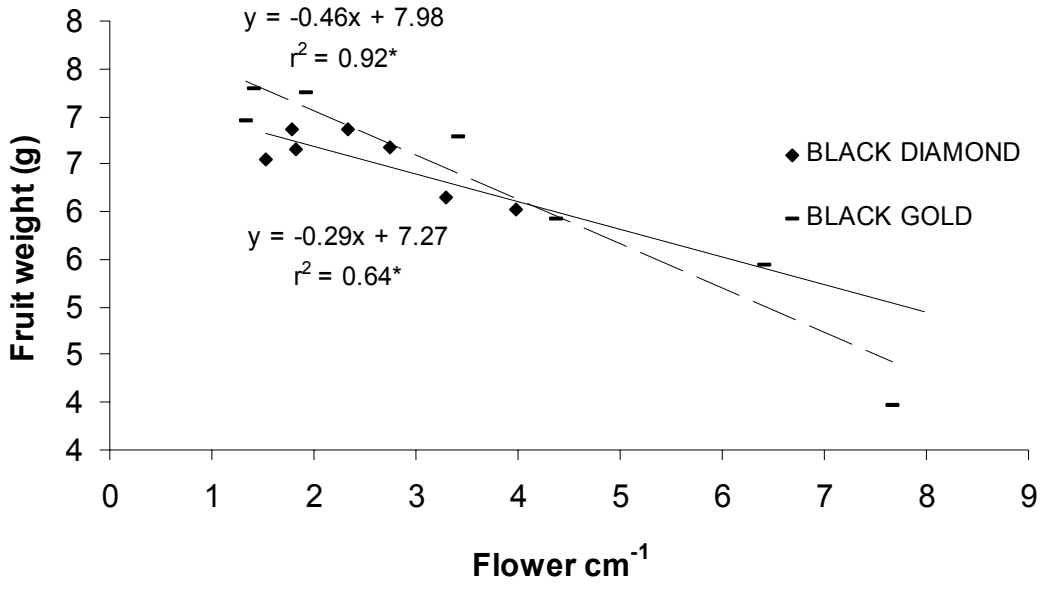

Figure 6.2: Weighted mean number of flowers cm^{-1} and fruit weight relationship at pit hardening in cv. 'Black Diamond' and cv. 'Black Gold' plums. Values for the spring of 2003. (*) Significant at $P \leq 0.05$.

CHAPTER 7

Short term effect of GA_3 spray on growth and chlorophyll fluorescence in young and adult trees of *Prunus persica* L. Batsch

7- Short-term effect of GA_3 spray on growth and chlorophyll fluorescence in young and adult trees of *Prunus persica* L. Batch

Abstract

The actual quantum yield of photosystem II (ΦPSII) significantly increased in young GA_3 treated trees at 3 days after treatment (DAT), suggesting a higher proportion of light absorbed by PSII chlorophyll. This is coincident with a significant decrease in the minimal fluorescence (Fo) and maximal fluorescence (Fm), indicating a potential energy dissipation process in these plants. No effect was evident on ΦPSII in adult treated trees throughout 4 weeks after treatment. Adult treated trees showed a significant decrease in Fv/Fm at 16 days after treatment, while only a slight decrease was evident in young plants in relation to controls. The decrease in the Fv/Fm ratio may indicate irreversible damage caused by photoinhibition. In the treated adult trees this effect was partially inhibited till harvest by the presence of fruit. The level of non photochemical quenching (NPQ) in young treated trees showed significant differences with respect to controls at 3 and 16 DAT. Adult treated trees showed increased NPQ levels at 16 DAT with respect to controls, suggesting an increase in heat dissipation. Although GA_3 significantly increases vegetative growth in adult trees during a 30 DAT period, there was no significant difference in the average shoot length and number or distribution of nodes between treatments, at the end of the growing season (114 DAT). Flower density decreased and sprouting increased in GA_3 treated trees with respect to controls, young trees being more sensitive than adult. The difference in maximum quantum yield (Fv/Fm) between young and adult trees seems to be related to the presence of fruit on adult trees. GA_3 appears to have a short term effect on photosynthesis when applied at flower bud induction in peach trees, but further research is needed to clarify its relation to carbohydrate, nitrogen and enzymatic metabolism.

Introduction

Gibberellins (GAs) have proved to be efficacious in controlling the flowering process in dicotyledonous species. Exogenous applications of gibberellic acid (GA_3) during flower bud induction result in a reduction of flowering in the following season in *Citrus*, *Prunus* and *Pome* species (Monselise and Halevy, 1964; Luckwill and Silva, 1979; Tromp, 1982; Taylor and Geisler-Taylor, 1998; Southwick and Yeager, 1991; Southwick and Fritts, 1995).

In the northern hemisphere, GA_3 sprays from late May to early July have successfully inhibited flower bud induction in deciduous fruit trees (Bradley and Crane, 1960; Li *et al*., 1989; Southwick *et al*., 1995; Southwick and Glozer, 2000). Under Mediterranean conditions, sprays with 50 mg L^{-1} GA_3 from 80 to 115 days after anthesis (DAA) were found to be optimal, reducing flowering of peaches and nectarines in the following season (Jourdain and Clanet, 1987; González-Rossia *et al*., 2003). It has also been used as an alternative to hand thinning, reducing costs in many *Prunus* species (Southwick and Glozer, 2000). However, to date little is known about the mechanism by which GA_3 interferes in the bud induction process. In addition, although the effect of GA_3 has been well verified in adult trees, not much work has been done in young trees (≤5 years old). Taylor and Geisler-Taylor (1998) have suggested that young trees are less sensitive to the effects of GA_3 sprays compared to vigorous mature trees.

Tromp (2000) suggested that since young leaves are rich sources of GAs, as long as the shoot growth continues and new leaves appear GAs inhibit flower bud formation, and thus a negative correlation between vegetative growth and flowering intensity is given.

It has been reported that GA_3 sprayed for inhibiting flower bud induction also enhanced photosynthesis rates in bougainvillea (Even-Chen and Sachs, 1980). This could be related to differences in dry matter accumulation (Biemelt *et al.*, 2004) or relative growth rate induced by GAs (Dijkstra *et al.*, 1990). Nevertheless, little information is available about the effects of GAs on photosynthesis. Moreover, it is not clear if there is any relationship between GA concentrations and the rates of photosynthesis, growth and respiration (Nagel and Lambers, 2002). The few available reports reveal contradictory results, concluding that application of GA_3 both stimulates and reduces the photosynthetic rate (Dijkstra *et al.*, 1990; Ashraf *et al.*, 2002; Biemelt *et al.*, 2004).

In recent years, chlorophyll fluorescence has become a powerful tool in plant ecophysiology studies (Maxwell and Johnson, 2000). This is a non destructive method used under field conditions which reveals the detailed properties of photosystem II (PSII), including the fraction of open PSII, energy dissipation via antennae, and photoinhibition of PSII (Björkman and Demming-Adams, 1994; Krause, 1994; Shreiber *et al*., 1994).

In this work attempts were made to elucidate the short term effect of GA_3 sprays on the photosynthetic and chlorophyll fluorescence parameters, stress occurrence and vegetative growth responses in peaches with regard to flowering response.

Material and methods

The study was conducted in an experimental orchard on three (young plants-YP) to ten (adult plants-AP) year old 'Springcrest' peach trees grafted onto 'GF677' rootstock, and located in the area of Valencia, Spain. AP trees were planted 6x4 m apart in a sandy-loamy soil; YP trees were planted in 160 litre pots filled with a sandy-loamy soil mixture. Trials were conducted in a randomized complete block design with 4 plots of one replication each. Plants were selected for their homogeneity and vigour. In both cases, plants were drip irrigated. The standard fertilization, pest management and cultural practices were appropriate for optimum development.

GA_3, at a concentration of 0 (control) and 100 mg l^{-1} (GA), was applied by handgun to whole trees as a cover spray to the point of runoff (3 and 5 l $tree^{-1}$ over YP and AP, respectively). A non ionic wetting agent (alkyl polyglycol ether; 20% w/v) at 0.05% was included in the treatment. Treatment was performed 105 DAA (days after anthesis), a date included in the period of sensitivity of *Prunus* to GA_3 as an inhibitor of flower bud induction (Bradley and Crane, 1960).

At 0, 3, 5, 8, 16 and 30 days after treatment (DAT) the leaf chlorophyll fluorescence was measured by a portable fluorometer Junior-PAM (Walz, Gademann Instruments, Germany). The fluorometer was connected to a leaf-clip holder (2030-B, Walz) and to a computer equipped with data acquisition software. Four leaves per treatment were labelled in order to take the measurements of the minimal fluorescence level (Fo) and the maximal fluorescence level (Fm) in dark adapted leaves, which were darkened for about 30 minutes before measurement. Each dark measurement was accompanied by a light adapted measurement (5 measures per leaf) in leaves exposed to daily light intensity. All measurements started at the same time each afternoon, in order to avoid hours with higher temperatures. The steady state value of fluorescence (Fs) and the maximal fluorescence (F´m) in light adapted leaves were recorded. By using the fluorescence parameters mentioned above, the following calculations were made: (1) the maximum quantum yield of PSII photochemistry (Fv/Fm); (2) variable fluorescence (Fv=Fm-Fo); (3) the photochemical quenching coefficient qP=(F´m-Fs)/(F´m-Fo); (4) the non-photochemical quenching coefficient measured through qN=(Fm-F´m)/(Fm-Fo), (5) and the actual quantum yield of PSII electron transport, ΦPSII = (F'm-Fs)/F'm.

Eight to ten homogeneous current season shoots per tree (20 cm long, approximately) were selected and tagged before treatment, and their length measured at 0, 8, 16, 30 and 114 (leaf fall) DAT. Average shoot length, shoot growth rate and number of nodes per shoot were evaluated at each day of measurement.

Ten current season-shoots were randomly selected on each tree in bloom, and their length measured. Each shoot was divided into three portions. The basal portion was termed *proximal*, the middle portion was termed *middle*, and the apical portion was termed *distal*. Each portion was selected with a similar number of nodes. The number of flowers and sprouts per shoot were counted at full bloom. Data was expressed as the number of flowers or sprouts per cm of shoot length.

Analyses of Variances were performed on the data, using Duncan's multiple range test for means separation.

Results

Effect on chlorophyll fluorescence. In young trees, 3 DAT GA_3 sprays significantly increased ΦPSII compared with control trees, but this effect declined afterwards and even reversed 8 DAT (Figure 7.1A). Adult plants showed no significant variation on ΦPSII due to GA_3 spray during 30 DAT (Figure 7.1B).

GA_3 scarcely affected Fv/Fm and only 30 DAT young treated trees showed values significantly lower as compared with control trees (Figure 7.2A). Adult trees showed significantly lower values for treated trees from 16 up to 30 DAT (Figure 7.2B). Due to tree age, different behaviour of control for both ΦPSII and Fv/Fm was recorded.

The photochemical quenching coefficient (qP) for GA_3 treated young plants was 0.90, at 3 DAT, which was significantly lower than that for the control value (0.99) (Figure 7.3A). It is important to note that this effect was coincident with the increase in ΦPSII for GA_3 treated young plants (Figure 7.1A). Adult GA_3 treated trees differed significantly from control adult plants 16 DAT, and levelled back to non significant values 30 DAT (Figure 7.3B).

NPQ in young GA_3 treated plants was 0.174 and 0.497 at 3 and 16 DAT, respectively, showing significant differences on both dates with regard to the control trees (0.423 and 0.211, respectively) (Figure 7.4A). Although there was a non clear evolution of NPQ for control adult trees

during the first week after treatment and no major changes were evident throughout the whole evaluation period, treated adult trees significantly increased NPQ with regard to control trees 16 DAT; no significant difference was observed 30 DAT (Figure 7.4).

Fo decreased 22% 3 DAT in GA_3 treated young trees with regard to control trees, however no significant differences were observed throughout the evaluation period (Table 7.1). On the other hand, in adult plants no differences were observed due to treatment during the first two weeks after treatment, but 16 DAT and 30 DAT, treated plants had a significant increment (63% and 51%, respectively) of Fo with respect to control trees (Table 7.2).

Fv/Fm differences between young control and GA_3 treated trees are due mainly to Fo values, which vary by between -2% to +18% from 16 to 30 DAT, whereas Fm value varies by between -17% to -6%, thus Fv (Fm-Fo) varies by between -21% to -16% and Fv/Fm by between -3% and -9% (Table 7.1). Similar results were recorded for adult trees, with increments of between +63% and 51% for Fo due to GA_3 from 16 to 30 DAT, by between +33% and 21% for Fm, +24% and +12% for Fv and -6% and -6% for Fv/Fm (Table 7.1).

Effects on vegetative growth. Shoot growth rate decreased gradually in the course of the evaluation period in all treatments (Table 7.3). GA_3 and young control plants had similar shoot growth rate during the first month after treatment, but differed significantly from 30 to 114 DAT. On the other hand, the shoot growth rate of GA_3 adult trees was significantly higher than that of adult control trees during the first month after treatment, but decreased significantly with respect to the control trees in the final period (Table 7.3). The effect of treatment on shoot growth was only statistically significant after the second week of treatment, whereas plant age had a significant effect during the whole period of evaluation.

The time-course of shoot growth of adult trees showed significant differences between control and GA_3 treated trees up to adult leaf fall, when no differences in the average shoot length was observed (Figure 7.5). There were no significant differences between control and GA_3 treated young trees throughout the experiment (Figure 7.5).

No significant difference in the number of nodes per shoot was found due to GA_3 irrespective of the age of trees at 0, 8, 16 and 114 DAT (data not shown). However, thirty days after treatment node density was significantly higher in the proximal section of young trees with regard to

adult trees, and significantly higher for treated adult trees in the middle shoot portion with respect to controls (Table 7.4). These results are coincident with a higher growth rate (Table 7.3) and time course of shoot growth (Figure 7.5) in GA_3 treated adult plants with respect to controls. Although no significant differences were observed in the total number of nodes per shoot due to GA_3 at 30 DAT, we observed significant differences between young and adult control trees (Table 7.4).

Effect on flower and sprout density. The long term effect of GA_3 sprays was verified at full bloom. Two-way ANOVA showed a significant effect of GA_3 treatment ($P \leq 0.05$) on flower m^{-1} and sprout m^{-1} in young and adult trees, but no significant effect was found for GA_3 treatment x tree age interaction (Table 7.5). GA_3 reduced flower density of young and adult trees by 57.4% and 47.5% respectively, with regard to control trees (Table 7.5). On the other hand, sprout density was increased by GA_3 both in young and adult trees, by 69% and 110%, respectively, with regard to controls.

Two-way ANOVA showed no significant effect ($P \leq 0.05$) of plant age or treatment on shoot length at full bloom (Table 7.5). This effect was consistent with the values measured at leaf fall (Figure 7.5).

Discussion

Photosynthetic behaviour has been widely studied in several *Prunus* species (DeJong, 1983; DeJong, 1986; Flore, 1992; Gucci *et al.*, 1991a; Gucci *et al*., 1991b Layne and Flore, 1992; Powles, 1984). However, there are no studies on the effect of GA_3 on photosynthetic activity during the induction period. From our results, the increase of the ΦPSII in young trees due to GA_3 treatment suggests a higher proportion of the light absorbed by chlorophyll associated with PSII. But this effect was only significant during the first 3 days after treatment. Afterwards, the tendency reversed and there was a decrease during the second week after treatment (Figure 7.1A).

The maximal fluorescence (Fm), variable fluorescence (Fv) and Fv/Fm ratio characterize the functional state of PSII in dark-adapted leaves and they are considered to be a measure of PSII effectiveness in the primary photochemical reactions. Slight changes of Fv/Fm ratio may occur with a much stronger decrease of Fm and Fv values. Such changes could be partly explained by some kind of disorder in the chloroplast ultra-structure (Buttler, 1977). A decrease in Fo (minimal fluorescence yield) may be due to energy dissipation processes within the chlorophyll pigment bed (Tsonev *et al*., 1999). Young treated trees showed a slight decrease in Fv/Fm during the 3 weeks after GA_3 treatment (Figure 7.2A), but Fo and Fm had fallen

sharply at the third day after treatment, indicating a potential energy dissipation processes (Table 7.1). However, at the end of the study period, there was an increase of Fo parallel to a reduction of Fv/Fm in young treated trees, suggesting an irreversible detachment of light-harvesting chlorophyll a/b protein complexes from reaction centre complexes of PSII to partly reversible inactivation of PSII (Schreiber and Armond, 1978; Yamane *et al*., 1997) and to dark reduction of plastoquinone QA (Havaux, 1996). Changes in the fluorescence components (Fo and Fv) have been used to determine whether a shift of Fv/Fm reflects a direct stress-induced damage of the PSII reaction centre (Powles, 1984; Krause and Weis, 1991; Öquist *et al*., 1992) or a photoprotective increase in non-radiative decay (Björkman and Demmig, 1987; Demmig-Adams *et al.*, 1998). The decrease in the Fv/Fm ratio of the dark-adapted sample in young trees may indicate the irreversible damage caused by photoinhibition. Increased irradiance and temperature at the last evaluation date could lead to increased photoinhibition in young treated trees, similarly to Griffin *et al.* (2004) results in *Illicium taxa*. In fact, it is accepted that a sustained decrease in dark adapted Fv/Fm and increase in Fo indicate the occurrence of photoinhibitory damage in response to high temperature (Gamon and Pearcy, 1989), low temperature (Groom and Baker, 1992), excess of photon flux densitiy (PFD) (Ögren and Sjöström, 1990) and water stress (Epron *et al*., 1992).

A decrease in qP (fraction of open PSII to total PSII) is a common response to some stresses, which might be linked to a limitation in carboxilation activity of Rubisco in some species (Kitao *et al*., 1998). A higher fraction of closed reaction centers denotes that light absorption exceeds the capacity of electron transport and CO_2 fixation. As a result, the part of non-photochemical energy conversion increases (Vassilev and Monolov, 1999). In our experiment, there was a significant decrease of qP at 3 DAT in young GA_3 treated trees which could be correlated with an increase in ΦPSII produced by a saturated photosynthesis by light. But at the same time, this effect was not accompanied by an increase in NPQ, linearly correlated to heat dissipation (Maxwell and Johnson, 2000), suggesting a possible effect of the treatment on the non-photochemical process. In adult trees, NPQ was only affected at 16 DAT while no effect on ΦPSII was observed due to GA_3. Thus, an increase in NPQ after harvest over 0.4, suggests an increase in leaf heat dissipation.

It is interesting to note that after harvest there was a significant increase in qP and Fo and a significant decrease in Fv/Fm. The increase in Fo and the decrease in Fv/Fm related to a photoinhibitory reaction in treated trees could be explained by an enlarged amount of open

Photosystems II without a parallel increase in the energy efflux to ATP through the ΦPSII. A disturbance of assimilates transport and of sink activity can affect the photochemical efficiency (Werner and Correia, 1996). Increased sink activity, either reproductive or vegetative, or reduction in source supplied, has been associated with an increase in rate of photosynthesis (Flore and Lakso, 1989). In our experiment, NPQ increased in adult treated trees after harvest, suggesting significant heat dissipation from treated trees compared to control trees. In GA_3 adult trees, Fv/Fm decreased significantly 10 DAT coinciding with harvest, showing partial inhibitory effect of the fruit over GA_3 effect on the maximum quantum yield of PSII. DeJong (1986) related this effect to a change in the stomatal behaviour and water status in the tree.

Taylor and Geisler-Taylor (1998) found that, in peach trees, 50 mg l^{-1} GA_3 increased long shoot length by 4.8% on average compared to control trees, but 100 mg l^{-1} sprays reduced shoot length by 3%. Our results showed that 100 mg l^{-1} GA_3 increased significantly shoot growth length of adult treated trees 30 DAT with respect to controls, but this was a temporary effect and no significant differences were observed at the end of the growing season (see Figure 7.5). The lack of effect on shoot development of young trees may be due to their greater vigour. Thus, no overall effect of GA_3 sprays on vegetative growth rate or shoot length was measured at the end of the growing season, which is in agreement with previous results from Ward (1993) and García-Pallas and Blanco (2001).

Differences in the number of nodes per shoot length are related to tree vigour. In sour cherry, as vigour decreases the number of nodes that have flower buds increases and the number of spurs decrease (Kenworthy, 1974). A similar response occurs in peach under drought conditions (Johnson *et al*., 1992). In agreement with Ward's results (1993), our study found no significant difference in the number or distribution of nodes at the end of the growing season between treatments, irrespective of the age of trees and treatment. However, flower density decreased in treated trees in comparison with controls, the young trees being more sensitive than adults, as was reported by Taylor and Geisler-Taylor (1998). This leads us to the conclusion that fewer flower buds per node reached the anthesis state in young trees. We also noted a tendency toward increased vegetative sprouting in the spring following treatment, as a result of the reduced flower budbreak from GA_3 treatments (see Table 5), as reported in apple (Palmer *et al*., 1991), peach (Southwick *et al*., 1995) and citrus (Guardiola *et al*., 1982).

In conclusion, GA_3 spraying appears to affect photosynthetic efficiency, causing photoinhibition and affecting the energy dissipation process in plants, when applied during the flower bud induction period. Young trees seem to be more sensitive than adult trees. The presence of fruit appears to partially inhibit the effect of GA_3 on the maximum quantum yield of PSII. The effect of GA_3 on shoot length, vegetative growth rate, number and distribution of nodes was compensated and no significant differences appeared at the end of the growing season. However, there was an increase in vegetative sprouting and an expected decrease in the number of flowers supported by mixed shoots.

References

Ashraf, M.; Karim, F. and Rasul, E. 2002. Interactive effects of gibberellic acid (GA_3) and salt strees on growth, ion accumulation and photosynthetic capacity of two spring wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Plant Growth Regulation 36: 49-59.

Biemelt, S.; Tschiersch, H. and Sonnewald, U. 2004. Impact of altered gibberellin metabolism on biomass accumulation, lignin biosynthesis, and photosynthesis in transgenic Tobacco plants. Plant Physiology 135: 254-265.

Björkman, O. and Demmig, B. 1987. Photon yield of O_2 evolution and chlorophyll fluorescence at 77k among vascular plants of diverse origins. Planta 170: 489-504.

Björkman, O. and Demming-Adams, B. 1994. Regulation of photosynthetic light energy capture, conversion and dissipation in leaves on higher plants. In: Schulze, E.D. and Caldwell, M.M. (Eds.). *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin, pp. 17-47.

Bradley, M.V. and Crane, J.C. 1960. Gibberellin-induced inhibition of bud development in some species of *Prunus*. Science 131: 825-826.

Buttler, W.L. 1977. Chlorophyll fluorescence: a probe for electron transfer and energy transfer. In: *Encyclopedia of Plant Physiology*. *Vol. 5*. Springer-Verlag, Berlin, pp. 149-167.

DeJong, T.M. 1983. CO_2 assimilation characteristics of five *Prunus* tree fruit species. Journal of the American Society of Horticultural Science Sci. 108: 303.

DeJong, T.M. 1986. Effects of reproductive and vegetative sink activity on leaf conductance and water potential in *Prunus persica* (L.). Batsch. Scientia Horticulturae 29: 131.

Demming-Adams, B.; Moeller, D.L.; Logan, B.A. and Adams, W.W. III. 1998. Positive correlation between levels of retained zeaxanthin + antherxanthin and degree of photoinhibition of shade leaves of *Schefflera arboricola* (Hayata). Merrill. Planta 205: 367-374.

Dijkstra, P.; Reegen, H.T. and Kuiper, P.J.C. 1990. Relation between relative growth rate, endogenous gibberellins, and the response to applied gibberellic acid for *Plantago major*. Physiologia Plantarum 79: 629-634.

Epron, D.; Dreyer, E. and Bréda, N. 1992. Photosynthesis of oak trees (*Quercus petraea* (Matt) Liebl.) during drought stress under field conditions: diurnal course of net CO_2 assimilation and photochemical efficiency of photosystem II. Plant, Cell and Environment 15: 809-820.

Even-Chen, Z. and Sachs, R.M. 1980. Photosynthesis as a function of short day induction and gibberellic acid treatment in Bougainvillea "San Diego Red". Plant Physiology 65: 65-68.

Flore, J.A. 1992. The influence of summer pruning on the physiology and morphology of stone fruit trees. Acta Horticulturae 322: 257.

Flore, J.A. 1994. Stone fruit. In: Schaffer, B. and Anderson, P.C. (Eds.). *Handbook of environmental physiology of fruit crops, Vol. 1, Temperate crops*. CRC Press, Inc., Boca Raton, Florida, pp. 233-270.

Flore, J.A. and Lakso, A.N. 1989. Environmental and physiological regulation of photosynthesis in fruit crops. Horticultural Review 11: 111.

Gamon, J.A. and Pearcy, R.W. 1989. Leaf movement, stress avoidance and photosynthesis in *Vitis californica*. Oecologia 79: 475-481.

García-Pallas, I.; Val, J. and Blanco, A. 2001. The inhibition of flower bud differentiation in 'Crimson Gold' nectarine with GA_3 as an alternative to hand thinning. Scientia Horticulturae 90 (3/4): 265-278.

González-Rossia, D.E.; Juan, M.; Almela, V.; Mesejo, C.; Martinez-Fuentes, A.; Gariglio, N. and Agustí, M. 2003. La inhibición de la floración en melocotoneros y ciruelos como técnica indirecta de aclareo de frutos. Agrícola Vergel 124-133.

Griffin, J.J.; Ranne, T.G. and Mason Pharr, D. 2004. Photosynthesis Chlorophyll Fluorescence and Carbohydrate content of *Illicium taxa* grown under varied irradiance. Journal of the American Society of Horticultural Science 129 (1): 46-53.

Groom, Q.J. and Baker, N.R. 1992. Analysis of light-induced depressions of photosynthesis in leaves of a wheat crop during the winter. Plant Physiology 100: 1217-1223.

Guardiola, C.; Monerri, C. and Agustí, M. 1982. The inhibitory effect of gibberellic acid on flowering in Citrus. Physiologia Plantarum 55: 136-142.

Gucci, R.; Petracek, P.D. and Flore, J.A. 1991a. The effect of fruit harvest on photosynthetic, starch content and chloroplast ultrastructure in leaves of *Prunus avium*. Advances in Horticultural Sciences 5:19.

Gucci, R.; Xiloyannis, C.; Flore, J.A. 1991b. Diurnal and seasonal changes in leaf net photosynthesis following fruit removal in plump. Physiologia Plant 83: 497-505.

Havaux, M. 1996. Short-term responses of Photosystem I to heat stress. Induction of a PSII-independent electron transport through PSI fed by stromal component. Photosynthesis Research 47: 85-97.

Johnson, R.S.; Handley, D.F. and DeJong, T.M. 1992. Long-term response of early maturing peach trees to postharvest water deficits. Journal of the American Society of Horticultural Science 117: 881.

Jourdain, J.M. and Clanet, H. 1987. L'eclaircissage. Fruit et Legumes 41: 16-20.

Kenworthy, A.L. 1974. Sour cherry tree vigor as related to higher yields and better fruit quality. Mich. Agr. Expt. Sta. Rpt. pp. 223.

Kitao, M.; Lei, T.T. and Koike, T. 1998. Application of chlorophyll fluorescence to evaluate Mn tolerance of deciduous broad-leaved tree seedlings native to northern Japan. Tree Physiology 18: 135-140.

Krause, G.H. 1994. Photoinhibition induced by low temperature. In: Baker, N.R. and Bowyer, J.R. (Eds.). *Photoinhibition of Photosynthesis: From Molecular Mechanisms to the Field*. BIOS Scientific Publishers, Oxford, pp. 331-348.

Krause, G.H. and Weis E. 1991. Chlorophyll fluorescence and photosynthesis: the basics. Annual Review of Plant Physiology and Plant Molecular Biology 42: 313-349.

Luckwill, L.C. and Silva, J.M. 1979. The effect of daminozide and gibberellic acid on flower initiation, growth and fruiting of apple cv. Golden delicious. Journal of Horticultural Science 54: 217-223.

Layne, D.R. and Flore, J.A. 1992. Photosynthetic compensation to partial leaf area reduction in sour cherry. Journal of the American Society of Horticultural Science 117: 279.

Li, W.H.; Bussi, C., Hugard, J. and Clanet, H. 1989. Critical period of flower bud induction in peach trees associated with shoot length and bud position. Gartenbauvissenschaft 54: 49-53.

Maxwell, K. and Johnson, G.N. 2000. Chlorophyll fluorescence-A practical guide. Journal of Expterimental Botany 51: 659-668.

Monselise, S. P. and Halevy, A. H. 1964. Chemical inhibition and promotion of citrus bud induction. Proceedings of the American Society of Horticultural Science 84: 141-146.

Nagel, O.W. and Lambers, H. 2002. Changes in the acquisition and partitioning of carbon and nitrogen in the gibberellin-deficient mutants A70 and W335 of tomato (*Solanum lycopersicum* L.). Plant, Cell and Environment 25: 883-891.

Ögren, E. and Sjöström, M. 1990. Estimation of the effect of photoinhibition on the carbon gain in leaves of a willow canopy. Planta 181: 560-567.

Öquist, G.; Anderson, J.M.; McCaffery, S. and Chow, W.S. 1992. Mechanistic differences in photoinhibition of sun and shade plants. Planta 188: 422-431.

Palmer, J.W.; Cai, Y.L. and Edjamo, Y. 1991. Effect of part-tree flower thinning o fruiting, vegetative growth and leaf photosynthesis in 'Cox's Orange Pippin' apple. Journal of Horticultural Science 66: 319-325.

Powles, S.B. 1984. Photoinhibition of photosynthesis induced by visible light. Annual Review of Plant Phisiology 35:15.

Schreiber, U and Armond, P.A. 1978. Heat-induced change of chlorophyll fluorescence in isolated chloroplasts and related heat-damage at the pigment level. Biochimica et Biophysica Acta 502: 138-151.

Shreiber, U.; Bilger, W. and Neubauer, C. 1994. Chlorophyll fluorescence as a non-destructive indicator for a rapid assessment of in vivo photosynthesis. Ecological Studies 100: 49-70.

Southwick, S.M. and Fritz, R. 1995. Commercial chemical thinning of stone fruit in California by gibberellins to reduce flowering. Acta Horticulturae 395: 135-147.

Southwick, S.M. and Glozer K. 2000. Reducing flowering with gibberellins to increase fruit size in stone fruit trees: Applications and implications in fruit production. HortTechnology 10 (4): 744-751.

Southwick, S.M.; Weis, K.G. and Yeager, J.T. and Zhou 1995. Controlling cropping in 'Loadel' Cling Peach using Gibberellin: effect on flower density, fruit distribution, fruit firmness, fruit thinning and yield. Journal of the American Society of Horticultural Science 120 (6): 1087-1095.

Southwick, S.M. and Yeager, J.T. 1991. Effects of postharvest gibberellic acid application on return bloom of 'Patterson' apricot. Acta Horticulturae 293: 459-466.

Taylor, B.H. and Geisler-Taylor, D. 1998. Flower bud thinning and winter survival of 'Redhaven' and 'Cresthaven' peach in response to GA_3 sprays. Journal of the American Society of Horticultural Science 123 (4): 500-508.

Tromp, J. 1982. Flower bud formation in apple as affected by various gibberellins. Journal of Horticultural Science 57: 277-282.

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

Tsonev, T.; Velikova, V.; Lambreva, M. and Stefanov, D. 1999. Recovery of the photosynthetic apparatus in bean plants after high and low induced photoinhibition. Bulgarian Journal of Plant Physiology 25 (3-4): 45-53.

Vassilev, A. and Monolov, P. 1999. Chlorophyll fluorescence of barley (*H. vulgare* L.) seedlings grown in excess of Cd. Bulgarian Journal of Plant Physiology 25 (3-4): 67-76.

Ward, D.L. 1993. Reducing flower bud density of 'Redkist' peach with GA_3. M.A. thesis. Southern Illinois Univ., Carbondale.

Werner, C. and Correia, O. 1996. Photoinhibition in cork-oak leaves under stress: influence of the bark-stripping on the chlorophyll fluorescence in *Quercus suber* L. Trees 10: 288-292.

Yamane, Y.; Kashino, Y.; Koike, H. and Satoh, K. 1997. Increases in the fluorescence Fo level and reversible inhibition of Photosystem II reaction centre by high-temperature treatment in higher plants. Photosynthesis Research 52: 57-64.

Table 7.1: Fluorescence parameters of dark-adapted leaves of control and GA_3 treated young trees of 'Springcrest' peach trees. Values are the average of 4 leaves per replication tree and 5 measurements per leaf. ± SE.

Days after treatment	0		3		5	
Parameters	Control	GA_3	Control	GA_3	Control	GA_3
Fo	536.6±68.9	536.6±68.9	360.3±28.6	279.5±21.4	338.4±38.6	330.6±11.9
Fm	1017.1±99.9	1017.1±99.9	1195.4±67.4	868.4±37.7	1106.9±48.8	1052.6±76.0
Fv=Fm-Fo	500.5	500.5	835.1	588.9	768.5	722
Fv/Fm	0.474	0.474	0.690	0.678	0.696	0.700
Days after treatment	8		16		30	
Parameters	Control	GA_3	Control	GA_3	Control	GA_3
Fo	367.6±38.6	334.6±14.9	266.14±18.0	260.1±25.2	261.7±15.5	308.3±35.7
Fm	1537.3±69.4	1285.7±63.6	1149.3±32.6	953.5±81.7	911.6±62.9	852.6±110.3
Fv=Fm-Fo	1169.7	951.1	883.2	693.4	649.9	544.3
Fv/Fm	0.757	0.743	0.760	0.740	0.707	0.618

Table 7.2: Fluorescence parameters of dark-adapted leaves of control and GA_3 treated adult trees of 'Springcrest' peach trees. Values are the average of 4 leaves per replication tree and 5 measurements per leaf. ± SE.

Days after treatment	0		3		5	
Parameters	Control	GA_3	Control	GA_3	Control	GA_3
Fo	333.3±7.2	333.3±7.19	244.7±17.8	260.8±47.4	360.3±34.2	350.0±23.4
Fm	1406.2±42.0	1406.2±42.0	1051.2±69.8	1144.0±170.8	1535.8±81.9	1567.3±131.3
Fv=Fm-Fo	1072.9	1072.9	806.5	883.2	1175.5	1271.3
Fv/Fm	0.765	0.765	0.766	0.773	0.766	0.776
Days after treatment	8		16		30	
Parameters	Control	GA_3	Control	GA_3	Control	GA_3
Fo	378.0±31.1	273.0±16.4	269.0±25.6	439.5±40.1	292.0±36.7	441.7±33.8
Fm	1655.5±141.9	1305.0±17.0	1230.0±116.9	1629.8±54.1	1279.5±84.6	1548.0±78.7
Fv=Fm-Fo	1277.5	1032.0	961.0	1190.3	987.5	1106.3
Fv/Fm	0.780	0.790	0.781	0.730	0.775	0.730

Table 7.3: Effect of GA_3 sprays (100 mg l^{-1}) on the time course of shoot growth rate (cm day^{-1}) of young and adult trees of 'Springcrest' peach. Values are the mean of 40 shoots per treatment.

		Growth Rate (cm day^{-1})			
Plant Age (A)	Treatment (B)	0 – 8 DAT[1] period	8 – 16 DAT[1] period	16 – 30 DAT[1] period	30 – 114 DAT[1] period
Young Plants	GA_3	0.809 abA	0.571 abA	0.510 aA	0.005 cB [Z]
	Control	1.093 aA	0.713 aAB	0.512 aB	0.001 dC
Adult Plants	GA_3	0.475 bcA	0.4 bA	0.514 aA	0.009 bB
	Control	0.181 cA	0.106 cAB	0.078 bB	0.080 aB
ANOVA	'A' effect	*	*	*	*
	'B' effect	Ns	ns	*	*
	'A x B' interaction	*	*	*	ns

[Z] Means followed by different lower-case letters in the same column and by different capital letters in the same line differ significantly ($P \leq 0.05$).
[ns] Non significant at $P \leq 0.05$
(*) Significant at $P \leq 0.05$
[1] Days After Treatment

Table 7.4: Effect of GA_3 spray (100 mg l^{-1}) on the number of nodes per shoot in the proximal, middle and distal portion of an average shoot, from young and adult plants of 'Springcrest' peach cultivar 30 days after treatment. Values are the mean of 40 shoots per treatment.

		Mean number of nodes			
Plant Age (A)	Treatment (B)	Proximal	Middle	Distal	Total nº nodes
Young Plants	GA_3	9.417 abB	8.083 aB	9.583 aB	27.083 abA[Z]
	Control	10.083 aB	8.333 aB	10.250 aB	28.667 aA
Adult Plants	GA_3	6.200 cC	9.400 aB	10.000 aB	25.600 abA
	Control	6.700 bcC	4.700 bC	10.500 aB	21.900 bA
ANOVA	'A' effect	*	ns	ns	*
	'B' effect	Ns	*	ns	ns
	'A x B' interaction	Ns	*	ns	ns

[Z] Means followed by different lower-case letters in the same column and by different capital letters in the same line differ significantly ($P \leq 0.05$).
[ns] Non significant at $P \leq 0.05$
(*) Significant at $P \leq 0.05$

Table 7.5: Plant age and GA_3 (100 mg l^{-1}) treatment effect on shoots length (cm), sprouts per meter and flowers per meter of shoot at full bloom, in peach (*Prunus persica* L. Batsch) cv. Springcrest, during the year following treatment.

Plant Age (A)	Treatment (B)	Length (cm)	Sprouts m^{-1}	Flowers m^{-1}
Young Plants	GA_3	34.233	24.024 b	7.285 b [Z]
	Control	31.813	14.249 a	17.117 a [Z]
Adult Plants	GA_3	34.924	26.212 b	9.731 b [Z]
	Control	32.895	12.470 a	18.555 a [Z]
ANOVA	'A' effect	ns	ns	ns
	'B' effect	ns	*	*
	'A x B' interaction	ns	ns	ns

[Z] Mean separation within a column was appropriate by Duncan at 5% level.
[ns] Non significant at $P \leq 0.05$
(*) Significant at $P \leq 0.05$

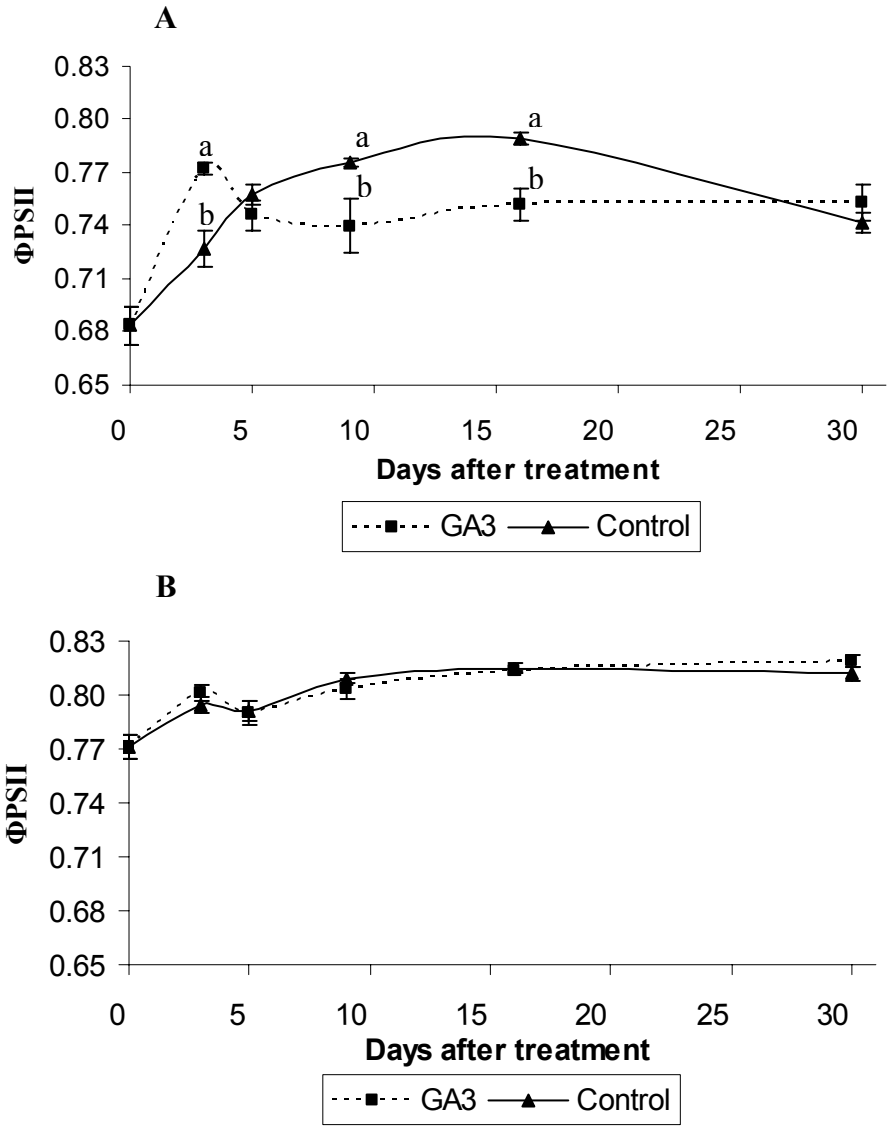

Figure 7.1: Effect of GA_3 sprays (100 mg l^{-1}) on the time course of the actual quantum yield of PSII electron transport, ΦPSII=ΔF/F`m, in young (A) and adult trees (B) of 'Springcrest' peach trees. Standard errors are given as vertical bars. Values are the average of 4 leaves per replication tree and 5 measurements per leaf. Different letters in the same date indicate significant difference ($P \leq 0.05$).

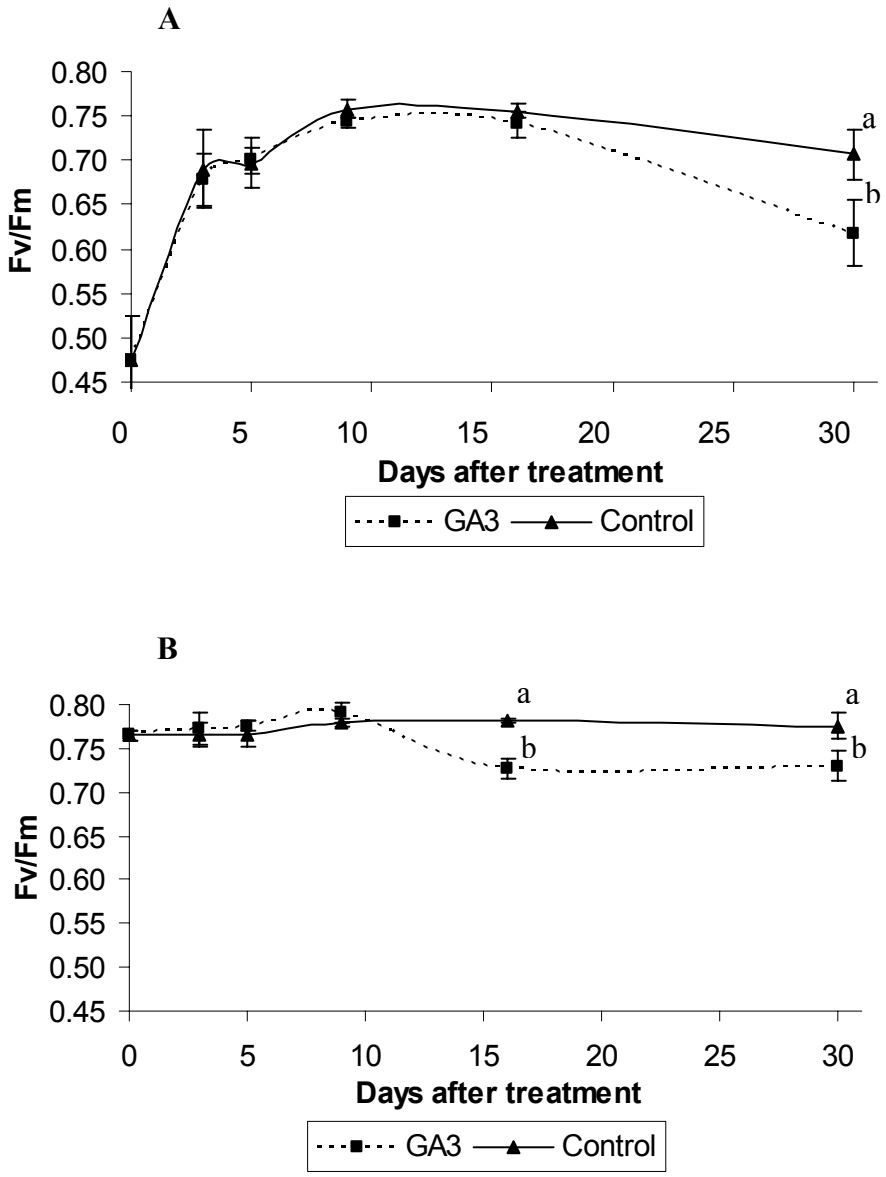

Figure 7.2: Effect of GA_3 spray (100 mg l^{-1}) on the time course of the maximum quantum yield of PSII photochemistry (Fv/Fm) in young (A) and adult trees (B) of 'Springcrest' peach. Values are average of 4 leaves per replication tree and 5 measurements per leaf. Standard errors are given as vertical bars. Different letters in the same date indicate significant difference ($P \leq 0.05$).

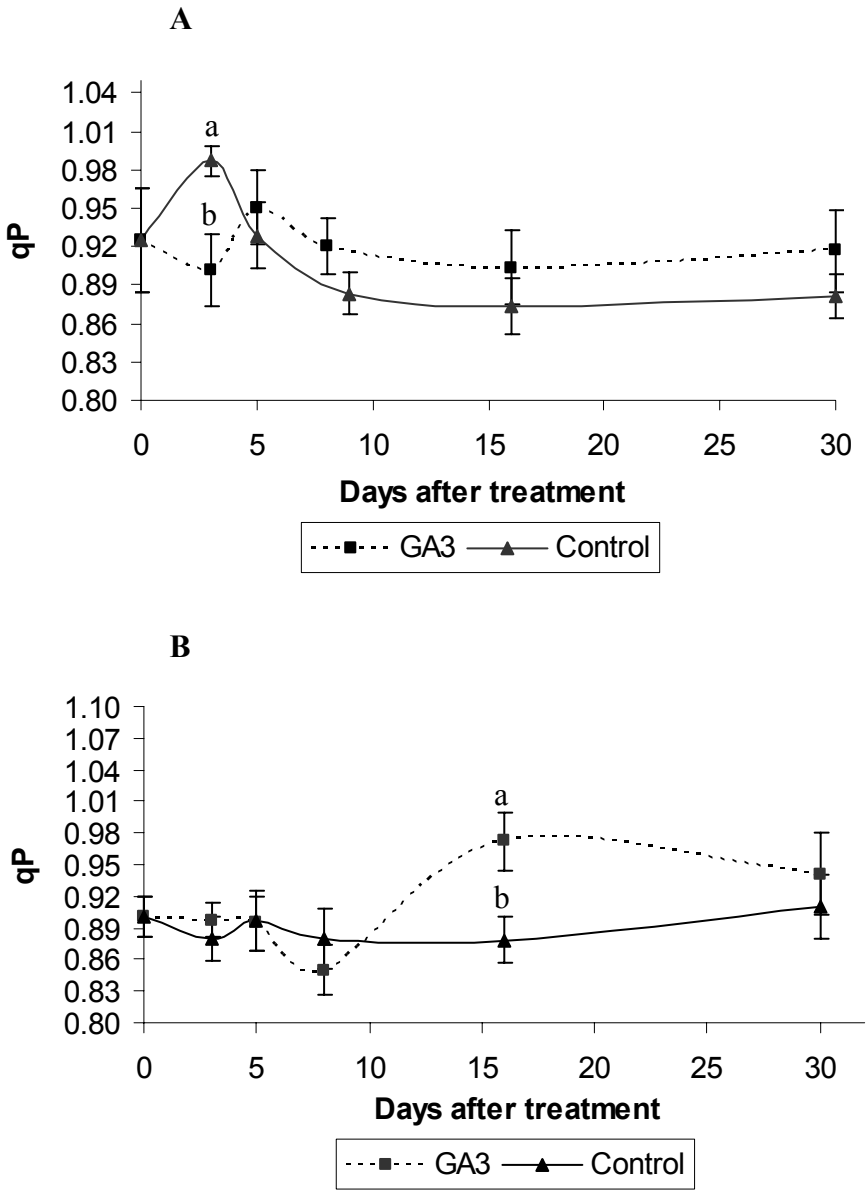

Figure 7.3: Effect of GA_3 spray (100 mg l^{-1}) on the time course of photochemical quenching (qP) in young (A) and adult trees (B) of 'Springcrest' peach trees. Values are average of 4 leaves per replication tree and 5 measurements per leaf. Standard errors are given as vertical bars. Different letters in the same date indicate significant difference ($P \leq 0.05$).

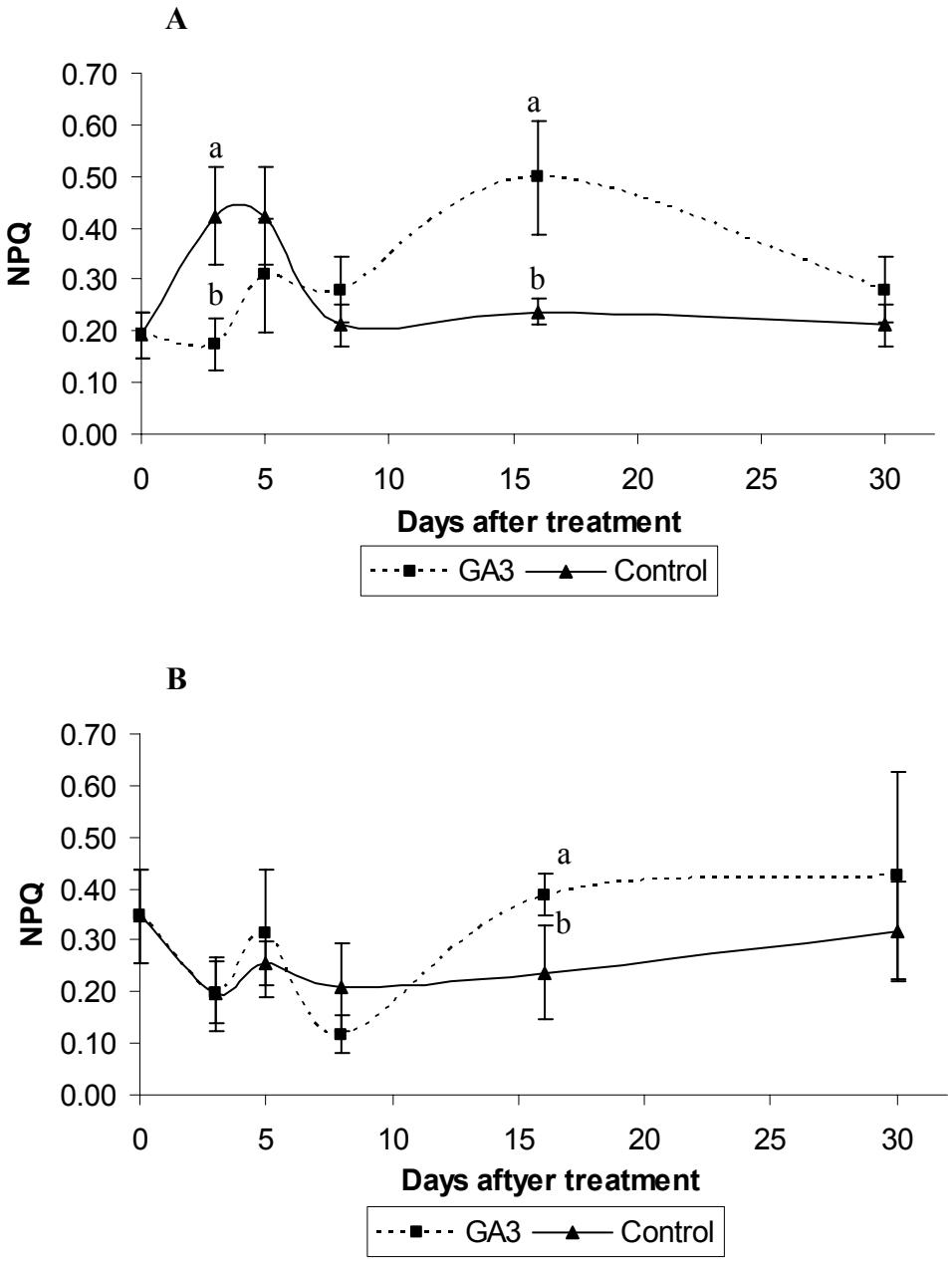

Figure 7.4: Effect of GA_3 spray (100 mg l^{-1}) on the time course of non-Photochemical quenching (NPQ) in young (A) and adult trees (B) of 'Springcrest' peach trees. Values are average of 4 leaves per replication tree and 5 measurements per leaf. Standard errors are given as vertical bars. Different letters in the same date indicate significant difference ($P \leq 0.05$).

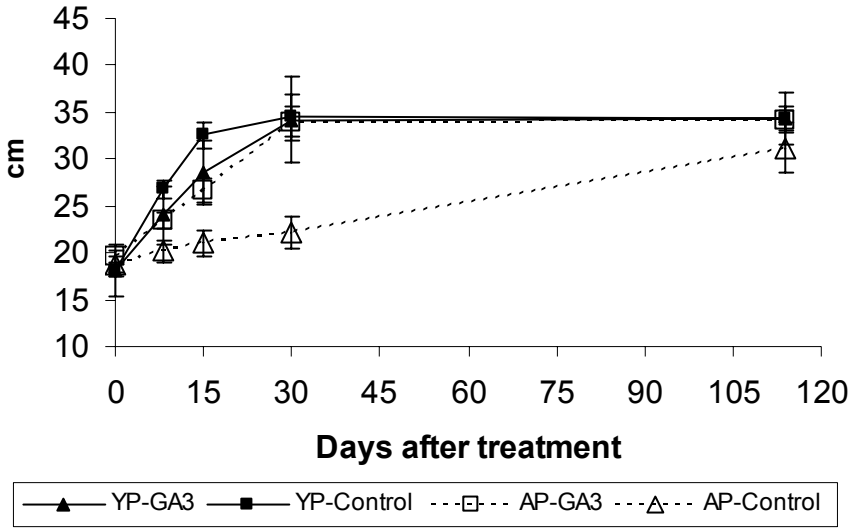

Figure 7.5: Effect of GA_3 sprays (100 mg l^{-1}) on the time course of shoot growth (cm) of young (YP) and adult trees (AP) of 'Springcrest' peach trees. Values are the average of 40 shoots per treatment. Standard errors are given as vertical bars.

CHAPTER 8

Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots

8- Effect of artificial chilling on the depth of endodormancy and vegetative and flower budbreak of peach and nectarine cultivars using excised shoots

Abstract

Stem cuttings were obtained from 12 peach and nectarine cultivars during leaf fall, placed in plastic bags at 3.0 ± 0.1ºC to simulate 0 to 800 h of chilling, and forced to budbreak at 20.0 ± 1.0°C for a period of 6 weeks. Some cultivars showed high blooming and leafing without exposure to chilling; chilling enhances leafing and blooming but the percentage increment was higher in leaf buds. In general, maximum budbreak was reached with less chilling accumulation (<100-200 h) in flower buds compared with leaf buds; excessive chilling caused a reduction of the percentage budbreak in flower but not in leaf buds. Additionally, chilling modified the proportion of blooming that occurred before leafing. In non-chilled shoots, blooming occurred earlier than leafing, except in cv. 'San Pedro 16-33' but the proportion of blooming before leafing decreased significantly with chilling in most cases. By studying the mean time to budbreak, we conclude that the flower bud generally has a lower intensity of rest; the intensity of rest declines at a slower rate in flower than in leaf buds with chilling; flower buds had greater heat requirements than leaf buds when the chilling requirement had been covered, so that each peach cultivar had a point of critical chilling accumulation below which blooming tended to occur earlier, and above which leafing tends to occur first. Flower and leaf buds had different depths of endodormancy but similar chilling requirements in the majority of peach and nectarine cultivars studied. Finally, different varieties with similar chilling requirements showed different responses to chilling. Therefore, the cutting test measuring the response of vegetative and floral buds provides considerable information on the characterisation of the variety, compared with the sole and traditional data of chilling requirements.

Introduction

Dormancy is a phase of development that occurs annually in deciduous fruit trees and other temperate woody perennial (Saure, 1985). Although the biological basis of dormancy is still not clearly understood (Arora *et al.*, 2003), it has long been known that the main factor in breaking bud endodormancy is low temperature (Balandier *et al.*, 1993, Rouland and Arora, 1997) and that insufficient chilling causes abnormal patterns in budbreak and development in temperate zone fruit trees cultivated in warm climates (Bonhomme *et al.*, 2005). The interest in understanding the

mechanism of dormancy set and release is mainly based on the necessity of manipulation of the dormant period in order to avoid spring frost damage, or to increase budbreak and obtain even flowering in areas of low chilling accumulation (Faust *et al.*, 1997). Furthermore, in warm climates, where the cold requirements cannot be satisfied, an evaluation of the intensity of endodormancy is essential for determining when rest-breaking treatments are necessary (Dennis, 2003).

Precise determination of the temperature requirements for breaking dormancy is nearly impossible under field conditions, where solar radiation, diurnal fluctuations in temperature and other factors cannot be controlled (Dennis, 2003). Thus, the measurement of dormancy ´depth´ and duration in diverse experimental contexts by isolation of buds as one-node cuttings (Balandier *et al.*, 1993) or excised shoots (Dennis, 2003) is an appropriate method to advance the study of dormancy. The mean time required for bud-burst is used for comparison of endodormancy in experimental or standard forcing conditions and its results are confirmed by biochemical tests (Crabbé and Barnola, 1996). The excised-shoots method was mainly used to study the evolution of leaf bud endodormancy in winter, collecting twigs every 2 or 3 weeks (Balandier *et al.*, 1993) and their response was paralleled to those observed in whole plants of sweet cherry (Arias and Crabbé, 1975). Balandier *et al.* (1993) used the excised-shoot method not only to study the evolution of endodormancy in winter, but also to calculate the mean time to budbreak corresponding to the end point of endodormancy, exposing the excised shoot to low temperature conditions. Later, Citadin *et al.* (2001) exposed excised shoots to low temperatures to simulate two chilling accumulation treatments in order to calculate the heat requirements for blooming and leafing in peach. To our knowledge, they were among the first authors to observe the behaviour of leaf and flower buds using the excised-shoots method. In this work, we studied the effect of artificial chilling on the depth of endodormancy and the percentage of floral and leaf budbreak, using the excised-shoot method, in several peach and nectarine cultivars.

Material and methods

During leaf fall (8 November in Valencia, Spain and 3 April in Esperanza, Argentina) and before chilling accumulation begins, 50 twigs were randomly collected from 10 adult trees of each variety of peach and nectarine (*Prunus persica* L. Batsch). Cultivars studied were 'Flordaprince', 'Flordastar', 'Earlygrande', 'San Pedro 16-33', 'Flordaking', 'Tropic Snow', 'Hermosillo' and 'Maycrest' (from Esperanza, Argentina), and 'Baby Gold', 'Spring Belle', ‘Sun Red’ (or ‘Rubro sol’), 'September Free' and 'Armking'

from an experimental orchard situated near the town of Lliria, Spain (39º 39,281' N - 000º 38,344' W; 180 m above sea-level). In the Spanish orchard, the trees were planted at 6x4 m (416 trees/ha) in a sandy-loamy soil, and grafted onto 'GF 677' rootstock, while in the Argentinean orchard (31° 26' S - 60° 56' W; 40 m above sea-level), the trees were planted at 5x5 m (400 trees ha^{-1}) in a silty-loamy soil, and grafted onto 'Cuaresmillo' rootstock.

Twigs were cut into segments 15 cm long obtaining 400 stem cuttings for each cultivar. Only three axilary buds from a typical node with one central leaf bud and two flower buds at 2 cm from their apical were conserved, while the other buds were eliminated. A group of five cuttings were then placed in plastic bags and exposed to a low temperature (3.0 ± 0.1ºC) (Balandier *et al.*, 1993) to simulate different chilling accumulation treatments: 0, 100, 200, 300, 400, 500, 600, 700 and 800 h. Treatments represent 0 to 800 chilling hours, according to Weinberger (1950).

After chilling, the shoots were placed with their basal tip in water and forced in a phytotron at 8-h photoperiod (22.5 μmol m^{-2} s^{-1}) (Citadin *et al.*, 2001) and 20.0 ± 1.0°C for 6 weeks. The basal ends of the shoots were cut (Balandier *et al.*, 1993; Citadin *et al.*, 1998) weekly and water was replaced daily. Flower and vegetative budbreak were observed three times a week. The number of buds that reached the balloon or green tip stage was recorded (Citadin *et al.*, 2001). Results were expressed as the percentage of budbreak or as mean time to budbreak (MTB) in days (arithmetic mean of each four group of five excised shoots) (Balandier *et al.*, 1993).

Trials were conducted in a complete randomized design with four replicates of five cuttings per treatment and cultivar. We also calculated the standard error or the ANOVA for each treatment using the Tuckey test for means separation. Percentages were analysed after arc sine transformation.

Results

Leaf budbreak was highly affected by chilling accumulation (Table 8.1). ´Flordaprince´, ´Earlygrande´, ´Tropic Snow´, ´Sun Red´ and ´San Pedro 16-33´ showed leaf budbreak even without chilling accumulation, with a high percentage of leafing (≈50%) in the last two varieties (Table 8.1). Except in ´Sun Red´, leaf budbreak increased significantly with chilling (Table 8.1). ´Flordaprince´, ´Flordastar´, ´Earlygrande´ and ´Hermosillo´ did not show a significant increase in the percentage of budbreak after 200 h of chilling; ´Tropic Snow´ and ´Flordaking´ after 300 h; ´San Pedro 16-33´ and ´September Free´ after 400 h; ´Armking´ after

600 h and, ´Maycrest´, ´Spring Belle´ and ´Baby Gold´ after 700 h (Table 8.1).

Flower budbreak showed differences compared with leaf budbreak in its response to chilling (Table 8.2). High flower budbreak (>45%) was observed in shoots not exposed to chilling in seven cultivars (´Flordaprince´, ´Flordastar´, ´Earlygrande´, ´Tropic Snow´, ´Sun Red´, ´San Pedro 16-33´ and ´Flordaking´), and only three varieties (´Maycrest´, ´Spring Belle´ and ´Baby Gold´) did not show flower budbreak for non-chilling treatment (Table 8.2). Except in the case of ´San Pedro 16-33´, a significant increase in flower budbreak was observed in response to chilling but, generally, maximum budbreak was reached with less chilling accumulation (<100-200 h) compared to leaf buds (Table 8.1). High chilling caused a significant reduction in the percentage of flower budbreak (Table 8.2), but not in vegetative budbreak. As shown in Table 8.2, flower budbreak diminished significantly in shoots chilled for 300 h or more in ´Flordastar´ and ´Tropic Snow´; ≥400 h in ´Flordaprince´ and ´Hermosillo´; ≥600 h in ´Earlygrande´ and ´Spring Belle´ and for ≥700 h in ´Sun Red´, ´San Pedro 16-33´ and ´Armking´. The exceptions were ´Flordaking´, ´Maycrest´, ´Baby Gold´ and ´September Free´ (Table 8.2) for which no significant reduction in flower budbreak caused by high chilling was observed. This phenomenon was not observed in vegetative budbreak (Table 8.1).

Chilling also modified the proportion of blooming that occurred before leafing (Table 8.3). In non-chilled shoots, blooming occurred earlier than leafing, except in 'San Pedro 16-33', which showed a similar proportion of blooming both before and after leafing. As the chilling exposure increased, the proportion of shoots that showed blooming before leafing decreased significantly. In 'San Pedro 16-33', 'Tropic Snow', ‘Armking’, ‘Spring Bellee’ and ‘September free’ in shoots chilled for 600 h or more, blooming always occurred after leafing (Table 8.3).

'San Pedro 16-33' had the lowest MTB value, both for leaf and flower buds, when shoots did not receive chilling (Figure 8.1A, Table 8.4). Furthermore, it was the only cultivar in which the MTB for leaf buds was not higher than the MTB for flower buds (Figure 8.1, Table 8.4). Chilling reduced MTB values both in leaf and flower buds, reaching the stability when shoots were chilled for 600 h in 4 and 6 days for leaf and flower buds, respectively (Figure 8.1A).

Although 'Earlygrande' (Figure 8.1B) had a similar chilling requirement to 'San Pedro 16-33' (Figure 8.1A), the evolution of MTB

values with chilling accumulation was different. The MTB value for non-chilling shoots was double in 'Earlygrande' and was at least 50% higher for all chilling treatments. The MTB value of vegetative buds was higher than that of flower buds for non-chilled and shoots chilled for 100 h, whereas it was lower after 200 h of chilling. Besides, the MTB value was stabilized in shoots chilled for 400 h compared with 500 h in 'San Pedro 16-33' (Figure 8.1A, Figure 8.1B). The point where the MTB for vegetative buds equals the MTB value for flower buds is defined as the point of critical chilling accumulation (PCCHA), which can be seen in Figure 8.1B.

In 'Maycrest', MTB was not measurable for non-chilling or shoots chilled for 100 h since no leafing or blooming was observed after 45 days at 20.0°C (Figure 8.1C, Table 8.1, Table 8.2). The MTB value for flower buds declined up to 600 h of chilling while the MTB for vegetative buds did not reach its minimum value in our experiment (Figure 8.1C). The MTB value of ´Flordaprince´, ´Flordastar´, ´Tropic Snow´, ´Hermosillo´, ´Flordaking´ and ´Armking´, showed particular changes with chilling but with the same pattern of evolution observed for 'Earlygrande' (Table 8.4; Figure 8.1B) reaching stable values between the range of chilling treatments used in this experiment, whereas ´Sun Red´, ´Baby Gold´, ´September Free´ and ´Spring Belle´, did not reach a stable MTB value, as occurred in ´MayCrest´ (Table 8.4, Figure 8.1C).

A comparison between the suggested chilling requirements and the range of chilling accumulation needed to reach a percentage of budbreak higher than 50% for both flower and vegetative buds is shown in Table 8.5.

Discussion

The increase in budbreak and endodormancy release caused by chilling observed in this work is well known (Erez *et al.*, 1979; Faust *et al.*, 1997; Fuchigami and Wisniewsky, 1997; Dennis, 2003). However, we noted that some low chilling cultivars had a relatively high budbreak (≈50%) and a low MTB value without exposure to low temperature, mainly in flower buds as compared to vegetative buds. Under insufficient chilling conditions, buds open but do not develop further, and flowers fail to set fruits (Dennis, 2003). The results using the cutting test may consider these limitations to predict field behaviour.

Excessive chilling diminished the percentage of flower budbreak (see Table 8.2) in low chilling cultivars (<400 h). These symptoms were also observed in shoots of low chilling requirement cultivars from the Southern Brazil breeding program, which were chilled for 800 h and the effect was

attributed to a physiological injury of buds and shoots caused by longer exposure to low temperatures (Citadin *et al.*, 2001). These symptoms may be observed in cultivars of high chilling requirement at higher chilling accumulation treatments not considered in this trial.

Except in cv. 'San Pedro 16-33', the MTB value of the vegetative buds for non-chilled shoots was higher compared with that of flower buds. The intensity of rest was therefore higher in vegetative than in flower buds at the beginning of the rest, explaining why the percentage of floral budbreak was higher compared with leaf buds at low chilling accumulation treatments (see Table 8.1 and Table 8.2), why less chilling was generally necessary to reach maximum budbreak in flower buds, and why blooming occurred earlier than leafing in low chilling treatments (see Table 8.3, Table 8.4 and Figure 8.1). Additionally, the occurrence of blooming after leafing in non-chilled shoots of 'San Pedro 16-33' (see Table 8.3), could be explained by the MTB value of the flower and leaf buds being equal in non-chilled shoots (see Figure 8.1A). This is an additional reason for using the method of measuring the time required to reach a specific stage of bud opening vs. the percentage of budbreak within a fixed time interval for measuring the response to chilling (Dennis, 2003). Furthermore, we observed that chilling caused a continuous decline in the intensity of rest, whereas no increase in the depth of dormancy was observed during the early phases of the rest, as observed in other trials (Arias and Crabbé, 1975; Walser *et al.*, 1981).

MTB value of leaf buds decreases faster than floral buds with chilling and consequently, the MTB value of leaf buds was lower than flower buds for high chilling accumulation treatment (see Figure 8.1 and Table 8.4). Thus, each cultivar showed a point of chilling accumulation at which the MTB value was equal for both flower and leaf buds. When chilling accumulation was lower than the point of critical chilling accumulation (PCCHA) blooming tended to occur first, whereas when chilling accumulation was higher than PCCHA, leafing tended to occur earlier. Plum showed the same response to chilling as that described for peach, but not apricot, in which the MTB value of leaf buds was always higher than flower buds in the three cultivars studied (data not shown). This evidence, together with the decrease in flower budbreak and the increase in the proportion of shoots leafing before blooming with the increase of chilling exposure, is in accordance with the conclusion of Citadin *et al.* (2001), who observed that prolonged chilling enhances leafing more than blooming in low chill requirement cultivars, and that after years of higher chilling accumulation, leafing can occur before blooming. In fact, in cv. 'San Pedro 16-33' leafing occurs before or together with blooming under the field

cultivation conditions of the central-east area of Santa Fe Province (Argentina) (unpublished data) because the depth of rest of leaf buds was always equal or lower (but not higher) than flower buds (see Figure 8.1A). Similar observations were made in the area of Valencia Spain, in the 'Springcrest' and 'Zincal 5' cultivars, when winter chilling was higher than the average year (>600 h of chilling).

Citadin *et al.* (2001) observed differences in the heat requirement between flower and vegetative buds in peach cultivars with low chilling requirements. This observation also occurred in our experiment, since when the MTB reached a stable low value and longer exposure at low temperature did not produce further MTB decrease, we therefore considered that the end of rest had occurred (Balandier *et al.*, 1993), and that the number of days to blooming or leafing was the time necessary to cover the heat requirement. Consequently, in all the peach cultivars studied, the heat requirement for leafing was lower than for blooming when sufficient chilling was accumulated (see Figure 8.1 and Table 8.4).

It is interesting to note that different varieties with similar chilling requirements showed different responses to chilling; thus, the cutting test measuring the response of vegetative and floral buds gives a great deal of information about the characterization of the cultivar compared with the sole and traditional data of chilling requirement. For example, 'Flordastar' and 'Flordaprince', or 'September Free' and 'Baby Gold' had similar chilling requirement but 'Flordaprince' and 'September Free' showed a higher percentage of budbreak in a wide range of chilling accumulation treatments, compared with 'Flordastar' and 'Baby Gold', respectively (Table 5). On the other hand, ´MayCrest´ needed more chilling to reach a higher percentage of budbreak compared with its chilling requirement (Table 8.5).

It is known that flower buds require less chilling than leaf buds (Tabuenca, 1965). However, both flower and leaf buds reached the end point of endodormancy with the same chilling requirement in at least four of the cultivars studied (see Figure 8.1 and Table 8.4) indicating that their chilling requirements are the same. This is more in accordance with the work of Guerriero *et al.* (1986), who proposed that vegetative buds would have a lower chilling requirement than flower buds. The fact that their depth of dormancy was different could possibly cause some confusion. To avoid it, the figure that represents the MTB evolution of leaf and floral bud with chilling, clarifies the concepts of intensity of rest measured as MTB, chilling requirement, CH H required for a constant MTB and heat requirement to sprout (constant MTB multiplied by daily heat accumulation).

Finally, the method of excised shoots is at present used to study the evolution of endodormancy in leaf buds, extracting twigs at different times during winter (Balandier *et al.*, 1993; Dennis, 2003). Also, recent works have used this method to simulate artificial chilling under controlled conditions (Citadin *et al.*, 2001; Dennis, 2003). Our results confirm the utility of the method and suggest that rest of both leaf and flower buds, and not that of leaf buds only, may be studied to clarify the physiological process of dormancy.

The main conclusions of this works are that some cultivars showed a high blooming and leafing without exposure to chilling; chilling enhances leafing and blooming but the percentage of increment was higher in leaf buds; excessive chilling caused a reduction of the percentage budbreak in flower but not in leaf buds; generally, flower buds have a lower intensity of rest; the intensity of rest declines more slowly in flower than in leaf buds with chilling; flower buds had higher heat requirements than leaf buds when the chilling requirement had been covered. Consequently, each peach cultivar studied had a point of critical chilling accumulation below which blooming tended to occur earlier and above which leafing tended to occur first.

References

Agustí, M. 2004. *Fruticultura*. Ediciones Mundi-Prensa, Madrid, Spain. pp 493.

Arias, O. and Crabbé, J. 1975. Les gradients morphogénétiques du rameau dún an des végétaux ligneux en repos apparent. Données complementaires fournies par létude de *Prunus avium* L. Physiologie Végetale 13: 69-81.

Arora, R.; Rowland, L.J. and Tanino, K., 2003. Induction and release of bud dormancy in woody perennials: a science comes of age. HortScience 38: 911-921.

Balandier, P.; Bonhomme, M.; Rageau, R.; Capitan, F. and Parisot, E. 1993. Leaf bud endodormancy release in peach trees: evaluation of temperature models in temperate and tropical climate. Agricultural and Forest Meteorology 67: 95-113.

Barbosa, W.; Ojima, M.; Dall'Orto, F.A.C.; Martins, F.P.; De Castro, J.L. and Dos Santos, R.R. 1997. Avalição de Pessegueiros e Nectarineiras introduzidos no Brasil, procedentes da Flórida, EUA. Scientia Agricola 54: 152-159.

Bonhomme, M.; Regeau, R.; Lacointe, A. and Gendraud, M. 2005. Influences of cold deprivation during dormancy on carbohydrate

contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batsch). Scientia Horticulturae 105: 223-240.

Citadin, I.; Raseira, M.C.B. and Quezada, A.C. 1998. Substrato para conservacão de ramos destacados de pessegueiro, *Prunus persica* L. (Batsch). Agropecuária Clima Temperado 1: 55-59.

Citadin, I.; Raseira, M.C.B.; Verter, F.G. and Baptista da Silva, J. 2001. Heat requirement for blooming and leafing in peach. HortScience 36: 305-307.

Crabbé, J. and Barnola, P. 1996. A new Conceptual approach to bud dormancy in woody plants. In: Lang, G.A. (Ed.). *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. CAB International, pp. 83-113.

Dennis Jr., F.G. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38: 347-350

Erez, A.; Couvillion, A. and Hendershott, C. 1979. The effect of cycle length on chilling negation by high temperature in dormant peach leaf buds. Journal of the American Society of Horticultural Science 104: 573-576.

Faust, M.; Erez, A.; Rowland, L.J.; Wang, S.Y. and Norman, H.A. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32: 623-629.

Fuchigami, L.H. and Wisniewsky, M. 1997. Quantifying bud dormancy: Physiological approaches. HortScience 32: 618-623.

Guerriero, S.E.P.; Indiogine, G. and Scalabrelli, G. 1986. Variación dei rapporti tra le percentuali di schiusura delle gemme a legno ed a fiore nel pesco, in funzione dell condizioni sperimentali di raffreddamento nel corso della dormienza. Rivista di Ortoflorofrutticoltura Italiana 70: 247-258.

Okie, W.R. 1998. *Handbook of peach and nectarines varieties*. USDA Agriculture Handbook Nº 714.

Powel, A. 1998. Suggested nectarine varieties for Alabama. Alabama Cooperative Extension System.
URL: http://www.aces.edu/pubs/docs/A/ANR-1046/, pp. 4.

Rouland, L.J. and Arora R. 1997. Proteins related to endodormancy (rest) in woody perennials. Plant Science 126: 119-124.

Saure, M.C. 1985. Dormancy release in deciduous fruit trees. Horticultural Reviews 7: 239-300.

Tabuenca, M.C. 1965. *Influencia del clima en plantaciones frutales*. Estación Experimental de Aula Dei, Zaragoza.

Valentini, G. 2002. *Variedades de nectarinos y durazneros para el NE de la provincia de Buenos Aires*. INTA. Estación Experimental Agropecuaria San Pedro.
URL: http://www.inta.gov.ar/sanpedro/info/doc/fru/gv_003.htm

Walser, R.H.; Walker, D.R. and Seeley, S.D. 1981. Effect of temperature, fall defoliation, and gibberellic acid on the rest period of peach leaf buds. Journal of the American Society of Horticultural Science 106: 91-94.

Weinberger, J.H. 1950. Chilling requirements of peach varieties. Proceedings of the American Society of Horticultural Science 56: 122-128.

Table 8.1: Effects of chilling accumulation at 3.0 ± 0.1°C on the percentage of vegetative budbreak of excised shoots of different peach and nectarine (*Prunus persica* L. Batsch) cultivars forced at constant temperature (20.0°C) for 45 days.

Cultivar	CH H[1]	Vegetative budbreak (%)[8]								
		Chilling accumulation at 3.0 ± 0.1°C								
		0	100	200	300	400	500	600	700	800
Flordaprince	150[2]	33 cB	46 bA	93 aA	100 aA	100 aA	100 aA	100 aA	92 aA	86 aA
Flordastar	250[2]	0 cC	13 bB	100 aA	100 aA	95 aA	90 aA	93 aA	94 aA	100 aA
Earlygrande	260[2]	26 cB	40 bA	100 aA	93 aA	100 aA	100 aA	93 aA	90 aA	87 aA
Tropic Snow	250[2]	24 cB	27 cB	79 bB	100 aA	94 aA	88 aA	88 aA	88 aA	88 aA
Sun Red	250[5-7]	50 aA	50 aA	50 aC	40 aC	30 aB	30 aC	30 aC	32 aB	35 aB
San Pedro 16-33	297[2]	44 dA	57 cA	71 bB	72 bB	100 aA	100 aA	100 aA	100 aA	92 aA
Hermosillo	350[2]	0 cC	20 bB	83 aB	93 aA	93 aA	100 aA	100 aA	100 aA	100 aA
Flordaking	450[2]	0 dC	13 cB	47 bC	93 aA	93 aA	90 aA	100 aA	100 aA	93 aA
Maycrest	600[2]	0 eC	0 eC	6 eD	20 dD	26 dB	45 cC	66 bB	85 aA	100 aA
Spring Belle	650[3]	0 cC	0 cC	0 cD	0 cE	0 cC	35 bC	50 bB	82 aA	100 aA
Armking	600[6-7]	0 dC	0 dC	0 dD	20 cD	30 cB	70 bB	100 aA	100 aA	91 aA
Baby Gold	850[4-7]	0 dC	0 dC	0 dD	0 dE	0 dC	40 cC	66 bB	91 aA	100 aA
September Free	850[7]	0 cC	0 cC	0 cD	40 bC	100 aA	100 aA	100 aA	100 aA	90aA

[1]Chilling Hours: chilling requirement of the cultivar.
[2]Valentini, 2002; [3]adjusted according to its behaviour in Valencia, Spain; [4]Agustí, 2004; [5]Barbosa et al., 1997; [6]Powel, 1998; [7]Okie, 1998.
[8]Measured as the percentage of excised shoots that reached the green tip stage.
Means followed by different lower-case letters in the same line and by the different capital letters in the same column differ significantly ($P \leq 0.05$).

Table 8.2: Effects of chilling accumulation at 3.0 ± 0.1°C on the percentage of flower budbreak of excised shoots of different peach and nectarine (*Prunus persica* L. Batsch) cultivars forced at constant temperature (20°C) for 45 days.

Cultivar	CH H[1]	Floral budbreak (%)[8]								
		Chilling accumulation at 3.0 ± 0.1°C								
		0	100	200	300	400	500	600	700	800
Flordaprince	150[2]	46 cB	46 cC	86 aA	80 aA	66 bB	68 bB	66 bB	62 bC	60 bB
Flordastar	250[2]	53 bB	67 bB	87 aA	47 bB	33 cC	38 cC	40 cC	35 cD	40 cC
Earlygrande	260[2]	53 cB	93 aA	93 aA	86 aA	86 aA	80 aA	70 bB	65 cC	60 cB
Tropic Snow	250[2]	72 bA	82 aA	100 aA	76 bA	52 cB	59 cB	58 cC	55 cC	53 cC
Sun Red	250[5-7]	62 cB	60 cB	75 bB	77 bA	75 bA	85 aA	100 aA	70 bB	50 cC
San Pedro 16-33	297[2]	85 aA	88 aA	100 aA	85 aA	80 aA	81 aA	80 aA	71 bB	65 bB
Hermosillo	350[2]	26 cC	36 cC	86 aA	93 aA	66 bB	65 bB	63 bB	61 bB	60 bB
Flordaking	450[2]	46 cB	60 bB	100 aA	100 aA	93 aA	85 aA	85 aA	87 aA	93 aA
Maycrest	600[2]	0 dE	0 dD	40 cC	46 cB	66 bB	75 bB	80 aA	91 aA	100 aA
Spring Belle	650[3]	0 dE	0 dD	37 cC	48 cB	87 aA	81 aA	71 bB	68 bB	67 bB
Armking	600[6-7]	12 dD	31 cC	87 aA	100 aA	91 aA	89 aA	87 aA	60 bB	25 cD
Baby Gold	850[4-7]	0 cE	0 cD	0 cD	0 cC	12 bD	20 bC	37 aC	38 aD	40 aC
September Free	850[7]	12 cD	13 cC	12 cC	50 bB	71 aB	81 aA	75 aB	71 aB	68 aB

[1]Chilling Hours: chilling requirement of the cultivar.
[2]Valentini, 2002; [3] adjusted according to its behaviour in Valencia, Spain; [4]Agustí, 2004; [5]Barbosa et al., 1997; [6]Powel, 1998; [7]Okie, 1998.
[8]Measured as the percentage of excised shoots that reached the green tip stage.
Means followed by different lower-case letters in the same line and by different capital letters in the same column differ significantly ($P \leq 0.05$).

Table 8.3: Effect of chilling accumulation at 3.0 ± 0.1°C on the proportion of blooming that occurred before leafing, measured from excised shoot of different cultivars of peach and nectarine (*Prunus persica* L. Batsch) forced at constant temperature (20.0°C) for 45 days.

Cultivar	Values expressed as % of blooming that occurred before leafing								
	Chilling accumulation at 3.0 ± 0.1°C								
	0	100	200	300	400	500	600	700	800
Flordaprince	100±0	71±3	69±4	25±3	1±0.2	12±1	10±1	13±1	11±1
Flordastar	100±0	100±0	46±2	42±2	20±1	19±1	22±1	25±2	33±2
Earlygrande	100±0	89±4	42±3	23±2	22±2	14±1	8±1	15±1	18±1
Sun Red	100±0	100±0	100±0	100±0	100±0	100±0	83±1	53±2	0±0
San Pedro 16-33	52±3	40±3	5±1	18±1	20±1	8±1	0±0	0±0	0±0
Tropic Snow	100±0	83±4	21±2	15±1	11±1	10±1	0±0	0±0	0±0
Hermosillo	100±0	100±0	69±4	7±0	20±1	19±1	20±1	18±1	20±1
Flordaking	100±0	100±0	93±5	93±4	64±3	48±3	33±2	30±2	28±1
Maycrest			100±0	100±0	100±0	100±0	92±5	80±4	66±3
Spring Bellee			100±0	100±0	100±0	41±3	0±0	0±0	0±0
Armking	100±0	100±0	100±0	92±0	71±0	32±0	0±0	0±0	0±0
Baby Gold					100±0	66±1	33±2	18±1	0±0
September Free	100±0	100±0	100±0	100±0	100±0	74±2	0±0	0±0	0±0

Table 8.4: Effect of chilling accumulation at 3.0 ± 0.1°C on the mean time to budbreak (MTB) of vegetative (V) and floral buds (F) from excised shoot of different cultivars of peach and nectarine (*Prunus persica* L. Batsch) forced at constant temperature (20.0°C) for 45 days.

Cultivar	Bud	MTB (days)								
		Chilling accumulation at 3.0 ± 0.1°C								
		0	100	200	300	400	500	600	700	800
Flordaprince	V	35±1.0	32±1.2	16±0.8	10±0.3	8±0.5	7±0.4	7±0.4	7±0.3	7±0.4
	F	31±0.8	29±0.9	15±0.5	12±0.4	10±0.4	9±0.5	9±0.2	9±0.3	9±0.2
Flordastar	V		29±0.3	13±0.3	9±0.3	7±0.3	6±0.2	6±0.2	6±0.3	6±0.2
	F	27±0.7	25±0.4	14±0.6	11±0.7	10±0.4	9±0.4	9±0.5	9±0.4	9±0.4
Tropic Snow	V	25±0.6	14±0.5	8±0.3	6±0.3	5±0.2	5±0.2	5±0.3	5±0.3	5±0.2
	F	20±0.6	14±0.4	11±0.5	9±0.4	8±0.3	8±0.2	8±0.3	7±0.2	7±0.1
Sun Red	V	34±0.8	28±0.7	24±1.7	19±0.5	16±0.6	15±0.5	13±0.5	12±0.4	10±0.4
	F	27±1.0	25±0.2	22±1.2	19±0.4	18±0.4	16±0.2	15±0.2	13±0.4	12±0.3
Hermosillo	V		28±0.1	14±0.3	8±0.2	7±0.3	7±0.7	7±0.2	7±0.3	7±0.2
	F	27±1.3	20±1.7	12±0.3	9±0.5	9±0.2	9±0.7	9±0.5	9±0.3	9±0.2
Flordaking	V		41±1.0	27±0.7	17±0.7	11±0.7	9±0.2	8±0.2	8±0.3	8±0.1
	F	30±0.7	26±0.5	19±1.0	14±0.2	10±0.3	10±0.4	10±0.2	10±0.2	10±0.1
Spring Belle	V					25±0.7	21±0.6	18±0.5	15±0.4	13±0.3
	F			30±0.3	27±0.2	22±0.2	19±0.3	18±0.7	16±0.4	15±0.3
Armking	V				21±0.8	17±0.7	15±0.5	13±0.4	9±0.3	9±0.3
	F	24±0.3	22±0.2	19±1.0	18±0.3	16±0.2	12±0.2	12±0.3	15±0.4	15±0.2
Baby Gold	V					28±0.4	25±0.4	22±1.7	18±1.1	15±0.7
	F					22±0.6	21±0.4	19±1.1	18±0.3	16±0.2
September Free	V				30±0.5	27±0.5	22±0.6	19±0.4	10±0.4	9±0.2
	F	31±0.7	30±0.4	27±0.3	22±0.7	21±0.8	21±0.4	18±0.7	18±0.4	16±0.3

Table 8.5: Range of chilling accumulation at which different peach cultivars showed a percentage of floral and vegetative budbreak higher than 50%.

Cultivar	CH H[1]	Range of chilling accumulation with bud break >50%[8]
Flordaprince	150[2]	200-800
Flordastar	250[2]	200-300
Earlygrande	260[2]	200-800
Tropic Snow	250[2]	200-800
Sun Red	250[5-7]	0-300
San Pedro 16-33	297[2]	100-800
Hermosillo	350[2]	200-800
Flordaking	450[2]	300-800
Maycrest	600[2]	600-800
Spring Belle	650[3]	600-800
Armking	600[6-7]	500-700
Baby Gold	850[4-7]	+800
September Free	850[7]	400-800

[1] Chilling Hours: chilling requirement of the cultivar.
[2] Valentini, 2002; [3] adjusted according to its behaviour in Valencia, Spain; [4] Agustí, 2004; [5] Barbosa et al., 1997; [6] Powel, 1998; [7] Okie, 1998.
[8] Measured as the percentage of excised shoots that reached the green tip stage.

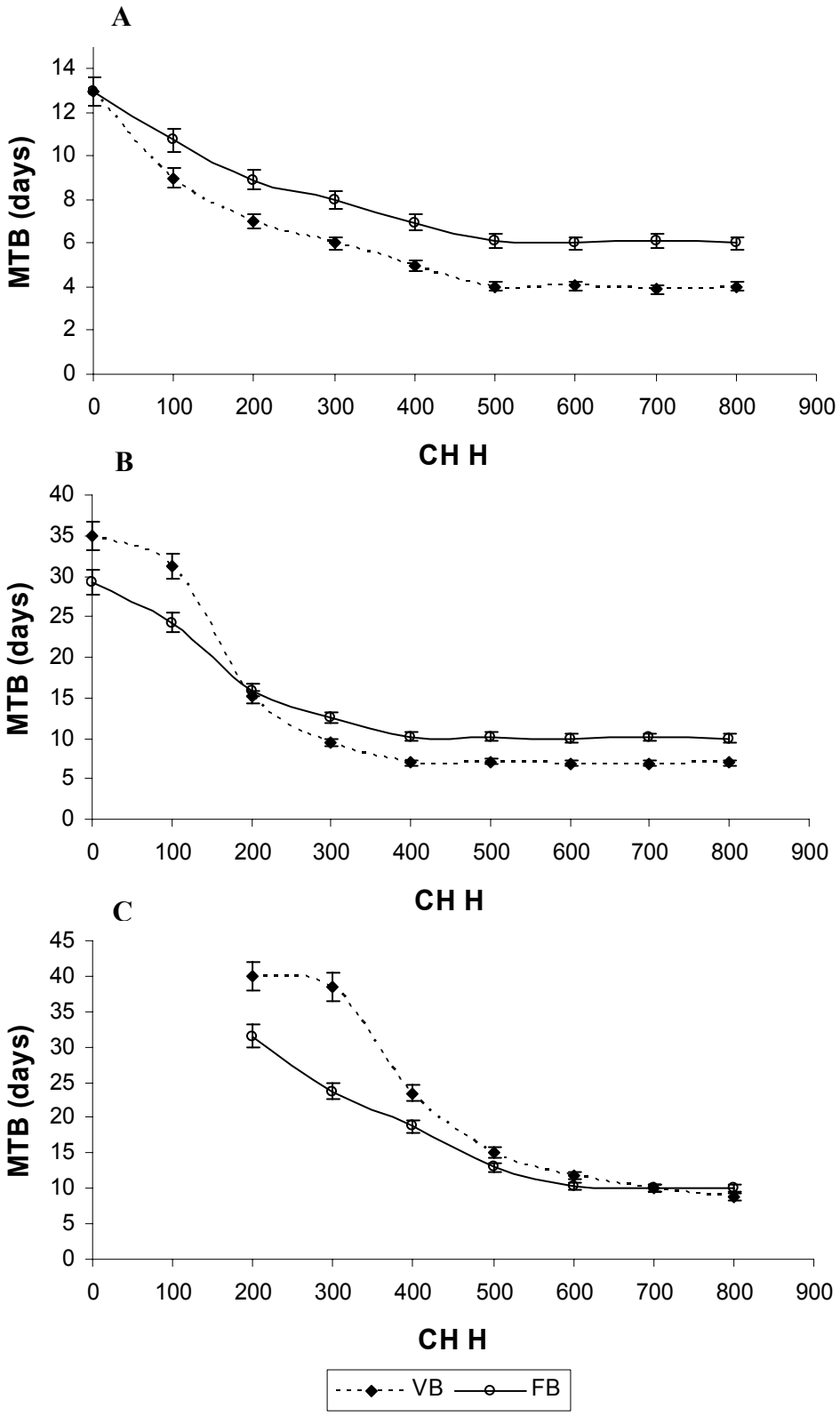

Figure 8.1: Effect of chilling accumulation (CH H) at 3.0 ± 0.1°C on the mean time to budbreak (MTB) of vegetative (VB) and flower buds (FB) from excised shoots of peach (*Prunus persica* L. Batsch), cv. 'San Pedro 16-33' (A) 'Earlygrande' (B) and 'Maycrest' (C), forced at constant temperature (20.0°C) for 45 days. MTB standard error for each sample is represented by the vertical bar.

CHAPTER 9

Effect of different chilling and heat conditions on the release of bud dormancy of 'Flordastar' peach (*Prunus persica* L. Batsch)

9- Effect of different chilling and heat conditions on the release of bud dormancy of 'Flordastar' peach (*Prunus persica* L. Batsch)

Abstract

In order to study the effects of different chilling conditions on budbreak and heat accumulation for blooming, a low chilling requiring peach (*Prunus persica* L. Batsch) cultivar was selected. In an excised shoots experiment, stem cuttings were obtained from 12 trees during leaf fall, placed in plastic bags at 3.0 ± 0.1ºC to simulate 0 to 800 h of chilling and forced to budbreak at 20.0 ± 1.0°C during a period of 6 weeks. Additionally, 18 adult trees were selected and submitted to 3 different temperature conditions from leaf to full bloom, during the 2002/03 and 2004/05 growth cycles, under greenhouse conditions. The treatments were: 1) natural conditions (NC); 2) natural conditions forced (NCF) and 3) chilling deprivation (CD). The percentage of flower and vegetative budbreak was highly affected by chilling accumulation. Leaf budbreak was highly affected at 0 and 100 h of chilling, but had no further differences after 200 h of chilling. For longer chilling (>400 h) a significant reduction on the percentage of flower budbreak was observed. The occurrence of dormancy was not avoided when low or non chilling accumulation conditions were set, in both field and excised shoot experiments. The cultivar studied showed a point of critical chilling accumulation, which according to the MTB was between 400 and 500 h of chilling. Below this point blooming tends to occur early, and above it leafing tends to occur before flowering. A negative correlation between the number of heat units accumulated from "green tip stage" to full bloom and the chill hours received till "green tip stage" was found. The bud weight at the green tip stage for floral and vegetative buds increased 2.7 and 3.3 times, respectively, compared to the initial bud weight at the beginning of the rest period. Finally, the flower density was highly affected by low chilling conditions.

Introduction

Dormancy has been described as a complex, dynamic, multi-faceted phenomenon that probably evolved as a survival strategy during plants' evolution (Van der Schoot, 1996).

Many kinds of dormancy exist, and even coexist, in a tree, and their primary cause may begin at a distance from the potentially dormant meristem and be related to unspecific correlative or environmental effects (Crabbé and Barnola, 1996).

Prunus buds go through three distinct stages of dormancy that Lang *et al.* (1987) have defined as paradormancy, when buds are dormant because of the inhibitory influences of other plant parts; endodormancy, related to inhibitory factors inside the bud that can only be overcome after a certain chilling requirement has been satisfied; and ecodormancy, when buds are able to resume growth once favourable environmental conditions have been met.

Nevertheless, in all types of dormancy, the primary causes of growth arrest are commonly sought outside the bud, even when, as in endodormancy, some endogenous factor within the bud is involved (Crabbé and Barnola, 1996). In stone fruits, the end of the rest period is preceded by a signal originating in the plant itself after chilling exposure has been completed, which unchains the biochemical changes needed for eliminating the repression of the active meristematic growth in the buds (Horvath *et al.*, 2003).

It has long been known that the main factor in breaking bud dormancy is low temperature (Parker, 1963) and that the amount of chilling required to overcome endodormancy is theoretically a varietals constant (Balandier *et al.*, 1993). In temperate-zone deciduous fruit trees, the most effective factor in releasing dormancy is the accumulation of a certain amount of cold and only part of this cold requirement can be substituted by other means (Faust *et al.*, 1997). During dormancy, lack of chilling is an important limiting factor in temperate zones and at latitudes near the equator. The lack of chilling associated with mild winter conditions, results in an abnormal pattern of budbreak and development in temperate fruit trees (Erez and Couvillon, 1987; Dennis, 1987; Mauget and Rageau, 1988; Lam Yam, 1989).

It has been suggested that budbreak is not affected by soil temperature (Hammond and Seeley, 1978; Young and Werner, 1985) and that temperature is locally perceived by the bud (Flore, 1994). Anderson *et al.* (1975) reported that the cooling of buds by sprinkling irrigation delayed bloom. Spiegel-Roy and Alston (1979) found that chilling requirement during dormancy (endodormancy) of pear (*Pyrus* sp.) and heat requirement in after rest-period (ecodormancy) are highly related to time of bloom. On the basis of these results, Wang and Faust (1987) suggested that trees also have a heat requirement that must be fulfilled before bloom occurs. Thus, vegetative and reproductive buds may have different chilling requirements or different temperature thresholds for growth, but once endodormancy is completed after sufficient chilling temperatures have occurred, the

initiation of bud growth and subsequent budbreak is regulated by heat and the amount required varies with species, physiological conditions of the plant, stage of development and geographical location of the trees (Flore, 1994).

Models of endodormancy release leading to budburst have been formulated (Couvillon and Erez, 1985; Hanninen, 1990), but all of them are based on phenological data giving a superficial description of the black box and on just a few clues regarding the basic mechanisms involved in dormancy.

According to Dennis (2003), the precise determination of the temperature requirements for breaking dormancy is nearly impossible under field conditions, where solar radiation, diurnal temperature fluctuations and other factors can not be controlled. As a consequence, chill unit model are not realistic and their accuracy in detecting end points may be confined to specific environments (Hanninen, 1995). The measurement of dormancy ´depth´ and duration in diverse experimental contexts by isolation of buds such as one-node cuttings (Balandier *et al.*, 1993) or excised shoots (Dennis, 2003) has been shown to be an adequate method to improve the study of dormancy. Therefore a combined methodology of phenological data under field conditions and excised shoot experiments could lead to a better understanding of the mechanism that controls bud dormancy and should be carried out prior to further physiological and molecular studies.

The objective of this work was to study the effects of different chilling conditions on budbreak and heat accumulation for blooming using a low chilling requiring peach cultivar which could over satisfy its requirements under temperate climatic conditions. In addition, complementary excised shoot experiments were carried out and a possible relationship with phenological data was investigated.

Material and methods

Excised shoot experiment. During leaf fall (3 April in Esperanza, Argentina) and before chilling accumulation, twigs were randomly collected from ten adult trees of peach (*Prunus persica* L. Batsch) cv. ‘Flordastar’ (chilling requirements: 250 h below 7.2ºC). Trees were located in an experimental orchard in Esperanza, Argentina (latitude 31° 26’ S; longitude 60° 56’ W; altitude 40 m), planted 5x5 m apart (400 trees ha^{-1}) on a silty-loamy soil, and grafted onto 'Cuaresmillo' rootstock. Twigs were cut into segments 15 cm long obtaining 400 stem cutting. Only three axillary

buds, of a typical node with one central leaf bud and two flower buds, located 2 cm from the upper cut were preserved. A group of five cuttings was placed in plastic bags and exposed at low temperature (3.0 ± 0.1ºC) (Balandier *et al.*, 1993) to simulate different treatment of chilling accumulation: 0, 100, 200, 300, 400, 500, 600, 700 and 800 h. Treatment represented 0 to 800 chilling hours, according to Weinberger (1950). After chilling, the shoots were placed with their basal tip in water and forced in a phytotron at 8-h photoperiod (22.5 μmol m^{-2} s^{-1}) (Citadin *et al.*, 2001) and 20.0 ± 1.0°C during a period of 6 weeks. The basal ends of the shoots were cut (Balandier *et al.*, 1993; Citadin *et al.*, 1998) weekly and water was replaced daily. Flower and vegetative budbreak were observed three times a week. The number of buds that reached the 'balloon' or 'green tip' stage was recorded (Citadin *et al.*, 2001). Results were expressed as the percentage of budbreak ("green tip stage") or as mean time to budbreak (MTB) in days (arithmetic mean of each four group of five excised shoots) (Balandier *et al.*, 1993). Trials were conducted in a completely randomized design with four replicates of five cuttings per treatment and cultivar. For each treatment, we also calculated the standard error or the ANOVA using the Tuckey test for means separation.

Field experiment. The experiment was conducted on adult trees of peach, cv. 'Flordastar', located in an experimental orchard in Carlet, Spain (latitude 39º 8' N; longitude 00º 18' W; altitude 180 m), planted 6x4 m apart (416 trees ha^{-1}) on a sandy-loamy soil, and grafted onto 'GF 677' rootstock. Eighteen adult trees were selected during two non consecutive growth periods (2002/03 and 2004/05), with different climatic conditions each. In order to modify the effect of chilling and the accumulation of heat units for budbreak and blooming during both growth cycles, treatments were designed as follows: 1) 'Natural Condition' (NC) treatment: 6 trees consecutive in the same line were left under natural conditions; 2) 'Natural Condition Forced' (NCF): 6 trees were initially under natural condition and then submitted to controlled temperature (≥5.0ºC from 30 Dic. for 2002-03 and ≥7.2ºC from 8 Jan. for 2004-05 growth cycle) under greenhouse conditions for budbreak induction; and 3) 'Chilling Deprivation' (CD) treatment: 6 trees in a consecutive line were covered under greenhouse conditions, with temperature maintained ≥7.0ºC, from leaf fall (between the 1 and 7 Nov. in Carlet, Spain), when no chilling had yet been accumulated, until the beginning of bloom (< 50% flowers in anthesis). In the NCF, the forcing dates were selected each year depending on the evolution of chilling hours (CH H) accumulation and attempts were made to force the plants when CH H accumulation was around the requirements of the selected cultivar (inferior in the 2002-03 and greater in the 2004-05 growth cycles).

Climatic data was recorded at 30 minutes intervals using 2 data loggers (Testostor 175) per treatment, placed at a height of 1.8 meters in the canopy area, from leaf fall to full bloom; accumulation of hours below 7.2ºC (chilling hours – CH H) and the maximum, minimum and mean air temperatures were calculated. The chilling requirement was considered fulfilled when budbreak ("green tip stage") reached the 50% of total buds; this is indicated in Figure 9.1 by continuous arrows. The heat units (HU) accumulation necessary to reach full bloom was calculated from the completion of chilling to full bloom by the addition of differences between daily mean temperatures and a base temperature of 6.0ºC, according to the Tabuenca and Herrero (1966) method (cited by Agustí, 2004). In addition, the number of HU from leaf fall to "green tip stage" was also calculated.

Fifteen to twenty shoots per tree were marked and the percentage of budbreak was calculated from leaf fall (50% leaf drop) until "green tip stage", during the 2002/03 and 2004/05 growth cycles.

Additionally, in order to follow the fresh bud weight evolution during rest and based on Tabuenca 's (1964) methodology criteria, floral buds (FB) and vegetative buds (VB) from the middle part of twenty stems were pool collected and weighed at intermittent periods, from November to mid January of the 2004/05 rest period. The mean bud weight to reach green tip stage in both kinds of buds was calculated. A significant difference (increase) in fresh weight indicates the endodormancy release.

Temperature effect on flowering was evaluated at full bloom, during the 2002/03 and 2004/05 growth cycle, by randomly selecting ten homogeneous current season shoots per tree. The shoot length and the number of flowers per meter of shoot length were measured. Full bloom is indicated in Figure 9.2 by dotted arrows.

Statistical analysis. The Analyses of Variances, Turkey's multiple range test and linear regressions were performed using Statgraphics (5.1 version) software. Percentages were analysed after arc sine transformation.

Results

Excised shoots. The percentage of floral and vegetative budbreak was affected by chilling accumulation. Our results show a significant increase in floral budbreak up to 200 h in response to chilling, but higher chilling (>400 h) caused a significant reduction in the percentage of floral budbreak compared with the natural requirement of the cultivar - 250 h according to Valentini (2002) and Okie (1998). This effect of chilling on floral budbreak

contrasts with that on vegetative budbreak; vegetative budbreak significantly increased up to 200 h of chilling, reaching the maximum (100 % of budbreak) and with no significant difference for higher chilling (Table 9.1). In addition, chilling modified the proportion of blooming that occurred before leafing, being the highest for less than 100 h, but dropping to less than 40 % when chilling was equal or longer than 400 h. Accordingly, the proportion of blooming before leafing decreased significantly as chilling exposure increased (Table 9.1).

The MTB value of vegetative buds was higher than that of floral buds for shoots chilled for 100 h, equal for 200 h and significantly lower when chilling was longer than 200 h. The stabilization point for MTB was reached between 400 and 500 h of chilling (Figure 9.1). The MTB value for non chilled vegetative buds is not shown, since no budbreak had taken place during the 45 - day period of evaluation.

Climatic data, phenology and chilling-heat unit accumulation studies. In the following description, unless otherwise specified, we always refer to floral buds when budbreak ("green tip stage") or flowering is mentioned. Temperature regimes and chilling accumulation during the 2002/03 and 2004/05 rest periods show remarkable differences among the three main field treatments (Figure 9.2). For the NCF treatment minimal temperatures remained above 7.0ºC and maximal temperatures rose over 25.0ºC in both seasons after forcing. Additionally, the difference between maximal and minimal temperature increased from 8.0ºC in 2002/03 and 12.0ºC in 2004/2005 before forcing to 20.0ºC after forcing. With the exception of the NC 2002/03 situation, budbreak occurred after a period of 5 to 8 days with maximal temperatures over 20.0ºC. During both seasons, minimal temperatures reached values below zero before budbreak in NC and NCF treatments (Figures 9.2A, 9.2B, 9.2C and 9.2D).

Temperature regimes of the three experiments yielded different kinetics of chilling accumulation (Table 9.2). As expected, chilling requirement satisfaction was achieved before budbreak only under NC in both years (318 and 556 CH H for 2002/03 and 2004/05 rest periods, respectively) and NCF 2004/05 (328 CH H), and only partially achieved for the NCF 2002/03 (200 CH H). In both years, the CD treatments yielded very small amounts of chilling (0 and 12 CH H for 2002/03 and 2004/05 rest periods, respectively) before budbreak occurred (Table 9.2). The same treatment had two days with minimal temperature below 5.0ºC between the period of budbreak and full bloom in 2002-03, and 3 days with minimal temperature below 7.2ºC before budbreak in 2004-05 (Figures 9.2E and 9.2F). Chilling hours to budbreak and HU accumulation from leaf fall to

budbreak for vegetative buds is shown in Table 9.2. In both years, the higher the CH H, the lower was the HU needed to flower budbreak, giving rise to a negative relationship between the both variables ($r^2 = 0.94$) (Figure 9.3). Although no data was collected during the 2002/03 season about vegetative budbreak, in 2004/05 the time lapse was very similar to that observed in floral buds, having less need of HU to reach the "green tip stage" when more chilling had been accumulated (Table 9.2).

Both for CD and NC experiments, "green tip stage" for floral buds was reached at 3.66±0.04 g fresh weight, the former reached on 3 Jan., the latter on 17 Jan. For vegetative buds, "green tip stage" was reached at 1.70±0.10 g fresh weight, that is, on 10 Jan. for CD and 14 Jan. for NC. NCF treatment evolved similarly to NC and CD conditions and changes were evident halfway between these two extreme treatments. The mean bud weight at the "green tip stage" for floral and vegetative buds was 0.036 g^{-1} and 0.017 g^{-1}, which is a 2.7 and a 3.3 fold increase, respectively, compared to the initial bud weight at the beginning of the rest period (Figure 9.4).

Considering a common range of chilling accumulation from 0 to 600 CH H, it is possible to correlate the number of heat units, counted from leaf fall to "green tip stage", with the amount of chilling accumulation (Figure 9.5). Similar slopes were obtained in different field conditions and excised shoot experiment of -0.42 and -0.45, respectively. Nevertheless, there was approximately 240 HU difference between the two straight lines (Figure 9.5). It is important to note that in the excised shoot experiment we counted the exact HU needed to "green tip stage", whereas in the experiment under field conditions HU and CH H were accumulated concomitantly, and this is the reason why we started to count HU and CH H from leaf fall.

The number of flowers per unit of shoot length was significantly lower in the CD experiments in relation to the NC in both seasons. The NCF 2004/05 differed significantly with NC 2004/05 and also with the NCF 2002/03 (Table 9.3).

Discussion

A relatively high floral budbreak (>50%) and a low MTB value were observed in the excised shoot experiments when no chilling was accumulated, compared to vegetative buds. But an excessive chilling accumulation lessened the percentage of floral budbreak (see Table 9.1) which, according to Citadin *et al.* (2001), could be attributed to a physiological injury to the buds caused by longer exposure to low temperature. Insufficient chilling is known to provoke an abnormal pattern

of budbreak and development in temperate zone fruit trees cultivated in warm climates (Bonhomme *et al.*, 2005). As under insufficient chilling conditions, buds open but do not develop further, and flowers fail to set fruits (Dennis, 2003). These results correlate with the decrease in the number of flowers per meter of shoot length in the CD treatments for both seasons (Table 9.3), which represented a 26.6% and 28.3% decrease with respect to the NC for 2002/03 and 2004/05, respectively. Although a high chilling accumulation was achieved in the NC 2004/05 treatment (see Table 9.2), this did not seem to be enough to affect the number of flowers per meter that reached anthesis, although a lower fruit set was measured (data not shown).

In the excised shoot experiment, the MTB value of the vegetative buds was higher compared with that of floral buds when ≤200 CH H were accumulated. Moreover, the MTB value of vegetative buds decreased faster with chilling compared with flower buds and consequently, the value of vegetative buds was lower than flower buds for high chilling accumulation treatments (see Figure 9.1). We observed that chilling caused a continuous decline in the intensity of rest, whereas no increase in the depth of dormancy was observed during the early phases of rest, as was observed in other trials (Arias and Crabbé, 1975; Walser *et al.*, 1981).

Vegetative and reproductive buds do not always break at the same time. At locations nearer the equator reproductive growth usually occurs first, followed by vegetative growth, but may coincide with vegetative development at distances farther from the equator (Keller and Loescher, 1989). This concurs with our results in the field experiments, where vegetative buds broke 7 days later than floral buds at CD 2004/05 treatment and 3 days earlier at the NC 2004/05 (see Figures 9.2F and 9.2B), with chilling accumulation of ≈12 CH H and ≈550 CH H, respectively, for both treatments (see Table 9.2). The NCF 2004/05 vegetative and floral buds broke approximately on the same date (13 Jan. 2005) having accumulated till then 328 CH H (see Figure 9.2D and Table 9.2). This evidence, together with the decrease of flower budbreak and the increase of the proportion of shoots leafing before blooming with the increase of chilling exposure shown in the excised shoots experiment, is in accordance with Citadin *et al.* (2001) who observed that prolonged chilling enhances leafing more than bloom in low chill requirement cultivars and that under years of higher chilling accumulation, leafing occurs before blooming.

Many authors agree that chilling increases the percentage of budbreak and favours endodormancy release (Erez *et al.*, 1979; Faust *et al.*, 1997; Fuchigami and Wisnewsky, 1997; Dennis, 2003). Nevertheless, our results

showed that low and non chilling accumulation conditions, like those for the CD experiments during 2002/03 and 2004/5 (see Figure 9.2 and Table 9.2), did not prevent the onset of dormancy, which agrees with the results found by Dennis (1994). Our results are also in agreement with Richardson *et al.* (1975) who said that resting or partially chilled plants require much more heat accumulation than plants for which chilling has been satisfied.

Considering that the end of rest starts when a stable low MTB value has been reached (Balandier *et al.*, 1993), the number of days to blooming or leafing is the time necessary to cover the heat requirement to reach the "green tip stage". Thus, the heat requirement for vegetative budbreak was lower than for floral budbreak when sufficient chilling was accumulated (see Figure 9.1).

According to Scalabrelli and Couvillon (1986), increasing the number of chill units does not reduce the heat requirements to budbreak, but increases the uniformity in chilling requirements among buds. But our results under field conditions showed a negative correlation between the number of heat units accumulated from "green tip stage" to full bloom and the chill hours received till "green tip stage" during the two seasons studied (see Figure 9.3). These results agree with those obtained with excised shoot experiments, and with Citadin *et al.* (2001) and Couvillon and Hendershott (1974) who found that extra chilling after completion of rest further reduces the heat accumulation requirement for bloom.

Assuming that MTB is the mean number of days needed to budbreak at a certain temperature, which in our experiment was 20.0 ± 1.0°C, it is possible to estimate the number of HU needed to reach the "green tip stage" by multiplying the MTB times the difference between 20.0ºC the base temperature of 6.0ºC (Tabuenca and Herrero, 1966). In this way, we correlated the number of heat units accumulated from leaf fall to "green tip stage" and the chilling hours needed to reach the "green tip stage" for flower buds in the excised shoot experiment and under field condition experiment, as is shown in Figure 9.5. The similarity between both slopes and the differences in the HU needed to budbreak can be explained by the fact that the ending of endodormancy is not a discrete, but a gradual and quantitative process (Citadin *et al.*, 2001), and according to Lavarenne *et al.* (1980) any temperature, with different efficiencies with respect to its range, can stimulate budbreak after release from dormancy. These results also agree with Felker and Robitaille (1985) who found that physiological maturation of cherry (*Prunus cerasus* L.) buds in the fall, chilling accumulation in deep rest, and heat accumulation leading to budbreak are overlapping processes that are very difficult to separate.

Zimmerman and Faust (1969) found that the fresh weight of buds increases rapidly towards spring but their dry weight remains constant, indicating the movement of water into the buds during bud development. Cole *et al.* (1982) suggested that the time of bloom depends on the rate of bud development before, during and after dormancy, which is mainly influenced by temperature. He also said that bud growth starts immediately after the beginning of heat unit accumulation. In our work, the evolution of bud weight was highly influenced by temperature during the rest period (see Figure 9.4). Moreover, vegetative and floral bud weight significantly increased at the same time as the percentage of buds at the green tip stage was over or equal to 50% during both seasons for flower buds, but only in the 2004-05 season for vegetative buds (see Figure 9.2 and Figure 9.4). Budbreak begins when bud weight triples the initial weight at leaf fall in both floral and vegetative buds. Additionally, the bud weight time lapse follows an exponential function (see Figure 9.4), which is in agreement with Corgan and Martin (1971) who found the same growth pattern in floral cups.

To summarise, we conclude that in peach chilling above the threshold requirement (250 CH H for the 'Flordastar' peach cultivar in our experiments) reduces heat requirement to reach the "green tip stage", there is a negative correlation between the number of chill hours received till "green tip stage" and the number of heat units accumulated from then to full bloom, with vegetative buds being more sensitive than flower buds. Also, excessive chilling causes a reduction in the percentage of budbreak in flower but not in vegetative buds. Thus, the number of flowers per meter of shoot length was highly affected by different chilling accumulation conditions. Further, under high chilling accumulation conditions, leafing can occur before blooming under field conditions. Finally, our results confirm the utility of the combined methodology of excised shoots and phenology studies under different field conditions for the study of the complex process of dormancy.

References

Agustí, M. 2004. *Fruticultura*. Ediciones Mundi-Prensa, Madrid, Spain. pp 493.

Anderson, J.L.; Ashcroft, G.L.; Richardson, E.A.; Alfaro, J.F.; Griffin, R.E.; Hanson, G.R. and Keller, J. 1975. Effects of evaporative cooling on temperature and development of apple buds. Journal of the American Society of Horticultural Science 100: 229-231.

Arias, O. and Crabbé, J. 1975. Les gradients morphogénétiques du rameau dún an des végétaux ligneux en repos apparent. Données

complementaires fournies par létude de *Prunus avium* L. Physiologie Végetale 13: 69-81.

Balandier, P.; Bonhomme, M; Rageau, R.; Capitan, F. and Parisot, E. 1993. Leaf bud endodormancy release in peach trees: evaluation of temperature models in temperate and tropical climate. Agricultural and Forest Meteorology 67: 95-113.

Bonhomme, M.; Regeau, R.; Lacointe, A. and Gendraud, M. 2005. Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batsch). Scientia Horticulturae 105: 223-240.

Citadin, I.; Raseira, M.C.B. and Quezada, A.C. 1998. Substrato para conservacão de ramos destacados de pessegueiro, *Prunus persica* L. (Batsch). Agropecuária Clima Temperado 1: 55-59.

Citadin, I.; Raseira, M.C.B.; Verter, F.G. and Baptista da Silva J. 2001. Heat requirement for blooming and leafing in peach. HortScience 36: 305-307.

Cole, M.E.; Solomons, T. and Faust, M. 1982. Growth and respiration of dormant flower buds of *Pyrus communis* and *Pyrus colleryana*. Journal of the American Society of Horticultural Science 107: 226-231.

Corgan, J.N and Martin, G.C. 1971. Abscisic acid levels in peach floral cups. HortScience 6 (4): 405-406.

Couvillon, G.A. and Erez A. 1985. Influence of prolonged exposure to chilling temperatures on budbreak and heat requirement for bloom of several fruit species. Journal of the American Society of Horticultural Science 110: 47-50.

Couvillon, G.A. and Hendershott, C.H. 1974. A characterization of the "after–rest" period of flower buds of two peach cultivars of different chilling requirements. Journal of the American Society of Horticultural Science 99: 23-26.

Crabbé, J. and Barnola, P. 1996. A new Conceptual approach to bud dormancy in woody plants. In: Lang G.A. (Ed.). *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. CAB International, pp. 83-113.

Dennis Jr., F.G. 1987. Producing temperate-zone fruits at low latitudes: an overview. HortScience 22: 1226-1227.

Dennis Jr., F.G. 1994. Dormancy – What We Know (and Don't Know). HortScience 29 (11): 1249-1255.

Dennis Jr., F.G. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38: 347-350.

Erez, A. and Couvillon, A. 1987. Characterization of the influence of moderate temperatures on rest completion in peach. Journal of the American Society of Horticultural Science 112 (4): 77-680.

Erez, A.; Couvillion, A. and Hendershott, C. 1979. The effect of cycle length on chilling negation by high temperature in dormant peach leaf buds. Journal of the American Society of Horticultural Science 104: 573-576.

Faust, M.; Erez, A.; Rowland, L.J.; Wang, S.Y. and Norman, H.A. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32: 623-629.

Felker, F.C. and Robitaille, H.A. 1985. Chilling accumulation and rest of sour cherry flower buds. Journal of the American Society of Horticultural Science 110: 227-232.

Flore, J.A. 1994. Stone fruit. In: Schaffer, B. and Anderson, P.C. (Eds.). *Handbook of environmental physiology of fruit crops, Vol. 1, Temperate crops*. CRC Press, Inc., Boca Raton, Florida, pp. 233-270.

Fuchigami, L.H. and Wisniewsky, M. 1997. Quantifying bud dormancy: Physiological approaches. HortScience 32: 618-623.

Hammond, M.W. and Seeley, S.D. 1978. Spring bud development of *Malus* and *Prunus* species in relation to soil temperature. Journal of the American Society of Horticultural Science 103: 655.

Hanninen, H. 1990. Modelling bud dormancy release in trees from cool and temperate regions. Acta Forestalia Fennica 213: 1-47

Hanninen, H. 1995. Effects of climatic change on trees from cool and temperate regions; an ecophysiological approach to modelling of bud burst phenology. Canadian Journal of Botany 73: 183-199.

Horvath, D.P.; Anderson, J.V.; Chao, W.S. and Foley, M.E. 2003. Knowing when to grow: signals regulating bud dormancy. Trends in Plant Science 8 (11): 534-540.

Keller, J.D. and Loescher, W.H. 1989. Nonstructural carbohydrate partitioning in perennial parts of sweet cherry. Journal of the American Society of Horticultural Science 114: 969.

Lavarenne, S.; Barnola, P. and Champagnat, P. 1980. Climats artificiels et dormance des bourgeons. I. Températures et dormance automnale chez le frêne (*Fraxinus excelsior* L.). Comptes-Rendus de l'Académie d'Agriculture de France 20: 92-106.

Lam Yam, L. 1989. Contribution à l`étude de la croissance et de la fructification du pêcher (*Prunus persica* L. Batsch) dans les conditions climatiques de type tropical de l`île de la Réunion. Fruits 44 (12): 669-680.

Lang, G.A.; Early, J.D.; Martin, G.C. and Darnell, R.L. 1987. Endo-, para- and eco-dormancy: physiological terminology and classification for dormancy research. Hortscience 22: 371-378.

Mauget, J.C. and Rageau, R. 1988. Bud dormancy and adaptation of apple tree to mild winter climates. Acta Horticulturae 232: 101-108.

Okie, W.R. 1998. *Handbook of peach and nectarines varieties*. USDA Agriculture Handbook Nº 714.

Parker, J. 1963. Seasonal changes in cold resistance and free sugars in some hardwood barks. Forest Science 8:225.

Richardson, E.A.; Seeley, S.D.; Walker, D.R.; Anderson, J.L. and Ashcorft, G.L. 1975. Phenoclimatography of spring peach bud development. HortScience 10: 236-237.

Scalabrelli, G. and Couvillon, G.A. 1986. The effect of temperature and bud type on rest completion and the GDHºC requirement for budbreak in 'Redhaven' peach. Journal of the American Society of Horticultural Science 111: 537-540.

Spiegel-Roy, P. and Alston, F.H. 1979. Chilling and post-dormant heat treatment as selection criteria for late-flowering pears. Journal of Horticultural Science 64: 115-120.

Tabuenca, M.C. 1964. Necesidades de frío invernal de variedades de albaricoquero, melocotonero y peral. Annals Aula Dei 7: 113-132.

Tabuenca, M.C. and Herrero, J. 1966. Influencia de la temperatura en la época de floración de los frutales. Annals Aula Dei 8: 115-153.

Van der Schoot, C. 1996. Dormancy and symplastic networking at the shoot apical meristem. In: Lang G.A. (Ed.). *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. CAB International, pp. 59-81.

Valentini, G. 2002. *Variedades de nectarinas y durazneros para el NE de la provincia de Buenos Aires*. INTA. Estación Experimental Agropecuaria San Pedro.
URL: http://www.inta.gov.ar/sanpedro/info/doc/fru/gv_003.htm.

Walser, R.H.; Walker, D.R. and Seeley, S.D. 1981. Effect of temperature, fall defoliation, and gibberellic acid on the rest period of peach leaf buds. Journal of the American Society of Horticultural Science 106: 91-94.

Wang, S.Y. and Faust, M. 1987. Metabolic activities during dormancy and blooming of deciduous fruit trees. Israel Journal of Botany 37: 227-243.

Weinberger, J.H. 1950. Chilling requirements of peach varieties. Proceedings of the American Society of Horticultural Science 56: 122-128.

Young, E. and Werner, D.J. 1985. Effects of shoots, root and shank chilling during rest in apple and peach growth resumption and carbohydrates. Journal of the American Society of Horticultural Science 110: 769-774.

Zimmerman, R.H. and Faust, M. 1969. Pear bud metabolism: seasonal changes in glucose utilization. Plant Physiology 44: 1273-1276.

Table 9.1: Effects of chilling accumulation at 3.0 ± 0.1°C on the percentage of vegetative budbreak and floral budbreak of excised shoots of peach (*Prunus persica* L. Batsch) cv. 'Flordastar', forced at constant temperature (20.0 ± 1.0°C) for 45 days, and percentage of blooming that occurred before leafing.

	Chilling accumulation at 3.0 ± 0.1°C								
	0	100	200	300	400	500	600	700	800
Floral budbreak (%)*	53b	67b	87a	47b	33c	38c	40c	35c	40c
Vegetative budbreak (%)*	0c	13b	100a	100a	95a	90a	93a	94a	100a
% of blooming that occurred before leafing	100±0	100±0	46±2	42±2	20±1	19±1	22±1	25±2	33±2

* Measured as the percentage of excised shoots that reached the "green tip stage".
Means followed by different lower-case letters in the same line differ significantly ($P \leq 0.05$).

Table 9.2: Amount of chilling hours (CH H) accumulated to budbreak and heat units (HU) counted from leaf fall to budbreak ("green tip stage") for floral and vegetative buds of adult trees (field experiments) of peach, cv. 'Flordastar'.

Growth Cycle	Climatic conditions	Floral buds		Vegetative buds	
		CH H	HU	CH H	HU
2002/03	NC	318	567	nd[(1)]	nd
	NCF	200	559	nd	nd
	CD	0	655	nd	nd
2004/05	NC	556	322	520	312
	NCF	328	407	328	407
	CD	12	452	12	536

NC: Natural Condition; NCF: Natural Condition Forced; CD: Chilling Deprivation.
(1) nd: not determined.

Table 9.3: Effect of Natural Condition (NC), Natural Condition Forced (NCF) and Chilling Deprivation (CD) treatments on flowers per meter of shoot at full bloom (50 % flowers in anthesis) in peach (*Prunus persica* L. Batsch) cv. 'Flordastar', during two growth cycles (2002-03 and 2004-05). Values for field experiment.

Cultivar	Climatic conditions	Flowers m^{-1}			
Flordastar		2002-2003		2004-2005	
	NC	86.9	a	92.2	a
	NCF	80.3	abA	61.7	bB
	CD	63.8	b	66.1	b

Means followed by different lower-case letters in the same column and by different capital letters in the same line differ significantly ($P \leq 0.05$).

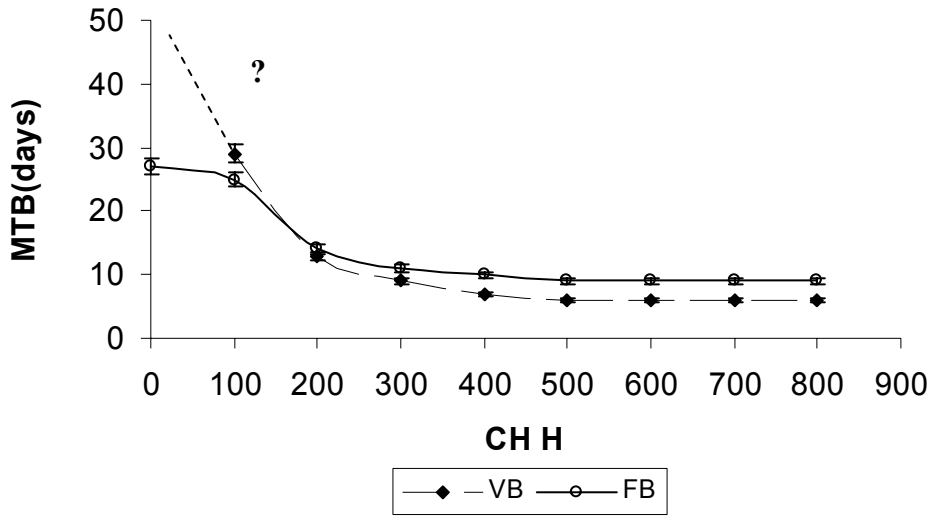

Figure 9.1: Effect of chilling accumulation (CH H) at 3.0 ± 0.1°C on the mean time to budbreak (MTB) of vegetative (VB) and floral buds (FB) from excised shoots of peach *(Prunus persica* L. Batsch) cv. 'Flordastar', forced at constant temperature (20.0 ± 1.0°C) for 45 days. Standard errors are given as the vertical bars.

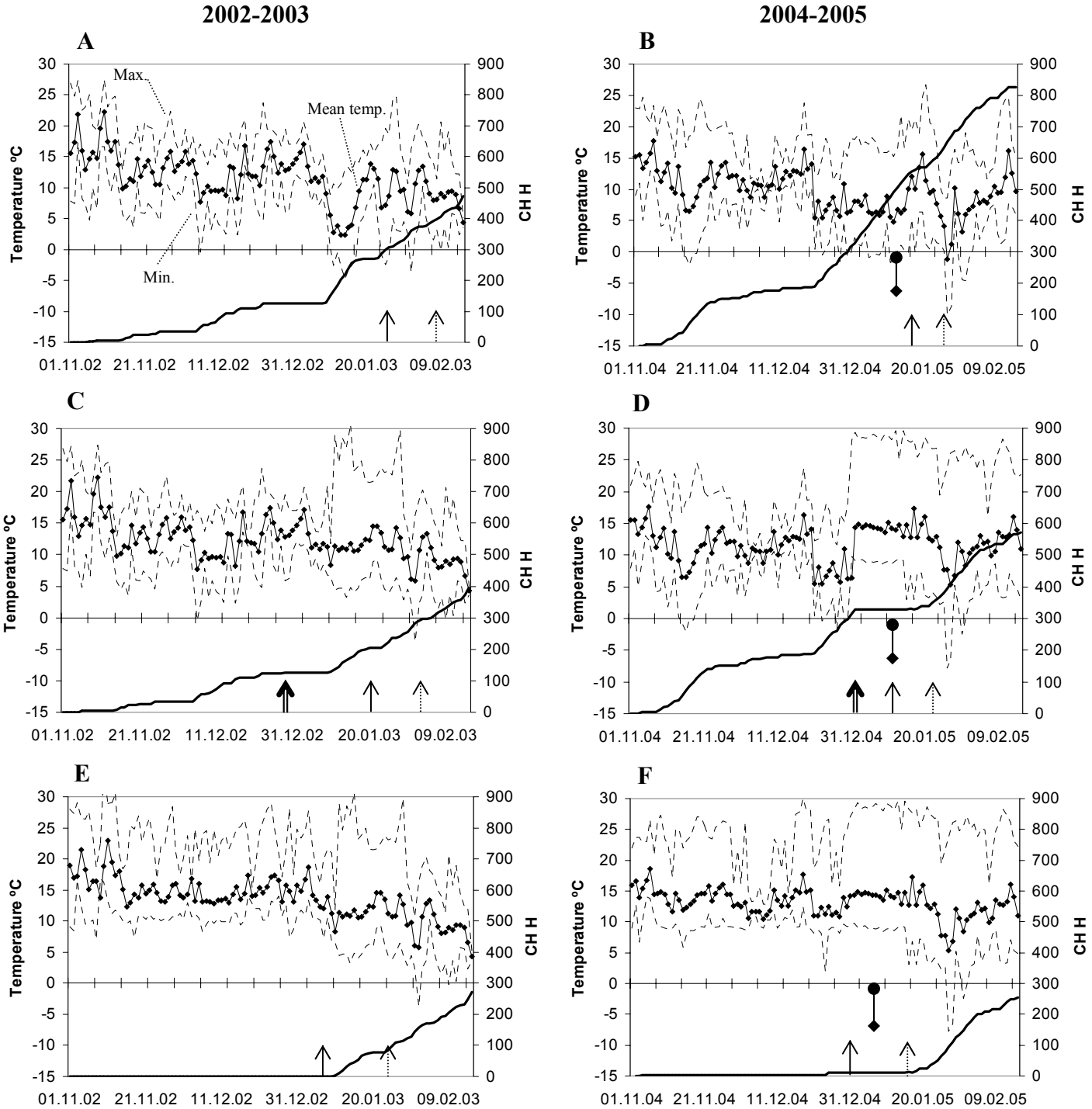

Figure 9.2: Climatic data: maximum, minimum and average air temperatures, cumulative hours with temperature below 7.2 ºC. (A) Natural Condition (NC) 2002-2003; (B) NC 2004-2005; (C) Natural Condition Induced (NCF) 2002-2003; (D) NCF 2004-2005; (E) 2002-2003 Chilling Deprivation (CD) and (F) CD 2004-2005. (⟶) Indicates flower budbreak (>50% at the "green tip stage"). (⇢) Indicates full bloom (>50% flowers). (●⟶◆) Indicates vegetative buds budbreak. (⟹) Indicates the date submitted to the greenhouse conditions (≥5.0ºC for 2002-03 and ≥7.2ºC for 2004-05 growth cycle). 'Flordastar' peach chilling requirements: 250 h.

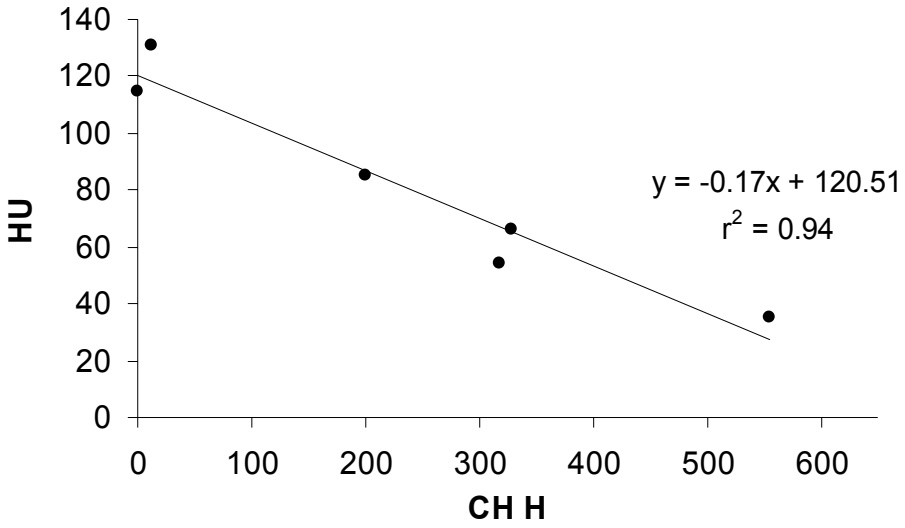

Figure 9.3: Relationship between the chilling hours (CH H) received to budbreak and the number of heat units (HU) accumulated from "green tip stage" to full bloom, in peach (*Prunus persica* L. Batsch) cv. 'Flordastar', during two growth cycles (2002-2003 and 2004-2005). Values corresponding to field experiment.

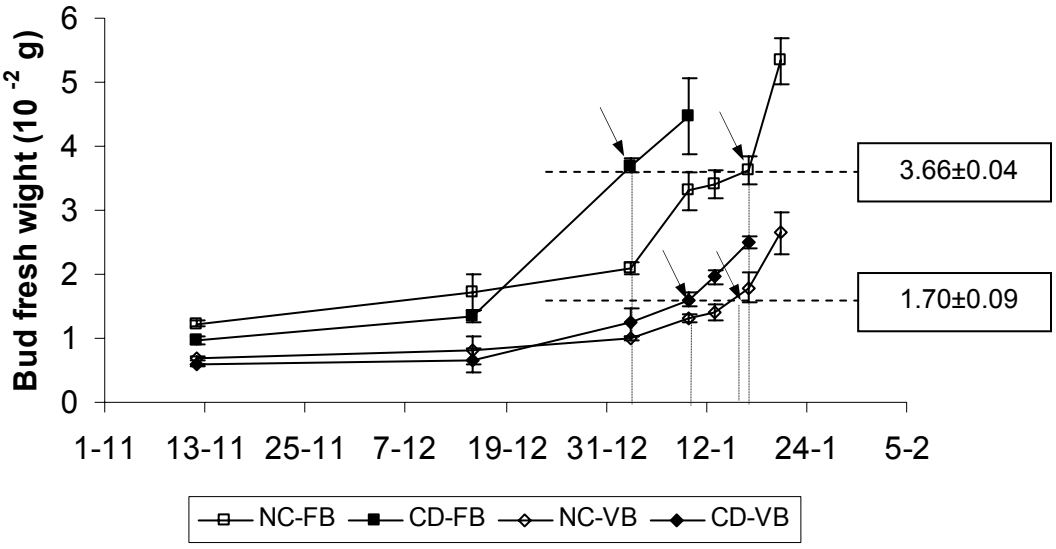

Figure 9.4: Effect of Natural Condition (NC) and Chilling Deprivation (CD) on the evolution of floral buds (FB) and vegetative buds (VB) fresh weight (g x 10^{-2}) of peach (*Prunus persica* L. Batsch) cv. 'Flordastar', during the rest period (2004-2005 growth cycles). Arrows show the date when more than 50% of buds reached the "green tip stage". Values corresponding to field experiment. Standard errors are given as vertical bar.

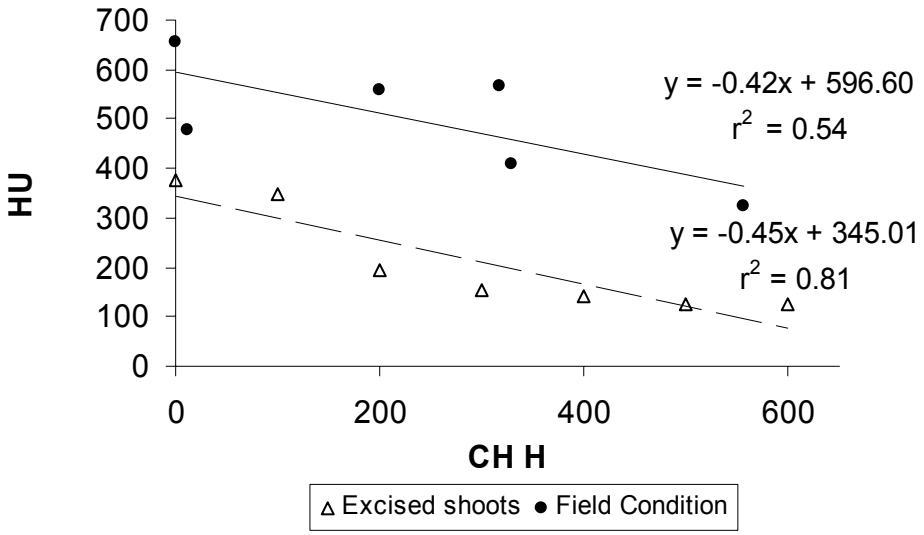

Figure 9.5: Relationship between the chilling hours (CH H) received to reach the "green tip stage" and the number of heat units (HU) accumulated from leaf fall to "green tip stage" in excised shoots and adult trees (field experiment) of peach (*Prunus persica* L. Batsch) cv. 'Flordastar'.

CHAPTER 10

Effects of chilling on carbohydrates and nitrogen fractions content in bark tissues of different *Prunus* species

10- Effects of chilling on carbohydrates and nitrogen fractions content in bark tissues of different *Prunus* species

Abstract

Stem cuttings of peach, nectarine, plum and apricot, obtained from one-year-old shoots at leaf drop in autumn, were exposed at a constant 3.0 ± 0.1ºC to simulate different chill hours of treatment, as occurs naturally during the winter chilling accumulation. The bark tissue was then isolated and the sugars and starch content analyzed by HPLC. Sorbitol, fructose and glucose were the main sugars in bark tissues, while sucrose and raffinose present in the lowest quantities in all *Prunus* spp. studied. The response to the low temperature treatment varied between the sugars. Sorbitol was also the main transport sugar and its concentration decreased when 1000 chill hours (CH H) (peach, nectarine, plum) or 1600 CH H (apricot) were accumulated. Fructose and glucose increased significantly till 800 CH H in peach, nectarine and plum, or 1200 CH H in apricot; then sharply decreased for 1000 CH H or higher. Total sugar concentration increased significantly in all the species as a consequence of chilling, and major variations were observed in plums and apricot. Starch showed a significant decrease due to chilling and, at the same time, carbohydrate reserves showed an overall negative balance as a consequence of metabolic activity. High correlation in total sugar and starch was found between 0 and 800 CH H. Ammonium nitrogen increased significantly at 600 CH H with respect to no chilled treatment, while no significant changes were observed in protein nitrogen or nitric nitrogen. Our results suggest the possibility of using excised shoots to study quantitative changes in carbohydrates as a consequence of chilling and the metabolism of dormant tissues.

Introduction

Dormancy is defined by the lack of visible growth (Romberger, 1963; cited by Van der Schoot, 1996) that conferred various advantages to the plant, from developmental synchronization to survival of environmental stresses (Lang, 1996). However, there are many uncertainties concerning the physiological mechanisms of this concept (Lang *et al*., 1987; Shirazi, 2003).

In temperate-zone deciduous fruit trees, the most important factor in release dormancy is the accumulation of a certain amount of cold, and only a certain part of this cold requirement can be substituted by other means (Faust *et al*., 1997). Thus, bud development in the spring may occur once chilling temperatures have accumulated (Westwood, 1993; Faust, 1989)

and endodormancy has been broken (Lang *et al*., 1978). In horticulture, the quantification of cold requirements in order to determine the end of endodormancy and ecodormancy is based on chill unit accumulation, but this does not take into account the physiological status of the plant (Fuchigami and Wisniewsky, 1997; Dennis, 2003). Besides, precise determination of the temperature requirements for breaking dormancy is nearly impossible under field conditions, where solar radiation, diurnal fluctuation in temperature, relative humidity and other factors cannot be controlled (Dennis, 2003). As a consequence, chill unit models are not capable of general application and their accuracy in detecting end points may be confined to specific environments (Hanninen, 1995).

The ultimate endodormant condition of the bud depends on the chain of prior correlative events that had occurred in the previous season (Crabbé and Barnola, 1996), and the four major factors that affect the intensity of dormancy are: hormone balance in the bud or in the tree, the state of water within the bud, the structure of membranes affecting cold resistance and governing resumption of growth, and the anabolic potential of buds (Faust *et al*., 1997). Bonhomme *et al*. (2005) found significant changes in the carbohydrates of different tissues from vegetative and reproductive structures in peach trees submitted to chilling deprivation, compared to natural chilling conditions. Carbohydrates are the main source of energy for the metabolic changes that occurred during the dormant period and for spring sprouting and blooming (Flore and Layne, 1996; Sherson *et al.*, 2003). Moreover, soluble sugars (hexoses and sucrose) are recognized as being important signalling molecules and are involved in many processes in the life cycle of plants, including dormancy (Sheen *et al.*, 1999; Gibson, 2000; Smeekens, 2000).

Accordingly, the study of dormancy is nearly impossible under field conditions and temperature cannot work as a perfect ´signal' for the release of dormancy, since specific temperature receptors do not exist (Crabbé and Barnola, 1996). Thus, its effects are not expected to be clear, but rather variable and fluctuating from species to species and according to the physiological context. The temperature also affects the rate of enzymatic reactions and causes an upset of the metabolic balance (Crabbé and Barnola, 1996). These metabolic changes in the content of carbohydrates and other substances, such as nucleic acids, proteins, polyamines, amino acids, organic acids and changes in the respiration rate may be associated with bud break and the time of bloom (Wang and Faust, 1987).

To overcome field restrictions, the measurement of dormancy ´depth´ and duration by isolation of buds as one-node cuttings (Balandier *et al*.,

1993) or excised shoots (Dennis, 2003) is an appropriate method to investigate the process of dormancy. The mean time required for bud-burst is used for comparisons of endodormancy in experimental or standard forcing conditions and its results are confirmed by biochemical tests (Crabbé and Barnola, 1996). The exposure of excised shoots to artificial low temperature conditions has proved to be a useful method of simulating the response of buds to natural chilling conditions (Arias and Crabbé, 1975; Balandier *et al*., 1993; Citadin *et al*. 2001; Dennis, 2003).

To better understand the mechanism of bud development and evolution during rest, we studied the quantitative changes in carbohydrate and nitrogen fractions content in the bark tissue of different *Prunus* spp. exposed to low temperatures, using stem cuttings under controlled conditions.

Material and methods

Plant Material

Experiments were conducted in an experimental orchard located in the area of Lliria (Valencia, Spain), latitude 39º 39.281' N, longitude 000º 38.344' W and altitude 180 m. The peach (*Prunus persica* L. Batsch, *vulgaris*) cultivars studied were 'Baby Gold', 'Spring Crest' and 'Spring Belle'; the nectarine (*Prunus persica* L. Batsch, *leavis*) cultivars were 'September Free', 'Armking', 'Suncost' and 'Sunred'; the plum (*Prunus salicina* Lindl.) cultivars were 'Early Beauty' and 'Plumlate', and the apricot (*Prunus armeniaca* L.) cultivars were 'Stark Early Orange', 'Gold Rich' and 'Canino'. All four species were planted at 6x4 m on a sandy-loamy soil, drip irrigated and fertilized according to their individual needs, and given a vase-shaped canopy. Peaches and nectarines were grafted onto 'GF677' rootstocks, while plums and apricots were grafted onto 'GF-8-1' and 'seedling' rootstock, respectively. In each variety, 120 twigs (≈ 40 cm long) were randomly collected from 5 adult trees during leaf drop (10 November) and before any natural chilling accumulation had occurred. The twigs were cut into segments 15 cm long, obtaining 240 stem cuttings for each variety. A group of ten cuttings were then exposed to low temperature (3.0 ± 0.1ºC) for chilling accumulation treatments: 0, 200, 400, 600, 800 and 1000 Chill Hours (CH H) in peach, nectarine and plum, and 0, 400, 600, 800, 1200 and 1600 CH H in apricots (Weinberger, 1950). After the chilling treatments, the bark tissues of the stem cuttings, including the buds, were separated from the wood tissues, frozen, lyophilized, ground, and stored at -40ºC. Trials were conducted in a completely randomised design, with four replicates of ten cuttings per treatment and variety.

Analysis of carbohydrates

The carbohydrates were extracted according to the procedure described by Moing *et al*. (1992) and Mehouachi *et al.* (1995). Soluble sugars were extracted from powdered samples (100 mg dry wt) with 1.0 ml of 80% ethanol. A known amount of mannitol (Sigma Chemical, Madrid, Spain), a sugar absent in the phloem sap, was added to the sample as internal standard to determine the possible losses during extraction. The extract was then boiled for 5 min and centrifuged at 12000 rpm for 10 min. The supernatant was separated and the process was repeated twice on the pellet. The achieved supernatant was evaporated in a Speed-Vac at 40ºC to 0.5 ml, and the pellet saved and stored at -40ºC for further starch analysis. The ethylic solution of sugar extracts was dissolved in 1 ml of mQ water and then purified sequentially by cation exchange Dowex 50x8-100 (Sigma Química, Madrid, Spain) and anion exchange Dowex 1x4-400 (Sigma Química, Madrid, Spain) chromatography. The columns (1.0 ml volume) were pre-equilibrated with 2M HCl and 1M Na_2CO_3, respectively, and washed with approximately 5 column volumes with mQ water before sample application, to reach pH 4.5–5.0 and 7.5–8.0 for cationic and anionic columns, respectively. The eluted (1.0 ml extract and 2.0 ml water washings) were then passed through 0.45 μm nylon filter of 13 mm in diameter (Syringe filter, Termo Quest) and a C-18 Sep-Pak Cartridge (Waters-Millipore, Barcelona, Spain). Sugars were analyzed using a Spectra System® HPLC with a P2000 gradient Pump, a RI-150 differential refractometer and a ChromQuest Chromatography Data System. Twenty microlitre aliquots of the filtered extracts were injected into a Sugar-Pack I 300 x 6.5 mm column. Column temperature was kept at 85°C (Croco-Cil oven) and pure mQ water was used as solvent at a flow rate of 0.5 ml min^{-1}. Mannitol, fructose, glucose, sucrose, sorbitol and raffinose were identified by their retention times. Sugar content was determined using peak area calculation related to regression curves for known concentrations of sugar (Sigma Química, Madrid, Spain). The efficiency of the extraction process was determined firstly by calculating the mannitol recovery percentage factor for each sample, and then multiplying this factor by the concentration values obtained for each sugar, in order to correct any possible loss during extraction. Total sugar concentration was calculated by adding the glucose, fructose, sucrose and raffinose concentrations.

For starch determination, the solid pellet from the soluble sugars extraction was washed with 6 ml of mQ water into a Pirex-18 glass tubes. The tubes were covered with aluminium paper and then put in a P-Selecta Presoclave 30 autoclave for starch gelling, at 130ºC and 1.5 bars for 2 hours. After cooling down, the tubes were centrifuged (P- Selecta

Meditronic-BL) at 4500 rpm for 12 min. The surplus supernatant was separated with a micropipette, until a volume of approximately 2 ml (including the pellet) was left in the tubes. Then, 0.2 ml of lactose 30 mg ml^{-1} (internal standard), 0.5 ml from a buffer solution of sodium acetate-pH 4.5 (40% of acetic acid 0.2M and 60% of sodium acetate 0.2M) and 1 ml of amyloglucosidase enzyme 60 $mg.ml^{-1}$ solution from *Rhizopus* mold (Sigma Química, Madrid, Spain), were added to each tube. The enzyme was left to work in a shaking bath at 55ºC for 2 hours. Afterwards, the tubes were centrifuged at 4500 rpm for 30 min, and the supernatant was collected into Pirex-18 glass tubes and dried to a drop in a Speed-Vac at 35ºC. The sugar drop was then re-diluted with 1 ml of warm water (40ºC), centrifuged at 12000 rpm for 20 min, filtered through a 0.45 µm nylon filter of 13 mm in diameter (Syringe filter, Termo Quest) and then through a C-18/18% Octadecyl MiniSpe-ed SPE Cartridge (Applied Separations), and finally collected in a 0.5 ml eppendorf tube. The sample was then again dried to a drop in a Speed-Vac at 35ºC. The dry drop was dissolved in 500 µl of mQ water and analyzed, in triplicate, in a reverse phase HPLC Spectra System® as previously described. The results were expressed in mg of liberated glucose per g of dried sample (mg g^{-1} dw).

Total sugars were calculated as the sum of individual sugars, and transport sugars as the sum of sorbitol and sucrose.

Nitrogen fraction analysis

The protein nitrogen (N-prot), ammonium nitrogen ($N\text{-}NH_4^+$) and nitric nitrogen ($N\text{-}NO_3^-+NO_2^-$) analyses were based on the techniques described by AOAC (1984), Raigón *et al*. (1992), Levey *et al*. (2000) and Beljaars *et al.* (1994), with some modifications, as described below.

Protein Nitrogen (N-prot). To determine the amount of N-prot, 0.5 g sub-sample were dissolved in 10 ml of cold (4ºC) trichloroacetic acid 5% (TCA 5%), which precipitates protein, and homogenized by magnetic stirring (AGIMATIC-E) for 15 minutes. Thereafter, 30 ml of cold TCA 5% were added and the final solution was stored at 4ºC for 15 minutes. The sample was then filtered with a 90 mm Schleicher and Shvell filter paper and the residue, containing the N-prot, washed three times with 10 ml of cold TCA 5%. The filtered solution was completed to 100 ml with mQ water and stored at 4ºC for further $N\text{-}NH_4^+$ analysis with a FIAstar 5000 Analyser® (Flow injection system -FIA). The solid residue and the filter paper were digested by the micro-Kjeldahl method in order to obtain the N-prot fraction. Samples (two to four replicates per sample) were digested with 10 ml of concentrated sulphuric acid, 10 ml of H_2O_2 and a 3 g of a

mixture of K_2SO_4:$CuSO_4$:Se (10:1:0.1) as catalysers, at 450ºC for 30 minutes; distilled in a Foss Kjeltec 2200 Auto Destillation® with 40% w/v sodium hydroxide and a 2% boric acid solution (Laboratorios MALAB), and finally titrated with 0.1 N HCl factor 1. The percentage of N-prot was calculated according to the following equation:

$$\%\ \text{N - prot} = \frac{\text{ml HCl} * \text{HCl factor} * 0.1 * 100 * 14}{1000 * [\text{sample weight (g)}]}$$

Final results were expressed as mg of nitrogen per g of dried sample (mg N g^{-1} dw).

FIA method for N-NH_4^+ determination. The determination of N-NH_4^+ was carried out in a FIAstar 5000 Analyzer equipped with an ammonia cassette including a gas diffusion membrane, and a 5027 Auto-Sampler, using mQ water as carrier, sodium hydroxide 0.5 M as Reagent 1, and the acid-base indicator solution as Reagent 2. Then, 40 µl of the eluted solution from N-prot extraction were injected into a carrier stream merged with a sodium hydroxide stream, and the colour shift measured photometrically at 590 nm. Values were expressed as µg of nitrogen per g of dried sample (mg N g^{-1} dw).

FIA method for N-NO_3^-+NO_2^- determination. The N-NO_3^-+NO_2^- were extracted from 0.5 g of dry pounded bark from annual shoots, with 50 ml of mQ water, shaken for 30 minutes and filtered with a 90 mm Schleicher and Shvell paper. Then, 200 µl of the extracted solution were injected in the FIAstar 5000 Analyser equipped with a nitrite cassette, dialysis membrane and cadmium-reducing column. The colour shift was measured at 540 nm. Values were expressed as µg of nitrogen per g of dried sample (mg N g^{-1} dw).

Statistical analysis

Linear regressions and Analysis of Variance were performed on the data, and means separated using Duncan's multiple range test for means separation.

Results and discussion

The sugar and starch concentrations in the bark tissue of the different *Prunus* species studied varied significantly with chilling accumulation (Table 10.1).

Sorbitol, fructose and glucose were the main sugars in bark tissues of peach, nectarine, plum and apricot (Table 10.1), and their concentration tended to increase with increasing chilling and decrease for higher CH H treatments. Sorbitol concentration reached its maximum at 200 CH H in peach (+26.1% compared with non-chilling treatment), at 400 CH H in nectarine (+9.3%), at 800 CH H in plums (+19.0%) and at 1200 CH H in apricots (+38.2%). This increase of sorbitol with increased chilling agrees with the results obtained by Hansen and Grauslund (1978), who showed that sorbitol levels generally increase in the limb sap of apple trees when the temperature decreases during the dormant season. Sorbitol concentration decreased significantly in peach (-23.7%), nectarine (-33.5%) and plum (-40.1%) for higher CH H used, but not in apricot (+8.4%), compared with the non-chilling treatment.

Fructose concentration increased significantly with increased chilling up to 800 CH H treatment in peach, nectarine and plum, and up to 600 CH H in apricot (Table 10.1). However, exposures to 1000 CH H or higher caused a significant decrease of fructose concentration, reaching higher (peach, plum and apricot) or lower (nectarine) values compared with non-chilling treatments (Table 10.1). Glucose behaved similarly to fructose, increasing its concentration at 600 and 800 CH H and showed a decrease with 1000 or 1600 CH H (Table 10.1). But in this case, the final concentration was higher in peach and apricot, equal in nectarine and lower in plum, compared with the non-chilling treatment (Table 10.1).

Sucrose and raffinose were the sugars of least concentration in the bark tissue (Table 10.1). Similarly to sorbitol, sucrose changes caused by increased chilling showed different responses according to species, reaching the highest concentrations at 200 CH H in peach and nectarine (+16.7% and +22.2%, respectively with respect to non-chilling treatment), 400 CH H in plums (+90.5%) and 1200 in apricots (+302.6%) (Table 10.1). Raffinose, although reaching its mayor concentrations at 200 CH H in peach and nectarine and 400 CH H in plum and apricot, did not show a uniform tendency with chilling between species (Table 10.1).

As a consequence of changes in the sugar concentration caused by chilling, total sugar concentration increased significantly in all *Prunus* spp. at low and moderate CH H treatments (Figure 10.1A), and major variations were observed in plum and apricot. Sorbitol and sucrose are the main transport sugars in *Prunus* spp. (Bieleski, 1982; Lo Bianco *et al*., 1999), and their total concentration did not have a uniform tendency with chilling treatments (Figure 10.1B). Starch concentration showed a significant decrease due to chilling (Figure 10.1C). Major changes with respect to the

non-chilling treatment were observed at 800 CH H in peach and nectarine (≈-35.0%) compared with plum (-24.0%) and apricot (-30.0%). Similarly, starch decreased in the phloem tissue of apple trees during bud development and budbreak (Priestley, 1981; Wang *et al*., 1987).

In sweet cherry (*Prunus avium* L.), like the peach tree a sorbitol-synthesizing plant, Keller and Loescher (1989) observed that sucrose is the most predominant soluble carbohydrate during dormancy. In our experiment, the four *Prunus* species studied showed a minor concentration of sucrose compared with sorbitol, fructose and glucose. Moreover, sorbitol appeared to be the main carbohydrate in peach and nectarine, as was also found in other studies (Maurel *et at.*, 2004; Lo Bianco and Rieger, 1999).

The energy source for budbreak comes mainly from the mobilization of products stored in the perennial parts of the tree (Bonhomme, 1998; Brunel, 2001). On that score, starch is known to serve as an important carbohydrate reserve during an extended period that commences at the final leaf drop and continues until some weeks after budbreak (Flore and Layne, 1996). It is interesting to note from our results, that the final increase in starch concentration observed in the four species of the highest chilling treatment (1000 and 1600 CH H), is coincident with the decrease in total sugar concentration (see Figure 10.1A and 10.1B). Although these results could suggest a possible re-synthesis of starch when high chilling is accumulated, there is an overall negative balance in carbohydrate reserves as a consequence of metabolic consumption during the rest period and chilling accumulation. We thus found a significant relationship ($P \leq 0.01$) between starch and total sugars, fitting the following general linear model:

Total Sugar = (-0.81) x Starch + 99.51

Individual models for each species showed a better correlation when data from 0 to 800 CH H was considered in peach ($r^2 = 0.68$, $P \leq 0.01$), nectarine ($r^2 = 0.35$, $P \leq 0.01$), plum ($r^2 = 0.95$, $P \leq 0.01$) and apricot ($r^2 = 0.82$, $P \leq 0.01$). These results are in agreement with Wang and Faust (1987) who found a marked increase in amounts of sorbitol, fructose, glucose and sucrose in the bark tissue of apple shoots, from autumn to mid-winter, coinciding with a decrease in starch content. Moreover, in our experiment with stone fruits the time course of fructose, glucose and starch, from 0 to 800 CH H, showed the same tendency described by Wang and Faust (1987) for pome fruits. However, a significant decrease in total sugar and transport sugar concentration was observed when chilling was higher than 800 CH H (peach, nectarine and plum) or 1200 CH H (apricot). Similarly, Keller and

Loescher (1989) found a significant decrease just before budbreak in total non-structural carbohydrate in all perennial tissues of sweet cherry.

Recent studies under field conditions showed that cold temperatures during the rest period induce starch hydrolysis and an increase in soluble sugars due to amylase activity in poplar (Elle and Sauter, 2000) and peach (Bonhomme *et al*., 2005), resulting in better frost resistance (Sauter, 1988). Our results indirectly confirm this by using excised shoots under different chilling conditions.

In order to evaluate the differences in the sugar content between species, we calculated the mean concentration of the sugar irrespective of the chilling accumulation treatment (Figure 10.2A), using peach as the standard for comparison. Similarly, we calculated the mean total sugars, transport sugars and starch concentration of each species (Figure 10.2B).

Ammonium nitrogen ($N\text{-}NH_4^+$) increased significantly at 600 CH H with respect to no chill treatment, but decreased at 800 CH H in apricot and at 1000 CH H or higher in peach, nectarine and plum (Figure 10.3A). No significant changes were observed in nitric nitrogen ($N\text{-}NO_3^-+NO_2^-$) (Figure 10.3B) and protein nitrogen (N-prot) (Figure 10.3C) concentration with chilling accumulation. The increase of $N\text{-}NH_4^+$ may be related to the reported change in free amino acid content in dormant buds of *Pyrus calleryana* Decne (Zimmerman and Faust, 1969) and peach (Torrigiani *et al.*, 1978). Wang and Faust (1987) suggest that the nucleic acid concentration increases in buds of cherry and apple during the cooler months up to a certain threshold value, where plants come out of rest. The increase in $N\text{-}NH_4^+$ without an increase in N-prot could suggest a link between our results and the previous findings of Wang and Faust (1987).

Endodormancy was interpreted for many years as a balance of inhibitors and growth promoters (Crabé and Barnolá, 1996). However, the mechanisms controlling induction and release of bud dormancy are more complex and involve interactions among different kinds of compounds (Kozlowski and Pallardy, 1997). Thus, metabolic activity during dormancy includes the modification of plasmalemma fluidity (Pétel and Gendraud, 1996), changes in enzymatic activity (Pétel and Gendraud, 1987, 1993), the reestablishment of nutrient flux to the bud tissues (Pétel and Gendraud, 1996), etc., and carbohydrates are necessary as a source of energy for metabolic changes (Flore and Layne, 1996). In this work we demonstrated that in excised shoot of different *Prunus* species exposed to low temperatures, significant changes in sugar and starch concentration occurred, in agreement with previous observations in the storage tissues of

the whole apple tree during winter (Wang *et al*., 1987) or in the nearby structures of peach buds under field conditions (Bonhomme *et al*., 2005). Therefore, the excised shoot method, proposed for the study of the evolution of endodormancy (Dennis, 2003), could also be considered for studying the tendency in the quantitative changes that occur in different compounds involved in dormancy and bud release. Furthermore, despite the fact that dormancy is located in the buds (Van der Schoot, 1996), chilling also induces changes in the metabolism of the adjacent tissue, which occur independently of the presence of terminal buds, and which may have a direct or indirect effect on bud dormancy.

In conclusion, the exposure of stem cuttings of different *Prunus* species to low temperatures caused significant variations in sugar, starch and ammonium nitrogen contents. Starch concentration decreased and total sugar increased with low or moderate chilling treatments. On the other hand, with high chilling accumulation treatments (1000 or higher CH H), starch concentration increased while total sugar concentration diminished. This confirms the importance of starch as a mayor storage material in all *Prunus* spp. studied during the rest period, as had been shown earlier in peach (Stassen *et al.*, 1981) and sweet cherry (Keller and Loescher, 1989). In our work we confirmed the increase of ammonium nitrogen, with no modification in protein or nitric nitrogen levels with chilling, in all the species studied.

References

AOAC. 1984. Williams, S. (Ed.). *Official Methods of Analysis of the Association of Official Analytical Chemists*. Association of Official Analytical Chemists, Arlington, Virginia.

Arias, O. and Crabbé, J. 1975. Les gradients morphogénétiques du rameau dún an des végétaux ligneux en repos apparent. Données complémentaires fournies par l'étude de *Prunus avium* L. Physiologie Végetale 13:69-81.

Balandier, P.; Bonhomme, M.; Rageau, R.; Capitan, F. and Parisot, E. 1993. Leaf bud endodormancy release in peach trees: evaluation of temperature models in temperate and tropical climates. Agricultural and Forest Meteorology 67: 95-113.

Beljaars, P.R.; Van Dijk, R. and Van der Horst, G.M.J. 1994. Determination of Nitrate in Vegetables by Continuous Flow: Interlaboratory Study. Journal of AOAC International 77: 1522-1529.

Bieleski, R.L. 1982. Sugar alcohols. In: Loewus, E.A. and Tanner, W. (Eds.). *Plant Carbohydrates. I. Intracellular carbohydrates.*

Encyclopaedia of Plant Physiology, New Series, Vol. 13A. Springer-Verlag, Berlin, pp. 158-192.

Bonhomme, M. 1998. Physiologie des bourgeons végétatifs et floraux de pêcher dans deux situations thermiques contrastées pendant la dormance : capacité de croissance, force de puits et répartition des glucides. PhD thesis, Blaise Pascal University, France.

Bonhomme, M.; Regeau, R.; Lacointe, A. and Gendraud, M. 2005. Influences of cold deprivation during dormancy on carbohydrate contents of vegetative and floral primordia and nearby structures of peach buds (*Prunus persica* L. Batch). Scientia Horticulturae 105: 223-240.

Brunel, N. 2001. Etude du déterminsme de la préséance des bourgeons le long du rameau d'un an che le pommier (*Malus domestica* [L.] Borkh.). PhD thesis, Angers University, France.

Citadin, I.; Raseira, M.C.B.; Verter; F.G. and Baptista da Silva; J. 2001. Heat requirement for blooming and leafing in peach. HortScience 36: 305-307.

Crabbé, J. and Barnola, P. 1996. A new Conceptual approach to bud dormancy in woody plants. In: Lang, G.A. (Ed.). *Plant Dormancy: Physiology, Biochemistry and Molecular Biology*. CAB International, pp. 83-113.

Dennis Jr.; F.G. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38: 347-350.

Elle, D. and Sauter, J.J. 2000. Seasonal changes of activity of a starch granule bound endoamylase and a starch phosphorylase in poplar wood (*Populus x Canadensis* Moench "*robusta*") and their possible regulation by temperature and phytohormones. Journal of Plant Physiology 156: 731-740.

Faust, M. 1989. *Physiology of temperature zone fruit trees*. John Wiley & Sons, New York, USA.

Faust, M.; Erez, A.; Rowland, L.J.; Wang, S.Y. and Norman, H.A. 1997. Bud dormancy in perennial fruit trees: physiological basis for dormancy induction, maintenance, and release. HortScience 32: 623-629.

Flore, J.A. and Layne, D.R. 1996. *Prunus*. In: Zamski, E. and Schaffer, A.S. (Eds.). *Photoassimilate distribution in plants and crops. Source-sink relationships*. Marcel Dekker, Inc., pp. 797-823.

Fuchigami, L.H.; Wisniewsky, M. 1997. Quantifying bud dormancy: Physiological approaches. HortScience 32: 618-623.

Gibson, S.I. 2000. Plant sugar-response pathways. Part of a complex regulatory web. Plant Physiology 124: 1532-1539.

Hanninen, H. 1995. Effects of climatic change on trees from cool and temperate regions; an ecophysiological approach to modelling of bud burst phenology. Canadian Journal of Botany 73: 183-199.

Hansen, P. and Grauslund, J. 1978. Levels of sorbitol in bleeding sap and in xylem sap in relation to leaf mass and assimilate demand in apple trees. Physiologia Plantarum 42: 129-133.

Keller, J.D. and Loescher, W.H. 1989. Nonstructural carbohydrate partitioning in perennial parts of sweet cherry. Journal of the American Society of Horticultural Science 114: 969-975.

Kozlowski, T.T. and Pallardy, S.G. 1997. Physiological regulation of vegetative growth. In: Kozlowski, T.T. and Pallardy, S.G. (Eds.). *Growth control in woody plants*. Academic Press, pp. 73-165.

Lang, G.A. 1996. Plant dormancy. Physiology, biochemistry and molecular biology. CAB International, pp. 386.

Lang, G.A; Early, J.D.; Martin, G.C. and Darnell, R.L. 1987. Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. HortScience 22: 371-378.

Lang, D.K.; Schulze, E.D.; Evenari, M.; Kappen, L. and Buschbam, U. 1978. The temperature related photosynthetic capacity of plants under desert conditions. I. Seasonal changes in the photosynthetic response to temperature. Oecologia 17: 97-110.

Levey, D.J.; Bissell, H.A. and O´Keefe S.F. 2000. Conversion of nitrogen to protein and amino acids in wild fruits. Journal of Chemical Ecology 26 (7): 1749-1763.

Lo Bianco, R. and Rieger, M. 1999. Activities of sucrose and sorbitol metabolizing enzymes in vegetative links of peach and correlation with sink growth rate. Journal of the American Society of Horticultural Science 124 (4): 381-388.

Lo Bianco, R.; Rieger, M. and Sung, S-J.S. 1999. Carbohydrate metabolism of vegetative and reproductive sinks in the late-maturing peach cultivar 'Encore'. Tree Physiology 19: 103-109.

Maurel, K.; Sakr, S.; Gerbe, F.; Guilliot, A.; Bonhomme, M.; Rageau, R. and Pétel, G. 2004. Sorbitol uptake is regulated by glucose through the hexokinase pathway in vegetative peach-tree buds. Journal of Experimental Botany 55 (398): 879-888.

Mehouachi, J.; Serna, D.; Zaragoza, S; Agustí, M.; Talon, M. and Primo-Milo, E. 1995. Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. Plant Science 107: 189-197.

Moing, A.; Carbonne, F.; Rashad, M.H.; Gaudillère, J.P. 1992. Carbon fluxes in mature peach leaves. Plant Physiology 100: 1878-1884.

Pétel, G. and Gendraud, M. 1987. Activité d´un système oxydoréducteur au niveau du plasmalemme des cellules de parenchyme de topinambour

(*Helianthus tuberosus* L.) en relation avec les propriétés morphogénétiques des tubercules. Comptes Rendus de l´Académie des Sciences de Paris 305:51-54.

Pétel, G. and Gendraud, M. 1993. ATP- and NADH-dependent membrane potential generation in plasmalemma enriched vesicles from parenchyma of dormant and non-dormant Jerusalem artichoke tubers. Biologia Plantarum 35: 161-167.

Pétel, G. and Gendraud, M. 1996. Processes at the plasma membrane and plasmalemma ATPase during dormancy. In: Lang, G.A. (Ed.). *Plant Dormancy. Physiology, biochemistry and molecular biology*. CAB International, pp. 233-243.

Priestley, C.A. 1981. Perennation in woody fruit plants and its relationship to carbohydrate turnover. Annals of Applied Biology 98: 548-552.

Raigón, M.D.; Pérez-García, M.; Maquieira, A.; Puchades, R. 1992. Determination of available nitrogen (nitric and ammoniacal) in soils by flow injection analysis. Analisis 20: 483-487.

Sauter, J.J. 1988. Temperature induced changes in starch and sugars in the stem of *Populus x Canadensis "robusta"*. Journal of Plant Physiology 132: 608-612.

Sheen, J.; Zhou, L. and Jang, J.C. 1999. Sugars as signalling molecules. Current Opinion in Plant Biology 2: 410-418.

Sherson, S.M.; Alford, H.L.; Forbes, S.M.; Wallace, G. and Smith, S.M. 2003. Roles of cell-wall invertases and monosaccharide transporters in the growth and development of *Arabidopsis*. Journal of Experimental Botany 54: 525-531.

Shirazi, A.M. 2003. Standarizing methods for evaluating the chilling requirements to break dormancy in seeds and buds (Including Geophytes): Introduction to the workshop. HortScience 38: 334-335.

Smeekens, S. 2000. Sugar-induced signal transduction in plants. Annual Review of Plant Physiology and Plant Molecular Biology 51: 49-81.

Stassen, P.J.C.; Strydom, D.K. and Stindt, H.W. 1981. Seasonal changes in carbohydrate functions of young Kakamas peach trees. Agroplantae 13: 47-53.

Torrigiani, P.; Marino, G. and Bagni, N. 1978. In vitro protein synthesis by polyribosomes of peach flower buds at different physiological stages. Physiolia Plantarum 43: 237-241.

Van der Schoot, C. 1996. Dormancy and Symplasmic Networking at the shoot apical meristem. In: Lang, G.A. (Ed.). *Plant Dormancy. Physiology, biochemistry and molecular biology*. CAB International, pp. 59-81.

Wang, S.Y. and Faust, M. 1987. Metabolic activities during dormancy and blooming of deciduous fruit trees. Israel Journal of Botany 37:227-243.

Wang, S.Y.; Ji, Z.L. and Faust, M. 1987. Metabolic changes associated with bud break induced by thidiazuron. Journal of Plant Growth Regulation 6: 85-95.

Weinberger, J.H. 1950. Chilling requirements of peach varieties. Proceedings of the American Society of Horticultural Science 56: 122-128.

Westwood, M.N. 1993. *Temperate zone pomology*. Timber Press, Portland, Oreg.

Zimmerman, R.H. and Faust, M. 1969. Pear and peach metabolism: seasonal changes in glucose utilization. Plant Physiology 44: 1273-1276.

Table 10.1: Effects of low temperature exposure (3.0 ± 0.1°C) on sugar concentration (mg g^{-1} dw) in the bark tissue of different *Prunus* species.

	Chilling Hours (CH H)							
	0	200	400	600	800	1000	1200	1600
Sorbitol								
Peach	20.32 bAB	25.63 aA	24.22 abA	18.36 bcAB	21.5 abAB	15.50 cA	nd	nd
Nectarine	21.99 abA	24.04 aA	24.21 aA	19.22 bA	21.40 abAB	14.63 cA	nd	nd
Plum	16.07 abB	17.99 abA	17.71 abB	13.71 bB	19.13 aB	9.62 cB	nd	nd
Apricot	23.43 bA	nd (1)	26.01 bA	22.85 bA	25.99 bA	nd	32.38 a	25.40 b
Fructose								
Peach	14.45 bA	17.06 abA	17.17 abB	17.18 abB	20.77 aB	15.69 bA	nd	nd
Nectarine	14.46 bA	17.56 abA	18.02 abB	15.93 abB	19.62 aB	11.57 bB	nd	nd
Plum	13.96 dA	20.91 cA	28.35 abA	24.64 bA	31.10 aA	14.42 dAB	nd	nd
Apricot	16.96 bA	nd	25.94 aA	28.19 aA	27.63 aA	nd	26.01 a	25.21 a
Glucose								
Peach	15.62 cB	17.42 bcB	17.94 bcB	19.44 bB	22.98 aB	18.28 bcA	nd	nd
Nectarine	14.28 bB	16.44 abB	18.69 aB	16.93 abB	18.63 aB	14.66 bB	nd	nd
Plum	19.22 cA	25.44 bA	26.48 bA	30.03 aA	30.34 aA	18.84 cA	nd	nd
Apricot	20.03 dA	nd	25.37 cA	32.81 aA	29.80 abA	nd	29.43 b	27.59 bc
Sucrose								
Peach	2.16 abAB	2.52 aB	2.47abB	1.94 abBC	2.21 abB	1.23 bB	nd	nd
Nectarine	2.34 abAB	2.86 aB	2.35 abB	1.38 bC	2.34 abB	0.99 bB	nd	nd
Plum	3.35 cA	5.55 abA	6.38 aA	4.08 bcA	5.11 bA	3.72 cA	nd	nd
Apricot	1.15 cB	nd	3.57 abB	2.73 bB	2.71 bB	nd	4.63 a	2.20 bc
Raffinose								
Peach	0.38 bcAB	1.34 aAB	1.28 aB	0.59 bA	0.99 abB	0.36 cAB	nd	nd
Nectarine	0.30 bB	0.97 aA	0.38 bC	0.49 abA	0.43 abC	0.17 bB	nd	nd
Plum	0.90 cA	1.72 bA	2.60 aA	0.49 cA	2.63 aA	0.70 cA	nd	nd
Apricot	0.21 cB	nd	1.77 aB	0.21 cA	0.85 bBC	nd	0.93 b	0.47 bc

Means followed by different lower-case letters in the same line and by different capital letters in the same column differ significantly ($P \leq 0.05$).
(1) nd: not determined.

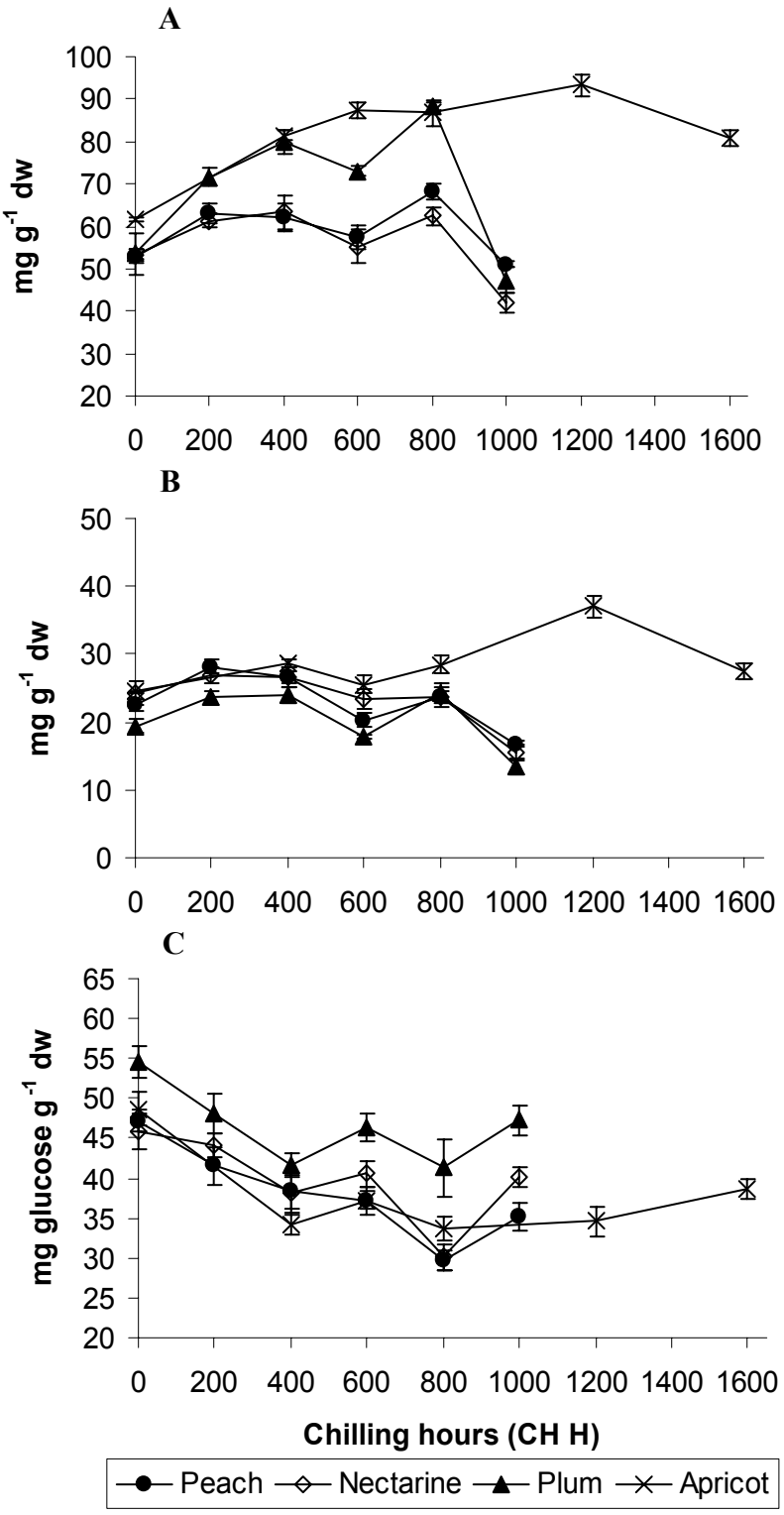

Figure 10.1: Total sugar (A), transport sugars -sorbitol+surcrose- (B) and starch (C) concentration changes caused by chilling (CH H) at 3.0 ± 0.1°C in stem cuttings of *Prunus* spp. Standard errors are given as vertical bar.

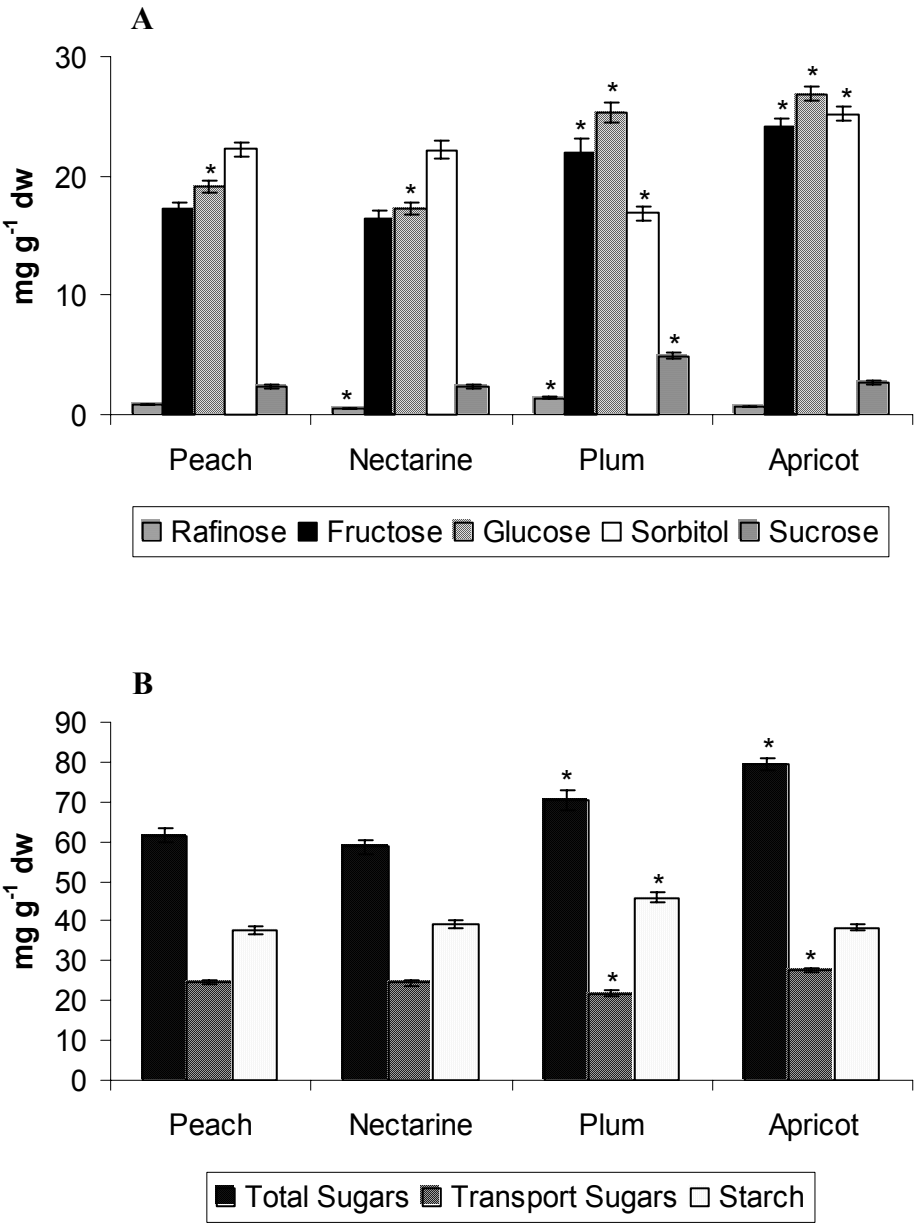

Figure 10.2: Sugar concentrations (A) and total sugar, transport sugar and starch (B) concentrations in the bark tissue of peach, nectarine, plum and apricot stem cuttings. Starch concentration is represented as mg of glucose liberated per g of dry sample. Sugar concentration standard error for each species is represented by the vertical bar. Asterisks denote statistical difference, at $P \leq 0.05$.

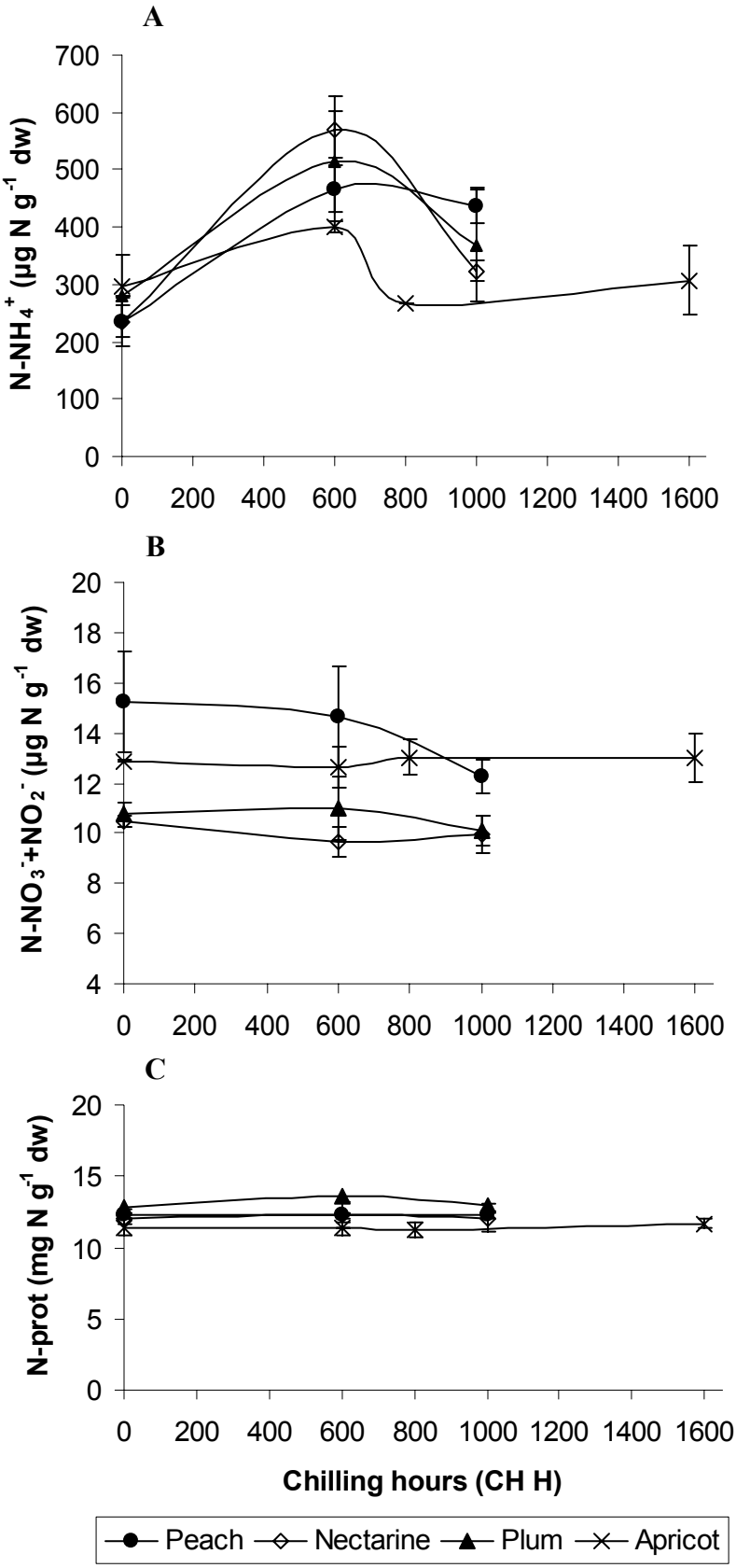

Figure 10.3: Ammonium nitrogen ($N\text{-}NH_4^+$) (A), nitric nitrogen ($N\text{-}NO_3^-+NO_2^-$) (B) and protein nitrogen (N-prot) (C) changes caused by chilling (CH H) at 3.0 ± 0.1°C in stem cuttings of *Prunus* spp. Standard errors are given as vertical bar.

CHAPTER 11

General discussion

11- General discussion

Our results demonstrate an inhibitory effect of fruit on the flowering process through a direct and indirect effect. Indirectly, by reducing the number of developing shoots, their length, number of nodes and, consequently, number of buds; directly, by reducing the number of floral buds. These effects have not been linked to any carbohydrate's requirements, except those related to energy availability to sprout, as has been shown for some woody species (García-Luis *et al.*, 1995; Davenport, 1986), but not for *Prunus* spp. The effect of fruit has been related to hormone synthesis, being the gibberellins, among them, which have been recognized as the hormones inhibiting flowering in woody perennials (Goldschmidt and Samach, 2004). They are synthesised in the seed and transported to the subjacent buds (Luckwill, 1970; Hoad, 1978; Pharis and King, 1985), which are, then, inhibited to flower.

Application of gibberellic acid during the floral induction period reduced significantly the number of developed floral buds both in peaches and plums, which confirms that the process of flowering can be interrupted by gibberellins, and it can be used as a useful technique to improve growers' returns. In fact, it reduces flowering by 40%-50% and, indirectly, the cost of hand thinning is also reduced by 45%, approximately. However, floral buds distribution along the shoot results asymmetrical by cause of gibberellic acid treatment, with a minor density of floral buds located in the proximal section, increasing their number closer to the distal shoot segment. It requires taking safety measures at the time of pruning in order not to remove distal segments of all branches. On the other hand, the magnitude of the response depended on the concentration applied, being 50 mg l^{-1} the most appropriate one according to our results; however, this concentration should be subjected to testing for specific cultivars and climatic conditions in order to avoid excessive depletion of the number of developing fruits and, consequently, of the final yield.

Since not all buds close to fruit or treated with gibberellic acid are inhibited, but all of them receive the same climatic signal to develop flowers, we hypothesize that flowering is not an inductive process but an inhibitory process, as has been suggested for other species (Goldschmidt and Samach, 2004; Martínez-Fuentes *et al*., 2004). That is, all buds have enough information to develop flowers but if any inhibitory factor acts they reverse to a vegetative bud. In this way, we have explored about the carbohydrates and nitrogen fractions contents in order to clarify if they are implicated in the floral process or not, and if they can explain differences between neighbouring buds. According to our results, neither soluble

sugars in phloem sap of shoots nor the carbohydrate reserves in fibrous roots have been associated to the flower bud initiation; however, evidences of some disturbance of the nitrate reduction process in trees tending to flower scarcely are shown. This is not different of that reported for other fruit species (García-Luis *et al*., 1995; Goldschmidt *et al*., 1985; Lovatt *et al*., 1988; Monselise *et al*., 1981). Further, the application of paclobutrazol, an inhibitor of gibberellins synthesis, did not modified flowering intensity, but significantly increased glucose and fructose and decreased sucrose in the roots. Why paclobutrazol failed increasing flowering on the current year was not the subject of this PhD Thesis and cannot be explained from our experiments. Concentration applied, date of treatment, difficulties to absorb and translocate into the tree, etc. are some questions to be answered from new experiments.

Experiments giving artificial chilling in the lab to cuttings gave rise to several conclusions about floral and vegetative buds development with regard to chilling hour concept. Thus, excessive chilling diminished the percentage of flower budbreak in low chilling cultivars, which may be due to physiological injury to the buds. We also demonstrated that MTB value of leaf buds decreased faster with chilling, compared with flower buds, up to a point of critical chilling accumulation (PCCHA) where MTB was equal for both, flower and leaf buds. On the other hand, there is a negative correlation between the number of chill hours accumulated till budbreak and the number of heat units accumulated from then to full bloom, being vegetative buds more sensitive than floral buds. Our results also prove that chilling induces metabolic changes, both in carbohydrates and nitrogen fractions, in the adjacent tissue of buds, which may have a direct or indirect effect on bud dormancy. And finally, the evolution of bud weight was highly influenced by temperature during rest, that is, budbreak starts when bud weight triples the initial weight at leaf fall in both floral and vegetative buds, following an exponential function.

The method of excised shoots is at present used to study the evolution of endodormancy in leaf buds, extracting twigs at different times during winter (Balandier *et al.*, 1993; Dennis, 2003), and our results confirm the utility of the method and suggest that rest of both leaf and flower buds, and not that of leaf buds only, may be studied to clarify the physiological process of dormancy.

References

Balandier, P.; Bonhomme, M.; Rageau, R.; Capitan, F. and Parisot, E. 1993. Leaf bud endodormancy release in peach trees: evaluation of temperature models in temperate and tropical climates. Agricultural and Forest Meteorology 67: 95-113.

Davenport, T.L. 1986. Avocado flowering. Horticultural Reviews 8: 257-289.

Dennis Jr., F.G. 2003. Problems in standardizing methods for evaluating the chilling requirements for the breaking of dormancy in buds of woody plants. HortScience 38: 347-350

García-Luis, A.; Kanduser, M. and Guardiola, J.L. 1995. The influence of fruiting on the bud sprouting and flower induction responses to chilling in *Citrus*. Journal of the American Society of Horticultural Science 70: 817-825.

Goldschmidt, E.E.; Aschkenazi, N.; Herzano, Y.; Schffer, A.A. and Monselise, S.P. 1985. A role for carbohydrate levels in the control of flowering in *Citrus*. Scientia Horticulturae 26: 159-166.

Goldschmidt, E.E. and Samach, A. 2004. Aspects of flowering in fruit trees. Acta Horticulturae 653: 23-27.

Hoad, G.V. 1978. The role of seed derived hormones in the control of flowering in apple. Acta Horticulturae 80: 93-103.

Lovatt, C.L.; Zheng, Y. and Hake, K.D. 1988. Demostration of a change in nitrogen metabolism influencing flower initiation in *Citrus*. Israel Journal of Botany 37: 181-188.

Luckvill, L.C. 1970. The control of growth and fruitfulness of apple trees. In: Luckvill, L.C. and Cutting, C.V. (Eds.). *Physiology of tree crops*. London, New York: Academic Press, pp. 237-254.

Martinez-Fuentes, A.; Mesejo, C.; Juan, M.; Almela, V. and Agustí, M. 2004. Restrictions on the exogenous control of flowering in *Citrus*. Acta Horticulturae 632: 91-98.

Monselise, S.P. and Goldschmidt, E.E. 1981. Alternate bearing in citrus and ways of control. Proceedings of the International Society of Citriculture 1: 239-242.

Pharis, R.P. and King, R.W. 1985. Gibberellins and reproductive development in seed Plants. Annual Review of Plant Physiology 36: 517-568.

CHAPTER 12

General conclusions

12- General conclusions

1) The fruit has an inhibitory effect on the flowering process indirectly by reducing the number of developing shoots, and directly by reducing the number of floral buds.

2) Carbohydrates are related to energy availability to sprout but are not to the flower initiation process.

3) Our results indicate some disturbance in the nitrate reduction process in trees tending to flower only slightly.

4) The application of GA_3 at flowering induction period reduces flowering by 40%-50%. GA_3 affects the photosynthetic efficiency and the energy dissipation process, causing photoinhibition, being young trees more sensible than adult trees. The presence of fruit inhibits partially the effect of GA_3 on the maximum quantum yield of PSII.

5) 50 mg l^{-1} GA_3 reduces the cost of hand thinning in peach, nectarine and plum by 45%-50%. The application, however, causes an asymmetrical distribution of floral buds along mixed shoots.

6) Paclobutrazol and prohexadione-Ca, two inhibitors of GAs biosynthesis, were ineffective in inducing flowering when applied at the period of flower bud induction, the following season.

7) Flower buds had generally a lower intensity of rest and greater heat requirements than leaf buds. Excessive chilling diminishes the percentage of flower budbreak in low chilling cultivars.

8) The MTB value of leaf buds decreases faster with chilling, compared with that of flower buds, up to a point of critical chilling accumulation where the MTB for both, vegetative and floral buds, coincides.

9) The cutting test has shown that chilling induces metabolic changes, in both, carbohydrate and nitrogen fraction concentrations.

10) The cutting test measuring the response of vegetative and floral buds provides considerable information on the study of dormancy.