

## The use of benzyladenine in orchard fruit growing: a mini review.

T. Bubán

*Research and Extension Centre for Fruit Growing, 4244 Újfehértó, P.O.Box 38, Hungary; \*Author for correspondence*

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### Abstract

Plant growth regulators (PGRs) can be well integrated into orchard production systems. Benzyladenine (BA) can be considered to be an endogenous-like compound which provides very little risk to the environment. A precondition of precocious bearing in young trees is the development of a canopy structure which has good cropping potential and this can be achieved by using BA. Fruit thinning with BA in mature trees can result in larger fruit size and increased return bloom the following year. However, the temperature dependence of the thinning response remains a problem to be resolved. The efficiency of PGRs is determined by the physiological age of trees, by the environmental conditions at application and by the application methods used.

*Abbreviations:* BA – benzyladenine, GA – gibberellic acid, IAA – indole-3-acetic acid

### Introduction

The cytokinins are a large group of plant hormones, defined by Letham (Koshimizu and Iwamura 1986) “as compounds which, in the presence of optimal auxin, induce cell division in tobacco pith or similar tissue cultures, and in other activities also resemble kinetin.” The isolation of kinetin by Miller and his group, 45 years ago (Helgeson 1968) provided an impulse to research into cytokinins. Although, at that time kinetin was discovered as an artifact (Helgeson 1968), 6-furfuryladenine (kinetin) formation from its precursor (furfural) in plant cell has been reported recently (Barciszewski et al. 1999).

As early as the 1960s, important properties of cytokinins were demonstrated, e.g. they were detected in ribosomal RNA and found as structural components of tRNA (Fox 1966; Helgeson 1968; Leopold and Kriedemann 1975). Their anti-senescence effect operates probably through inhibiting protein degradation, blocking increases in RNAase activity and by retention of chloroplast RNA (Hall 1973; Helgeson 1968) as well as by inhibition of the synthesis of mRNAs encoding degradative enzymes (Leshem

1973). Cytokinins are known to overcome acrotony and apical dominance by stimulating the growth of lateral and axillary buds, respectively (Faust 1989; Helgeson 1968; Leopold and Kriedemann 1975). Flower bud formation in apple trees is related to a gibberellin:cytokinin balance (Luckwill 1970), cytokinins are essential for flower bud development in grapevines (Lavee 1989) and, the cytokinin concentration in phloem is critical to the induction of flowering of the long-day-plant *Chenopodium murale* (Mahácková 1988). In investigations of the early drop of apple fruits it was found (Fukui et al. 1985) that in seeds of potential drop fruits the cytokinin activity was only 1/40 to 1/15 of that in seeds of persistent fruits. Cytokinins have also been implicated in bud regeneration (Leopold and Kriedemann 1975) and in root primordia initiation in cuttings (Sudeinaya 1986). All these processes are associated with a specific and well-regulated cytokinin level. According to the model proposed by Kaminek and his team (Kaminek 1988) cytokinins exert autoinductive effects on their own biosynthesis and accumulation (positive feedback) and degradation (substrate induction of cytokinin oxidase).

Due to the wide range of endogenous cytokinin effects it is not surprising that the production of synthetic compounds has assumed more interest. After providing a short review of synthetic cytokinins as well as biological effects of benzyladenine (BA), we describe two special possibilities for using BA: 1) regulation of the canopy structure in fruit trees to increase their cropping potential, 2) fruit thinning to obtain fruits of higher market value and to increase return bloom in the next season.

#### *Synthetic cytokinins*

The main group of synthetic cytokinins includes N6-substituted adenines like kinetin (6-furfurylamino-purine), benzyladenine (6-benzylaminopurine, BA), 6-benzylamino-9-(tetrahydropyran-2-yl)-9H-purine; PBA (Helgeson 1968; Leopold and Kriedemann 1975; Williams and Stahly 1969). A further group comprises phenylureas such as diphenylurea, DPU (Baldini et al. 1973; Helgeson 1968), N-(2-chloro-4-pyridyl)-N'-phenylurea, CPPU (Greene 1989b), N-phenyl-N'-(4-pyridyl)urea, 4-PU (Ogata et al. 1989) and N-phenyl-N'-1,2,3-thiadiazol-5-yl-urea, TDZ (Lin et al. 1989; Thomas and Katterman 1986).

The effects of synthetic cytokinins are consistent with the functions of the endogenous compounds. Following application, enhanced chloroplast differentiation and chlorophyll synthesis result in delayed senescence (Thomas and Blakesley 1987). Cytokinins have been implicated in apical dominance (Baldini et al. 1973; Weaver 1972; Williams and Billingsley 1970). The synthetic cytokinins usually stimulate flower induction (Leopold and Kriedemann 1975), although, some phenylureas like CPPU and TDZ frequently inhibited flower bud formation in apple trees (Greene 1989a; Greene 1993a). Fruitlet abscission brought about by synthetic cytokinins has been demonstrated both in *in vitro* cultured pears (Jonkers 1973) and in apple trees in field conditions (Edgerton et al. 1981; Greene 1989a; Greene 1989b). Nevertheless, kinetin applied at a low rate (12.5 ppm) increased fruit set in trees of the apple cv. 'Royal Delicious' (Mahadev and Jindal 1986). Shoot proliferation of grapevine in *in vitro* culture has also been reported (Goussard 1981).

#### *Development and production of plant growth regulators*

The production of growth regulators for the agriculture and horticulture industries is attractive commercially. Up to 1970, the chemical industry screened approximately 63000 chemicals for their properties as growth regulators, but only 11 products were further developed (Jonkers 1973). At the end of the eighties (Crovetti and Shafer 1989), the market share for plant growth regulators was 4% of the worldwide value (20 billions USD) of agricultural chemicals at the user level. As much as 15 % of plant growth regulator production was specifically for apple growing. Later on (in 1991, 1995), the share of plant growth regulators remained nearly unchanged: 3.4 and 3.7%, respectively Rademacher 1993; 1997. Production of chemicals with importance for fruit growing like ethephon (17 to 24%) and gibberellins (GAs, 7%) was less than that of defoliant and desiccants i.e. 40% of all the plant growth regulators produced.

#### *Considerations for the use of plant growth regulators*

Key factors in the use of plant growth regulators were suggested by Abbott (1986) as follows: "Plant growth regulators have limitations when used in inappropriate circumstances, the state of target is just as important as the correct composition, concentration and timing of spray. Furthermore: "more details regarding the physiological condition of the tree might reduce inconsistency and lead to more reliable treatment, thereby enhancing both the value and reputation of plant growth regulators in fruit production."

There is also a quite different, but no less important aspect of using plant growth regulators (Looney 1993). A growing number of consumers believe that fruits produced without pesticides are more "wholesome" i.e. healthy, therefore wholesomeness must be recognized as an increasingly important component of fruit quality. The fundamental differences between pesticides and plant bioregulators should be explained to the consumers. Eventually, according to the statement of the Fruit Section of International Society for Horticultural Sciences (ISHS), instead of "plant growth regulators" the term plant "bioregulators" should be preferred.

## Benzyladenine

6-benzyladenine (BA), 6-benzylaminopurine (BAP), N6-benzyladenine, N-(phenylmethyl)- 1H - purine - 6 - amine.

### *What is benzyladenine?*

Although, it was the first synthetic compound with high cytokinin activity (Koshimizu and Iwamura 1986), BA is described as a natural purine cytokinin (Thomas and Katterman 1986) and, recently it has been isolated as a natural cytokinin from a number of plants (van Staden and Crouch 1996). All the naturally occurring cytokinins are N6- substituted adenines having the ability to stimulate cell division in the presence of an adequate auxin supply (van Staden and Cook 1986). There are also naturally occurring derivatives of BA like o-hydroxybenzyladenine; OHBA, o-hydroxybenzyladenosine; OHBAR (Kuhnle et al. 1977), or oOH(9R)BAP (McGaw 1987). Benzyladenine and the very recently discovered highly active 6-(3-hydroxybenzylamino) purine with its trivial name *meta*-topolin, as well as the less active 6-(2-hydroxybenzylamino) purine as *ortho*-topolin and their derivatives from poplar leaves constitute the natural aromatic cytokinins, distinguishable from the isoprenoid cytokinins, like zeatin (Strnad 1998).

In terms of practical use of BA (see in sections 3 and 4) it should be emphasized, that BA is not toxic to several important predatory mite species used for biological control of phytophagous mites on apple and its toxicity to mammalian and arthropod species is low (Bound et al. 1997).

### *Physiological effects of benzyladenine*

During the expansion of cells in peach leaves sprayed with BA, the number of chloroplasts per cell and the amount of chloroplast DNA (ctDNA) increased with the cell size, after this phase the chloroplast number per cell continued to increase, but ctDNA per chloroplast remained almost constant (Nii and Kuroiwa 1986). Increased chlorophyll synthesis was also reported (Walker et al. 1988). Increased photosynthate influx to BA-treated organs (i.e. the "sink effect") has also been reported (Weaver 1972), with treatments at the earlier growth stages being more effective in promoting photosynthate efflux from (grapevine) leaves adjacent to the application sites (Dzagnidze et al. 1986).

BA and IAA act in a synergic way in increasing net synthesis of RNA and proteins (Brendan and MacLachlan 1972), on the one hand, while BA induces qualitative alterations in the protein spectrum of intact roots (Caldeira 1986) on the other hand. An increased mitotic index leading to near synchronization of the meristem cell population in G1 was shown in the long-day plant *Sinapis alba* after a single application of BA to the shoot apex of vegetative plants kept in short day conditions (Havelange et al. 1986). The enhanced ethylene production observed in BA-treated apple leaves and fruits (Greene 1989a) may be one of those factors involved in processes resulting in improved flower bud formation in treated trees (Bubán 1996).

However, BA-effects are usually limited because there is only slow translocation of small amounts of BA in apple trees (Kender and Carpenter 1972; Faust 1989; Friedrich et al. 1970; Sterrett and Hipkins 1980).

### *Benzyladenine and developmental processes*

BA is used in *in vitro* propagation of apple rootstocks (Nordström and Eliasson 1986) and apple cultivars (Castelli et al. 1986). Return bloom, i.e. flowering in the year after treatments, was increased by foliar sprays of BA in trees of some apple cultivars (McLaughlin and Greene 1984); there were more flowers within an inflorescence than in that of untreated trees (Tukey 1989). BA treatment via the roots, however, reduced the production of tendrils, inflorescences and leaf primordia in the buds of young pot-grown grapevines (Palma and Jackson 1989). Although its fruit thinning effect is well known, an increased fruit set was recorded after BA treatment in apple trees (Costa and Misericocchi 1988), in mango trees and grapevine (Weaver 1972), as well as in pear trees if combined with aminoethoxyvinyl glycine and GA<sub>4+7</sub> (Edgerton et al. 1981). Overcoming self-incompatibility and improving fruit set in olive trees has also been reported (Voyiatzis 1993). Stimulation of runner formation of strawberry plants was successful with BA plus GA (Kender et al. 1971) or by BA alone (Prits et al. 1986).

### **Special aspects of canopy formation in fruit trees**

Canopy formation is very important in fruit trees because the preconditions of early productivity in young

trees are (1) a reasonable lateral branching for training scaffold limbs, and (2) a proper balance between elongated and short shoots (e.g. spurs) which have high flowering potential.

These can be achieved using chemicals such as BA applied to newly planted trees or even in the fruit tree nursery. It is interesting that the use of BA for these purposes was not yet mentioned during two ISHS symposia on plant growth regulation in fruit production, held in 1972 (Jonkers 1973).

Two important aspects of canopy development are (1) branching i.e. shoot growth from dormant lateral buds, in the spring via prolepsis (proleptic shoot formation), and (2) feathering i.e. shoot growth from axillary buds of the current year shoot via syllepsis, i.e. sylleptic shoot formation (Cook et al. 1998a).

#### *Initiation and growth of new shoots*

##### *Proleptic branching*

In fruit trees proleptic branching is predominantly acrotonic i.e. those buds nearest to the tip of a one-year-old shoot (i.e. distal buds) have dominance to develop strong elongated shoots which retain their primary role during the whole season. Acrotony establishes a hierarchy among branches forming the canopy of the tree, the development itself of the trunk is a consequence of this acrotony. Acrotony is established under correlative influences within the shoot during dormancy and, it appears to develop via primigenic dominance of the apical bud established prior to bud burst in the Spring (Cook et al. 1998a). Cytokinins play a distinctive role in Spring bud burst (Faust et al. 1997), and this kind of relationship has been reviewed previously (Cook et al. 1998b). Findings of the same team (Cook et al. 1998, unpublished) indicate that shoot-derived cytokinin may have importance in the regulation of dominance phenomena that lead to acrotonic branching. Decrease in acrotony achieved by any methods (pruning, applying chemicals etc.) results in bud activity, i.e. shoot formation in the bottom half of one-year-old shoots, it is also being important in cropping potential of trees (Bubán 1996; Bubán and Faust 1982). Outgrowth of proleptic shoots is also associated with the cessation of dormancy.

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 dor-

mancy 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 artificial treatments. While searching for mechanisms of dormancy control in buds, it was shown (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. In reality buds are not fully dormant during the dormancy period because their primordia actively enlarge and their structure becomes more complex (Bubán and Faust 1995). It has been suggested recently (Erez et al. 1998) that bound water in buds of temperate zone fruit trees is correlated with cold tolerance rather than with the level of endodormancy.

When endodormancy weakens during shallow-endodormancy, buds respond to dormancy breaking agents (Faust et al. 1997), such as cytokinins. One of the first experiments with young apple trees (Plich et al. 1975) in which bud outgrowth was promoted by BA application, suggested that cytokinins were in short supply and that buds compete for them. Manifestation of acrotonic branching can also 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. The less acrotonic (mesotonic) habit of the Japanese plum cv. 'Rubynel' may also be the result of a less intense dormancy, i.e. lower chilling requirement (Cook et al. 1998c).

##### *Sylleptic shoot formation*

This is strongly controlled by apical dominance. Apical dominance is the active mechanism of inhibition of lateral shoots exerted by the growing terminal bud. The actively growing apex inhibits outgrowth of shoots from axillary buds of the same shoot, and this inhibition is attributed, at least in part, to the auxin deriving from the growing apex. A possible role of auxin in apical dominance is to control distribution and metabolism of cytokinins. However, various hor-

mones have a sequential role in releasing buds from apical dominance (Hall 1973). For example, split applications of BA and GA<sub>4+7</sub> induced more and longer axillary shoots in apples than simultaneous applications (Popenoe and Barritt 1988), and removal of the subterminal immature (auxin producing) leaves provide similar results. Cytokinins such as BA (Chvojka 1964) increase biosynthesis of nucleic acids and mitotic activity in apices of buds. Even strong inhibition of axillary buds following 6-methylpurine application could be released by BA (Sharpe and Schaeffer 1970).

#### *Control of canopy formation by BA treatment*

With the exception of some other cytokinin-active compounds like PBA (Williams and Stahly 1968) or TDZ (Faust 1989), BA is used most often for training canopies of nursery trees and young trees in newly planted orchards.

In nursery trees, the aim is to induce sylleptic (axillary) shoots which will be strong enough to meet the demands of fruit growers for good planting material. Treatments should be carried out during the period of most intensive growth of the main axis.

In young orchards the treatments should be made early in the spring (just after bursting of terminal buds of elongated shoots) resulting in proleptic shoot formation and weak acrotony. Moderation of acrotony leads to the development of a larger number of meristematic growing point and, the growth vigour is distributed among these growing points. These benefits are manifested in favourable strength and periodicity of shoot growth for generative development and increased susceptibility to flower induction in the axillary buds of elongated shoots (Bubán and Faust 1982).

Details (doses, timing of treatments) can be found in references cited (Table 1), usually 200 to 400 ppm (in nurseries) and 100 to 200 ppm (in young orchards) of BA is applied by successive spraying 3 to 4 times and 2 to 3 times, respectively. One of the beneficial side effects is the increased crotch angle in treated trees (Williams and Billingsley 1970; Jankiewicz et al. 1973; Koen et al. 1989), but this does not occur in all cases (Plich and Jankiewicz 1973). However, some hard-to-treat apple cultivars like 'Paulared' and 'Northern Spy' are less responsive to this kind of treatment.

#### **Fruit thinning by the use of benzyladenine**

The biennial bearing of apple trees usually starts with a too heavy "snow ball" flowering. A proportion of the young fruitlets on overloaded trees should be removed to obtain fruits with proper size providing a high market value at harvest, and to ensure better return bloom i.e. crop of the next year (Bubán 1996; Bubán and Faust 1982).

In addition to a number of commercial products and chemicals for fruit thinning, reviewed recently (Greene 1993b; Wertheim 1997; Greene 1997), BA has now become more acceptable. Effects of BA include abscission of fruitlets (i.e. fruit thinning) as a main effect (Greene and Miller 1984; Greene 1989a; Elfving 1989; Elfving and Cline 1993), and other effects such as increased size of fruit at harvest (Greene 1989a; Elfving 1989; Greene and Autio 1989), elongated shape of fruit (Williams and Stahly 1969; Stembridge and Morrell 1972; Unrath 1974) the latter being important in the marketing of 'Red Delicious' type apples, and increased return bloom (McLaughlin and Greene 1984; Elfving 1989; Greene 1989a), an important "carry over" effect.

When applying BA for fruit thinning, the timing of (a single) spraying and the concentration of BA depends on the cultivars to be treated. Generally speaking, BA is applied when fruitlets have a diameter of 7 to 12 mm (most often 10 to 12 mm) i.e. 14 to 21 days after full bloom. Concentrations of BA employed cover a range of 25 to 200 ppm, most often 50 to 100 ppm of BA, in the presence of a non-ionic surfactant, e.g. 0.1% of Tween-20. Treatments for fruit thinning were most often carried out using BA as an experimental compound, as well as by using commercial products like Accel (Ferree 1996; Greene and Autio 1994), or CyLex (Bound et al. 1997), or Paturyl 10 WSC (Basak 1996; Basak 1998; Basak and Rademacher 2000; Bubán and Lakatos 1997; Bubán and Lakatos 2000) which include BA as an active ingredient and in many cases by Promalin containing both GA<sub>4+7</sub> and BA.

The majority of papers cited in this section reported successful fruit thinning effect of BA, in terms of increase in fruit size (in the year of treatment) and enhanced return bloom (in the following year). Nevertheless, there are some apple cultivars of less responsiveness to fruit thinning treatments (like those belonging to the group of spur-type 'Red Delicious' cultivars). Another problem can be the temperature dependence of BA treatment effects.

Table 1. References of using BA for canopy formation

Site of application	Species	Materials used as		References	
		Experimental compound	products with a.i. of		
		BA	BA + GA <sub>4+7</sub>		
Fruit tree nursery	Apple	BA		(Edgerton 1981; Koike et al. 1983; Cody et al. 1985; Elfving 1985; Quinlan and Tobutt 1990)	
			Paturyl 10 WSC <sup>a</sup>		(Basak et al. 1993; Wertheim and Estabrooks 1994; Hrotkó et al. 1995)
			Promalin <sup>b</sup>		(Forshey 1982; Cody et al. 1985; Basak and Soczek 1986; Ferree and Rhodus 1987; Jaumien et al. 1993; Jacyna 1996)
	Pear			Cytolin <sup>c</sup>	(Jarassamrit 1989)
			Promalin <sup>b</sup>		(Sansavini et al. 1981; Cody et al. 1985; Jacyna 1996)
	Sweet cherry		Paturyl 10 WSC <sup>a</sup>	(Hrotkó et al. 1999)	
Christmas trees			Promalin <sup>b</sup>	(Cody et al. 1985)	
Young orchards	Apple	BA	Pro-Shear <sup>2</sup>	(Crovetti and Shafer 1989)	
					(Williams and Billingsley 1970; Kender and Carpenter 1972; Plich et al. 1975; Forshey 1982; Elfving 1984; Unrath and Shaltout 1985; Miller and Eldridge 1986; Greene and Miller 1988)
			Paturyl 10 WSC <sup>a</sup>		(Bubán et al. 1990; Basak et al. 1994)
			Promalin <sup>b</sup>		(Forshey 1982; Elfving 1985; Miller and Eldridge 1986; Ouellette et al. 1996)
	Pear		Cytolin <sup>c</sup>	(Koen et al. 1989)	
			Paturyl 10 WSC <sup>a</sup>	(Bist and Bubán 1997)	

<sup>a</sup>Hungary<sup>b</sup>USA<sup>c</sup>Australia

Very little is known as to how BA works as a fruit thinning agent. Penetration of BA into the fruit is considerably higher than penetration into the leaves through either the abaxial or adaxial surface (Greene 1993b). Nevertheless, leaves may play a primary role because BA applied to the leaves alone, or both to the leaves and fruits resulted in comparable thinning (Greene 1989a; 1993b). The direct application of BA to the fruit is important to influence flesh firmness (Greene 1993b). Penetration of BA into leaves through the adaxial surface was linear with increase in temperature from 15 to 35 °C and, it increased through the abaxial surface between 25 and 35 °C. Over this range of temperature the Q<sub>10</sub> values for penetration through the adaxial and abaxial surfaces were 1.7 and 6.1, respectively (Greene 1993b).

When spraying BA for fruit thinning, a maximum daily temperature of at least 15 °C is needed (Bound et al. 1997), in other words, it should be applied only when the temperature is 18 °C or more (Greene and Autio 1989; Bound et al. 1997). Results of experiments carried out over a three years period with the same apple cultivars (Bubán and Lakatos 1997; Bubán and Lakatos 2000) confirmed the temperature dependence of the fruit thinning effect in BA-treated trees (Table 2). When the average temperature during 10 days after treatments was higher than before treatment (in 1995), excellent treatments effects were recorded. However, if temperature decreased during the 10 days after treatments, there was a less reliable (in 1996), or hardly any fruit thinning (in 1997, for details see Bubán and Lakatos (2000)). The optimum range of temperature is between 24 to 27 °C in the 2

Table 2. Temperatures and treatments efficiency of BA\*, Bubán and Lakatos (2000)

Years	Temp.°C on days of treatments	Average of °C during 10 days		Differences of Averages	Treatment efficiency			
		before	after		Fruit set		Fruit size	
		treatments	treatments		G	W	G	W
1995								
min.°C	13.5	9.3	13.7	+4.4	-	-	+	+
max.°C	20.5	18.3	28.9	+ 10.6				
1996								
min.°C	20.0	15.0	11.8	-3.2	-	±	+	±
max.°C	27.0	26.1	22.0	- 4.1				
1997								
min.°C	12.5	8.1	4.7	- 3.4	-	0	0	0
max.°C	19.8	15.2	11.7	- 3.5				

\* as Paturyl 10 WSC. Rainfall within 24 hours after treatments: 1995 0.0 mm; 1996 4.7 mm; 1997 11.0 mm. - = decreased, + = increased, ± = inconsistent, 0 = no effect. G and W = apple cvs. 'Golden Li' and 'Wellspur Delicious', resp.

to 4 days following application of fruit thinning chemicals (Greene 1997). A rainfall of 10 mm or more during a period of 24 hours after treatment should be considered as a constituent of risk (Table 2 and Bubán and Lakatos (2000)).

After BA application, increased ethylene production (because of its magnitude) cannot be responsible for the thinning response (Greene 1989a), but it may have a function in processes leading to flower induction and/or flower bud formation (Bubán 1996). BA increases fruit size indirectly by reducing competition among fruits and, directly by stimulating cell division (Greene 1993b). The greatest activity of natural cytokinins during the period of intense cell division was already reported earlier (Williams and Stahly 1969). Furthermore, the increase in average fruit weight in BA-treated trees appeared to be greater than could be accounted for solely by a reduction in number of fruits (Greene 1993a).

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