

¹ CITA-DGA, Campus de Aula Dei, Zaragoza, Spain

² Estación Experimental de Aula Dei -CSIC, Campus de Aula Dei, Zaragoza, Spain

Flower bud drop and pre-blossom frost damage in apricot (*Prunus armeniaca* L.)

Carme Julian¹, Maria Herrero², Javier Rodrigo¹

(Received January 17, 2007)

Summary

Apricot (*Prunus armeniaca* L.) is a species particularly prone to erratic fruit set and flower bud drop has been repeatedly reported in different cultivars and growing conditions. A number of potential causes have been explored, but a clear main cause remains elusive. However, the fact that bud drop can vary depending on the year or site suggests that the process is somehow environmentally influenced. In this work bud development and bud damage have been related with frost temperatures during endo and ecodormancy. Two years of experiments showed that as it might be expected, no frost damage was recorded during endodormancy, when the buds are fulfilling their chilling requirements. Likewise, frost damage took place, when frost occurred after bud burst. But unexpectedly, flower buds were particularly vulnerable to frost damage when they enter in ecodormancy, which occurs once chilling requirements are fulfilled but prior to bud swelling. At this time, while no external symptoms were detected in an apparent still quiescent stage, internal damage was observed as perpendicular voids characteristics of ice formation that resulted in browning of tissues in the stamens, pistil or both. Vulnerability appeared concomitantly with the establishment of vascular connections first in the anthers and later in the pistil, and differences between buds in the stage of development explain differences in bud damage for a same frost episode. The proportion of frost damaged buds clearly contributed to the proportion of subsequent bud drop that occurred later. These results provide evidence for the contribution of frost damage to flower bud drop in apricot, showing that there is a stage particularly prone to frost damage, once the buds have completed their chilling requirements and prior to bud-burst, when the first xylem connections are established. The identification of the cause of a phenomenon that is widespread in this species and that is a limiting factor in cultivar adaptation will contribute to understand and select new cultivars for new areas.

Introduction

Apricot (*Prunus armeniaca* L.) is a species particularly prone to erratic productions and this behaviour has been related to the narrow adaptability of this species (LAYNE et al., 1996). Thus, most apricot cultivars are highly specific in their ecological requirements and low yields are often obtained whenever cultivars are grown in other regions. Climatic adaptation is one of the main objectives in most apricot breeding programmes (HORMAZA et al., 2006), but the causes behind this low adaptability are not clear. While no vegetative problems are usually recorded, crop failure is mainly related to pre- and post-blossom problems, since pollination and fertilisation are essential to set a fruit in apricot (RODRIGO and HERRERO, 1998) as well as in other *Prunus* species (DITTMANN and STOSSER, 1999). Thus, two main causes are behind fruit set failure after flowering: pollination problems (RODRIGO and HERRERO, 1996) and spring frost damage (GUNES, 2006). Prior to flowering, flower bud drop is the main limiting factor.

While flower bud drop in apricot has been repeatedly reported in different cultivars, the causes responsible for this behaviour are unclear and a variety of potential causes have been explored, as chilling

requirements (BROWN, 1953; LEGAVE, 1978a), irrigation (BROWN, 1953), type of shoot (ALBURQUERQUE et al., 2003), flower density (ALBURQUERQUE et al., 2004), climatic areas (LEGAVE et al., 1982; 1984) or early defoliation and high pre-blossom temperatures (MARTÍNEZ-GÓMEZ et al., 2002). While all these factors surely play a part, a clear main cause that explains flower bud abscission in apricot still remains elusive. The main limitation to elucidate this problem relies in that no apparent differences can be observed between the buds that are going to fall and those that carry on development, and also that since buds have differentiated some six months ahead quite a number of things have happened by the time bud drop is manifested. However, the fact that bud drop can vary not only between cultivars, but also for a same cultivar depending on the year or site (RODRIGO and HERRERO, 2002) suggests that the process is somehow environmentally influenced.

After dormancy and during the pre-blossom period, flower buds are exposed to variable climatic conditions from the end of winter to the beginning of spring. In this period frost temperatures can be easily registered in most temperate zones and the stage of development is the most important parameter in the resistance to frost injury (PROBSTING and MILLS, 1978). Thus, most of the work done on frost damage in reproductive organs of fruit trees is concentrated either during endodormancy (ASHWORTH and WISNIEWSKI, 1991) or in the post flowering period (RODRIGO, 2000). However, less is known about a transition period called ecodormancy, that occurs once chilling requirements are fulfilled but prior to any external apparent sign of bud swelling (LANG et al., 1987), when the capacity to deep supercool is gradually lost (ANDREWS et al., 1983). In this work flower bud drop is evaluated in relation to frost. For this purpose bud drop was characterised in orchard conditions along two years of experiments and analysed in relation to temperature records. The first frost injury symptoms have been characterized, examining the anatomical effects caused by frosts in flower buds during ecodormancy and evaluating their implication on the subsequent flower bud drop. Furthermore, an histological examination of buds with no apparent external differences has revealed the key factors that make buds vulnerable to frost damage.

Material and methods

Plant material

Eight mature apricot trees of cultivar Moniqui were selected from an experimental orchard planted in 1992, at 6 x 6 m and grafted on 'Montizo' plum rootstock. The orchard is located at the CITA-DGA in Montañana (Zaragoza, Spain), 41°44'30"N latitude, 0°47'00"W longitude, and 220 m altitude. Temperature records were registered in a meteorological station (OFICINA DEL REGANTE, 2006) located in the research centre. From dormancy -in December- to flowering in March, 4 to 6 one-year-old shoots 10 - 30 cm long were randomly collected once a week. To characterize flower bud abscission for the two years of experiments, over 1,500 flower buds were weekly counted in marked twigs from before budbreak to 3 weeks after blossom.

Determination of chill units

For two years of experiments, the time when the chilling requirements were covered were estimated. This cultivar has been reported as having requirements of 1050-1150 chill units (CU) (TABUENCA, 1979) and 779-956 hours below 7°C (TABUENCA, 1968). Daily records of temperature were obtained (OFICINA DEL REGANTE, 2006) and the number of chill units was determined according to the Utah model (RICHARDSON et al., 1974) adapted for apricot cultivars (TABUENCA, 1979).

Anatomical characterization

Fresh flower buds were dissected with the help of an ophthalmologic scalpel and observed under a Wild Heerbrugg M8 stereoscopic microscope. Observations were taken weekly on ten flower buds, and records on the stage of developments and on necrotic damage were taken on these buds. For the frost that occurred in 2004 once the cold requirements were fulfilled but prior to bud burst, a sample of 1,000 flower buds was also randomly collected and analysed to estimate the presence or absence of necrotic tissues.

To evaluate the internal structure of the flower bud and frost damage, and to detect the presence of xylem vessels and procambium connexions in flower buds, ten flower buds were weekly sampled and, after the removal of bud scales, fixed in FAA [70% ethanol: acetic acid: formaldehyde (18:1:1, v/v/v)]. Following fixation, the buds were dehydrated in a tertiary butyl alcohol series (70%, 85%, 95% and 100% v/v), embedded in paraffin wax and sectioned at 10 µm in a Reichert-Jung 1130/Biocut rotatory microtome (Reichert-Jung, Heidelberg, Germany). Prior to staining, the sections were rehydrated in HistoClear II (CellPath, Hemel, UK), and in an ethanol series (100%, 70%, 40% v/v). Sections from 5 to 10 flower buds per week were stained with 0.07% calcofluor white in distilled water for cellulose and observed under an Olympus BH2-RFCA microscope with UV epifluorescence using a BP-405 exciter filter and an Y455 barrier filter. Sections were also observed mounted in water for autofluorescence with the same filter combination. Photographs were taken with a Leica DC150 camera connected to the microscope.

Results

Flower bud differentiation started the previous summer and flower buds entered dormancy in stage A (BAGGIOLINI, 1952), but with the rudimentary sepals, petals, stamens and pistil differentiated. In the two years of experiments, the chilling requirements were fulfilled by mid-end January, depending on the year, bud burst occurred at the end of February and flowering at the middle of March. However, at flowering time a proportion of flower buds failed to burst and eventually dropped in the first two weeks following flowering (Fig. 1). While in both years frost episodes occurred during endodormancy prior to the chilling requirements were covered, subsequent frosts were different in both years. In the first year of experiment, frosts mainly occurred during ecodormancy prior to bud burst; while in the second year frosts mainly occurred following bud burst. As it might be expected, frost episodes at the time of bud-burst induced frost damage, which was easily identifiable by the browning of the pistils and sepals. In the first year of experiment, a number of frost episodes occurred while the flower buds were at stage A after fulfilling the chilling requirements but prior to bud burst. The dissection of 1,000 flower buds along 4 weeks showed that 522 remained at stage A at flowering. The examination of these buds revealed that 97% had necrotic symptoms, showing dark stamens (58%) or browning of both stamens and pistil (39%). Upon flowering, a proportion of the flowers presented the same dark stamens and pistil characteristic of early frost damage. This similar

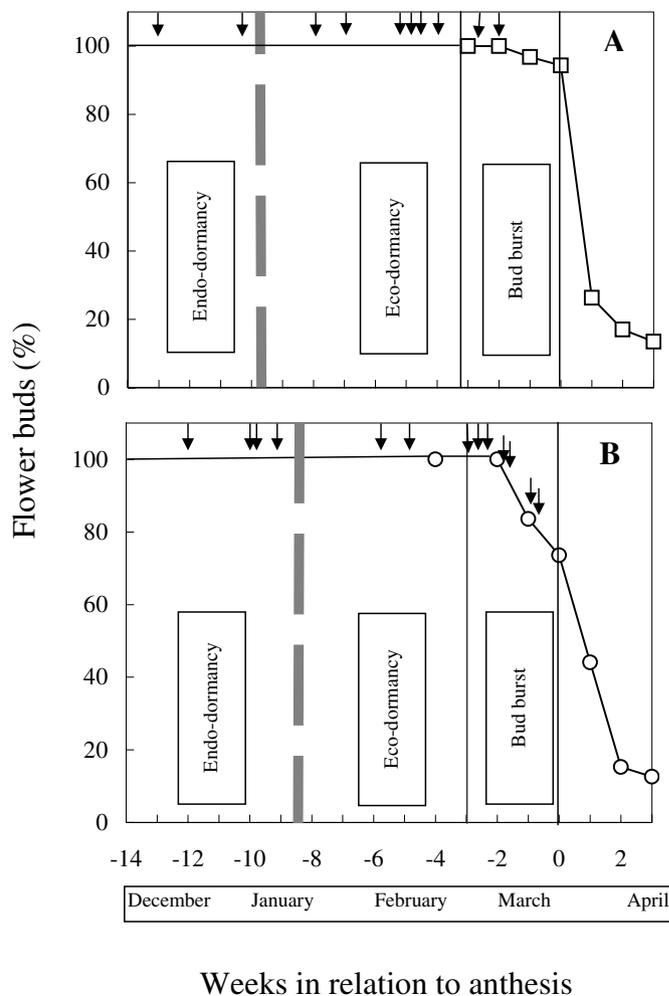


Fig. 1: Flower bud drop, frost episodes (arrows), and fulfillment of the chilling requirements (grey line) over two consecutive years in apricot cv Moniqui.

damage together to the fact that these pistils had the same size than those found in buds at stage A prompted to evaluate whether frost damage at this stage could contribute to this necrosis.

An examination of flower bud anatomy showed that while the first frost episodes during endodormancy did not cause flower bud damage, subsequent frost episodes caused a clear damage. While no external symptoms of frost injury were detected between affected and non-affected flower buds, the first symptoms of internal damage appeared as browning of stamens, pistils or both. Stamens were the first floral tissues to be injured by the early frost episodes after endodormancy, especially those sited in low positions into the hypanthium, whereas the first pistils damaged were detected between two and three weeks later. However, no damage was registered in non-reproductive organs as sepals and petals.

Histological examinations of undamaged (Fig. 2A, 2C, 2E, 2G) and damaged buds (Fig. 2B, 2D, 2F, 2H) of apparently the same stage A (BAGGIOLINI, 1952) show that damaged buds had developed xylem vessels (Fig. 2D). Before and during dormancy, xylem vessels were observed at the bottom of bud axis, and the flower primordia were connected to the bud axis through a procambium (Fig. 2G). Following endodormancy, xylem vessels developed first through the hypanthium up to the anthers (Fig. 2C) and later to connect the pistil. A close examination of the flower buds showed perpendicular voids cha-

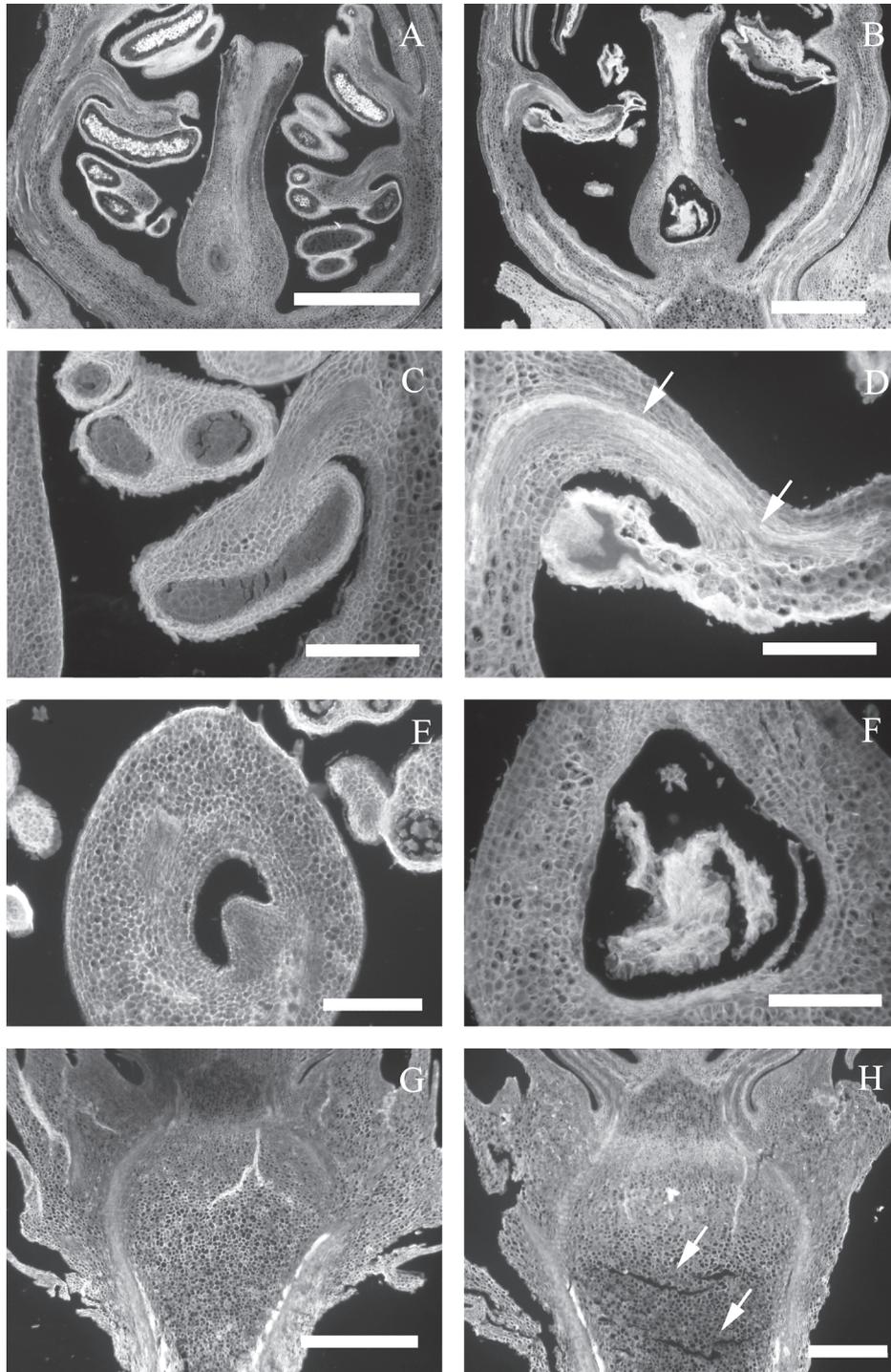


Fig. 2: Bud development before (Fig. 2A, 2C, 2E and 2G) and after frost damage (Fig. 2B, 2D, 2F and 2H). Flower primordia before (Fig. 2A) and after frost damage (Fig. 2B), showing xylems vessels in hypanthium and anthers. Detail of young xylem vessels (arrows) in anthers (Fig. 2D) and procambium in ovules (Fig. 2E and 2F) and absence (Fig. 2G) or presence (Fig. 2H) of voids (arrows) in flower bud receptacle. Sections stained with 0.07% (w/v) calcofluor in distilled water. Figures A, B, G, H: Scale bar = 500 μ m. Figures C, D, E and F: Scale bar = 150 μ m.

racteristics of ice formation in the bud axis and also a cytoplasmic dense zone in the pedicel that separated the flower primordia from the bud axis (Fig. 2H).

To evaluate whether flower buds became vulnerable to frost as vascular connections developed, buds of a same phenological stage A were sequentially examined for frost damage and vascular connections (Tab. 1). Results clearly show that, for a same frost temperature, when

no vascular connections were established, buds were undamaged. Interestingly, at the time of early xylem differentiation, frost damage could be observed in the areas where vascular connections were established (Fig. 2D). Vulnerability appeared first in the anthers (Fig. 2D) and later in the pistil (Fig. 2F), concomitantly with the development of xylem vessels connecting these structures. Moreover, since all flower buds within a tree are not synchronous in development, when the correspondence between frost damage and developmental stage was

Tab. 1: Minimum temperature registered in 2004 for the different frost episodes, and presence of frost damage and vascular connections in flower buds stage A of apricot cv Moniqui collected 2-6 days after each frost episode.

Frost episodes		T°C	Frost	Vascular
Nº	Date	Minimum	damage	connection
1	16 December	-2.3	-	-
2	25 December	-1.3	-	-
3	5-8 January	-3.4	-	-
4	16-17 January	-1.6	-	-
5	29-30 January	-2.6	+	+
6	8-14 February	-3.2	+	+
7	17-19 February	-3.0	+	+
8	22 February	-0.7	+	+
9	27-4 March	-4.8	+	+

Symbols: (-) Absence. (+) Presence.

evaluated, only the buds that had attained a stage with the vascular connections established were the ones that suffered frost damage. While those that were at earlier stages of development without vascular connections remained undamaged.

Discussion

Bud drop occurred in both years of experiments. While frost damage after bud-burst is expected, the occurrence of frost damage before bud-burst was unexpected and clearly contributed to bud drop. This was clear in our results, but a number of points suggest that this may well be a general problem that can explain situations of excessive bud drop previously reported in a good range of apricot cultivars with no apparent reasons for abscission (LAYNE et al., 1996). First, it is widely accepted that bud drop in apricot is environmentally influenced. Second, the anatomical damage recorded here is remarkably similar to the damage recorded in bud drop in other instances. Finally, results are fully compatible with what is known about frost sensitivity, since frost damage appeared at a time when xylem connections were established for rapid water pumping prior to bud-burst. However, the possibility that frost may contribute to bud drop has not been previously considered, perhaps because frost damage has been related to flowering time and injury at this early stage was unexpected, since it shows no external symptoms; still freezing temperatures of around -3°C are common at this time of the year in many apricot production areas.

Our observations of frost damage are based on the histological appearance of the tissues with voids characteristic of frost (RODRIGO, 2000 and references therein) and on the dates of apparition of damage concomitantly with frost temperatures once the flowers have broken the endodormancy and are in ecodormancy. The pattern of ice formation has been reported to be different during endodormancy and during de-acclimation. During endodormancy, freezing temperatures can damage the lower portion of the bud axis, which is more vacuolated and has more intercellular spaces, but the damage does not reach the upper dense zone at the flower primordia base (ASHWORTH et al., 1989; QUAMME et al., 1995). In contrast, results herein are in concordance with those reported for other species following de-acclimation, when the pattern of ice formation is different and large ice crystals can be formed in the whole bud axis, floral organs and peduncle (ASHWORTH et al., 1989).

Thus, our results show that apricot flower buds, prior to bud-burst, became sensitive to freezing temperatures within a week of difference.

This fits with the idea than as *Prunus* flower buds de-acclimate in the spring, the capacity to deep supercool is lost with the progressively warmer temperatures (ANDREWS et al., 1986).

While flower bud drop has been repeatedly reported in this species, it has been considered of a physiological rather than an accidental nature (LEGAVE et al., 1982). However, whenever the anatomy of these flower buds has been inspected (LEGAVE, 1978b; ALBURQUERQUE et al., 2003) they are remarkably similar to the observations reported here, showing necrotic pistils and anthers at the same phenological flower bud stage. While these abnormalities were clearly related to flower bud drop, a number of different possible causes have been hypothesised as failure in completing the chilling requirement (LEGAVE, 1978a) and it has been suggested that stamens necrosis could also be related to this failure (TABUENCA, 1971). However, in this experiment 'Moniqui', with an estimated requirement of 1,050-1,150 CU (TABUENCA, 1979), had already satisfied its chilling requirements before detecting necrosis on flower bud primordia and our observations show that the first reproductive organs to undergo frost injury are stamens, and for stamens and pistils, frost was the main factor inducing necrosis and contributed significantly to flower bud drop.

Ecodormancy has been referred as a time period (LANG et al., 1987), in which once the endodormancy requirement have been covered, a marked increase in the water content of the flower primordia takes place (QUAMME, 1983; ANDREWS and PROEBSTING, 1987; SUGIURA et al., 1995). Moisture content has been related to hardiness of flower buds (RODRIGO, 2000 and references therein), and water content has been reported as the main difference between endodormant and ecodormant flower buds (SUGIURA et al., 1995). The sequential examination of internal development showed differences in the frost sensitivity of buds. Although externally both resistant and sensitive buds were all similar, still closed and covered by scales, conspicuous changes related to the water content were observed internally. Thus, an important difference between flower buds during endodormancy and ecodormancy was observed: the absence or presence of xylem vessels in flower primordia. This was reflected in the hardiness of flower buds: while damaged buds had developed xylem vessels, undamaged buds had not completed the vascular connections. It has been put forward that during endodormancy the lack of xylem continuity is an important feature of flower buds which deep supercool to resist lower air temperatures, since ice could not propagate via the vascular system and nucleate the water within the primordia (ASHWORTH, 1984, SUGIURA et al., 1995). Once flower buds de-acclimate, xylem vessels continuity and the loss of the capacity to deep supercool occur concomitantly (ASHWORTH et al., 1989) and ice nucleation may propagate along xylem vessels and damage reproductive organs of flower primordia (WISNIEWSKI et al., 1997).

The analysis of the buds collected each week showed differences in the establishment of xylem connection not only between sampling dates, but also between buds collected the same day. The coexistence of different flower bud developmental stages for a particular sampling data has also been reported for other *Prunus* species (LAMP et al., 2001). The fact that after a frost episode damaged buds were the ones that had developed xylem vessels support the idea that this stage is particularly vulnerable to frost.

The relationship between frost damage and development of xylem elements found among buds is put forward also in particular damaged buds by the fact that stamens are the first organs to develop xylem connections and are the first organs that appear damaged by frost. This indicates that frost hardiness is highly dependent not only on the phenological stage of the flower buds (PROEBSTING and MILLS, 1978; ANDREWS et al., 1983), but also on the internal stage of xylem vessels development in the different floral structures.

Taken together these results provide evidence on the contribution of frost damage to flower bud drop in apricot, showing that there is a stage particularly prone to frost damage, once the buds have completed their chilling requirements and prior to bud-burst when the first xylem connections are established. Furthermore, the asynchrony reported here in the establishment of vascular connections among buds of the same age and its relationship with frost sensitivity could explain variable proportions of flower bud drop in different circumstances. The identification of the cause of a phenomenon that is widespread in this species and that is a limiting factor in cultivar adaptation will contribute to understand and select new cultivars for new areas.

Acknowledgements

We thank Reyes López, Rosa Fustero and Olga Frontera for technical assistance. Financial support for this research was provided by Ministerio de Ciencia y Tecnología-FEDER (Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica, Project grant: CICYT AGL2003-05318-C02-00 and CICYT AGL-2006-13529-CO2-00/AGR) and by Grupo consolidado de Aragón A-43 (Gobierno de Aragón). C. J. was supported by a fellowship of INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria).

References

- ALBUQUERQUE, N., BURGOS, L., EGEE, J., 2003: Apricot flower bud development and abscission related to chilling, irrigation and type of shoots. *Sci. Hortic.* 98, 265-276.
- ALBUQUERQUE, N., BURGOS, L., EGEE, J., 2004: Influence of flower bud density, flower bud drop and fruit set on apricot productivity. *Sci. Hortic.* 102, 397-406.
- ANDREWS, P.K., PROEBSTING, E.L., 1987: Effects of temperature on the deep supercooling characteristics of dormant and deacclimating sweet cherry flower buds. *J. Am. Soc. Hortic. Sci.* 112, 334-340.
- ANDREWS, P.K., PROEBSTING, E.L., GROSS, D.C., 1983: Differential thermal analysis and freezing-injury of deacclimating peach and sweet cherry reproductive-organs. *J. Am. Soc. Hortic. Sci.* 108, 755-759.
- ANDREWS, P.K., PROEBSTING, E.L., GROSS, D.C., 1986: Ice nucleation and supercooling in freeze-sensitive peach and sweet cherry tissues. *J. Am. Soc. Hortic. Sci.* 111, 232-236.
- ASHWORTH, E.N., 1984: Xylem development in *Prunus* flower buds and the relationship to deep supercooling. *Plant Physiol.* 74, 862-865.
- ASHWORTH, E.N., WISNIEWSKI, M.E., 1991: Response of fruit tree tissues to freezing temperatures. *Hortscience* 26, 501-504.
- ASHWORTH, E.N., DAVIS, G.A., WISNIEWSKI, M.E., 1989: The formation and distribution of ice within dormant and deacclimated peach flower buds. *Plant Cell Environ.* 12, 521-528.
- BAGGIOLINI, M., 1952: Les stades repérés des arbres fruitiers à noyau. *Rev. Romande Agr. et Vit.* 8, 1-8.
- BROWN, D.S., 1953: The effects of irrigation on flower bud development and fruiting in the apricot. *Proc. Amer. Soc. Hort. Sci.* 61, 119-124.
- DIETMANN, K., STOSSER, R., 1999: Development of ovules in relation to premature fruit drop in *Prunus*-species. *J. Appl. Bot.* 73, 86-98.
- GUNES, N.T., 2006: Frost hardiness of some Turkish apricot cultivars during the bloom period. *Hortscience* 41, 310-312.
- HORMAZA, J.I., YAMANE, H., RODRIGO, J., 2007: Apricot. In: Kole, C. (ed.), *Genome Mapping & Molecular Breeding*. Chapter 7, 173-189. Springer, Heidelberg, Berlin, New York, Tokyo.
- LAMP, B.M., CONNELL, J.H., DUNCAN, R.A., VIVEROS, M., POLITO, V.S., 2001: Almond flower development: Floral initiation and organogenesis. *J. Am. Soc. Hortic. Sci.* 126, 689-696.
- LANG, G.A., EARLY, J.D., MARTIN, G.C., DARNELL, R.L., 1987: Endodormancy, paradormancy, and ecodormancy – physiological terminology and classification for dormancy research. *Hortscience* 22, 371-377.
- LAYNE, R.E.C., BAILEY, C.H., HOUGH, L.F., 1996: Apricots. In: Janick, J., Moore, J.N. (eds.), *Fruit breedings Volume I: Tree and Tropical Fruits*, 79-109. John Wiley & Sons Inc. New York, Chichester, Brisbane, Toronto, Singapore.
- LEGAVE, J.M., 1978a: Attempt at understanding of flower necroses before blooming on apricot tree in relation to a study of chilling requirements for breaking dormancy. *Ann. Amélior. Plantes* 28, 593-607.
- LEGAVE, J.M., 1978b: Some aspects of flower necroses before blooming on apricot tree. *Ann. Amélior. Plantes* 28, 333-340.
- LEGAVE, J.M., GARCIA, G., MARCO, F., 1982: Some descriptive aspects of drops process of flower buds, or young flowers observed on apricot tree in south of France. *Acta Hortic.* 121, 75-83.
- LEGAVE, J.M., GARCIA, G., MARCO, F., 1984: Interference of temperature conditions and the varietal requirements in cold and heat, on the determination of the end of dormancy, then the blooming, of diverse varieties of apricot trees in a French cultivation area. *Fruits* 39, 399-410.
- MARTÍNEZ-GÓMEZ, P., DICENTA, F., RUIZ, D., EGEE, J., 2002: Flower bud abscission in apricot: Competition between vegetative and flower buds, and effects of early defoliation and high pre-blossom temperatures. *J. Hortic. Sci. Biotechnol.* 77, 485-488.
- OFICINA DEL REGANTE, 2006: Datos meteorológicos. <http://192.168.0.43/pw/noticias/mnoticia.php> 2003-05.
- PROEBSTING, E.L., MILLS, H.H., 1978: Synoptic analysis of peach and cherry flower bud hardiness. *J. Am. Soc. Hortic. Sci.* 103, 842-845.
- QUAMME, H.A., 1983: Relationship of air-temperature to water-content and supercooling of overwintering peach flower buds. *J. Am. Soc. Hortic. Sci.* 108, 697-701.
- QUAMME, H.A., SU, W.A., VETO, L.J., 1995: Anatomical features facilitating supercooling of the flower within the dormant peach flower bud. *J. Am. Soc. Hortic. Sci.* 120, 814-822.
- RICHARDSON, E.A., SEELEY, S.D., WALKER, D.R., 1974: A model for estimating the completion of rest for 'Redhaven' and 'Elberta' peach trees. *Hortscience* 9, 331-332.
- RODRIGO, J., 2000: Spring frosts in deciduous fruit trees – morphological damage and flower hardiness. *Sci. Hortic.* 85, 155-173.
- RODRIGO, J., HERRERO, M., 1996: Evaluation of pollination as the cause of erratic fruit set in apricot 'Moniqui'. *J. Hortic. Sci.* 71, 801-805.
- RODRIGO, J., HERRERO, M., 1998: Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). *Sex. Plant Reprod.* 11, 86-93.
- RODRIGO, J., HERRERO, M., 2002: The onset of fruiting in apricot (*Prunus armeniaca* L.). *J. Appl. Bot.* 76, 13-19.
- SUGIURA, T., YOSHIDA, M., MAGOSHI, J., ONO, S., 1995: Changes in water status of peach flower buds during endodormancy and ecodormancy measured by differential scanning calorimetry and nuclear-magnetic-resonance spectroscopy. *J. Am. Soc. Hortic. Sci.* 120, 134-138.
- TABUENCA, M.C., 1968: Necesidades de frío invernal en variedades de albaricoquero. *An. Aula Dei* 9, 10-24.
- TABUENCA, M.C., 1971: Caída de yemas de flor en melocotonero. *An. Aula Dei* 11, 69-97.
- TABUENCA, M.C., 1979: Duración del periodo de reposo a distintas temperaturas y evaluación de las necesidades de frío en variedades de albaricoquero y almendro. *An. Aula Dei* 14, 519-531.
- WISNIEWSKI, M., LINDOW, S.E., ASHWORTH, E.N., 1997: Observations of ice nucleation and propagation in plants using infrared video thermography. *Plant Physiol.* 113, 327-334.

Address of the authors:

Carme Julian (corresponding autor, email: cjulianla@aragon.es) and Javier Rodrigo, Unidad de Fruticultura, Centro de Investigación y Tecnología Agroalimentaria (CITA)-DGA, Campus de Aula Dei, Apdo. 727, 50080 Zaragoza, Spain
 Maria Herrero, Departamento de Pomología, Estación Experimental de Aula Dei -CSIC, Campus de Aula Dei, Apdo. 202, 50080 Zaragoza, Spain