# Contributions to the resumption of growth in ecodormant buds of apple

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INTERNATIONAL JOURNAL OF HORTICULTURAL SCIENCE

AGROINFORM
Publishing House, Hungary

***Key words:*** flower bud, bound water, dormancy, xylem differentiation, frost damage, acrotony

***Summary:*** The resumption of development in ecodormant buds in terms of establishing a functional vascular connection between the inflorescence primordia and spur tissues in apple trees was investigated. Differentiation of the xylem elements could be observed first in the pedicel of the flower primordium, in the middle of January. Much later (at the beginning of April) there were mature xylem vessels in the wall of the receptacle and, merely a procambial strand for the ovule primordium which was at this time an undifferentiated protrusion of meristematic cells, only. As for phenological development of buds incubated at a temperature of 20 °C, it was the slowest in buds sampled in January, faster in buds sampled in the middle of February and, buds from the middle of March responded very quickly. The function of temperatures needed both for xylem differentiation and for the flower primordium to achieve maturity is pointed out. The nature of frost damage in vessel elements, as well the relationship between chilling requirement and growth features of apple cultivars will be discussed.

## Introduction

The rest period of temperate-zone woody plants is controlled by a chill-related dormancy mechanism which exerts a commanding influence on growth and development (*Powell,* 1987), including the time of flowering (*Powell,* 1986). A model of winter dormancy has been proposed (*Faust* et al., 1995) which is made up of three overlapping segments: para-, endo- and a second paradormant period based on the reaction of buds to hormonal control. More recently (*Faust* et al.,1997), the endodormant period has been divided into deep endodormancy characterized by the inability to induce the buds to grow under natural conditions and shallow endodormancy, in which latter stage endodormancy can be overcome by artifical treatments. While searching for mechanisms of dormancy control in buds, it was shown (*Faust,* 1991, *Faust* et al., 1991) by magnetic resonance imaging (MRI) that in dormant buds of apple the water is mostly bound and is freed only when accumulation of cold units is sufficient for breaking dormancy.

The differentiation of certain flower parts in the buds of pome fruits (apple, pear, quince) is continued during winter months (*Zeller* 1955, 1960c, *Reichel* 1964b, cit. *Bubán* 1996), although there are some exceptions, e.g. *Malus baccata* (*Zeller* 1955), the winter development occurs in most cultivated forms. Biochemically, the buds do not show rest *Faust* 1989), nucleic acid and protein synthesis both also indicated that buds of fruit trees do not rest (*Zimmerman* et al., 1970, cit. *Faust* 1989). During winter, the differentiation, called qualitative development, is accompanied by considerable growth in size of flower primordia. The diameter of the terminal and lateral flower primordium of the apple inflorescence between October and early December incresed by 23 to 26%. By the middle of February, the growth was slower, 16 to 17% and 6 to 18%, respectively, but following this from mid-February until the middle of March the flower primordia more than doubled (*Bubán* et al., 1979). The sudden growth in the middle of February makes it probable that by then the bound water in the tissues of flower buds is already converted to free water (*Bubán & Faust,* 1995). Namely, processes involved in satisfying chilling requirement are also involved in converting water in buds from a bound to a free form (*Faust* et al., 1991).

As for the stone fruit species we cannot generalize (see papers cited by *Bubán,* 1996). There is hardly any further development in the flower buds of the cherry and peach during the winter according to *Zeller* (1995), nevertheless, *Feucht* (1955, cit. *Reichel,* 1964b) observed a quantitative growth. *Tarnavschi* et al. (1963) as well as *Cociou & Bumbac* (1973) have reported on growth in certain flower parts or in tissues and archesporium of the apricot, mainly by cell elongation. With respect to the cold hardiness in buds of *Prunus* species, xylem continuity between the flower primordium and adjacent tissues did not seem to be established until buds had deacclimated in trees of the peach cv 'Harbrite'. The appearance of mature xylem vessels appeared to parallel the loss of hardiness. However, it was insufficient to establish causality (*Ashworth,* 1982). Furthermore, an absence of mature xylem vessel elements in the upper bud axis and primordia of 'Meteor' tart cherry was generally associated with deep supercooling (*Callan,* 1990).

The aim of this study was to investigate resumption of bud development in terms of establishing a functional vascular connection between the inflorescence primordia and spurs in apple trees.

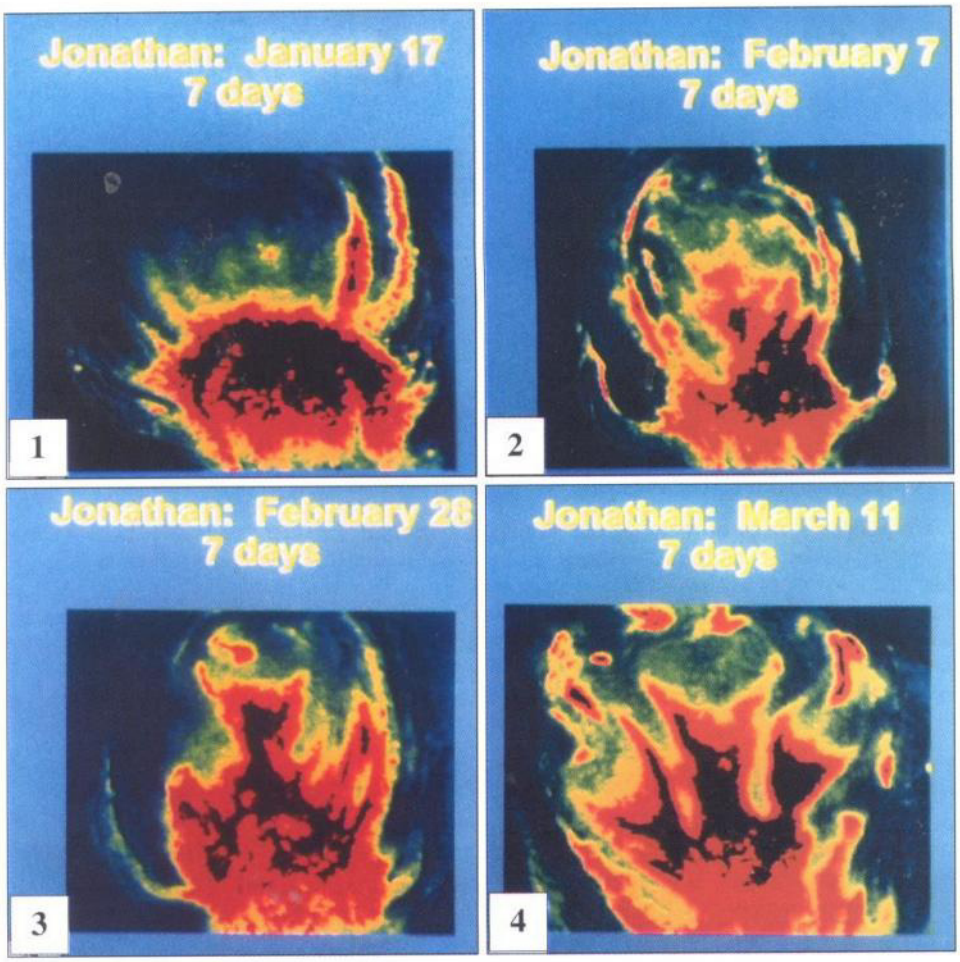


***Photo 1 to 4*** Spectral images of azosulfamide staining to detect water movement into the inflorescence primordia in buds incubated for 7 days at 20 °C (date of bud sampling see in the photos) (Azarenko et al., 1995)

## Material and methods

Two-year-old twigs from trees of apple cv 'Jonathan' were excised, the length of these twigs were about 15 cm, with 5 to 6 spurs on each. Sampling was done every 10 days from 7th of January to 21st of March. The cut surface of twigs were placed into azosulfamide dye solution (1%, in water) than incubated at 20 °C, in dark. Developmental stages of buds were estimated at the 1st, 3rd, 7th, 10th and 20th day after sampling, by a scale of 0 to 5 (0= dormant, 1= bud swelling, 2= green tip, 3= early mouse ear, 4= budbreak, 5= early green cluster). Another set of terminal buds of spurs were bisected longitudinally and the location of the red dye was observed by a dissecting microscope after the 1st and 7th day of incubation, respectively, and photos were taken (by the microscope) on color slides. Using an imaging spectrophotometer (*Azarenko,* et al. 1995a, 1995b, *Ning* et al., 1995) spectral images of these slides obtained by monochromator set at 553 nm (green) and 650 nm (red) were analysed by MATLAB software.

Histological investigations were carried out in an additional study (*Nyakas* et al., 1996). Terminal buds of spurs were embedded in paraffin and then longitudinally sectioned by a Reichert-type microtome. After serial sectioning the sections were triple-stained with astrablau, auramin and safranin (*Maácz & Vágás,* 1961).

## Results

Within the buds sampled on 7th and 17th of January, most of the red color accumulated only at the base of the bud, even following

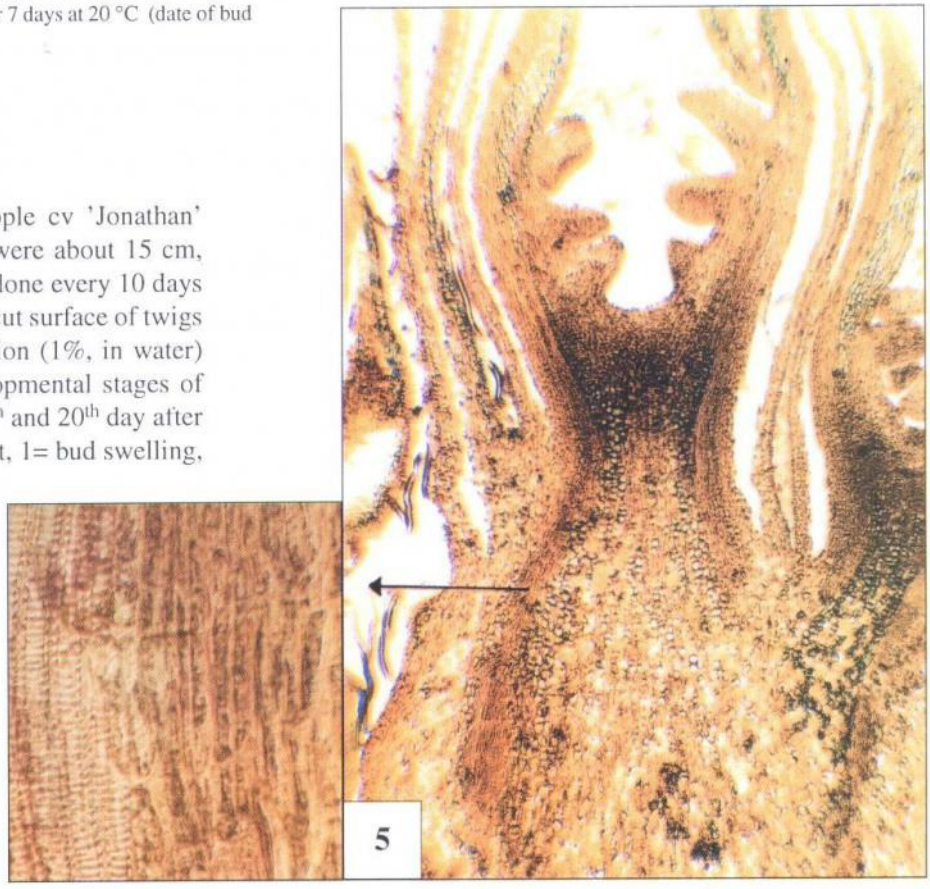


***Photo 5*** Histological section of an inflorescence primordium and appearance of xylem vessel elements below the terminal flower primordium in mid-January. (Nyakas et al., 1996)

incubation of 7 days (*Photo* 1). It means that the water soluble dye was readily translocated through the xylem vessels of the spur, but not into the inflorescence primordium. In other words, there was no functional xylem continuity to the inflorescence primordia in buds sampled during the first half of January.

It is worth to emphasize that during the whole period of investigations (till mid-March) there was no movement of dye into the bud, i. e. dye limited to the base of buds incubated for 24 hours only. Incubating them for seven days, however, dye moved more and more intensively into the inflorescence primordium as time goes on.

At the 7th day of incubation in buds sampled on

- 28th of January: there was an uptake of dye into the bottom part of the axis of inflorescence primordium and bracts inserted lowest,
- 7th to 18th of February: staining in the whole length of the axis up to the terminal flower primordium within the inflorescence (*Photo* 2),
- 28th of February: dye flow has reached the sepal primordia of the terminal flower primordium (*Photo* 3),
- 11st of March: dye distributed throughout the inflorescence primordium (*Photo* 4).

As for phenological development of buds from stage 0 to 5, it was the slowest e.g. on 28th of January, faster in buds sampled on 18th of February and, buds from 11st of March responded very quickly in terms of development at 20 °C (*Fig.* 1a to 1c).

Differentiation of the xylem elements could be observed first in the pedicel of the flower primordium (*Photo* 5), in the middle of January. Much later (at the beginning of April) there were mature xylem vessels in the wall of the receptacle and, merely procambial strand for the ovule primordium which was at this time an undifferentiated protrusion of meristematic tissues, only.

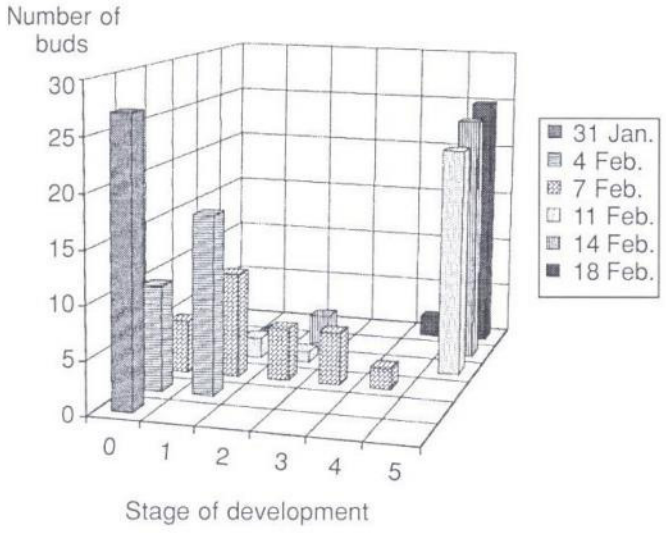


*Figure 1a*

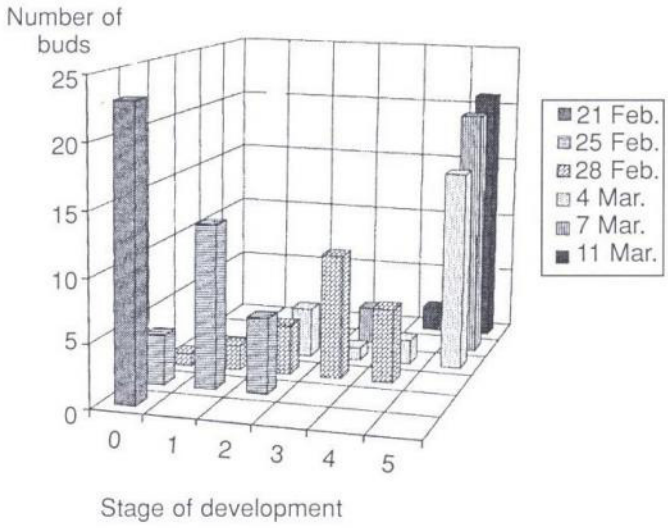


*Figure 1b*

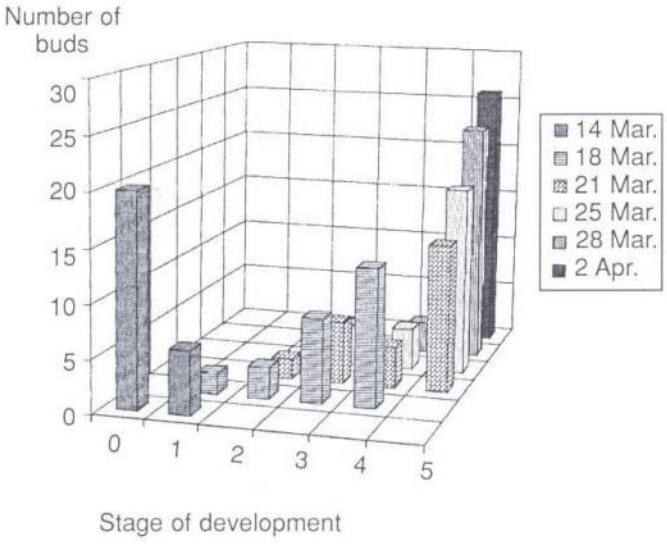


*Figure 1c*

***Figures 1a to 1c*** Development of terminal buds on spurs of apple cv 'Jonathan' forced at a temperature of 20 °C (Azarenko et al., 1995)

## Discussion

Based upon *Hatch* and *Walker* (1969, cit. *Callan,* 1990) endodormancy in buds of our investigations was considered complet, i.e. they already became ecodormant because budbreak occured within 2 weeks. In these ecodormant buds, however, there was no xylem connection to the inflorescence primordia after 24 hours incubation at 20 °C (or there was a barrier inhibiting the movement of water into the buds, *Azarenko*, pers. comm.). As time goes on (during the second

half of winter), buds respond to the 7 days incubation of 20 °C more rapidly as it can be noticed by the increase in the amount of dye in the bud (*Photo* 1 to 4) and also how fast they developed (*Fig.* 1a to 1c).

The cells in the vascular bundles of dormant buds proved to be procambial ones, then we identified the first xylem elements in the middle of January just below the flower primordium, in the pedicel (*Nyakas* et al., 1996). According to *Mackenzie & Costa Tura* (1991) differentiation of the xylem within carpellary bundles of apple flowers spreads acropetally from a point 900–1400 µm below the base of the locules. At the same time, another wave of lignification spreads basipetally from a point just below the stigma. Xylem vessel elements were observed by *Ashworth* (1982) later in the spring in peach buds that had visibly swollen, it was reported the same as regards 'Italien' prune flower buds (*Hanson & Breen,* 1985). We have induced bud swelling by incubation at a temperature of 20 °C. Taking into consideration that an intensive azosulfamide translocation into the inflorescence primordium could be observed not earlier than in the first half of February, it takes a rather long time from appearance of the first xylem elements (in mid-January) and establishing a proper functional vascular connection to the inflorescence primordium. Within the leafless period of woody plants these processes could be realized in a heat requiring i.e. paradormant period, following endodormancy (*Anderson* et al. 1975, *Lang* et al 1987, cit. *Faust & Wang,* 1993).

The hypothesis that we have now (*Azarenko* et al.,1995a) could be as it follows. Warm temperatures are required to induce xylem development to the flower bud. As the bud continues its slow rate of development in winter, there is no xylem connection. However, coincident with the buds receiving more chilling (which now would be growing temperatures), the rate of bud development increases once they have been exposed to warm temperatures (20 °C) and functional xylem becomes present. Therefore, the growing degree day requirement for budbreak is not simply growing temperature needed for the flowers to achieve maturity but also for xylem differentiation and development to occur.

For understanding dormancy a number of research problems are to be solved regarding physiological transduction of environmental signals (*Lang,* 1989). Budbreak is thought to be regulated by endogenous plant growth substances like gibberellins and root-produced cytokinins (*Donoho & Walker* 1957, *Jones,* 1973, *Torrey* 1976, cit. *Cutting* et al., 1991). In apple trees, zeatin-type cytokinins in the xylem sap rose steadily during the 6 weeks before budbreak, however, with rest-breaking chemicals it was possible to shift the the time of the cytokinin increase up to 5 weeks earlier and change the rate of the increase relative to the control (*Cutting* et al., 1991). During endodormancy the lipase activity was studied in low- and high-chilling-requiring apple cultivars 'Anna' and 'Northern Spy', respectively (*Liu* et al., 1991). Lipase activity greatly increased in bud axes when the chilling requirement of buds was almost satisfied in 'Anna' after 400, in 'Northern Spy' after 2600 chilling units. This corresponded with an increase in budbreak at 22 to 24 °C and with the release of water in buds from the bound to free form. Nevertheless, the authors were unable to determine whether this relationship was correlative or causative. Investigating vegetative buds of peach trees it was pointed out (*Marquat* et al., 1999) that during dormancy in buds there was a low absorption potential to carbohydrates and an increased sucrose concentration by starch hydrolisis which improved their freezing protection. During dormancy release, however, the buds were able to absorb carbohydrates. Biochemical processes concerning resumption of growth in fruit trees were reviewed by *Faust* and *Wang* (1993).

The winterhardiness, hereby the possibility of fruit production under the actual ecological circumstances is determined by the length of endodormancy i.e. the chilling requirement of fruit species and/or cultivars. The chilling requirement of apple cultivars (it means: the number of hours with temperature below +7 °C) is at a range of 200 to 2000 hours (*Soltész,* 1997). According to the investigations at 119 fruit production areas in Europe (*Kronenberg,* 1979, cit. *Soltész,* 1997), fulfilment of 1000 "cold" hours becomes due between 17$^{th}$ of November and 12$^{nd}$ of March, in Hungary it can most likely be from 10$^{th}$ of January to 22$^{nd}$ of January. A relationship between satisfying chilling requirement and changes in water status of bud tissues has already been mentioned above (*Faust* et al., 1991).It has recently been reported, however, that the bound water in buds of temperate zone fruit trees is correlated with the level of cold resistance rather than with the level of endodormancy (*Erez* et al., 1998). Dehydrin proteins, due to their high hydrophilicity, are possible candidates to bind water (*Muthalif* & *Rowland* 1994, cit. *Faust* et al., 1997). Dehydrin levels are more closely associated with cold hardiness transitions than with the development and maintance of dormancy (*Rowland* et al., 1999).

Regarding the risk of frost damage in winter, it is of no minor importance that water is free form in the cambium and in the xylem even during the period in which the buds of apple trees are endodormant (*Faust* et al., 1991). Water in xylem vessels freezes above –10°C (*Levitt* 1980, cit. *Ashworth* and *Rowse* 1982), ice formation in trees of 'Columbia' nectarine was detected at a range of temperatures –0.6 to –2.6 °C following an only moderated deep supercooling (*Ashworth* et al., 1985). Freezing behaviour of bark and xylem tissues, however, proved to be quite different both in peach (*Ashworth* et al., 1983) and apple trees (*Ashworth* et al., 1988). Water in bark freezes extracellulary and its cells become damaged by dehydration, while water in xylem ray parenchyma and pith cells may supercool prior the freezing intercellulary. Investigating the recovery of winter injuries in apple trees *Gogoleva* (1995) found that death of xylem mother cells accelerated the initiation of cambial activity, but death of phloem mother cells delayed the reactivation of cambium. It is worth

mentioning that hormonal reactivation of the cambium in 1-year-old shoots of 'Brompton' plum has been induced more earlier and intensively by using gibberellic acid than 1-naphthalene acetic acid or benzylaminopurine (*Jewer,* 1979).

It is not widely known, however, there is an interesting and well provable relationship between the chilling requirement and growth feature of apple cultivars. Manifestation of acrotonic branching can be related to the high chilling or low chilling character, e.g. the apple cv 'Anna' has a very short endodormancy and weak acrotony, whereas 'Northern Spy' has a long endodormant period and strong acrotony (*Faust* et al., 1995). Unfortunately, in the paper cited (*Faust* et al., 1995) the term apical dominance is used instead of acrotony. There are less spurs – and by this means fewer terminal buds i.e. the prefered places of flower initiation – in canopies of high chilling apple cultivars (*Giesberger,* 1972, cit. *Dennis,* 1997). A lack of adequate winter chilling modifies the normal pattern of spring budburst in apples, in other words, an increased basitonic tendency or enhanced basal dominance is observed (*Cook & Jacobs,* 1999). The less acrotonic (mesotonic) habit of the Japanese plum cv 'Rubynel' may also be result of a less intense dormancy i.e. lower chilling requirement (*Cook* et al., 1998).

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