# Dormancy in peach (*Prunus persica*) flower buds. V. Anatomy of bud development in relation to phenological stage

**Herminda Reinoso, Virginia Luna, Richard P. Pharis, and Rubén Bottini**

**Abstract**: Anatomical changes in the peach (*Prunus persica* (L.) Batsch.) flower buds were defined and then assessed and correlated with the phenological stage from early dormancy through to flower opening. The peach flower bud, unlike the vegetative bud, shows a continuous anatomical development during the late autumn and winter dormancy period, even though there are no obvious macroscopic changes. Sterile whorls differentiate rapidly in late summer through early autumn. In contrast, fertile whorls develop very slowly during winter; their rapid development begins in late winter and continues through early spring. The androecium develops throughout the winter, while the gynoecium develops in late winter. By late winter, the anthers begin to undergo microsporogenesis and microgametogenesis and the ovaries have formed ovules. Vascular connections between flower primordia and branch wood are complete by late winter, when rapid phenological changes begin. At this point in time, the peach floral bud enters a "rapid maturation phase" that ends in flower opening. Thus, for the peach flower bud at least, the concept of dormancy as "a temporary suspension of visible growth of any plant structure containing a meristem" that was proposed by earlier researchers appears inappropriate. Rather, cell division, enlargement, and differentiation, which lead to organogenesis, take place throughout the entire "dormancy" period.

*Key words*: dormancy, floral bud anatomy, floral bud phenology, peach, *Prunus persica*.

**Résumé** : Les auteurs ont défini les changements anatomiques des bourgeons floraux de la pêche (*Prunus persica* (L.) Batsch.), puis les ont évalués et corrélés avec les stades phénologiques, du début de la dormance à l'ouverture des fleurs. Le bourgeon floral de la pêche, contrairement au bourgeon végétatif, montre un développement anatomique continu au cours de la période de dormance de fin d'automne et d'hiver, même s'il n'y a pas de changements macroscopiques évidents. Les verticilles stériles se différencient rapidement de la fin de l'été jusqu'au début de l'automne. Au contraire, les verticilles fertiles se développent très lentement au cours de l'hiver et leur développement rapide débute à la fin de l'hiver, de façon continue jusqu'au début du printemps. L'androcée se développe tout au long de l'hiver, alors que le gynécée se développe à la fin de l'hiver. Vers la fin de l'hiver, les anthères commencent à subir la microsporogénèse et la microgamétogénèse et les ovaires ont formé les ovules. Les connexions vasculaires entre les primordiums floraux et le bois des rameaux se complètent vers la fin de l'hiver, lorsque surviennent les changements phénologiques rapides. A ce moment, le bourgeon floral de la pêche entre dans une « phase rapide de maturation » qui se termine avec l'anthèse. Ainsi, du moins dans le cas du bourgeon floral de la pêche, le concept de dormance comme « suspension temporaire de croissance visible de toute structure végétale contenant un méristème », proposé antérieurement, semble inapproprié. Au contraire, la division, le gonflement et la différenciation des cellules conduisant à l'organogénèse s'effectuent tout au long de la période de « dormance ».

*Mots clés* : dormance, anatomie du bourgeon floral, phénologie du bourgeon floral, pêche, *Prunus persica*.

[Traduit par la Rédaction]

## Introduction

During plant development, cell growth and differentiation are coordinated in a process known as morphogenesis, and differentiation and specialization processes are part of the morphogenetic processes (Esau 1982). Many woody species have developed a mechanism called dormancy within their morphogenesis. This evolutionary achievement allows them to survive adverse environmental conditions during winter and favours the synchronization of vegetative bud break and flowering in the spring (Carl 1996).

Dormancy was defined as the "opposite" of morphogenesis by van der Schoot (1996). Morphogenesis thus denotes "organization of development" and dormancy indicates a temporary absence of development. Hence, neither concept

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implies specific physiological mechanisms; rather, they denote two different states of a dynamically organized system (van der Schoot 1996). During morphogenesis, this dynamic system follows a developmental course, and during dormancy the developmental trajectory has a vector of zero, or almost zero. Therefore, one can further describe dormancy as the stationary phase of a series of physiological networks that impart pattern to the organ.

It is well known that this steady-state phase is a consequence of seasonal photoperiodic and thermoperiodic rhythms that influence bud behaviour in trees, thereby causing a winter rest period followed by the reinitiation of metabolic activity that leads to bud break. Phillips (1962) has suggested that while buds of deciduous trees develop and differentiate during the active growing season, they become dormant in autumn because of an accumulation of growth inhibitors, the latter being synthesized in leaves as days get shorter. In winter, an accumulation of low temperature units leads to a destruction of these inhibitors and (or) enhances the synthesis of bud break promoters (such as gibberellins (GAs)), thereby leading to dormancy release. An excellent discussion of the physiology of how low temperatures can control such processes is given in Lang (1965; see especially Fig. 35, p. 1488). Experimental evidence for this broad hypothesis has been obtained over the years using buds from a range of different species but without distinguishing between floral and vegetative buds (reviewed in Luna et al. 1990, 1991).

Previous work suggests that peach (*Prunus persica* (L.) Batsch.) flower buds have a resting mechanism that differs from that of vegetative buds. This conclusion is based primarily on their different responses to exogenous treatments with $GA_3$ (Hatch and Walker 1969; Walker 1970) and their different requirements with regard to the duration of the cold period required for uniform bud break (Samish and Lavee 1982).

According to Erez (1987), peach flower buds probably have a gradual and prolonged development during morphogenesis, since their organogenesis is generally not completed until just before flower anthesis. In contrast, Baggliolini (1952) has proposed that there are a series of phenological stages in peach flower buds, and he has given these a nomenclature. Then, once the bud is established, there follows a long period with no apparent change and this is designated as stage A (winter bud). The first morphological indication of dormancy release that can be observed in stage A buds is swelling, e.g., stage B (swollen bud). Then, the protective bracts begin to separate gradually and the sepals become visible (stage C, visible calyx). Later stages occur rapidly and in just a few days the flower has opened. Baggliolini's (1952) description was reaffirmed by Gil-Albert Velarde (1991). However, although the phenology of peach floral bud development has been described, the anatomical changes accompanying this phenology have not been documented.

Thus, the objective of the present work was to precisely describe the anatomical changes that occur in the peach flower bud during and subsequent to the low temperatures of winter and prior to flower opening.

## Materials and methods

Each year from 1992 until 1996, flower buds were detached from 1-year-old limbs of peach trees, cv. Novedad de Córdoba, in an orchard at Río Cuarto, Argentina. Collections were made periodically starting in early fall (April) and concluding with flower bud opening in late winter (first week of September). Phenological stages were determined and recorded according to the basic nomenclature of Baggliolini (1952): stage A, winter resting bud; stage B, swollen bud where the calyx cannot yet be seen; stage C, the calyx can be observed but petals are not yet visible; stage D, petals are visible; stage E, the young flower is partially open and stamens are visible; stage F, petals have expanded.

A vernier caliper was used to determine the size of stage A buds in situ in order to evaluate subsequent bud growth. On each collection date, 12–15 buds were taken and fixed in FAA (95% formaldehyde – glacial acetic acid – ethanol – water; 10:5:50:35, v/v/v/v).

To prepare buds for histological examination, scale leaves were first carefully removed prior to further processing. The buds were then dehydrated in a graded ethanol series. Xylene was used as a transitional fluid prior to paraffin infiltration and embedding (Johansen 1940). A series of transverse and longitudinal sections 10 μm thick were obtained using a rotary microtome. The sections were triple-stained with hematoxylin, safranin, and fast green (Luna et al. 1990). The histological preparations were assessed with a Standard Zeiss model 16 microscope and photomicrographs were taken with a Zeiss Axiophot microscope using black and white Kodak Plus PAN X-36 125 print film.

## Results

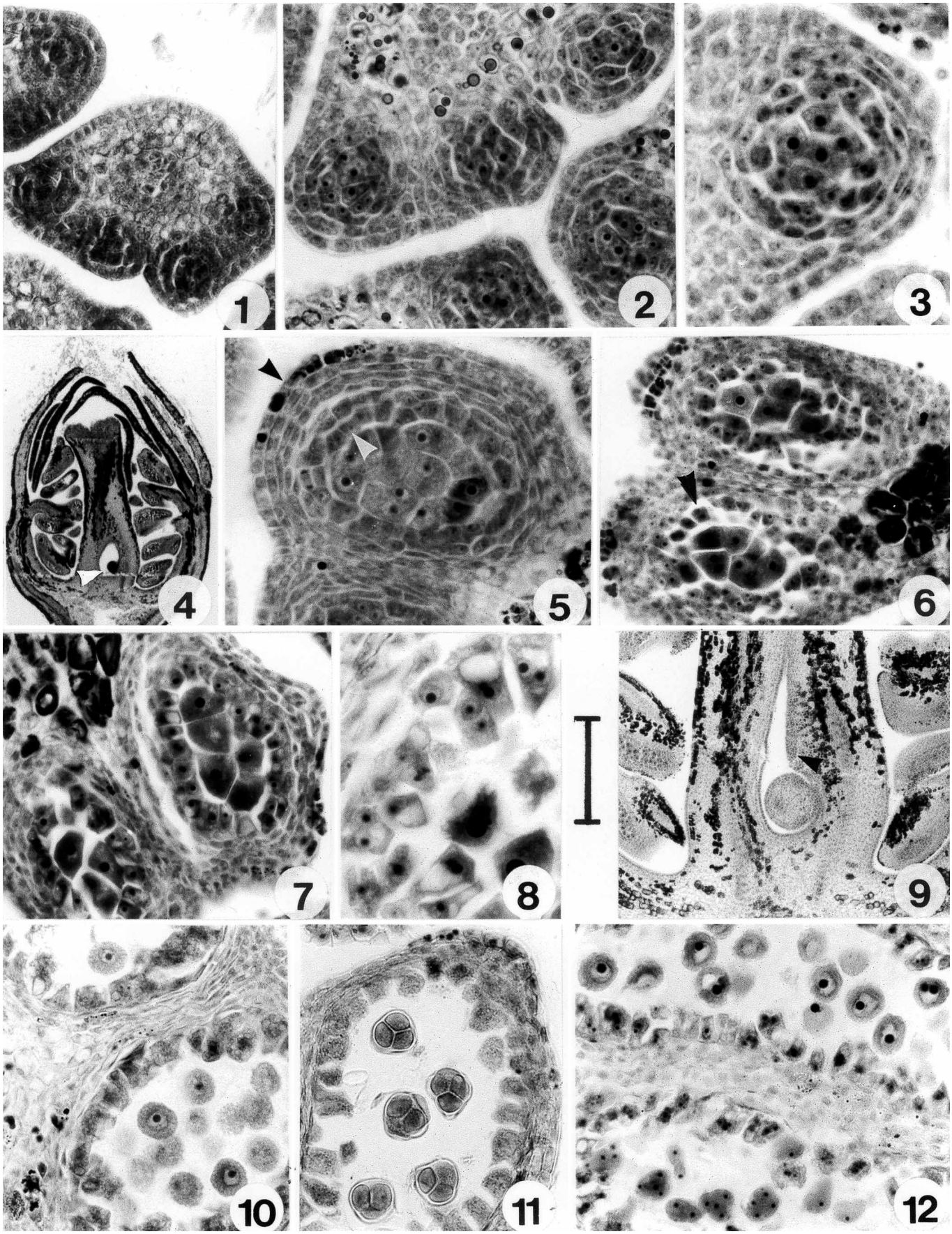
In the temperate region of the southern hemisphere, peach flower buds begin to differentiate in midsummer (January), and by the end of April, they reach stage A (winter resting bud). Stage A extends through approximately mid-August, depending on ambient temperatures. During stage A, there is minimal variation in bud size, e.g., buds are on average 6 mm in length by 8 mm in diameter at the end of April and 8 mm in length by 9 mm in diameter in mid-August.

However, during the stage A period, gradual and progressive anatomical changes are occurring in the whorls. These developmental changes during floral bud maturation are readily observed with the dissecting microscope, especially in the androecium during microspore development and pollen grain formation. We thus used modifications in the androecium to establish the different anatomical stages for each phenological stage.

### Anatomical stages of winter buds (phenological stage A)

#### *Stage 1*

Bud whorls begin to develop between the end of April and the end of May (mid-autumn, Fig. 13). The outer whorls, i.e., sepals and petals, begin to differentiate earlier than the inner whorl organs, i.e., stamens and gynoecium. The anthers are tetrasporangiate with the two locules in each of the two lobes being joined by connective tissue (Fig. 1). The gynoecium is the last organ to form, as it is located at the centre of the developing floral bud.



**Figs. 1–12.** Photomicrographs of anatomical stages 1–6 of peach flower buds. Fig. 1. Anatomical stage 1 (phenological stage A). Detail of a transverse section of a very young tetrasporangiate anther. Scale bar = 45 μm. Fig. 2. Anatomical stage 2 (phenological stage A). Transverse section of an anther showing developing microsporangium walls and the locules containing sporogenous tissue. Scale bar = 45 μm. Fig. 3. Anatomical stage 2 (phenological stage A). Detail of a microsporangium of the anther shown in Fig. 2 in the center of which large sporogenous cells with a dense cytoplasm, large nuclei, and distinct nucleoli can be seen. Scale bar = 24 μm. Fig. 4. Anatomical stage 3 (phenological stage A). Longitudinal section of a young bud where an ovule primordium (arrowhead) appears in the ovary. Scale bar = 800 μm. Fig. 5. Anatomical stage 3 (phenological stage A). Transverse section of a microsporangium wall showing identifiable layers, e.g., epidermis (black arrowhead), endothecium, three to four middle layers, and the tapetum (white arrowhead). Scale bar = 60 μm. Fig. 6. Anatomical stage 4 (phenological stage A). Transverse section of a microsporangium where the intact tapetum cells exhibit an intensely stained cytoplasm (arrowhead). Scale bar = 45 μm. Fig. 7. Anatomical stage 5 (phenological stage A). Transverse section of a microsporangium where the PMC have started to separate. The tapetal cells have enlarged and the middle layers of the microsporangium walls appear to be stretched. Scale bar = 45 μm. Fig. 8. Anatomical stage 5 (phenological stage A). Detail of the tapetum cells showing uni- or binucleated cells whith large vacuoles. Scale bar = 24 μm. Fig. 9. Anatomical stage 6 (phenological stage A). Longitudinal section of the ovary. The obturator (arrowhead) appears over each ovule. Scale bar = 200 μm. Fig. 10. Anatomical stage 6 (phenological stage A). Transverse section of a microsporangium where PMC are very close to division and show their typical round shape and granular cytoplasm. Scale bar = 45 μm. Fig. 11. Anatomical stage 6 (phenological stage A). View of a microsporangium after PMC meiosis showing the resulting tetrads. Scale bar = 45 μm. Fig. 12. Anatomical stage 6 (phenological stage A). View of two microsporangia from the same anther showing PMC close to division in one locule and PMC that are undergoing cell division in the other. Scale bar = 45 μm.

### *Stage 2*

From the end of May until mid-June (almost the end of autumn, Fig. 13), only the epidermis can be clearly identified as a differentiated tissue in the microsporangium wall; other cell layers are still developing (Fig. 2). Large distinct sporogenous cells are present. These cells have a dense cytoplasm with large nuclei and distinct nucleoli (Fig. 3).

### *Stage 3*

From mid-June until the beginning of July (early winter, Fig. 13), within each ovary, meristematic protuberances appear that will subsequently develop into ovules (Fig. 4, arrowhead). The microsporangium wall has identifiable layers, e.g., epidermis (Fig. 5, black arrowhead), endothecium, three to four middle layers, and the tapetum (Fig. 5, white arrowhead). The sporogenous cells have increased in number and have begun to differentiate into pollen mother cells (PMC).

### *Stage 4*

In buds collected during the first 15 days of July (early winter, Fig. 13), the tapetum becomes more distinct as the tapetal cells have a dense cytoplasm and these cells form the innermost layer of the microsporangium, enclosing the developing PMC (Fig. 6, arrowhead). No significant changes are observed in the gynoecium during this period.

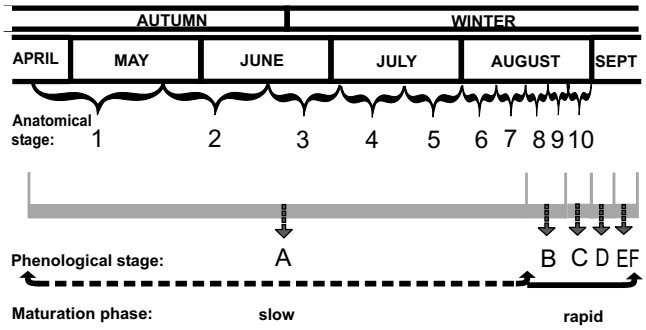
### *Stage 5*

By the end of July (almost midwinter, Fig. 13), the middle layers of the microsporangium walls appear stretched due to the expansion of the anther (Fig. 7) and the tapetal cells enlarge as vacuolation occurs (Figs. 7 and 8). Some of the vacuolated tapetal cells are binucleate (Fig. 8). In addition, the PMC have gradually enlarged and are beginning to separate from one another (Fig. 7).

### *Stage 6*

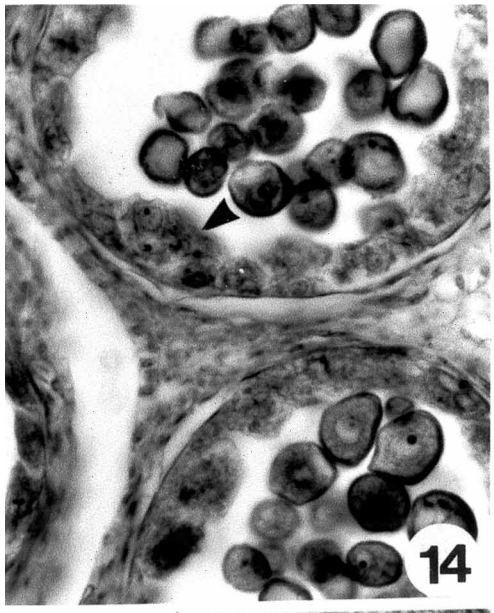
Substantial developmental changes are observed in the flower buds during the first week of August (midwinter, Fig. 13). The hypanthium has overgrown the ovary, resulting in the characteristic perigynous flower. Within the ovary, an obturator appears over each ovule (Fig. 9, arrowhead). Meiotic divisions are not, however, synchronous among anthers of the same flower. Within the locules of the microsporangium, some of the PMC take on a round shape, with a granular cytoplasm, a prominent nucleus, and a thin cell wall. These PMC are in meiotic prophase, ready to undergo meiotic division (Fig. 10). In other anthers, PMC have already undergone meiosis and this results in the simultaneous formation of tetrahedral tetrads with a thick wall (Fig. 11). Furthermore, different stages of meiotic division can be found in different locules of the same anther (Fig. 12). Thus, meiotic division is asynchronous at this stage.

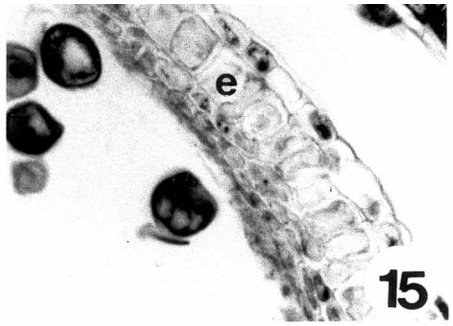
**Fig. 13.** Correlation between phenological stages and anatomical development in peach flower buds during their ontogeny.

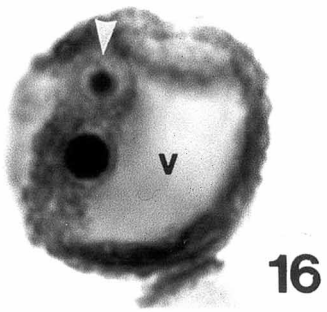


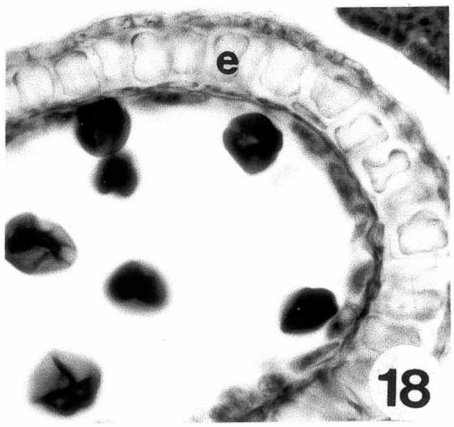

### *Stage 7*

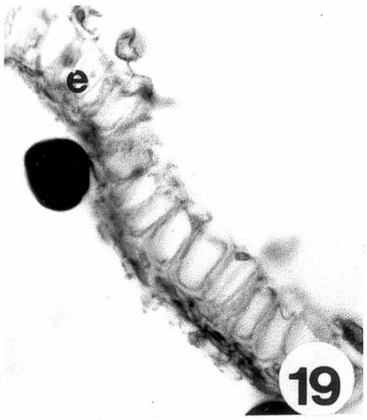
By the second week of August, vascular tissues in all bud whorls are recognizable. This vascular connection between the bud and the branch determines the end of phenological stage A as shown in Fig. 13. The end of stage A coincides with the beginning of microgametogenesis inside the anthers, and recently liberated microspores 15–20 μm in diameter and their haploid nucleus can be seen through the young, thin cellulose wall (Fig. 14). In the microsporangium, the tapetum degenerates gradually (Fig. 14, arrowhead). Middle layers then start to compress further and no subsequent change in the size of the endothecium cells is observed, relative to stage 6.

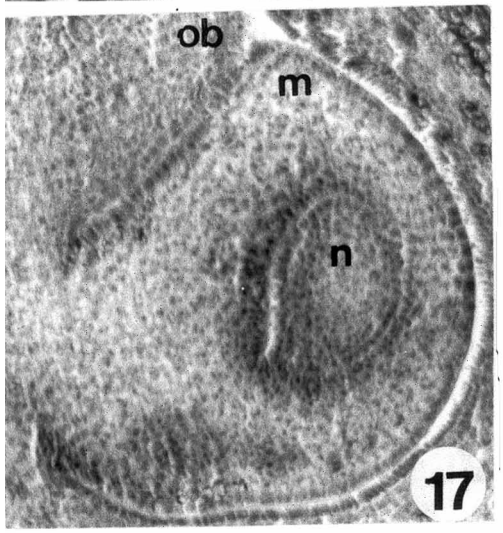


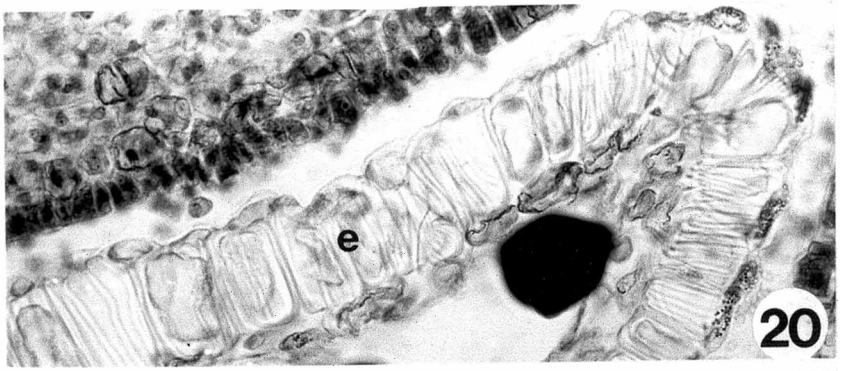



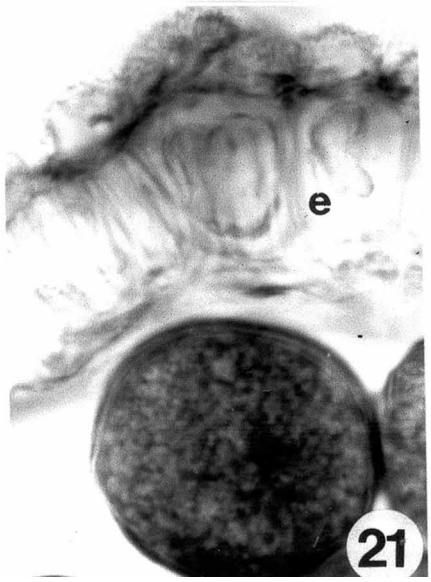



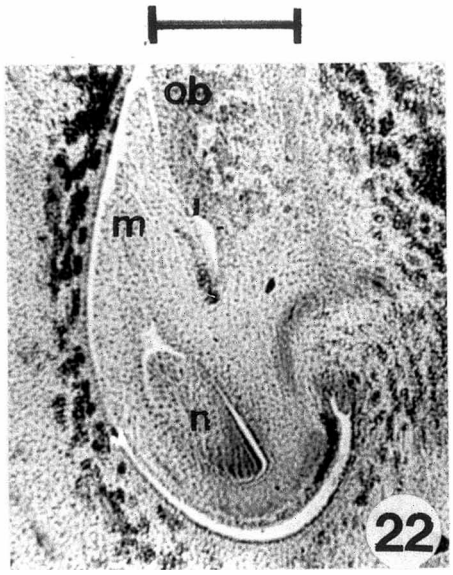



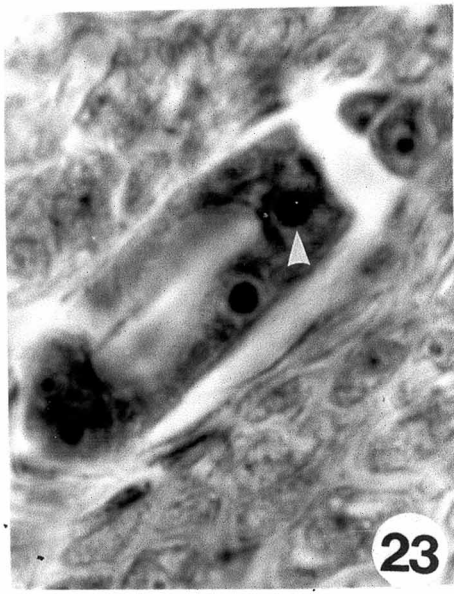



**Figs. 14–23.** Photomicrographs of anatomical stages 7–10 of peach flower buds and flower gametophytes. Fig. 14. Anatomical stage 7 (phenological stage A). Transverse section of a microsporangium showing progressive breakdown of the tapetum (arrowhead) and the locule containing young microspores. Scale bar = 45 μm. Fig. 15. Anatomical stage 8 (phenological stage B). View of a microsporangium wall section showing tapetum residues and middle layers. The endothecium cells (e) have just begun to enlarge. Scale bar = 45 μm. Fig. 16. Anatomical stage 8 (phenological stage B). Young bicellular pollen grain (arrowhead indicates the generative cell nucleus). v, vacuole. Scale bar = 12 μm. Fig. 17. Anatomical stage 9 (phenological stage B). Longitudinal section of a developing ovule where the nucellus (n), micropyle (m), and part of the obturator (ob) zone can be distinguished. Scale bar = 100 μm. Fig. 18. Anatomical stage 9 (phenological stage B). Transverse section of a microsporangium showing pollen grains with a thick wall. Remnants of the middle layers of the microsporangium wall adhere to the inner surface of the endothecium. Scale bar = 45 μm. Fig. 19. Anatomical stage 10a. (phenological stage C). Detail of endothecium cells showing developing secondary wall thickenings in the anticlinal and inner tangencial cell walls. Scale bar = 45 μm. Fig. 20. Anatomical stage 10b (phenological stage C). Detail of endothecium cells showing a highly increased number of secondary wall thickenings as well as pollen grains noticeably larger than in stage 10a. Scale bar = 45 μm. Fig. 21. Open peach flower (phenological stage F). Detail of endothecium cells of a dehiscent microsporangium and mature pollen grains ready to be released. Scale bar = 24 μm. Fig. 22. Open peach flower (phenological stage F). Longitudinal section of the ovary showing the mature anatropous ovule. Scale bar = 24 μm. Fig. 23. Open peach flower (phenological stage F). Polygonum-type gametophyte where the egg cell can be observed (arrowhead). Scale bar = 24 μm.

### Anatomical stages of swollen buds (phenological stage B)

#### *Stage 8*

After the second week of August, tapetum cells are difficult to discern. The most important characteristic of this stage is the beginning of expansion of the endothecium cells (Fig. 15). Also, some microspores have undergone mitosis within the microsporangium, indicating the formation of a microgametophyte. A distinct generative cell (Fig. 16, arrowhead) is located within the vegetative cell cytoplasm. Pollen would thus be released from the dehiscent anther as a two-celled structure.

#### *Stage 9*

Stages 8 and 9 are both present after the second week of August. However, during the third week of August, numerous buds have a noticeable nucellus within the ovules and the micropyle has become more distinct and is located near the obturator (Fig. 17). In the microsporangium wall, the endothecium is easily recognized and remnants of the middle layers are adhering to the inner surface of the endothecium (Fig. 18). By stage 9, pollen grains have developed a thick wall and their contents cannot be seen (Fig. 18).

### Anatomical stages of buds where the calyx is already visible (phenological stage C)

#### *Stage 10*

From the last week of August until anthesis in mid-September, the perianth continues to grow and differentiate, acquiring quite specific characteristics, e.g., within the sepals, numerous chloroplasts develop. Vacuoles within the parenchyma of the petals begin to show anthocyanin accumulation. In the ovary, ovules continue their development. The endothecium of the microsporangium wall has developed bands of secondary wall thickenings on both anticlinal and inner tangential walls (Fig. 19). Because of this latter morphology, we have differentiated stage 10 into two substages: stage 10a where most pollen grains are 28 to 30 μm in diameter (Fig. 19), and stage 10b where most pollen grains are 34–36 μm in diameter and the endothecium shows a very increased number of bands of thickening walls (Fig. 20).

### Last phenological stages

The last phenological changes occur rapidly and are easily observed. Therefore, we made no anatomical analysis of these buds during their rapid development toward an open flower. Descriptively, phenological stage D is initiated when the corolla can be seen as a small red circle in the apex of the bud. Stage D is thus divided into two substages (Fig. 24): $D_1$ where the petals form a "conical structure" that emerges 4 mm from the calyx (by this time, sepals have acquired their characteristic green–brownish colour) and $D_2$ where the conical structure emerges 8 mm from the calyx but stamens are not visible.

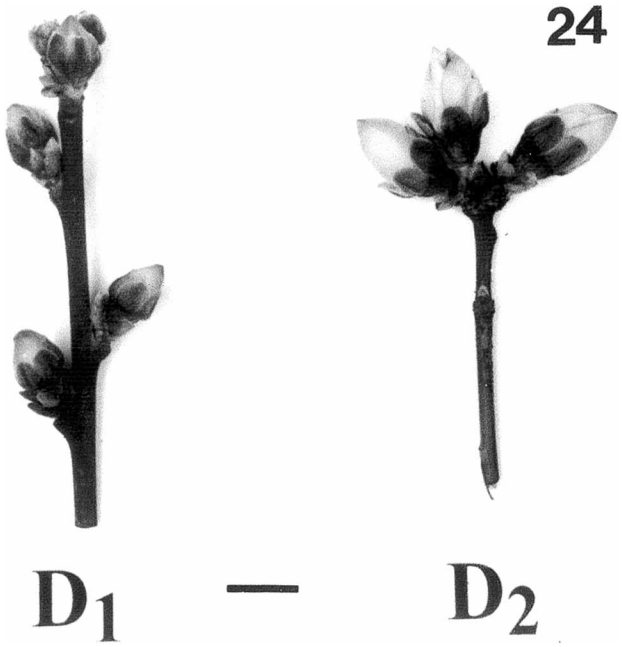
Petals continue their rapid growth and double in length over 4 days, by which time the young flower is partially open, with stamens apparent (phenological stage E). Subsequent development is a very rapid and continuous process and stage F (open flower) is reached in 5–6 h.

In stage F, the anatomy of fertile whorls was again assessed. In the androecium, anthers are dehiscent and released pollen grains average 43 μm in diameter (Fig. 21). In the gynoecium, the stigma has developed a large surface covered with secretory papillae. In the ovary, the pedunculate and anatropous ovules (Fig. 22) already have a fully differentiated female gametophyte, with an organization pattern that corresponds to the polygonum type (Maheshwari 1950). The egg cell can just be observed in stage F (Fig. 23, arrowhead).

## Discussion

Several studies have examined various aspects of anatomical changes in developing peach flower buds (Di Césare 1974; Luna et al. 1990, 1991; Luna 1993; Basconsuelo et al. 1995; Reinoso 1998). However, a detailed description of the anatomical features that characterize each phenological stage from the start of dormancy through to flower opening has not been published, and this was the objective of the present study. During the periods when our field sampling and phenological bud assessments were carried out (Province of Córdoba, Argentina, 1992–1996), monthly mean temperatures were representative of the long-term averages. Hence, we have been able to establish an average duration (in weeks) for each phenological stage.

**Fig. 24.** Phenological stage D. The section of the young branch on the left shows flower buds in substage $D_1$ in which petals are a conical structure that emerges 4 mm from the calyx. The section of the young branch on the right shows flower buds in substage $D_2$ in which the conical structure has emerged 8 mm from the calyx but stamens are not yet visible. Scale bar = 10 mm.




In a previous study, we observed in *P. persica* that the way in which the anther wall originates corresponds to the basic type described by Davis (1966), i.e., one parietal stratum has a common origin with the endothecium and the other with the tapetum (Reinoso 1998). The presence of tetralocular dithecic anthers, secretory tapetum, simultaneous microspore formation, and pollen grain liberation at the bicellular stage are some of the characteristics observed in peach that are also present in other members of the order Rosales (Johri et al. 1992).

We also observed a well-developed obturator in the pistils at anthesis, in concurrence with Arbeloa and Herrero (1987). It extends along the ovary suture line between the base of the style and ovule micropyle. A chemotropic and trophic role has been postulated for the obturator with regard to nutrition of the pollen tube and orienting and guiding its growth towards the ovary in the peach flower (Arbeloa and Herrero 1987).

The gynoecium of *P. persica* is unicarpellate, as for other *Prunus* species, e.g., *Prunus cerasus* (Bradbury 1929), *Prunus domestica* (Sterling 1964), and *Prunus armeniaca* (Szujko- Lacza 1982). In the ovary, the locule contains two anatropous ovules with micropyles curved toward the style. The ovules are crassinucellate and the functional megaspore initiates a polygonum type embryo sac (also see Johri et al. 1992). Finally, abortion of one of the ovules occurs after fertilization, a developmental event that is a characteristic of all *Prunus* species (Szujko-Lacza 1982).

The above results confirm the suggestion made earlier by Erez (1987), namely that peach flower buds must go through a series of developmental stages in organ formation before flowering can take place. Thus, while the developmental pattern of peach flower buds does resemble the phenological development of peach vegetative buds (Luna et al. 1991), the floral buds show continuous development that was not obviously visible, although readily seen upon anatomical assessment. Hence, even though all whorls were differentiated by May (early autumn, Fig. 13), only the sterile whorls had reached an advanced degree of development. The sterile whorls thus passed through autumn–winter in a dormant state until 2 or 3 weeks before flowering, when the buds began to swell, increased their pigmentation, and began to show the presence of vascular bundles. The androecium developed throughout the winter, but the gynoecium showed structural changes only toward the end of winter.

In July, anther development had proceeded almost to microsporogenesis and ovules were being formed. After the vascular connections between the flower primordia and the adjacent woody tissue of the branch were complete, in August, rapid changes in phenological development began to take place. This "rapid maturation phase" (Luna 1993) was characterized by an increase in cell enlargement, differentiation, and specialization of all bud tissues. Flower "maturity" occurred a few days before rapid flower opening. However, for sterile whorls, the speed of differentiation and development was most rapid toward the end of summer and beginning of fall, with tissues in the fertile whorls developing slowly during winter (anthers excepted, see above). Then, in late winter, there was an appreciably increased growth and differentiation, which continued right through to full bloom.

Hence, as summarized in Fig. 13, for the peach floral bud at least, the old concept (Lang et al. 1987) of dormancy as "a temporary suspension of visible growth of any plant structure containing a meristem" seems too simplistic. Rather, there is a combination of ongoing cell division, enlargement, and differentiation that results in organogenesis during the entire dormancy period. This is a process that is more appropriately called a "slow maturation phase", corresponding to phenological stage A. From mid-August on (end of winter), phenological stages B–F proceed in very rapid succession, a process one could call a "rapid maturation phase".

In comparison, peach vegetative buds are fully differentiated by late summer and progressively enter a dormant state in autumn. During winter (May–July), peach vegetative buds are unable to break dormancy even if isolated leaf buds are placed under favourable conditions. However, if these vegetative buds are treated with $GA_3$, or further chilled (Luna et al. 1991; Luna 1993), bud break will occur. In contrast, before or during deep dormancy (anatomical states 7–10), peach floral buds do not respond to any of these treatments with normal bud break and flower opening. An exception, however, is that some floral buds will show sepal and petal emergence but only with concomitant abortion of the fertile whorls, apparently because of their gynoecial and androecial immaturity.

In summary, this work provides a developmental timetable for peach flower bud formation and in doing so also provides useful information for future experimental studies on sexual reproduction in peach. In this context, an example of such an experimental physiological study is given in Reinoso et al. (2002).

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