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Abscission of young apple fruits (*Malus domestica* Borkh.): a review

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Abscission is a morphogenetic process, which is genetically regulated and plays several rules in plant life. Abscission of young apple (*Malus domestica* Borkh.) fruits is especially important to prevent alternate bearing and to improve several quality parameters of mature fruits. For stimulation of fruitlets abscission different chemical substances can be used. Chemical substances act by prevention of pollination/fertilization (bloom thinning) or by stimulation of fruitlets drop (fruit thinning). Although the chemical thinning is well known technological measure in fruit growing, the mode of action of different chemical thinners is still unclear. Researchers believed that chemicals can act by interfering hormonal state in plant, inhibiting assimilate production, or affect on polar auxin transport (PAT). However, until now none of these theses have been fully confirmed. Since abscission is genetically controlled process, several experiments were designed to indentify genes related to abscission in the last years. Until now groups of genes related to ethylene biosynthesis, to auxin efflux/influx carriers, to carbohydrate metabolism and to hydrolytic enzymes were identified. All mentioned genes are differently down- or up regulated during the abscission process and to clarify the role of these genes in abscission more experiments have to be designed.

Key word: apples, abscission, fruit thinning, ethylene, AZ

INTRODUCTION

In optimal conditions and adequate care and nutrition most of the fruit species form abundance of flowers. In consequence the formation of many fruitlets occurred, usually more than is needed for an optimal fruit set. This is typical also for apples (*Malus domestica* Borkh.). If inadequate crop load regulation is performed there is an abundant yield of small, poor quality, low price fruits. At the same time this may lead to another problem of alternate bearing and is expressed differently in apple cultivars. Alternate bearing is an occurrence in which a year of extremely heavy blooming (on year) is followed by a year of extremely low blooming (off year) (Byers 2003). The reason for low blooming in the `off year` is a small flower bud formation which occurred in the former year. Results from Črnko (1981) and Črnko and Marn (1985) prove that non thinned trees exhibited alternate bearing. Based on a number of investigations Greene (2002) believes that regulation of fruit set is the only practical way to break this biennial bearing cycle. Fruit thinning is done to reduce the limb breakage, increase the fruit size, improve the color and quality, and stimulate the floral initiation for the next year crop (Westwood 1992, Arteca 1995).

In the modern fruit growing, chemical thinning is used for the regulation of crop load. Chemical thinning products increase the fruitlet shedding, although their mode of action is still unclear. The efficiency of chemical thinners is also very variable and dependent on environmental factors (temperature, humidity, light) and tree condition (Byers 2003). It has been considered that fruit drop is connected with hormonal changes in plants. From this perspective, the role of ethylene as plant growth regulator which promotes abscission and the role of auxins (indol acetic acid) as inhibitor are important. Furthermore, the possibility of some still unknown factor responsible for the abscission of fruitlets can not be ruled out.

CHEMICAL THINNING

First experiments with chemical substances as a potential tool for fruit set regulation were done in the 30`s of the past century (Faust 1989). Bagenal (1925; in Dennis 2000) compared several fungicide mixtures for the control of apple scab (*Venturia inaequalis*). Along with that they found out that sprays containing lime-sulfur induced an abnormal fruit drop. Later on they started to test different chemical substances such as sodium polysulfide, $CuSO_4$, $NaNO_3$, $ZnSO_4$, and oil emulsion, but no treatment was completely satisfactory. Chemicals that killed all blossoms at the same time injured twigs and leaves (Dennis 2000). In 1940, MacDaniels and Hildebrand reported the effect of dinitro-o-cresylate (DNOC or Elgetol) in preventing pollen germination when applied to the stigmata of apple flowers. The use of DNOC was spread over USA in a few years and gave satisfactory results. The production of DNOC, which contained heavy metals, was discontinued in 1989 because of environmental concerns and the high costs of re-registration (Dennis 2000).

Nowadays substances for chemical thinning can be divided in two groups. In the first group there are substances

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for plant protection with fruit drop side-effect (carbaril, metiocarb). These are insecticides and, because of toxicity, they are ecologically less suitable. Although the effect of carbaril (Sevin) was very satisfactory it was not acceptable for growers because of its toxic effect on bees. Second group are substances with the effect of interfering with the endogenous hormonal system of plant (Bangerth 2000). These are the naphtaleneacetic acid (NAA), ethephon, benzyladenine (BA), naphtaleneacetamid (NAAm, NAD) (Bangerth 2000). This group of bioregulators is ecologically more acceptable. The problem of bioregulators lies in an inconsistent effectiveness (Črnko 1981). In some years the thinning effect is too low while in the others over-thinning may occur, although the concentration was the same.

Chemical fruit set regulation can be done by two principles of action. Chemicals used for thinning either prevent the fruit set (blossom thinning) or increase the proportion of fruits that fall in the "June" drop (fruit thinning) (Dennis 2002). Fruit thinning chemicals act by interfering with endogenous hormonal state of plants (Wertheim 2000). The biggest problem of fruit thinning with these substances is their inconsistent effectiveness, due to the numerous external factors that can influence the substance activity. Temperature, humidity (Wertheim 2000), cultivar, location, orchard design, tree condition (Byers 2003), concentration and time of application are just some basic factors that can influence the success of thinning. In addition, the optimal spray distribution and optimal water volume are also important for an efficient thinning (Unrath 2002).

MECHANISMS OF THINNING AGENTS ACTION

The mechanism of action of different fruit thinning agents has not been cleared yet. The physiologists who research this topic have still a different opinion about the effect of applied chemicals on phloem transport, endogenous hormone content/biosynthesis, seed development and other physiological processes (Dennis 2002). On the basis of their experiments, scientists obtained different results on the mode of action of chemical agents. Dennis (2002) has made a summary of important articles over the past few years and has found that there are at least eight potential mechanisms of action of thinning chemicals. According to his data the potential mechanisms are the following:

1. Inhibition of seed development
2. Delayed abscission (increasing competition among fruits)
3. Blocked transport of nutrients to fruit
4. Reduced sink strength of fruit
5. Reduced auxin synthesis in seeds
6. Reduced auxin transport from fruit
7. Stimulation of ethylene biosynthesis
8. Inhibition of photosynthesis.

Individual theses are differently accepted among researchers. In the last period, the so-called "hormonal" theory of abscission and the "assimilate" one are the two most established and most studied theories. The assimilate theory is based on the principle saying that abscission of fruit is a consequence of insufficient supply of assimilate to fruitlets as a result of limited assimilate production and/or allocation to the fruit, while the hormonal theory is based on the hypothesis saying that abscission is a consequence of hormonal changes in a plant (Bangerth 2000). Just in quick review of some results of different studies indicate quite contradictory data.

The first results of studying the impact of NAA on seed development indicated that NAA affects a smaller seed number in a fruit. Fruits with smaller number of seeds are not so competitive for nutrients. By proving this mechanism Dennis (2002) also found some contradictions such as the effect of NAA on seed number can not be proven in all cultivars and NAA can thin seedless fruits. The fact that seedless fruit can be thinned by NAA casts further doubt on the role of seed abortion as the mechanism of action (Byers 2003). With more certainty can be stated that the application of NAA influenced the lower auxin synthesis in fruits and lowered the bazipetal IAA transport as reported by Bangerth and Ebert (1981, 1982, Dennis 2002).

The correlation between ethylene and abscission was first reported by Dennis (2002), although he could not prove this hypothesis. The evidence of correlation between ethylene and fruit drop was demonstrated 25 years latter by Walsh and Williams who had observed the influence of ethephon and NAA on ethylene concentration. On the other hand, Ebert and Bangerth (1981, 1982) reported strong ethylene enhancement after ethephon treatment, while NAA treatment had small effect on ethylene concentration. Later it was also reported that NAA and BA had little impact on ethylene biosynthesis (Greene 1992). In the case of BA, Byers (2003) and Greene (1992) found positive correlation between the application of BA and ethylene biosynthesis, but they mentioned that the increase of ethylene initiated by BA was not so strong to affirm that the ethylene was the main promoter of abscission. On the basis of these results it could be concluded that ethylene is not the primary factor which controls the fruit drop.

There are also reports about the mechanisms of action through inhibition of photosynthesis. It was found that shadowing of trees or application of chemicals that inhibit photosynthesis (terbacil) strongly accelerate fruit drop (Dennis 2002). Application of NAA inhibits the carbon assimilation for 25 %, while BA has no effect on photosynthesis (Stopar et al. 1997). Based on the data collected, Dennis (2002) concluded that in fact no hypothesis corresponded all the substances which were used for chemical fruit thinning.

FRUIT ABSCISSION PHYSIOLOGY

Abscission is a process which can occur during different developmental stages in the time of vegetation. Abscission may be defined as the separation of a plant part, such as leaf, flower, fruit, seed, stem, or other from the parent plant (Arteca 1995). Under defined circumstances, fruit abscission or fruit drop can occur in the period from pollination to ripening. In apples, three periods of increased fruit drop are known. The first one occurs soon after flowering (1-2 weeks) and is usually the consequence of incomplete pollination. The second one comes 4-6 weeks after flowering and witnesses the most intensive fruit drop, also called "June drop", and the third period is the shedding of almost mature fruits.

From the physiological point of view, abscission includes two important processes: separation and protection. During abscission both processes usually occur almost simultaneously, although there are cases where they occur separately (Arteca 1995). The preliminary condition for fruit abscission is formation of a layer where separation can occur. Separation of organs (flowers, fruits) can occur only on pre-determinate layers of specialized cells called abscission zone (AZ) (Roberts et al. 2002). The abscission zone is made of one or more layers of thin-walled parenchyma cells extending across the petiole and excluding the vascular bundle (Arteca 1995). The differentiation of AZ appears to occur long before organ separation is destined to take place. The formation of abscission zone is also hormonally regulated, and it is assumed that ethylene induced the formation of AZ, whereas the indol acetic acid (IAA) dictated where it would be sited (Roberts et al. 2002). According to the previous studies, we can state that the key factors that affect the evolution of abscission are plant growth regulators ethylene and auxin IAA, or the relationship between them. Shedding of young apple fruits depends on hormonal changes in AZ, which is located on the base of the peduncle. A simplified definition could say that the abscission is inhibited by auxins and promoted by ethylene (Wertheim 2000). The role of IAA is to maintain AZ insensible to ethylene. It was found for the IAA that in the first stage it inhibits the abscission, but when the abscission is induced, IAA has a positive effect on the abscission evolution. Application of ethylene in the first stage, when AZ is insensible of ethylene, has no effect on abscission process, while application after induction has a strong accelerating effect (Arteca 1995). There are several reasons for the reduction of IAA concentration at AZ or for lowering the so called basipetal IAA transport, but when the concentration of IAA at AZ drops under the threshold value, the process of abscission can start. In the first stage of separation layer formation, the middle lamella between cells located farthest from the stem (distal region) is digested. This results by `de novo` produced polysaccharide-hydrolyzing enzymes such as cellulases and pectinases, which are secreted from the cytoplasm into the cell wall. Along with the digestion of the cell wall, there is a burst of respiration and ethylene production in cells within the abscission zone closest to the stem (proximal region). The proximal cells of the abscission zone increase in length and diameter, while cells located distally to this region do not. The combination of degradation of middle lamella and of mechanical forces which are generated by different cell growth results in the separation of two layers (Arteca 1995). In contrast to the final ethylene regulated part of the abscission process, the "desensitizing" of the AZ caused by IAA is little understood. IAA is considered the main factor in controlling the sensitivity of the AZ to ethylene, but it may not be the only one. There are periods during fruit development when the AZ is insensitive to ethylene even though IAA transport to these AZ during that period may be low (Bangerth 2000). Abscission of senescent leafs or ripened fruit is regulated during ethylene production. In senescent leaves and ripened fruits there is a big ethylene production, which has an impact on IAA transport reduction. For this reason AZ loses insensitivity to ethylene and the abscission process can start. Based on these facts Bangerth (2000) and Dennis (2002) consider that abscission of young vigorously growing fruit, however, cannot be compared with senescing leaves or ripening fruit and the regulation of their abscission in particular periods may be significantly different.

Despite of a good explanation of leaf abscission process, there are experts who doubt that ethylene is a primary factor that induces the abscission of fruit. It is presumed that negative regulation of IAA is the consequence of some "other" factor and not the consequence of ethylene, like in leaf abscission. Bangerth (2000) believes that there is a "correlative dominance effect" of adjacent fruit or nearby shoot tips. With regard to this theory the decision about which fruit will abscise depends on the strength of its IAA transport. At the "junction" where the strong polar IAA transport pathway of dominant fruit meets the weaker IAA pathway of a dominated fruit the latter is inhibited by the former (Li and Bangerth 1999). The degree of dominance of one fruit over another as compared to a dominated fruit largely depends on:

- The difference (in hours or days) in fruit set between dominant and dominated fruit
- The number of seeds/fruit
- The proximity and vigour of nearby vegetative shoot tips
- The number of fruits in a cluster.

All these dominance effects are cumulatively reflected in the IAA export rates of a particular fruit (Figure 1). It is well-known that king flower (KF) blooms earlier than lateral flower (LF); for this reason KF is in advantage in development regarding LF, and because of that, its polar IAA transport is dominant. The number of seeds in fruit has also an impact on the strength of IAA pathway. In this case the fruits with higher number of seeds are dominant compared to the fruits with lower seed number (Bangerth 2000). The next factor is proximity and vigour of nearby vegetative shoot tips, which can inhibit the polar IAA transport pathway of fruits.

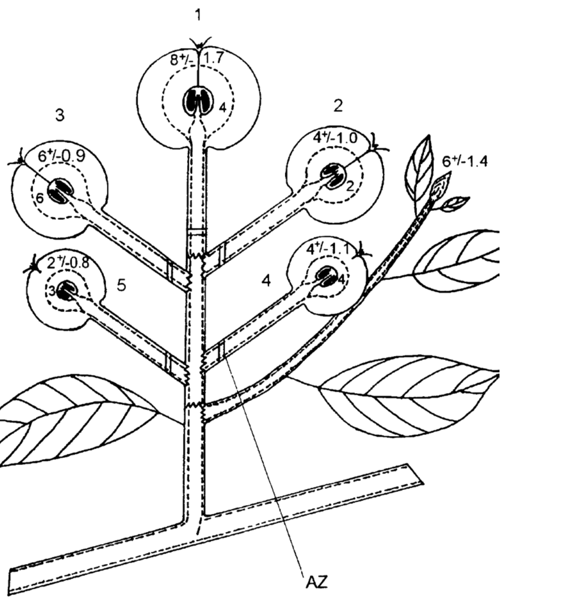

Figure 1: Scheme of an apple cluster. Numbers outside the fruit represent the ranking in PD (pathway dominance) hierarchy. Numbers inside the fruit give ng IAA export per 20h ± standard error and, as an example, the number of seeds (Bangerth 2000).

The final effect of the resultant correlative dominance on auxin transport of dominated fruits leads to a situation where the auxin transport of most dominated fruit falls below a certain "threshold value" at which stage it no longer inhibits sensitization and activation of the AZ by ethylene, which then results in the shedding of that fruit (Bangerth 2000). Beside the formerly mentioned factors, the quantity of sun radiation and temperature or interaction between them, also have an impact on fruit abscission. It has been reported that temperature during and following application is the most important environmental factor affecting fruit abscission response to ethephon. Low temperature following the application results in a poor fruit abscission response whereas high temperature may lead to over-thinning (Yuan 2007). There are several studies reporting that lowering of photosynthetic active radiation (PAR) influenced the fruit abscission (Greene 2002). Especially interesting is the information saying that a 5 days long complete shading of apple trees before June drop, 100% fruit drop can be induced, but if the shading is performed after June drop, no fruit will abscise (Berueter and Droz 1991). Byers (2003) reported that with a 2-3 days reduction of photosynthetic active radiation (PAR), the fruit retention can be influenced, if the measure is performed 14-28 days after blooming. The reduction of the lighting mainly lowers the photosynthesis, leading to the lack of assimilates and the plant is no longer able to provide enough energy for all fruits, so they start shedding. Although there are several experiments with shade induced apple fruitlet abscission, little is known about molecular nature of this process. However, in recent study Zhou et al. (2008) reported that at least 26 genes are up-regulated after shade treatment on apple tree. Most of these genes are related to carbohydrate metabolism.

Irrespective of different findings it is clear that in case of abscission, ethylene is always involved. Ethylene is considered to be the regulatory hormone in abscission process. It was found that the biosynthesis of ethylene in organs that will become detached in many cases increases before abscission, although there are examples where abscission is made without the presence of ethylene (Ruperti et al. 1998). Bangerth (2000) also agrees that ethylene is essential in the last stage of fruit abscission, but in contrast to the abscission of senescent/ripened fruit, ethylene is not the initial factor for the beginning of abscission of young fruitlets. There are also researchers reporting that external application of ethylene can accelerate abscission and the application of ethylene inhibitors, can inhibit the abscission (Ruperti et al. 1998). All these data show that ethylene is probably the factor that leads the entire course of abscission of leaves and mature fruit, and that most likely it has no impact on the initiation of abscission of young fruitlets at the time of fruit setting.

GENETIC ASPECT OF ABSCISSION

In the case of fruit abscission the greater part of genetic research is related to the expression of genes responsible for ethylene biosynthesis. In ethylene synthesis there are at least three groups of genes involved: 1-aminocyclopropane-1-carboxylate synthase genes (ACS), 1-aminocyclopropane-1-carboxylate oxidase genes (ACO), and ethylene receptors genes (ETR, ERS) (Wiersma 2006). Although ACS and ACO genes were found in early 90's, there are only few reports about their expression. For apples, MdACS1 gene was the first reported by Lay-Yee and Knighton (1995), MdACS2 and MdACS3 by Rosenfield et al. (1996), MdACS5B and MdACS5A by Sunako et al. (2000).

ACS and ACO enzymes participate in the biosynthesis of ethylene; it was found that the activity of both of them was strongly related to the amount of ethylene. In one experiment carried out on ripening apple fruit, it was found that the amount of ethylene produced by the fruit correlated with the level of transcription of the ACS genes (Harada et al. 2000). In the same experiment it was found out that the allelotype of ACS gene had a significant impact on the fruit abscission rate. Dal Cin et al. (2006) noted ethylene accumulation in apple fruits before abscission, which was connected with an increased MdACO gene expression. There are few data about the correlation between MdACO and MdACS genes, and ethylene synthesis, however, the correlation with MdACO gene is stronger. MdACO gene expression is specific in different tissues and is regulated through the environmental and hormonal factors, including ethylene. In one experiment the last four weeks of apple fruit ripening were observed. In the fruits of cultivars 'Golden Delicious' and 'Sunrise' the expression of MdACS gene increased 1.000-fold, while the expression of MdACO gene increased 10.000-fold, but just a small change of expression of ethylene receptors (ETR, ERS) was noted (Wiersma et al. 2006). Ruperti et al. (1998) and Dal Cin et al. (2009a) also agree with the conclusion that accumulation of

MdACO and related transcripts coincides with the evolution of ethylene. MdACS and MdACO genes are induced by ethylene at the beginning of ripening and then they are involved in autocatalytic biosynthesis of ethylene (Dal Cin et al. 2006).

It is known that beside to the ethylene also auxin (especialy IAA) has a very important role, when fruit abscission is concerned. However little is known about expression of different genes related to the IAA. But in recent study Dal Cin et al. (2009a) found several genes related to the polar auxin transport (PAT). It was reported that seven auxin efflux carriers (MdPIN) and three auxin influx carriers (MdLAX) are up- or down regulated during apple fruit abscission, stimulated by BA application (Dal Cin et al. 2009b).

ENZYMATIC ASPECT OF ABSCISSION

All the processes that have been mentioned in previous chapters (AZ determination, sensitizing AZ, expression of genes related to ethylene, increased ethylene synthesis) are followed by key part that is necessary for the execution of abscission. In the final stage changes are related to AZ cells in which the separation of tissue will occur. In this process two enzymes have the major role, namely endo-ß-1,4-glucanase (EG) and polygalacturonase (PG) (Sexton 1995). These are two hydrolytic enzymes causing changes in cells and resulting in shedding of fruit (Bonghi et al. 2000). In the process of cell separation beside the PG and EG, some other enzymes like pectate lyases, pectin methylesterases, and xyloglucan endotransglycosylases participate as well (Kim et al. 2006). As a consequence of their action, the cell wall extensibility and cell-cell adhesion can be altered leading to cell wall loosening that results in cell elongation, sloughing of cells at the root tip, fruit softening, and fruit decay. Correlation between PG and cell separation was first reported for ripening fruit (Roberts et al. 2002). Activity of EG in fruit abscission was reported for apples, avocado, citrus, peaches, pepper, raspberry (Bonghi et al. 2000). It was found that the increased activity of these enzymes is defined to abscission layer, where cell separation occurs, although no significant increase in activity on distal or proximal side of AZ is noted (Bonghi et al. 2000). External application of ethylene can accelerate the PG and EG activity. Pandita and Jindal (1991) reported higher PG and EG activity in cultivar 'Golden Delicious' after ethephon treatment. Beside higher activity of enzymes, also higher expression of PG and EG genes was noted in AZ tissue, but not in non-AZ tissue (Ruperti et al. 1998).

In the past, several genes which expression is increased during abscission have been discovered (Roberts et al. 2002), some of them also responsible for the synthesis of hydrolytic enzymes. PG is controlled by multigene family. For the variety '*Arabidopsis thaliana*' there are at least 66 genes linked to PG enzyme (Kim et al. 2006). Expression of PG and EG genes can be accelerated by ethylene application or inhibited by the application of auxin IAA (Roberts et al. 2002).

CONCLUSION

The role of different plant regulators, especially ethylene, is not totally clarified. Variability of results from different research investigations in the case of ethylene is very high. In some cases ethylene is mentioned as a key factor for the initiation and regulation of abscission, while there are opinions saying that ethylene is not even necessary for the abscission. Somehow, the opinion that ethylene is not included in the initial stage has prevailed, but there is still no evidence that would completely eliminate the need for ethylene in the abscission process of young fruits.

As we have mentioned through the article abscission or fruit shedding is a complex process that may take place in different periods of vegetation. Its complexity is mainly due to the relatively poor knowledge of abscission physiology, and many factors that influence its course. The poor knowledge we have in mind is primary related to the initial stage of abscission. Still, it is not clear, if one factor or sums of different factors are responsible for the initiation of abscission. The knowledge of this response would be crucial for further development of the research on this topic. Better understanding of fruit abscission can bring development of new substances for fruit thinning, with better effectiveness and reliability, and also, with smaller ecological impact, as a very important fact.

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