

## Cytohistological and cytochemical features of the seeds of *Malus domestica* Borkh exposed to spring frosts

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**Abstract** — *Malus domestica* Borkh cv. Golden Delicious trees, exposed to spring frost, showed the occurrence of seeds with white (W), spotted (S) and black (B) integuments, with a different distribution, amount and damage in shedding and non-shedding fruits at several times after anthesis. While B seeds were completely degenerated, the S seeds showed histological and cytochemical features that included a precocious embryo development stage, an embryonal cells shrinkage, an absence of reserves in the suspensor, endosperm and integuments, and a probable block of the routing of reserves, as a consequence of spring frost damages. All these features are the same occurring in seeds undergoing to natural abscission phenomenon and in the W seeds of shedding fruits observed in previous years. The S seeds damage is certainly attributable to meteorological events, because the S seeds were never found until the occurrence of the spring frosts. On the contrary, the damage in the W seeds, which do not show external symptoms in shedding fruits, may be due on the one hand to meteorological stress, and on the other hand to endogenous stress caused by natural abscission process. At 60 days post anthesis, at the end of fruit-set, the ripening fruits show a mean number of five well developed W seeds in normal cytohistological conditions. The data are discussed in relation to the general problem of natural abscission of immature fruits.

**Key words:** abscission, fruit set, *Malus domestica*, seeds, spring frost.

### INTRODUCTION

Perception of changes in the natural environment is of great importance for all living organisms. This is especially valid in the case of plants, which are sessile organisms showing a great capacity to adapt to changes in the surrounding environment (KEVEI and NAGY 2003). Potentially adverse environmental conditions (stressors) affect plant growth and development and trigger a wide range of responses, from altered gene expression and modifications in cellular metabolism to changes in growth rate and crop yield (KACPERSKA 2004). Signalling pathways operate in stress-affected cells and different models of stress-responsive transduction pathways have been proposed to link the perception of a stress signal with the stress-induced modifications (XIONG and ZHU 2002). Climate changes can directly affect crops, as well as their interaction with microbial pests (ROSENZWEIG *et al.* 2000). Extreme local seasonal

changes too (as altered precipitation pattern, great variations of temperature in few hours, fall in relative humidity and chiefly frosts) can reasonably change crop productivity, affecting the fruit-set. The effects of spring frosts on reproductive organs of deciduous fruit trees are highly variable and depend on the characteristics of both the freezing stress and the plant material; their anatomical consequences result in internal and external morphological abnormalities that influence the normal development of the fruit or even cause its abscission (RODRIGO 2000). The information available on the influence of late spring frosts on reproductive organs of deciduous fruit trees, in temperate climate, has been reviewed by RODRIGO (2000). In this review frost damage has been examined at cellular level as well as for its anatomical and morphological consequences in flowers and fruits. Very few are the data on the influence of the frost at the cytohistological level on the seeds during the different developmental stages of the fruits. The number, the structural, physiological and morphological features and the developmental stage of the seeds in every fruit are the principal parameters controlling the fruit set (MARRO 1982, RAGAZZINI and SANSAVINI 1982;

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RASCIO 1985; AVANZI *et al.* 1988; VERNIERI *et al.* 1992; FORINO *et al.* 2000). In this context we have programmed a research to evaluate the number and the cytohistological characters of *Malus domestica* fruit seeds, picked in different developmental fruit stages with the aim to relate these parameters either to immature fruit abscission or to spring frosts happened in the 2000 year at experimental farm of San Piero a Grado where apple trees were grown.

Meteorologic data of the 1999 and 2000 years were accomplished by Avanzi Center.

## MATERIALS AND METHODS

*Plant materials* - Fruits of *Malus domestica* Borkh cv. Golden Delicious were sampled from trees grown at the experimental farm of San Piero a Grado (University of Pisa). Two sample of 10 fruits were picked at 30, 40, 50 and 60 days post-anthesis (d.p.a.): 1) fruits, which were non-shedding and tightly attached to the trees, and 2) shedding fruits, which were not yet shed from the tree but were easily detached by slightly shaking the branch.

*Cytohistological analysis* - Isolated seeds were numbered, morphologically characterized, fixed in FAA (10% formaldehyde, 5% acetic acid, 45% ethanol), dehydrated through a graded ethanol series (50, 75, 95%, 5 h each step), incubated overnight in 100% ethanol and embedded in LR White resin (Sigma). The material was polymerized at 60°C for 24 h. Sections 3- $\mu$ m thick were cut with a LKB Ultratome Nova. For detection of insoluble polysaccharides the sections were stained with periodic acid-Schiff's reagent (PAS) (O'BRIEN and McCULLY 1981) and with 0.05% toluidine blue O (TBO) (BDH) in 0.1 M acetate buffer pH 4.4 (FEDER and O'BRIEN 1968) for the localization of polyphenolics and as generic dye for DNA, cytoplasm and some cell wall components, then air dried and mounted in DPX (BDH).

Observations were made with LEITZ DIAPLAN light microscope. Photomicrographs were made with a LEICA DC 300F. Images were mounted using an ADOBE PHOTOSHOP 7.0 software package.

*Meteorological data* - Fig. 1 evidences daily minimum and maximum temperature in March and in the 1<sup>th</sup> ten days of April in the 1999 and 2000 years (A) and daily minimum and maximum relative humidity in the same time intervals (B). By

comparison of A and B it is possible to note that in the 1<sup>th</sup> ten days, in the 3<sup>th</sup> ten days of March and in the 1<sup>th</sup> ten days of April the minimum temperature is near to 0°C, (indicated by an arrow), maximum temperature is near 15°C and a fall in relative humidity from 40% to 23% occurs in the same periods. It is worth of note that the Avanzi center, where temperatures were recorded, is not localized near enough to experimental orchard to reflect the real condition. Minimum temperatures may vary across a zone, even an orchard, depending on several factors such as air drainage or tree row orientation. Therefore, as experimental orchard was exposed, repeatedly, to high "Libeccio" wind, it is reasonable that the minimum temperature (Fig. 1) dropped below freezing and thus the necessary conditions happened in order to cause the frost, without ice formation, named black frost. Although ice crystal may not be formed on solid surfaces in these conditions, ice can form in plant tissue. In the 1999 year the above mentioned conditions did not occur.

## RESULTS

The number and the external characteristics of the seeds belonging to every sample of shedding and non-shedding fruits of *Malus domestica*, during the collection times, are reported in Fig. 2 (A, B).

The figure shows the occurrence of W, S and B seeds in every sample of shedding and non-shedding fruits, in all the stages of development. From the comparison of the Figures 2A and 2B it is evident:

- 1) the number of W seeds is higher at every stage of development in non-shedding than in shedding fruits;
- 2) a decrease of W seeds in both samples and in the various developmental stages;
- 3) an increase of S and B seeds in shedding fruits in all developmental stages in regard to non-shedding fruits;
- 4) the distribution of S and B seeds is rather heterogeneous in both shedding and non-shedding fruits and in the various developmental stages.

The spots of S seeds consist in a localized external brown accumulation of phenolic compounds together with dead cells. In the integuments of S seeds, phenolic compounds are present as inclusions of spherical shape, variable in size and in considerable amount; they progressively decreased from S, W seeds of shedding to non-

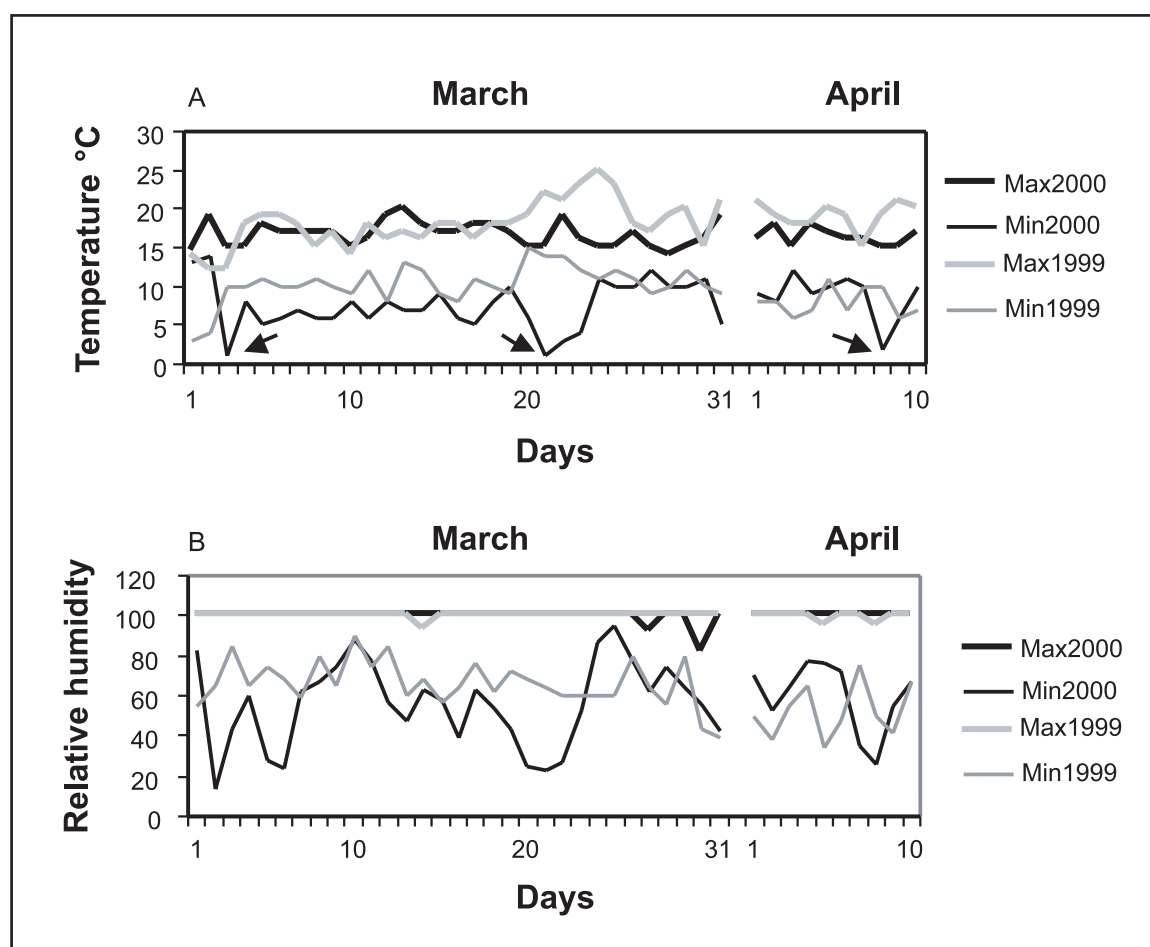


Fig. 1 — Time development of minimum and maximum daily temperature (A) and of minimum and maximum daily relative humidity (B) during March and April (1<sup>th</sup> ten days) in the 1999 and 2000 years.

shedding fruits (Figs. 3A, 3B and 3C). The B seeds result not analysable. At 30 d. p.a., at early developmental stage, the seeds of shedding and non-shedding fruits are undistinguishable; however, the S and W seeds are present, and the differences between them are showed in Fig. 4. While the W seeds evidence an initial globular embryo stage with nuclear endosperm (Fig. 4A), the S seeds show either absence of embryo and endosperm together with degenerated and wrinkled synergids with a deeply stained filiform apparatus (Fig. 4B) or the presence of embryo at globular stage with large cells and wrinkled cell walls (Fig. 4C). Starch grains are lacking in all the sections. A comparison between W and S seeds in non-shedding fruits, at 40 d.p.a., is shown in Fig. 5. The W seeds evidence classical cytological features; they exhibit only a light heterogeneity of developmental embryo stages, being the embryo in Fig. 5B (late globular stage surrounded by cellular endosperm) more developed than in Fig. 5A (globular stage

surrounded by nuclear endosperm). Both the Figs. 5A and 5B show starch reserves in the suspensor and in the cellular endosperm at the late globular embryo stage (Fig. 5B).

The S seeds of non-shedding fruits show:

1) an heterogeneity of developmental stage of precocious globular embryo (Figs. 5C, 5D and 5E)

2) a shrinkage of embryonal cells more emphasized in Fig. 5D. The Fig. 5E shows a globular embryo stage with large cells that evidence abnormalities in cytoplasm, chromatin and wall, and disorganized nuclear endosperm.

The W seeds of shedding fruits evidence a precocious and disorganized developmental stages of embryo structure like (Fig. 5F), while the S seeds exhibit either the same disorganized embryo structure like W seeds (Fig. 5G) or its absence (Fig. 5H) together with heavy damage at level of gametophyte. In both the above samples (W and S seeds) the endosperm is lacking.

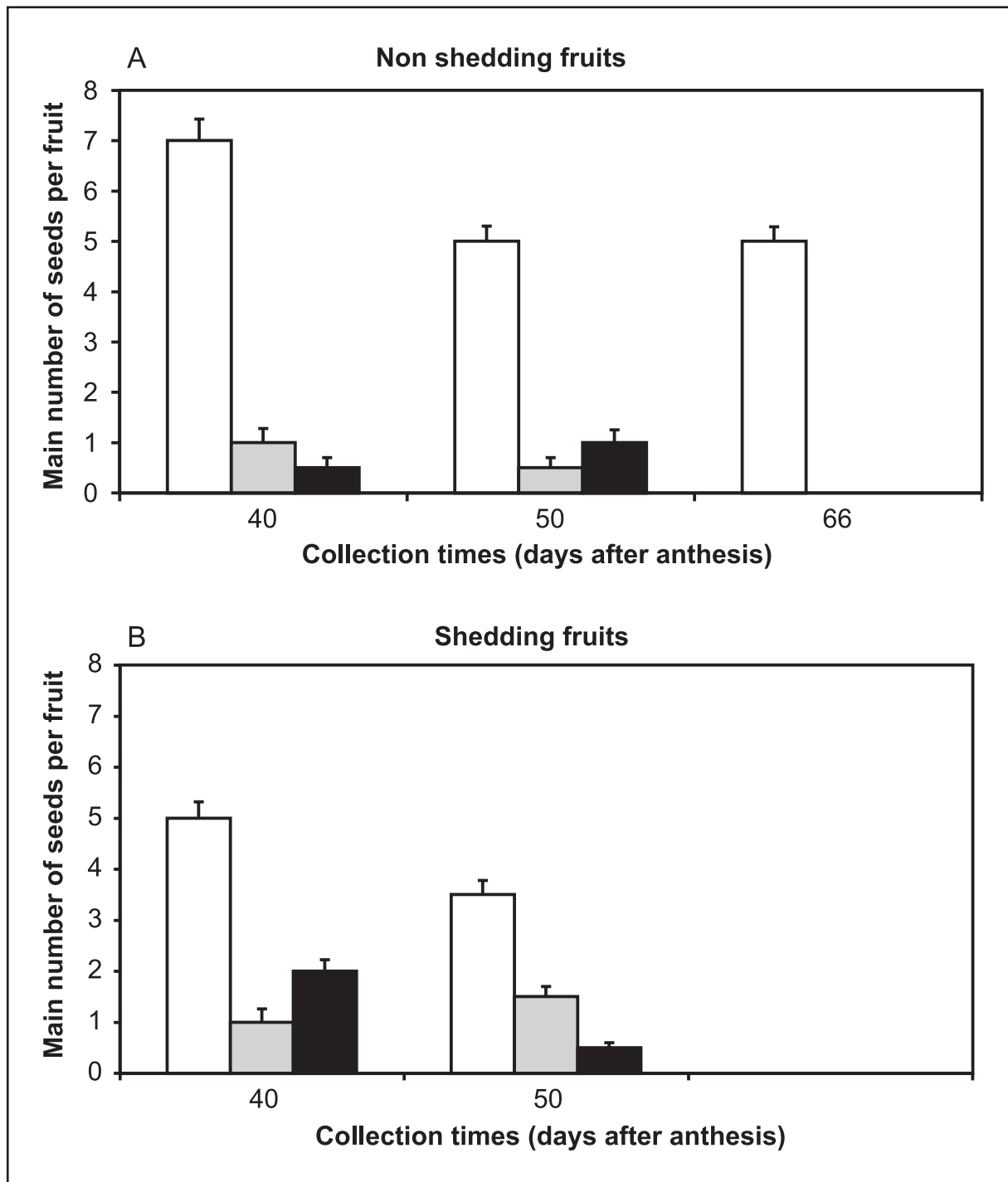


Fig. 2 — Mean number ( $\pm$  standard error) of seeds per non-shedding fruit (A) and of seeds per shedding fruit (B). White integuments seeds (W): empty columns. Spotted integuments seeds (S): dotted columns. Black integuments seeds (B): solid columns.

At 50 d.p.a. the embryos in W seeds of non-shedding fruits show an heterogeneity of development with initial heart-shaped stage together with a well organized cellular endosperm surrounding the embryo (Fig. 6A) or cotyledonary stage with

almost completely digested cellular endosperm (Fig. 6B). While low amounts of little starch grains are present only in the suspensor cells of micropilar region, when the embryo is at precocious developmental stage (Fig. 6A<sub>1</sub>), consider-

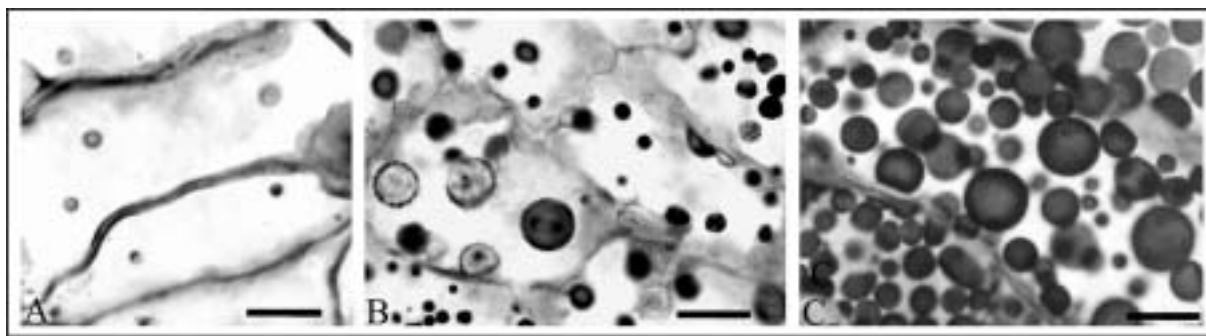


Fig. 3 — Integuments sections. Integument cells in W seeds of non shedding fruits (A), in W seeds of shedding fruits (B) and in S seeds (C) at 40 d.p.a.. Staining with toluidine blue for polyphenolics. Note the presence of scarce polyphenolics in A and their abundance in B and chiefly in C. Scale bar = 9  $\mu$ m.

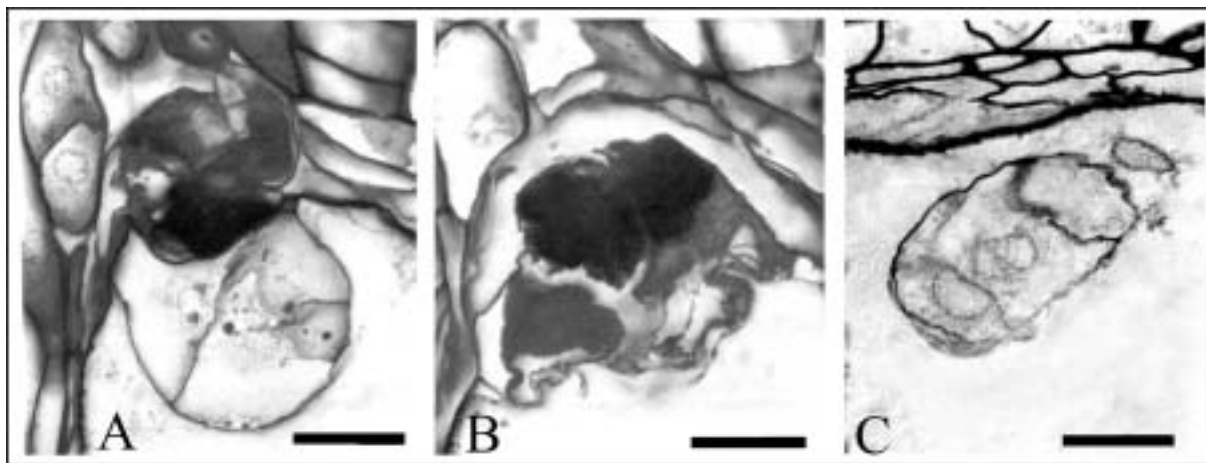


Fig. 4 — Sections of fruit seeds at 30 d.p.a. stained by TBO + PAS. W. - Embryo at initial globular stage with a nuclear endosperm (A). S. - Degenerated and wrinkled synergids with deeply stained filiform apparatus. Absence of embryo and endosperm (B). Globular embryo with large cells and wrinkled cell walls (C). Note the absence of starch grains in all the sections. Scale bar = 16  $\mu$ m.

able amounts of large starch grains are present in cells of the whole suspensor (Fig. 6B<sub>1</sub>), when the embryo is at late developmental stage. The S seeds of non-shedding fruits evidence the same heterogeneity in regard to the developmental embryo stage of W seeds of non-shedding fruits. Moreover, they exhibit a cellular wall shrinkage, a disconnection of protoplast from the wall more accentuated at the embryonal boundary (Figs. 7A<sub>1</sub> and 7B<sub>1</sub>) both in the embryo and suspensor (Figs. 7A, 7B, 7A<sub>1</sub> and 7B<sub>1</sub>), and the absence of starch grains in both the suspensors (Figs. 7A<sub>1</sub> and 7B<sub>1</sub>). It is observable a suspensor region (Figs. 7A<sub>1</sub>, 7B<sub>1</sub>) stained intensively by TBO, where some cellular components are modified to constitute a block of the routing of reserves. The W and S seeds of shedding fruits evidence a disconnection of protoplast from the wall in micropilar embryo

region (Figs. 8A, 8B and 8C), and chiefly in the suspensors which are lacking of starch grains (Fig. 8C<sub>1</sub>). The S seeds evidence, moreover, an heterogeneity of developmental stage being the embryo globular (Fig. 8A) or initial heart-shaped (Fig. 8B) in regard to W seeds which exhibit only a cotyledonary embryo stage.

At 60 d.p.a. non-shedding fruits W seeds show a late cotyledonary stage embryo with normal cytological features (Fig. 9).

## DISCUSSION

Many factors play a role in the vulnerability or resistance of flowers to spring frosts, namely related to the genotype, the developmental stage and the nutritive status of the pistil (RODRIGO

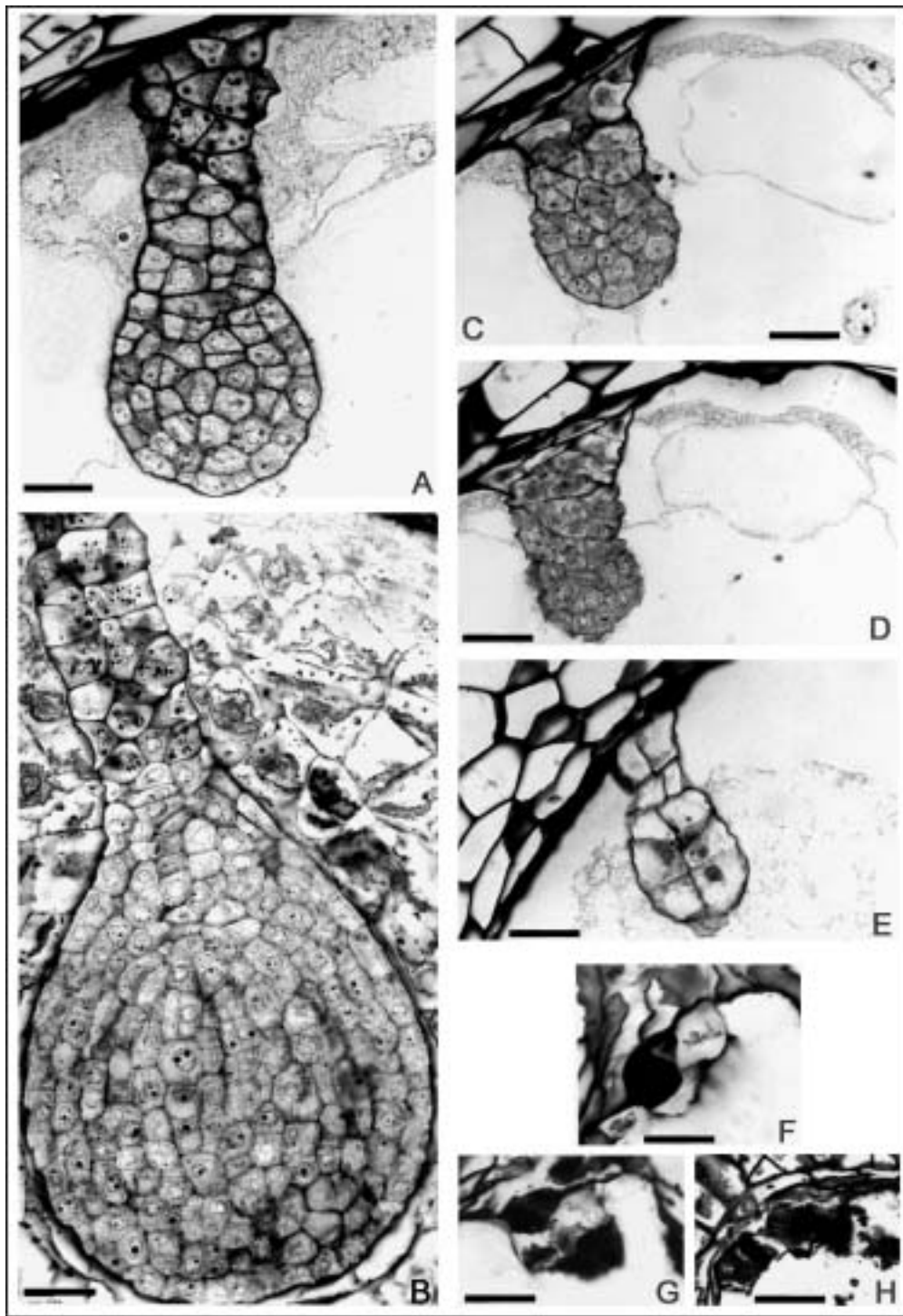


Fig. 5 — Sections of non-shedding (A, B, C, D, E) and shedding (F, G, H) fruits seeds at 40 d.p.a. stained by PAS + TBO. W. - Embryo at globular stage with nuclear endosperm and with starch grains, near to micropilar region of the suspensor (A) and at late globular stage with cellular endosperm. Note the presence of starch grains in the endosperm and chiefly in the suspensor (B). S. - Embryo at globular stage with nuclear endosperm (C, D, E). Embryonal cells evidence a shrinkage of cell walls, more emphasized in D and traces of chromatin, wall degeneration, increasing of cellular size and a little development of disorganized nuclear endosperm (E). Absence of starch grains in suspensor, embryo and endosperm. W. - Note a precocious and disorganized developmental stage of embryo structure like. Absence of endosperm (F). S. - Note the same disorganized structure (G) as in F and its absence in H, the lacking of endosperm and presence of heavy damage at level of gametophyte (H). Scale bar = 16  $\mu$ m.

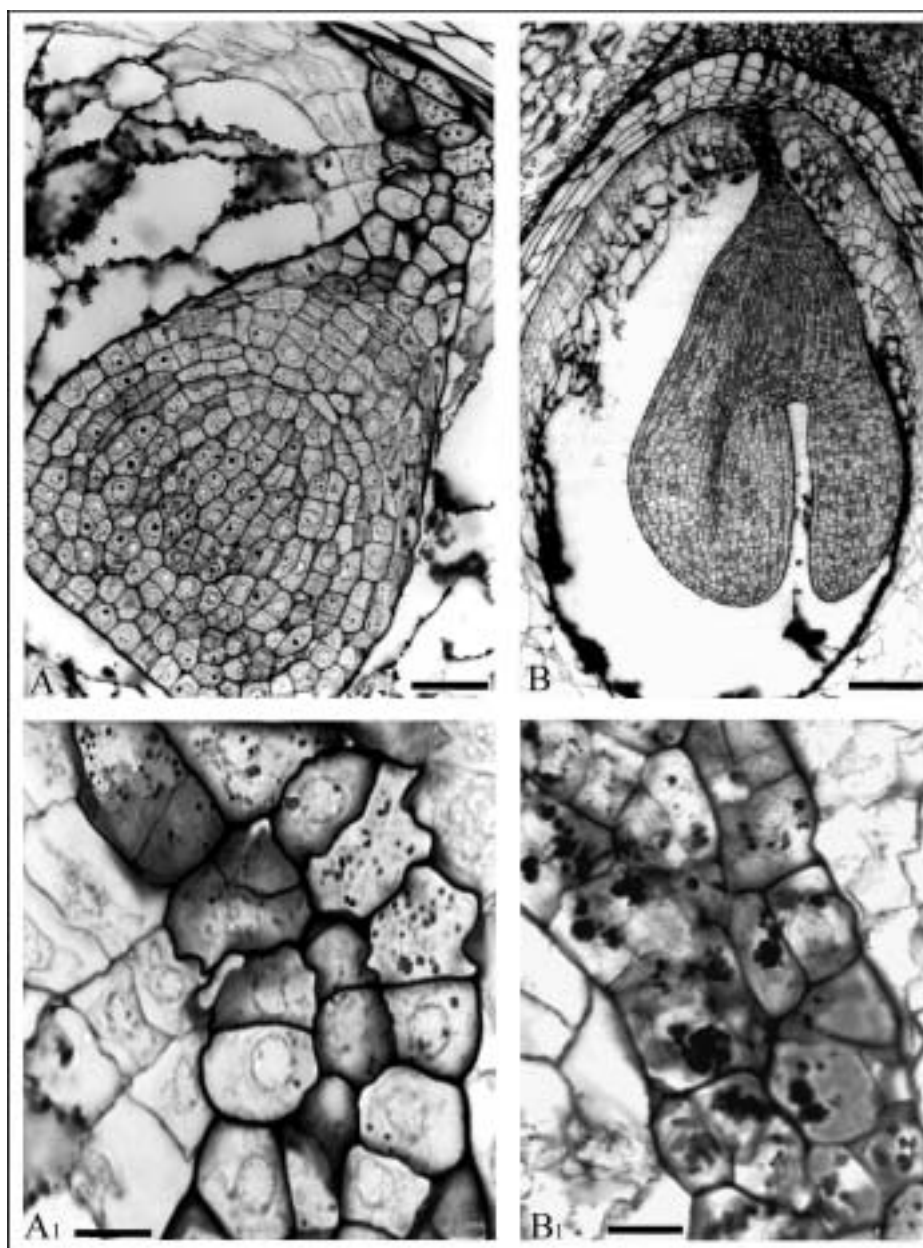


Fig. 6 — Sections of non-shedding fruit seeds at 50 d.p.a. stained by PAS + TBO. W. - Developing embryo at initial heart-shaped stage with a well organized cellular endosperm surrounding the embryo (A) and at cotyledonary stage with, almost completely digested, cellular endosperm (B).  $A_1$  and  $B_1$  suspensor details of A and B respectively. While low amounts of little starch grains are present only in the suspensor cells of micropilar region ( $A_1$ ), considerable amounts of large starch grains are present in cells of the whole suspensor ( $B_1$ ). Scale bar = 16  $\mu\text{m}$  (A), 60  $\mu\text{m}$  (B), 9  $\mu\text{m}$  ( $A_1$ ,  $B_1$ ).

2000). These factors are the same involved in the control of the fruit set (FORINO *et al.* 1987; VERNIERI *et al.* 1992; FORINO *et al.* 2000). From dormancy to fruit set, the flower bud undergoes a number of developmental events that are associated with a progressive increasing vulnerability of the pistil to low temperature (PROEBESTING and MILLS 1961; PROEBESTING 1963). Thus, the early

developmental fruit phase is the most vulnerable stage (PROEBESTING and MILLS 1978). Apparently, similar flowers in the same developmental stage and in similar position, often present differences in cold resistance and while some of them are killed under a spring frost, others can continue their development, indicating internal attributes that would make flowers more sensitive than oth-

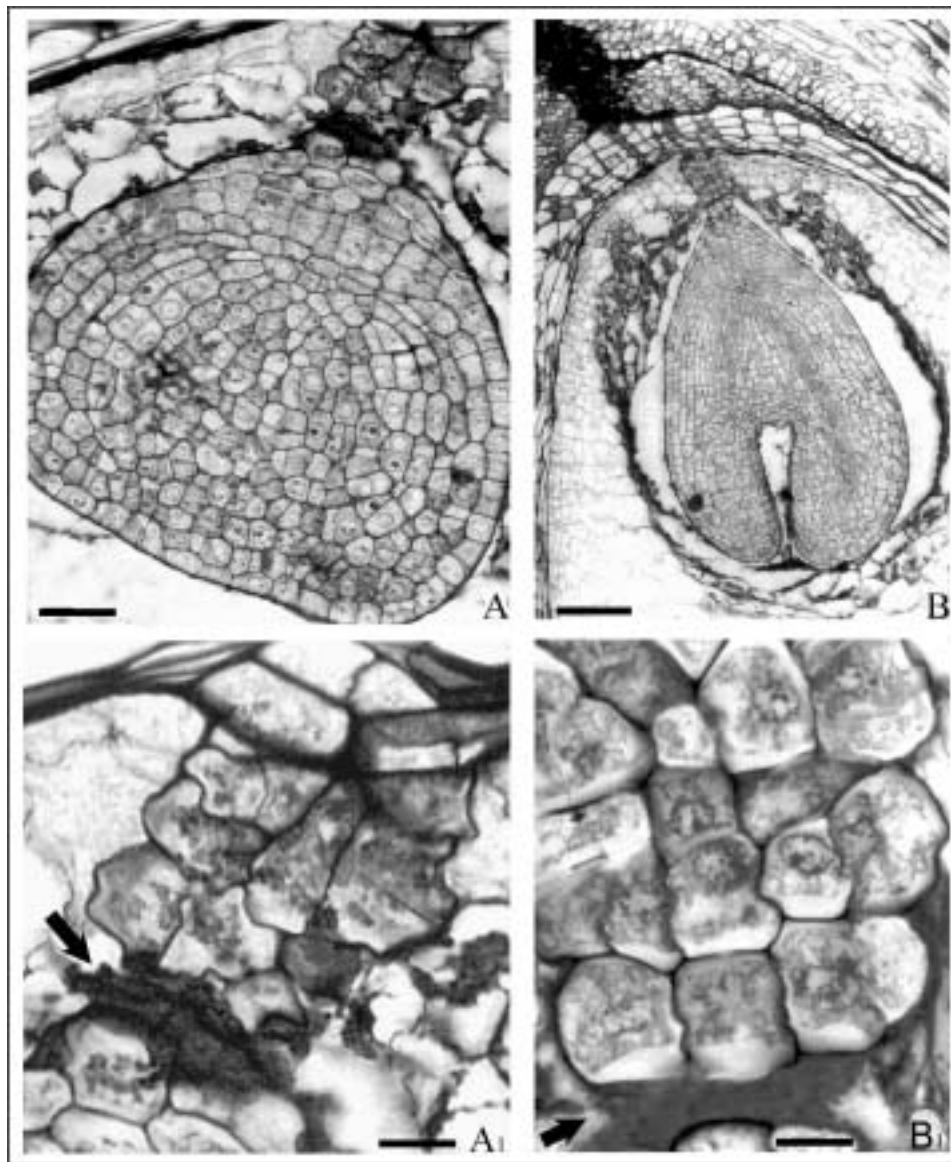


Fig. 7 — Sections of non-shedding fruit seeds at 50 d.p.a. stained by PAS + TBO. S. - Developing heart-shaped (A) and cotyledonary embryo stage (B). A<sub>1</sub> and B<sub>1</sub> suspensor details of A and B. It is evidenced a cellular wall shrinkage and a disconnection of protoplast from the wall, more accentuated at embryonal boundary (A<sub>1</sub>, B<sub>1</sub>) in the embryo and suspensor (A, B, A<sub>1</sub>, B<sub>1</sub>) and the absence of starch grains in both the suspensors. Note in A<sub>1</sub> and B<sub>1</sub> (arrows) a suspensor region, where cellular components, chiefly the wall, are probably modified to constitute a block for the routing of the reserves. Scale bar = 16 (A), 60 (B), 9 μm (A<sub>1</sub>, B<sub>1</sub>).

ers (RODRIGO 2000). In this context FORINO *et al.* (1987) evidenced in *Malus domestica* that the King Flower (K.F.) was generally better conformed, and considering the flowers of each single corymb, was the earliest to be completely structured, therefore had the highest probability to be fertilized. It, moreover, showed the high number of gametophytes among the flowers of an inflorescence. These features would make the K.F. proof against the spring frost in regard to the flowers of the same corymb.

Among the large number of morpho-physiological events that must be spatially and temporally expressed in order to control the fruit set, there is the number and histo-physiological conditions of the seeds in every fruit (SANSAVINI unpublished data, VERNIERI 1992; FORINO 2000). If these mentioned conditions do not happen, the fruits are subjected to the phenomenon of abscission which is a naturally occurring process that enables plants to shed their organs (KOSTENYUK and BURNS 2004). A number of molecular and physi-



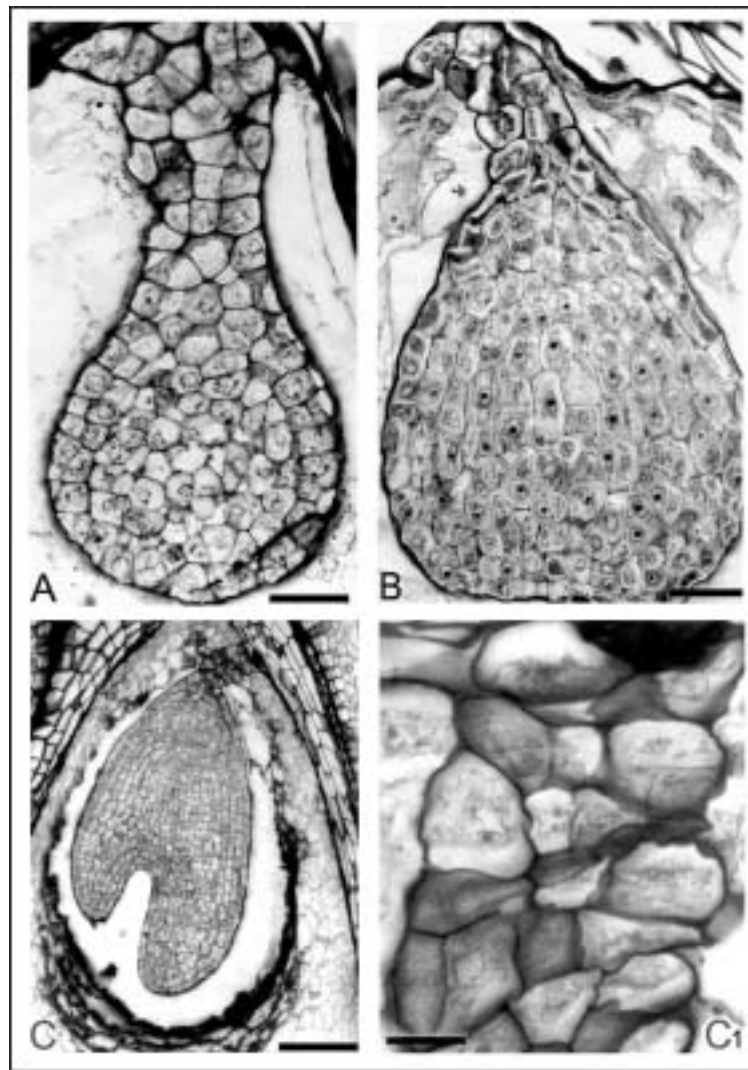


Fig. 8 — Sections of shedding fruit seeds at 50 d.p.a. stained by PAS + TBO. S. - Developing embryo at globular (A), initial heart-shaped stage (B). Note the disconnection of protoplast from the wall in the embryos. Both the suspensors are lacking of starch grains and both the endosperms are disorganized and lacking of starch grains. W. - Developing embryo at cotyledonary stage (C); C<sub>1</sub> suspensor detail of C. Note the cellular endosperm partially digested in the calazal region (C) and the disconnection of protoplast from the wall chiefly in the suspensor (C<sub>1</sub>) which is lacking of starch grains. Scale bar = 16 (A, B), 60  $\mu$ m (C), 9  $\mu$ m (C<sub>1</sub>).

ological events occur during abscission that begin with the perception and propagation of an appropriate signal and culminate in organ separation (ROBERTS *et al.* 2002). Abscission can be triggered by a range of conditions, including excessive water loss, various forms of wounding (CHEONG *et al.* 2002) and mechanical injury (BLUNDELL and PEART 2000; KLINGEMAN *et al.* 2001). We use “abscission” in broad sense: this term could be applied to the shedding of any plant organ, regardless of the process involved (SEXTON *et al.* 1982). It is reasonable to think that the severe climatic events happened in March 2000 have negatively influenced flowering and anthesis, and the next

stress in April made worse both the fertilization of ovules and the development of seeds; all these conditions have negatively influenced the fruit set. At least the S and B seeds damage is attributable to meteorologic events because this kind of seeds was never found in previous years in the samples of seeds in both non-shedding and shedding fruits, before the happening of spring frosts (VERNIERI 1992; FORINO 2000). Our data evidence the appearance of S seeds early at 30 d.p.a., when shedding and non-shedding fruits are undistinguishable, and their persistence together with differently damaged S and W seeds, until the end of fruit set in non-shedding and in shedding fruits.

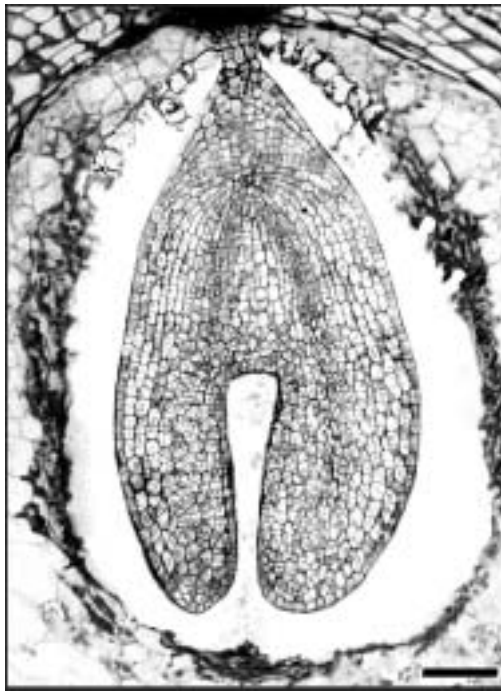


Fig. 9 — Section of a W seed of non-shedding fruit at 60 d. p.a. stained by PAS + TBO. Embryo at cotyledonary stage showing not altered cytohistological features and partially digested cellular endosperm. Scale bar = 60  $\mu$ m.

The absence of well conformed embryo in S seeds, states the precocious damage at level of gametophyte before the fertilization. At 40 d.p.a., the embryo, when present, is at globular developmental stage and exhibits a precocious cytological differentiation as an increase of cellular size and symptoms of cellular degeneration as the turgor loss, the attaining shrinkage of the cellular walls and chiefly the absence of starch reserves in the suspensor and integuments. All these features, together with a disconnection of protoplast from the wall, the incipient degeneration of chromatin and, probably, the block in the routing of the reserves, are found at the late development, when the embryo is at heart shaped or cotyledonary stage in S seeds of non-shedding fruits or at heterogeneous and more precocious stage (globular, initial-heart shape) in S seeds of shedding fruits. The cytological and developmental features of S seeds are the same occurring in W seeds of shedding fruits in the 2000 year and in shedding fruit seeds undergoing natural abscission phenomenon in previous year, as reported by FORINO *et al.* (2000). It is impossible, therefore, to state if W seeds damage in shedding fruit is attributable to natural abscission phenomenon or to meteorological stress, not showing external symptoms. In

this connection the apple flower buds, without external symptoms, can also present internal damage in the style, ovary and/or stamens during spring frosts (MODLIBOWSKA 1957). The uncertain attribution of W seeds damage in shedding fruits to meteorological stress or to natural abscission is not surprising just recalling that this latter phenomenon is fundamentally caused by endogenous stress. If both the frost and the abscission might be share the same involvement, it is worth mentioning that the pathway of natural abscission may be performed even though there are a few fruits under unfavourable environment. The W seeds of non-shedding fruits, evidencing only a lightly heterogeneity in developmental stage not found by FORINO (2000) in the previous year, appear cytologically “healthy”, but decreased in number, since the S and B seeds appearance. It is probable that this heterogeneity may be the first physiological symptom of the damage due to meteorological events. In conclusion, the differential damage among W, S and B seeds may be caused, on the one hand, by the peculiar feature of freezing stress of the successive frosts, by the differential developmental stage of the ovule/seed, by their different position inside the tree (RODRIGO 2000), by the previous damage due to the natural abscission, and, on the other hand, to actual proceeding natural abscission pathway. The S seeds are characterized on outer integuments by wrinkled brown areas where there is probably a localized accumulation of phenolic compounds. The general browning is reported by RODRIGO (2000) to be an immediate external symptom, occurring in the de-acclimated apple flower buds killed by spring frost. The S seeds contain, chiefly in the integuments, an high level of phenolic substances which, in B seeds, are likely polymerized into other secondary metabolites as tannins. In *Malus domestica* leaves and fruits, indeed, phenolic compounds may be changed in biosynthesis and accumulation, under various types of stresses caused by pathogenes or adverse environmental conditions (TREUTTER 2001). Phenolic compounds comprise a large and varied group of secondary metabolites commonly found in plants and having various functions (STEFANOWSKA and ZOBEL 2003). Their protective role is well known under stress conditions (DIXON and PAIVA 1995; SOLECKA 1997; ZOBEL and BROWN 1993). High level of phenolic substances are linked, on the one hand, to unfavourable conditions of mineral nutrition (CHAPIN 1980) and, on the other hand, together with early differentiation, to the process of tissue degeneration (MICHAX-FERRIERE *et al.*

1992; NOUR *et al.* 1993). The poor condition in S seeds could be related, as in seeds of shedding apples (FORINO *et al.* 2000) to such a phenomenon, considering the unfavorable stress environment caused by a spring frost. This stress, probably, influences the hormone level that, in turn, influences the availability of mineral nutrients. The lacking of starch reserve in the integuments and suspensor, in every developmental stage of S seeds, may be a consequence of an altered supply of nutrients from maternal tissue and/or an interruption in the nutrient routing and/or inability of seed storage tissue to synthesize some storage compounds. The probable barrier to nutrients supply, observed in suspensor region of the S seeds, seems of the same kind, even if it is differently localized, in comparison to the findings of FORINO *et al.* (2000), who found this barrier at level of the "window" at chalazal region of the natural shedding apple fruit seeds. It may be possible, also in this case, an involvement of some polyphenols, since a close relation exists between them and cell wall suberification (KOLATTUKUDY 1977; SCOTT and PETERSON 1979), even if the above mentioned modification of the wall has not been investigated in the suspensor region. The change in accumulation and routing of reserve compounds seems to be interrelated with a complex hormonal control. In plants subjected to a mild stress, increased synthesis and accumulation of ABA take place, the hormone being engaged in cell signalling during cold, drought and salt stresses (XIONG *et al.* 2002). HETHERINGTON and QUATRANO (1991) have reported that ABA levels trigger the transition of tissue from a state of active growth to one prepared to survive a developmentally or environmentally induced stress. High level of ABA, together with a loss of synthetic activity and precocious cell differentiation is reported by VERNIERI *et al.* (1992) in seeds of *Malus domestica* shedding fruits; they considered ABA to be one of the factors involved in fruit abscission in the early period of the fruit development. Very little is known about the role of ABA in our investigated processes. However, it has been demonstrated that ABA lowers the capacity of cell for water uptake, probably through a reduction in plasmalemma permeability (PARASASSI *et al.* 1990), and/or through the inhibition of extensibility of the cell wall (SCHOPFER and PACHY; 1985). These phenomena might explain the cellular shrinkage and the disconnection of protoplast from the wall, observed in S and in W seeds of shedding fruits. At 60 d.p.a., at the end of fruit set, five is the mean number of non-shedding fruit

seeds, at late cotyledonary stages, with white integuments and with normal cytological features. According to SANSAVINI (unpublished data), ripening fruits evidence generally at least five or six developed seeds in *Malus domestica*. In the 2000 year it was valued that the fruit-set of *Malus domestica*, grown in the experimental farm of S. Piero a Grado, were lower than in the previous year. The successive frosts causing a decreasing of the number of the flowers, ovules, gametophyte per ovule, and finally, of the seeds quantity per fruit, have lowered the fruit set. In regard to the number of the seeds per fruit there is a loss of "healthy" W seeds, due, probably, as above mentioned, to the one hand, to meteorological events and on the other hand, to natural abscission phenomenon. Such a loss would not allow to reach the number and the normal development of W seeds for a correct comparison of the fruit set to those of the previous year. Among the "healthy" W seeds of non-shedding fruits is to count of the K. F. seeds which, on the basis of above reported considerations, may show the highest fruit set detectable in every corymb. In conclusion, our data are in agreement to SANSAVINI (unpublished data) in regard to the presence of the number of seeds in ripening fruits; moreover our cytohistological analyses make clear the seed condition in order to occur the fruit-set: a *Malus domestica* fruit, at 50 d.p.a., containing more than 30% of damaged seeds is destined to drop. Concerning the damage of the W seeds in shedding fruits, attributable either to natural abscission or to the frost, our data point out some questions about the general aspects of abscission. If there is a little number of flowers and fruits, the drop of immature fruits occurs; then, the natural fruit abscission may be, mainly, related to genetic causes; the immature fruits may be able to self-destruct in response to environmental changes and cell signalling, in order to allow the survival only of the fruits in the best genetic condition.

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