Development of 6-Benzyladenine as an Apple Thinner

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Abstract. The road from discovery through evaluation, field testing, and then marketing of 6-benzyladenine (6-BA) is one of the most interesting and longest of any of the presently used plant growth regulators. Its ability to stimulate cell division in the calyx end of apples resulted in the development of a commercial product to elongate apples that also contained an equal amount of GA₄₊₇. Abscission is not a physiological characteristic that is normally attributed to cytokinins. However, it was soon discovered that this proprietary product to elongate apples (Promalin®) could cause some thinning. Initially, it was assumed that the thinning was due to the GA₄₊₇ component, since GAs were known to cause some apple fruit thinning. It was subsequently shown that 6-BA could cause apple fruit abscission. 6-BA was evaluated alone as an agent to stimulate lateral branch development on young apple trees. It was during this evaluation process that the potential of 6-BA as an apple thinner was recognized. Not only did it thin apples but it also increased fruit size and enhanced return bloom for a crop the following year. The first 6-BA product to be released as an apple thinner was not successful because it contained a small amount of GA₄₊₇. It was not until several years later when an all 6-BA product was introduced that 6-BA was accepted and adopted by the apple industry as an important and impactful chemical thinner.

Chemical Thinners

The benefits of removal of some fruit from heavily set fruit trees were recognized before the time of Christ (Petracek et al., 2003). It was suggested that all thinning be done early (Dennis, 2000); soon after June drop. The thinning was done with the idea of increasing fruit size, improving fruit quality and to reduce limb breakage. Although fruit quality was improved by thinning at this time, the biennial bearing characteristic of pome fruit was largely accepted as an unfortunate fact of nature. It was not until the early decades of the 20th century that meaningful progress was made in the art and science of fruit thinning. Slowly, a body of information started to build up that revealed that fruit removal very early was a key component in influencing flower bud formation and achieving return bloom. The first chemical thinners were caustic materials that burned flowers thus achieving thinning by preventing set of a percentage of the fruit on a tree. The first hormone-type thinners were identified in the 1930s, soon after the discovery of the first class of plant hormones, auxins (Dennis, 2000). Over the following few years

naphthaleneacetic acid, naphthaleneacetamide, carbaryl, and ethephon emerged as thinners following a somewhat traditional pathway of development to commercial use.

The road from discovery, through evaluation, field testing, and the marketing of 6-BA is one of the longest and most interesting of any plant growth regulator. This process occurred over a 35-year period. There are at least three reasons for this very slow and deliberate process. First, abscission was not one of the major characteristics attributed to cytokinins thus an intuitive or science-based connection was not made between cytokinins and thinning. Second, the apple industry is constantly changing over time. As the industry evolved (training systems, rootstocks, cultivars, and strains), new needs emerged, which in turn created the opportunities for new uses of existing growth regulators. Third, there were challenges posed in formulating and packaging 6-BA that required time and formulation research to solve.

The Beginning

There is no unambiguous point in time that one can identify as the definitive starting time for the development of 6-BA as a thinner. However, a logical starting point may be the paper published by Williams and Stahly (1969), which closely followed the identification of transzeatin and the proliferation of cytokinin research. Williams and

Stahly (1969) showed that, all cytokinins evaluated caused elongation of ‘Delicious’ apples at the calyx end. The application was made during bloom when active cell division was occurring, especially in the calyx end of the fruit. They further showed that GA₄₊₇ caused fruit elongation and that the combination of a cytokinin and GA₄₊₇ caused more fruit elongation than when either was used alone.

There was an Industry Need

The apple cultivar profile for distribution of production in the United States in the early 1970s (Childers et al., 1995) revealed that the cultivar Delicious was the leading cultivar produced in the United States (Table 1). There were more than twice as many ‘Delicious’ produced than any other cultivar and the domination by ‘Delicious’ was even greater in Washington State. ‘Delicious’ is characterized by having an elongated shape with prominent calyx lobes. These traits are accentuated in Washington, due primarily to very cool nights during the bloom period. Growers in Washington used this environmental advantage in marketing apples by making an elongated ‘Delicious’ synonymous with quality and associated it with the Washington apple industry. The initial work of Williams and Stahly (1969) showed that the application of cytokinins and gibberellins could elongate ‘Delicious’. Stembridge and Morrell (1972) initiated field evaluation of GA₃, GA₄₊₇, and 6-BA to elongate ‘Delicious’ apples. They confirmed that both GA₄₊₇ and 6-BA elongated ‘Delicious’ and the combination of both was most effective. It was unclear which compound was more effective and this uncertainty persisted since results varied from 1 year to the next. There was interest by growers in other apple growing regions to have a product that could be used to elongate ‘Delicious’ to have an appearance similar to ‘Delicious’ grown in Washington.

Development of a Product

Abbott Laboratories initiated in the 1970s, a project to develop a product to elongate ‘Delicious’. They were aware that GA₄₊₇ could elongate ‘Delicious’ and the work of Williams and Stahly (1969) confirmed this. There were a number of naturally occurring cytokinins that were very active in apple fruit elongation; some more active than 6-BA. The naturally occurring cytokinins, in general, lost activity in solution over a very short period of time. Initially, they looked at the naturally occurring transzeatin but because of the instability, difficulties in making the material and high cost, further development was abandoned. They then turned their attention to 6-BA, because it was very stable and it was much easier and more economical to make. Commercial development focused on a product containing both GA₄₊₇ and 6-BA. The 6-BA posed real challenges and problems since it was not soluble in water or in commonly used

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organic solvents at concentrations up to 1000 mg·L⁻¹. A key to this development was the approval of tetrahydrofurfuryl alcohol (THFA) for agrochemical use in the early 1970s. Another key component in the process was the structure of GA₄₊₇. The special arrangement of GA₄₊₇ is buckled (not flat) due to the hydroxyl and carboxyl groups. The basic alkaline centers (nitrogens) of 6-BA came in contact with the acidic carboxyl groups of the GAs creating a salt form. This stable GA₄/GA₇/6-BA salt/complex allowed solubility in THFA. Thus the 1:1 mixture of GA and 6-BA was selected for both physiological and formulation reasons and a commercial formulation was prepared for field testing.

Testing of the Product

The combined GA₄₊₇ and 6-BA product was given the name Promalin® and was registered in 1979. Testing and an experimental use permit (EUP) leading up to registration occurred over a several year period before the registration. One of the responses noted by Unrath (1974) and others was that at rates of 50 mg·L⁻¹ or higher thinning could occur. The thinning response at this time was not generally embraced as an asset, but rather it was viewed as a side effect to be mitigated. The thinning component in this mixture was not known, but there was ample evidence in the literature to document fruit thinning by gibberellins, thus it was assumed by most that this response was caused by the GA₄₊₇. An experiment was initiated by Williams and Greene in 1977 in Wenatchee, WA to identify the thinning component in Promalin®. Treatments were applied 4 d after full bloom on 'Winesap' apples using 50 mg·L⁻¹ of GA₄₊₇ and 6-BA, alone and in combination. Final fruit set results taken at the end of June drop are presented in Table 2. 6-BA reduced final fruit set to the same level as the combination of GA₄₊₇ and 6-BA (Promalin®). While the mean for fruit set for the GA₄₊₇ was lower the

difference was not statistically significant. This clearly demonstrated that 6-BA was the primary thinning component.

Industry Evolution Continued in the 1970s

Several changes occurred in the industry during the 1970s. Production of 'Delicious' continued to increase its domination of the industry (Fisher and Ketchie, 1981). Planting of spur type trees expanded due to smaller tree size, ease of management horticulturally, and increased production. As frequently happens with new, very popular red variety of apples, the industry sought more highly colored mutations particularly with the spur-type growth habits (Ketchie, 1987). Although these trees were easy to manage horticulturally, they were slow to develop a good fruiting surface due to a limited number of spurs developing into productive lateral branches. This situation was further complicated by the use of the central leader training system on trees propagated on semidwarf rootstocks that were becoming increasingly popular at that time (Heinicke, 1975). These trees were trained and pruned to have limbs arranged in tiers with a relatively large space between tiers of branches. Clearly, it would be advantageous to have a method to increase branching on these trees.

The Bud Break Characteristic of Cytokinins.

The budbreaking characteristic of 6-BA was discovered early in cytokinin research. Williams and Billingsley (1970) demonstrated that cytokinins and GAs could cause branching on apple trees. Promotion of branching in young nursery trees was included on the original Promalin® label with rates allowed up to 500 mg·L⁻¹. It was widely used in the nursery industry as a branching agent on apple trees growing in the nursery. Promalin® was experimentally evaluated to determine if it could increase the surface bearing capacity of a tree, and thus the early yield of spur-type trees and other sparsely branching apple varieties. Rates between 200 and 500 mg·L⁻¹ were generally used. Increased branching was achieved, but it came at an unacceptable expense. Flower bud formation was inhibited by the GA thus dramatically reducing early fruit production (Greene, 2003; Miller, 1988). The influence of Promalin® and 6-BA on branching and flower bud formation is illustrated in Table 3. Both compounds caused a comparable amount of branching as a result of stimulating spurs to

elongate. However, Promalin® completely inhibited flower bud formation for the following year, whereas 6-BA had no effect. Branching studies comparing 6-BA alone with 6-BA plus GA₄₊₇ were carried out in the late 1970s and early 1980s by a number of investigators including Unrath (1989), Unrath and Shaltout (1985), Miller (1988), Miller and Eldridge (1986), Forshey (1982), Elfving (1984), Greene and Miller (1984) and many others. Application rates of 6-BA sufficient to stimulate lateral branching frequently resulted in near defruiting of these young trees. Although the thinning response was noted by a number of researchers, McLaughlin and Greene (1984) reported that 6-BA had potential as a chemical thinner on apples in since it satisfied the primary characteristics of a chemical thinner: caused fruit abscission, increased fruit size, and promoted return bloom.

The Apple Industry Continued to Evolve in the 1980s

The 1980s was a time of consumer change and increased product diversity offered to consumers in the produce department. Fruit and vegetables once available only seasonally became available for longer periods of time and consumers were introduced to a wide range of flavors, tastes, and choices. 'Granny Smith' was introduced in the early 1980s and it was soon followed by 'Gala', 'Fuji', 'Braeburn', and a host of other new and less-well-known varieties. The different varieties were eagerly accepted by the consuming public. Many had unique appearance, taste, texture, and flavor profiles that were unlike the standard apple cultivars and consumers showed a willingness to pay premium prices for these new cultivars. In response to demand and economic incentives, growers planted these more profitable cultivars. However, these new varieties presented new thinning challenges that were not completely met by existing thinning options. There clearly was a need for a new thinning chemistry, which was further exacerbated in 1989 with the loss of Elgetol® an extremely important blossom thinner for Washington state growers. There was very strong support and encouragement from the research community for the registration of 6-BA as a postbloom thinner.

Why Did It Take so Long to Pursue 6-BA as a Thinner?

It required at least 10 years to recognize the full potential that 6-BA had as a thinner. Promalin® was known to cause thinning

Table 1. Apple cultivars with the highest production grown in the United States in the 1969–71 time period (Childers et al., 1995).

Apple cultivar	Percent of total
Delicious	29.4
Golden Delicious	13.0
McIntosh	11.1
Rome	8.2
Jonathan	6.8
York Imperial	5.6

Table 2. Effect of 6-benzylamino purine (6-BA), gibberellins A₄₊₇ and Promalin® (6-BA + GA₄₊₇) applied 4 d after bloom on fruit set of 'Winesap' apples (M.W. Williams and D.W. Greene, unpublished data).

Treatment (mg·L ⁻¹)	Fruit set (%) ²
Control	98 a
6-BA + GA ₄₊₇ 50,50	40 b
GA ₄₊₇ 50	63 ab
6-BA 50	47 b

²Mean separation by Duncan's multiple range test, *P* = 0.05.

Table 3. Influence of 6-BA + GA₄₊₇ (Promalin®) and 6-benzylamino (6-BA) alone on lateral budbreak, shoot growth and return bloom on 'McIntosh'/M.26 apple trees in Wibraham, MA.

Treatment (mg·L ⁻¹)	1978		1979	
	Lateral shoot length (cm)	Lateral shoot no. per cm LCSA ^y	Spur per cm LCSA	Flower clusters per cm LCSA
Control	24.0 ² a	0.7 b	4.1 a	11.9 a
6-BA + GA ₄₊₇ 500	20.6 b	2.6 a	2.1 b	0.1 b
6-BA 500	17.0 c	2.3 a	2.7 b	13.8 a

LCSA = limb cross-section area.

²Mean separation Duncan's multiple range test, *P* = 0.05.

Table 4. Physiological effects that were identified during the development of 6-BA as an apple thinner.

Physiological effects	Journal citation
Promotes fruit abscission	Bound et al., 1991; Bound et al., 1993; Bound et al., 1997; Buban and Lakatos, 1998; Byers and Carbaugh, 1991; Elfving, 1989; Elfving and Cline, 1993a; Elfving and Cline, 1993b; Ferree, 1996; Greene and Autio, 1989; Greene et al., 1990; Greene et al., 1992; Greene and Autio, 1994; McArtney et al., 1995; Wertheim, 1997; Wismer et al., 1995
Increase fruit size/weight	Bound et al., 1991; Bound et al., 1993; Buban and Lakatos, 1998; Elfving, 1989; Elfving and Cline, 1993a; Elfving and Cline, 1993b; Ferree, 1996; Greene and Autio, 1989; Greene et al., 1990; Greene et al., 1992; Greene, 1993; McLaughlin and Greene, 1984; Wertheim, 1997; Wismer et al., 1995
Increase return bloom	Bound et al., 1991; Bound et al., 1993; Buban and Lakatos, 1998; Elfving, 1989; Elfving and Cline, 1993a; Elfving and Cline, 1993b; Greene and Autio, 1989; Greene et al., 1990; Greene, 1993; Greene and Autio, 1994; McArtney et al., 1995; McLaughlin and Greene, 1984; Wismer et al., 1995
Increase flesh firmness	Greene and Autio, 1989; Greene et al., 1990
Increase soluble solids	Greene and Autio, 1989; Greene et al., 1990; Wismer et al., 1995.
Weak thinner alone; strong thinner with carbaryl	Byers and Carbaugh, 1991; Elfving, 1989; Greene and Autio, 1989; Wertheim, 1997
Few postharvest effects—not attributed to increased fruit size	Greene and Autio, 1989; Greene, 1993; Greene and Autio, 1994
Increase branching	Elfving, 1984; Forshey 1982; Miller, 1988; Miller and Eldridge, 1986; Unrath, 1989; Unrath and Shaltout, 1985

when applied as a bloom spray. In retrospect, we now know that 6-BA is a very mild thinner when applied near bloom (Greene, 2002). Even when 6-BA was identified as a thinner on apples in 1977 (Table 2), the response was mild and deemed insufficiently potent to serve in its own right as a thinner. When 6-BA was applied 10–14 d after bloom (a traditional thinning time for postbloom thinners) to stimulate lateral branching, its true thinning strength was realized. In the 1980s, the apple industry was undergoing change and consumer demands were changing. Retailers and consumers were asking for larger fruit and but increased size was often difficult to achieve with available thinning options. Large fruit were generally the most profitable to growers. One of the new and emerging varieties especially on the east coast was the small-fruited cultivar 'Empire'. 6-BA was not only an effective thinner on 'Empire' it also increased the size of fruit to a much greater extent than could not be attributed solely to a reduction in crop load (Wismer et al., 1995). In 1989, the blossom thinner Elgetol® was withdrawn from the market. Although this was a blossom thinner and it was used primarily on the west coast, this provided an additional psychological impetus to register 6-BA.

A 6-BA Formulation for Thinning

The solubility problems of 6-BA, originally encountered during the development of Promalin®, also plagued the successful formulation of an all 6-BA product. The availability of THFA and the apparent marriage between GA₄₊₇ and 6-BA appeared to solve the solubility problem. The 6-BA alone product formulated for the branching studies once again posed solubility problems. It was reported by some researchers, including Elfving and Forshey (personal communication, 1982) that 6-BA precipitated out of solution soon after mixing at the higher concentrations. Before proceeding with an all BA product, the formulation problem had to be solved. A stable 6-BA product was developed in the early 1980s for branching of Christmas trees; ProShear®. It was this product or a similar formulation that were

used by researchers to develop the early thinning information for 6-BA on apples. The first citation in the literature suggesting 6-BA was a thinner was in 1984 (McLaughlin and Greene). There was a 5-year gap in published thinning reports using 6-BA, despite active research was going on during this period of time.

Defining the Characteristics of 6-BA

Starting in 1989 and for several years thereafter many research reports were published that documented the many characteristics and attributes of 6-BA. Listed in Table 4 are some of these attributes and some of the journal citations summarizing the major findings of research that focused primarily on 6-BA when used as an apple thinner.

The First 6-BA Thinning Product

The need for a new thinning product and the overwhelming body of research data showing efficacy of 6-BA as an apple thinner were the driving forces for the refinement of the 6-BA product. The 6-BA product formulated in the 1980s, contained components that would not be allowed for registration by the Environmental Protection Agency (EPA). To obtain a registration for a 6-BA-alone product it would require at least two years to obtain residue samples to satisfy registration requirements. As a consequence, an expedient response was to develop the Accel® formulation which was a modification of the Promalin® formulation. It contained primarily 6-BA but also reduced the amount of GA₄₊₇ by 90%. This formulation was approved by the EPA. Abbott Laboratories was granted provisional registration of Accel® in 1993 and the first commercial sales were in 1994. The final registration was granted in 1997.

The Performance of Accel® was Disappointing

The performance of the first 6-BA thinning product, (Accel®) did not live up to the expectations that had been buoyed and reinforced by over 10 years of thinning data and hundreds of thinning trials. Part of the initial problem could be attributed to the restrictions of

the amount of product that could be used based upon limitations imposed by the original Promalin® label. This limitation was eventually rectified by modifying the label to allow more product to be applied, but it proved only to be a partial solution. Based upon extrapolation of data gleaned from the literature, the amount of GA₄₊₇ present in the formulation was thought to be too small to be of major physiological significance, especially given the much higher percentage of 6-BA present. The GA₄₊₇ did appear to have an undesirable influence and it manifested itself by increasing the retention of small fruit and by reducing the abscission-promoting effect of 6-BA. It was not uncommon for some spurs treated with Accel® to retain multiple fruit and frequently one or more of these fruit were small.

The 6-BA-alone Thinning Products

The first commercial-scale trials of a pure 6-BA product were carried out in Australia in 1993–94 comparing the pure 6-BA product (Cylex®; Valent Biosciences Corp., Libertyville, IL) with the Accel® (Valent Biosciences Corp.) (product containing 6-BA and GA₄₊₇). The results clearly showed that Cylex® was a superior fruit thinner (Bound, 2006). The Cylex® formulation was registered in Australia in 1996. Australia was the first place for Abbott/Valent BioSciences to get a 6-BA product registered at high rates (up to 180 mg·L⁻¹). The Cylex® formulation was further modified and improved. The VBC-30001 formulation was an improved and modified Cylex® formulation was that tested in the United States. An EUP was granted in 2003 and the commercial product, MaxCel® (Valent Biosciences Corp.), was registered and commercially sold in 2004. This is the product that is now sold worldwide.

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