MANIPULATION OF CROP LOAD WITH BIOREGULATORS TO MITIGATE BIENNIAL BEARING IN APPLE

By

TORRANCE RAY SCHMIDT

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of TORRANCE RAY SCHMIDT find it satisfactory and recommend that it be accepted.

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Chair

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MANIPULATION OF CROP LOAD WITH BIOREGULATORS

TO MITIGATE BIENNIAL BEARING IN APPLE

Abstract

By Torrance Ray Schmidt, MS
Washington State University
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Chair: Donald C. Elving

Biennial bearing habits in apple (*Malus domestica* Borkh.) produce high yields of small, poor quality fruit in the "on" year and low yields of large fruit prone to physiological disorders in the "off" year. Flowering promoters such as ethephon may help bolster return bloom after an "on" year, and floral inhibitors such as gibberellic acids (GAs) may reduce bloom in the season following an "off" year, improving cropping consistency and orchard profitability. These studies conducted in commercially important cultivars prone to biennial bearing sought to: 1. Evaluate the influences of crop load, application timing, and spray concentration on the efficacy of ethephon and GA in apple. 2. Examine effective concentrations, application timings, and spray concentrations of several isomers of GA. 3. Investigate collateral effects of GA programs, including maturity of fruit present during spray application.

Crop load was manually adjusted on individual whole trees to three levels (100%, 50%, 0%) in six trials. In 2004 and 2005, 400 mg/L GA_{4+7} were applied to trees of each crop level at one of three timings to 'Cameo' 45 days after full bloom. In separate 2004

and 2005 ‘Cameo’ trials, four concentrations of ethephon were applied to trees of each crop level. In the two remaining crop-adjusted trials, ‘Honeycrisp’ (2004) and ‘Fuji’ (2005) trees were also sprayed with 300 mg/L GA_{4+7} at one timing. Return bloom was generally inhibited by GA and promoted by ethephon in all trials, but their effects were overwhelmed by the influence of initial crop load. A 2004 ‘Fuji’ trial found a dose response relative to GA_4 concentration and greatest floral inhibition (51-75%) at 10 mm timing. GA_3, GA_4, GA_{4+7}, and GA_7 reduced 2006 flowering by 53-90% in 2005 ‘Honeycrisp’ and ‘Fuji’ trials. Several concentrations of GA_{4+7} applied to ‘Cameo’ in 2004 and 2005 failed to clearly affect fruit maturity, but similar treatments accelerated ripening of ‘Honeycrisp’ in a 2004 trial by 2-5 days; fruit maturity effects from several GA isomers in a 2005 ‘Honeycrisp’ trial were not as clear. Our results indicate that bioregulators offer promise as tools for crop load management of apple.

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CHAPTER 1

Plant bioregulators and biennial bearing of apple

Keywords: alternate bearing, floral initiation, gibberellin, GA, ethephon, return bloom, crop load management, chemical thinning

Commercial apple (*Malus domestica*, Borkh.) growers rightly dedicate considerable resources to pest control, tree nutrition, and irrigation, but these issues rarely impact a typical orchard's fiscal bottom line as significantly as crop load management. To be financially viable in today's industry, apple plantings must produce consistent crops of large, quality fruit. Growers employ a wide range of horticultural tactics to achieve this goal, including pruning, training, nutrient management, chemical thinning, manual fruitlet thinning, and application of exogenous growth regulators. Yet despite the best efforts of even the most conscientious growers, many apple trees fall into crippling patterns of biennial (alternate) bearing. This trend has become increasingly common as traditional Red Delicious plantings have been replaced by new cultivars more prone to alternation.

While the national apple industry experiences ramifications of crop size in terms of market prices, individual growers endure the effects of biennial bearing on a block-by-block basis. Heavy crop loads tend to incur large hand-thinning bills, small fruit size, and poor fruit color. Light crops are often associated with rank vegetative growth (Looney et al., 1978), large fruit prone to storage disorders, and most obviously, low yields. Whether in the "on" or "off" phase of a biennial cycle, apple growers suffer when their trees are out of cropping balance.

History of crop load management research

For more than a century, horticulturists have sought solutions to biennial bearing. Beach (1903) described significant year-to-year swings in yields from individual 'Rhode Island Greening' apple trees in upstate New York, noting that "degree of productiveness is a variety characteristic, but it also seems to be a permanent characteristic of the individual tree." In an effort to improve the "regularity" of production in individual trees, Beach hand-thinned fruits to various degrees at several timings through the growing season and concluded that aggressive early thinning resulted in the highest crop yields in the year after treatment. These experiments provided early clues that something within apples themselves might inhibit the degree of flowering in the subsequent season.

Biennial cycles were not problems peculiar to the American industry; in 1931, Carne published a paper entitled "Heavy and Light Cropping in Alternate Years: A Serious Defect of the Australian Apple Industry," in which he estimated that the Australian industry routinely experienced 70% annual swings in crop yields, compared to 20% swings in the Western United States. He postulated that in years with excessive vegetative growth, trees build up tremendous reserves of carbohydrates, favoring flower development in the following season; in theory, he argued, these tendencies could be partially mitigated through application of nitrogen to help offset the effects of large carbohydrate reserves, or by thinning unopened flowers, which had not yet used their nitrogen reserves (Carne, 1931). As with Beach, Carne offered practical remedies to help promote annual bearing despite primitive knowledge of plant physiology. Growers might be well-served, he argued, to maintain roughly half of their trees in the on year of an alternating cycle, and the other half in the off year.

Interestingly, Carne also referenced an unknown series of events which had synchronized the bearing cycles of most apple trees in three separate Australian states in different years; the most feasible explanation offered was blossom infestation by flower thrips, whose feeding damage had caused significant crop abortion (Carne, 1931). While insect pressure rarely becomes severe enough to trigger widespread crop failures in today's industry, heavy spring frosts which have been know to synchronize and amplify biennial cropping cycles across production areas.

The case for thinning crops to promote annual bearing was further bolstered by Palmer and Fischer, who reported annual yield benefits from hand-thinning 'McIntosh', 'Delicious', 'Rome Beauty', and 'Newtown' in British Columbia (1937). They felt their results corroborated the claim of a 1915 pomology textbook by Gourley, which claimed "as a rule, the sooner the thinning is done after the June drop, the better will be the result, since by doing so the developing seeds are prevented from draining energy from the tree" (cited in Palmer and Fischer, 1937). Fischer (1941) later advocated for hand-thinning fruits to nine inches of separation, which, in his studies, resulted in relatively annual crops, without significantly sacrificing overall tonnage. In a 1948 literature review, Singh (1948a) found published reports of improved consistency in annual yields from a variety of techniques including nitrogen manuring, partial or complete defoliation, increasing soil moisture in arid climes, and branch ringing, but concluded the most effective approach was blossom thinning. He was unable to replicate positive results in his own research from defoliation, but did successfully improve yields in the year following treatment with bud rubbing, i.e. manual removal of flower buds as they began

to swell in early spring, and blossom thinning, i.e. manual removal of flower clusters shortly before anthesis, in the on year of a biennial cycle (Singh, 1948c).

Recognizing the high costs of labor, other researchers investigated use of chemicals to achieve apple fruit thinning. Auchter and Roberts (1933) tried thinning with calcium polysulphide (lime sulfur), sodium polysulphide, and copper sulfate in a series of experiments across Arkansas, Missouri, Georgia, and North Carolina, but had more success burning foliage and russeting fruit, than actually reducing fruit set. Harley and Moore (1939) were able to thin fruit with 35-70 gallons per tree of 2% tar oil solution sprayed to several cultivars in Wenatchee, WA. They also noted that "in addition to tar oil sprays being toxic to apple blossoms, they are also irritating to skin tissues" and "protective measures should be taken." Bomeke (1954) found that chemical thinning of apples promoted more annual yields, especially in the cultivar 'Klarapfel.' Goldwin (1986) suggested that applications of a tank mixture containing a gibberellin (GA_3), cytokinin (DPU), and auxin (NAA) could sustain high annual yields of large fruit in trees that lack cross-pollination. Waldner and Knoll (1998) reported chemical and hand-thinning of 'Fuji' helped maintain annual bearing, especially when completed no later than 45 days after full bloom (DAFB).

Potential sources of alternation

Theories regarding the causes of biennial bearing have been as diverse as suggestions of their remedies. Echoing Carne's theory regarding excessive carbohydrate reserves, Singh (1948b) found that heavy cropping inhibited trunk and root growth, further noting that leaf area:fruit spur ratio during the period of flower bud initiation was roughly 2x higher in off years than on years. Hansen and Grauslund (1980) found a

negative correlation between fruit:leaf area ratio and flowering density in the following season.

Individual apple cultivars have their own proclivities for alternation. Singh (1948b) observed that the relatively annual cultivars 'Ellison's Orange' and 'Ribston Pippin', produced flowers on the same spurs in consecutive years, while the biennial cultivars 'Miller's Seedling' and 'Blenheim Orange', did not. McLaughlin and Greene (1991), following up on work by Fulford, confirmed that individual cultivars form specific numbers of juvenile appendages, i.e. budscales, transition leaves, true leaves, and bracts, in bourse spurs, but no relationship between the number of appendages and biennial bearing habit could be detected, as had been suggested by others. Lespinasse and Delort (1993) asserted that acropetal cultivars with large bourses, such as 'Granny Smith' and 'Rome Beauty,' rarely alternate, while basipetal cultivars with short spur lengths, such as 'Golden Delicious,' often do, suggesting that terminal bourses exert considerable influence on the balance of vegetative and reproductive growth.

Currently, the most widely accepted theory of the primary physiological trigger for biennial bearing in apple is the role of gibberellins in suppressing flower bud initiation. A classic paper by Chan and Cain (1967) argued against the competitive nutrient sink model of flower development. They demonstrated that manual pollination of two parthenocarpic apple varieties, 'Spencer Seedless' and 'Ohio 3', reduced flowering in the following season, suggesting that the process of seed formation itself inhibits floral initiation; the primary agents of that inhibition are now believed to be gibberellins produced in juvenile seed endosperm (Elfving, 1996). Subsequent research has repeatedly demonstrated that application of exogenous GA reduces floral initiation in

apple (Bertelsen and Tustin, 2002a; Elfving, 1996; Greene, 1993; Looney, 1996; Marino and Greene, 1981; McArtney, 1994; Meador and Taylor, 1987; Tromp, 1982). Interestingly, Dennis (1967) found that application of seed-derived extracts of the parthenocarpic cultivar 'Wealthy' applied to unpollinated blossoms of the same variety actually promoted development of mature seedless fruit, implying that gibberellins can themselves increase fruit set.

This gibberellin-inhibited model of flower initiation has not gone unchallenged. Dennis and Neilsen (1999) argued for consideration of an alternative model in which juvenile fruit seeds might be strong sinks for florigen, the hypothetical hormone responsible for triggering flower bud differentiation; in theory, if trees carry large loads of fruits in one season, relatively little florigen would be available to induce flowering in the following season. Weinbaum et al. (2001) described strong floral inhibition from seedless 'Williams Bon Chretien' (Syn. 'Bartlett') pear (*Pyrus communis* L.) fruits, although seedless fruit of the same cultivar grown in France reportedly do not suppress return bloom. They concluded that "seed-derived hormonal inhibitors may not function consistently as the primary determinant of floral initiation" and "broad extrapolation of Chan and Cain's results to other apple cultivars and species may be inappropriate." These assertions were supported by Callejas and Bangerth (1997), who found increased diffusible levels of the auxin indolacetic acid (IAA) in fruits and shoot tips of 'Elstar' and 'Golden Delicious' during the period of floral initiation, especially following application of exogenous gibberellic acid (GA). This study did not report treatment effects on return bloom, but the impact of GA on auxin concentration during bud differentiation suggests a complex model of flowering involving multiple hormonal factors.

Overview of gibberellins

Research on gibberellins dates back to the 19th century, as excessive stem elongation in certain rice plants was reported in 1828 (Mander, 2002). Near the end of the century, Hori demonstrated that this lengthening effect could be induced by inoculating healthy plants with the "bakane fungus," *Gibberella fujikuroi.* Work by Sawada in 1912 and Kurosawa in 1926 confirmed that some component of the fungus was responsible for the growth response. In 1938, Yabata and Sumuki successfully isolated a crystalline material from the fungus that promoted spectacular growth in plants. Researchers began to describe the chemical features of GAs in the 1950s and evolving techniques such as X-ray crystallography facilitated conclusive identification of GA structure. To date, at least 25 GAs have been derived from their namesake fungus, while more than 100 have been isolated exclusively from higher plants (Mander, 2002).

Structurally, gibberellins are diterpene acids produced in the terpenoid pathway. Their synthesis typically begins in cell plastids, followed by modification on the endoplasmic reticulum, and is finally completed by enzymes in the cytosol (Taiz and Zeiger, 2002). Gibberellins induce a wide range of responses in a broad spectrum of plant production systems. Because young leaves synthesize GAs, they are generally associated with vigorous shoot growth (Westwood, 1990). Variation in the growth habits of apple trees on standard vs. dwarfing rootstocks may partially be explained by differences in the isomer profiles and concentrations of GAs in developing shoots (Steffens and Hedden, 1992). Guak et al. (2001) observed that GA_{4+7} can reverse the effects of shoot growth suppression from prohexadione-Ca, an inhibitor of GA synthesis. Although generally considered to be a floral inhibitor in apple, GA_{4+7} is known to have

little flowering effect on other woody angiosperms (Meilan, 1997) and to promote reproductive growth in gymnosperms (Pharis and King, 1985).

Gibberellins in tree fruits

Although gibberellins can trigger many varied responses in plants, they clearly inhibit flowering in most tree fruit species. Years of research have established that not only is GA_3 an inhibitor of flowering in citrus, but that growth retardants known to be antagonistic to gibberellins promote florigenesis (Goldschmidt et al., 1997). Chailakhyan and Nekrasova (1969) demonstrated that application of an unnamed gibberellic acid inhibited flowering, while the growth retardant chlorocholinchloride (CCC) enhanced flowering in both lemon (*Citrus limon*) and peach (*Prunus persica*).

Later work by Byers et al. (1990) confirmed that GA_3 reduced return bloom in peach when applied up to 47 days after full bloom (DAFB). Southwick et al. (1995) observed inhibition of return flowering in peach due to applications of GA_3 up to four weeks prior to harvest. In nectarine, Garcia-Pallas et al. (2001) found that flowering was reduced in a linear relationship with concentration of GA_3, with 200 mg/L producing fruit densities comparable to a commercially hand-thinned crop. GA_3 has also been shown to reduce flowering of sweet cherry (*Prunus avium* L.) (Lenahan et al., 2006), especially on first-year wood (Facteau et al., 1989).

Dennis et al. (1970) demonstrated decreased levels of flowering in the pome fruit 'Bartlett' pear (*Pyrus communis*) with 20-100 mg/L of GA_3. In 'Kosui' Japanese pear (*Pyrus pyrifolia* Nakai), June applications of GA_4 decreased flower bud formation, while August sprays of the same material increased flowering (Ito et al., 2000).

Gibberellins in apple

Apple growers often seek to promote annual cropping via chemical thinning in an effort to minimize potential sources of endogenous GAs. Dennis (1976) reported that peak GA activity, shortly after June drop, was 3000 times higher in developing seeds than in apple flesh (on a fresh weight basis). Interestingly, effective chemical thinning not only eliminates sites of GA synthesis, but inhibits diffusion of gibberellins from the surviving fruit (Ebert and Bangerth, 1981). Not surprisingly, the inhibitory effect decreases as distance from gibberellin sources, i.e. seeds, increases (Greene, 1996b).

Gibberellins are a diverse family of hormones and the comprehensive characterization of their chemistries is likely not yet complete in apple. Hedden et al. (1993) identified 33 gibberellic acids, five "GA-like compounds," and three kaurenoids in extracts from 'Cox's Orange Pippin', 'Dabinett', 'Sunset', and 'Tremlett's Bitter'. Ramirez (1995) found isomers GA_4, GA_7, GA_{12}, and GA_{20} in 8-10 week old seeds of both 'Golden Delicious' and 'Rome Beauty', with peak GA activity noted approximately 10 weeks after full bloom.

It should be no surprise that discrete apple cultivars have distinct profiles of endogenous gibberellins. Ramirez-Rodriguez et al. (2001) found considerable variation in the concentrations of gibberellins in flower petals and embryo sacs of 'Delicious', 'Golden Delicious', and 'Rome Beauty'. Some have suggested that differences in endogenous GA concentrations may in part account for varietal tendencies toward bienniality. In similar studies, Hoad (1978), Hoad and Ramirez (1980), and Ebert and Bangerth (1981) demonstrated that strongly biennial bearing varieties such as 'Laxton's Superb' and 'King of the Pippins' contained higher levels of GA than more annual

bearing varieties such as ‘Cox’s Orange Pippin’ and ‘Golden Delicious’, respectively. However, Prang et al. (1998) found no differences in endogenous GA concentrations of the biennial bearing variety ‘Elstar’ and the more annual cultivar ‘Golden Delicious’.

Recent research advances have begun to elucidate the dynamics of gibberellin transport and metabolism in apple. Using mass spectroscopy, Stephan et al. (1999) established export of several GAs from fruit during floral induction. Later work by the same group demonstrated translocation of radio-labeled GA, applied to developing fruit, into surrounding pedicels and bourses, providing direct evidence for translocation of gibberellins from fruit to adjacent spurs (Stephan et al., 2001).

Research has described the effects of exogenous gibberellins on apple flowering and fruit finish in many cultivars and locations. ‘Golden Delicious’ has been studied in British Columbia (Looney et al., 1992), Illinois (Meador and Taylor, 1987), Ontario (Elfving and Allen, 1987), Massachusetts (Greene, 1993), Mexico (Ramirez, 1995), and Europe (Prang et al., 1998). GA research has also been conducted on ‘Delicious’ in Massachusetts (Greene, 1993) and North Carolina (Unrath and Whitworth, 1991). New Zealand studies have included ‘Braeburn’ (Khurshid et al., 1997; McArtney and Li, 1998) and ‘Pacific Rose’ (Bertelsen et al., 2002b). Little or no work has been published regarding GA effects on strongly biennial varieties such as ‘Fuji’, ‘Cameo’, or ‘Honeycrisp’. While certain districts of British Columbia or New Zealand may be similar to Washington, little GA research has been conducted directly on cultivars or in conditions pertinent to the dominant apple industry of the United States. Many basic questions regarding GA in apples have already been explored, but the relevance of those results to Washington conditions and cultivars is uncertain.

Ethephon

While the ability of gibberellins to reduce floral initiation offers one approach to help manage crop load in apple, the converse strategy of promoting floral initiation can also be useful. Ethylene is known to thin fruit and promote reproductive growth in many tree fruit species (Westwood, 1990; Greene, 1996a), but it is unclear whether increased floral initiation is directly induced by the gas itself, or associated with increased availability of plant resources due to reduced vegetative growth (Walsh and Kender, 1982). When applied from balloon stage to four weeks after full bloom, ethephon, an artificial precursor of ethylene, thins apple crops and promotes return bloom (Bound et al., 1993; Byers, 1993; Byers and Carbaugh, 1991; Knight et al., 1987; Marini, 2004; Stopar, 2000a; Stopar and Zadravec, 2004) and is used widely by commercial apple growers around the world.

Ethephon can be a useful tool when increased florigenesis is desired without increased thinning of the current season's crop; Ferree and Schmid (2000) demonstrated these effects in 'Fuji' with weekly applications of 200 mg/L starting at 10 mm fruit size for durations of four or six weeks. Byers (1993) found that 1200 mg/L reduced current-season fruit size and trunk cross-sectional area, but increased return bloom of 'Starkrimson Delicious' when applied at 26, 61, and 103 DAFB. Return bloom has also been improved by applications of ethephon around June drop (35-50 DAFB) on 'Nured Delicious' (Byers and Carbaugh, 1991), 'McIntosh' and 'Melba' (Karaszewska et al., 1986), and 'Golden Delicious' and 'Cox's Orange Pippin' (Luckwill and Child, 1978). The crop protection guide for tree fruits in Washington (Washington State University,

2005) recommends application of 300 mg/L of ethephon after June drop has begun to promote flowering in bearing apple trees without excessive thinning of the current crop.

These effects, however, are not guaranteed; Meland (1997) found no clear thinning or return bloom effects of 150 mg/L ethephon on three apple cultivars, but suggested that higher rates and warmer weather during application might have yielded better results. Stopar (2000b) likewise found no promotion of return bloom on 'Golden Delicious' with 150 mg/L ethephon alone, but synergistic increases in flowering from tank mixes with benzyladenine (BA) or naphthalene acetic acid (NAA). As with gibberellin research, little has been published in popular journals regarding use of ethephon on commercial varieties in Washington-type conditions.

Apple growers are ultimately interested in finding practicable solutions to biennial bearing. Judicious pruning and aggressive chemical thinning can effectively alter reproductive:vegetative growth balance, but are often inadequate to counteract severe alternation. If gibberellins could be applied in an off year to suppress return bloom in the subsequent on year, and conversely, ethylene products applied in an on year to promote return bloom in the subsequent off year, growers might be better able to pull their blocks back into annual bearing. In order to do so, researchers must be prepared to suggest appropriate timings and concentrations for bioregulator programs that are customized to specific cultivars and cropping patterns of particular orchard blocks, if not individual trees themselves. Further, orchardists must consider the economic ramifications of these programs before spraying expensive materials which have the potential to dramatically alter their production for years to come.

The Washington apple industry's interest in use of plant growth regulators to help break biennial bearing cycles is high and growing. The research community has considerable work remaining before it can responsibly place these important tools in the hands of growers.

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CHAPTER 2

Influence of crop load dominates flowering response to ethephon and gibberellic acid in apple

Keywords: adjusted crop load, biennial bearing, alternate bearing, floral initiation, GA, ethephon, return bloom, shoot growth, Cameo, Honeycrisp, Fuji, bioregulator, PGR

Abstract

Potential strategies against biennial bearing in apple (*Malus domestica* Borkh.) include promotion of return bloom with an "on" year application of ethephon or inhibition of return bloom with an "off" year application of gibberellic acid (GA), but the influence of initial crop load on the efficacy of these bioregulators is poorly understood. In 2004 and 2005, six total trials were initiated in which whole trees were manually adjusted to one of three crop levels (100%, 50%, 0%) in 'Cameo', 'Honeycrisp', and 'Fuji'; GA_{4+7} was overlaid on trees of each crop level in four trials, and ethephon in two. In all trials, initial crop load was the primary determinant of return bloom; proportional influence on flower density, fruit density, and yield was generally most pronounced at the 50% crop level. GA_{4+7} consistently reduced floral initiation, while ethephon promoted it. Flowering responses from a historically alternating 'Cameo' trial site showed greater sensitivity to ethephon and less sensitivity to GA_{4+7} than did responses from parallel trials established in an annually bearing 'Cameo' block. Light crop loads and GA_{4+7} applications generally promoted shoot extension, while heavy crops and ethephon had the opposite effect. Implications for models of floral initiation in apple and practical crop load management are discussed.

Introduction

Effective crop load management of apple is critical to the viability of commercial orchard operations. Growers routinely employ a variety of strategies to produce consistent annual yields of high-quality fruit. Standard techniques such as targeted pruning, nitrogen management, and chemical thinning help maintain appropriate balance between vegetative and reproductive growth, yet many well-managed apple trees still fall into biennial bearing cycles.

The financial and horticultural costs of alternate bearing have been decried for decades in commercial apple industries around the world, including those of New York (Beach, 1903), the Pacific Northwest (Palmer and Fischer, 1937), and Australia (Bowman, 1932; Carne, 1931). Historically, research on biennial bearing focused on effective thinning techniques, but more recently, synthetic bioregulators have proven effective at either promoting or inhibiting floral initiation in apple. By applying a flowering promoter such as naphthaleneacetic acid (NAA) (Harley et al., 1958), naphthylacetamide (NAD) (Harley and Regeimbal, 1959), or ethephon (Byers and Carbaugh, 1991) in the "on" year and/or a flowering inhibitor such as gibberellic acid (GA) in the "off" year (Marino and Greene, 1981), the peaks and valleys of a biennial cycle can theoretically be diminished to achieve annual yields with less variability.

Chan and Cain (1967) demonstrated the importance of fruit seeds with respect to flowering in a classic experiment on the parthenocarpic apple cultivars 'Ohio 3' and 'Spencer Seedless'. Manual pollination of blossoms induced a marked reduction in flowering the following season, suggesting a direct correlation between seed development and floral initiation. Careful analyses of apple seed extracts have subsequently identified a variety of endogenous gibberellins (Ramirez, 1995) and

exogenous GAs have been shown to reduce flowering in apple the season after application (Bertelsen and Tustin, 2002; McArtney, 1994; Meador and Taylor, 1987; Tromp, 1982).

The presence or absence of gibberellins clearly influences flowering in apple, but the underlying mechanisms for that relationship are not well understood. Low return bloom in poorly thinned apple trees is often blamed on floral inhibition from endogenous gibberellins synthesized in the endosperm of developing seeds (Elfving, 1996), but the systematic model of flowering in apple may involve multiple metabolic mechanisms.

Pointing to the lack of direct proof of GA export from apple seeds, Dennis and Neilsen (1999) argued for consideration of an alternative model for floral initiation in which seeds have high demand for the presumed flowering promoter, florigen; if differentiating buds are poorer sinks for florigen than surrounding fruitlets/seeds, they would be less likely to become reproductive. Using radio-labeled GA and mass spectrometry, Stephan et al. did later offer evidence of GA export from apple fruits (1999) and transport of radio-labeled exogenous GAs to surrounding pedicels and bourses (2001). They further found that 'Spencer Seedless' exhibited quantities of GA similar to those of seeded cultivars 'Golden Delicious' and 'Jonica' and suggested that GA may be produced in the pericarp of 'Spencer Seedless'. Despite the findings of Stephan's group, the hypothesis posited by Dennis and Neilsen may still be valid; plant hormones other than gibberellins could logically play a role in floral initiation.

Conducting a similar study to Chan and Cain's on the facultatively parthenocarpic pear (*Pyrus communis* L.) cultivar 'Williams Bon Chretien' (Syn. 'Bartlett'), Weinbaum et al. (2001) found that return bloom was inhibited on spurs bearing fruit, whether or not

those pears contained seeds. While this phenomenon is common in California, where the study was conducted, in other geographic regions, seeded 'Bartlett' pears are believed to be more inhibitory to floral initiation than unseeded fruit. Because of this inconsistency among genotypes in different locations, the authors suggest that "seed-derived hormonal inhibitors may not function consistently as the primary determinant of floral initiation" and that "broad extrapolation of Chan and Cain's results to other apple cultivars and other species may be inappropriate" (Weinbaum et al., 2001).

Callejas and Bangerth (1997) found increased levels of diffusible indolacetic acid (IAA), an auxin, in fruits and shoot tips of 'Elstar' and 'Golden Delicious' during the period of floral initiation, especially following application of exogenous GA. The study did not report treatment effects on return bloom, but the impact of GA on transport of auxin during bud differentiation suggests a complex model of flowering involving multiple hormonal factors.

Ethylene is known to thin fruit and promote reproductive growth in many tree fruit species (Greene, 1996), but it is unclear whether increased floral initiation is directly controlled by the gas or associated with increased plant resources due to reduced vegetative growth (Walsh and Kender, 1982). Regardless of specific mechanisms, ethylene-inducing bioregulators such as ethephon and NAA are widely used by commercial orchardists to promote return bloom.

Ethephon can also be a useful tool when increased florigenesis is desired without increased thinning of the current season's crop; Ferree and Schmid (2000) demonstrated these effects in 'Fuji' with weekly applications at 200 mg/L starting at 10 mm fruitlet size for durations of four or six weeks. Byers (1993) found that 1200 mg/L of ethephon

reduced current-season fruit size and trunk cross-sectional area, but increased return bloom of 'Starkrimson Delicious' when applied at 26, 61, and 103 days after full bloom. Return bloom has also been improved by applications of ethephon around June drop (35-50 days after full bloom) on 'Nured Delicious' (Byers and Carbaugh, 1991), 'McIntosh' and 'Melba' (Karaszewska et al., 1996), and 'Golden Delicious' and 'Cox's Orange Pippin' (Luckwill and Child, 1978). The crop protection guide for tree fruits in Washington (Washington State University, 2005) recommends application of 300 mg/L of ethephon after June drop has begun, to promote flowering in bearing apple trees without excessive thinning of the current crop.

Considerable work has been published regarding effective isomers, rates, and timings for using GA and ethephon to manage bloom in apple, but little has been reported regarding the influence of initial crop on the efficacy of these bioregulators. Greene (1989) sprayed GA in split applications on 'Empire' trees of varying crop load. He found that GA_{4+7} decreased return bloom on de-cropped trees, increased return bloom on fully cropped trees, and had little effect on trees with moderate crop loads, results which do little to clarify the role gibberellins in floral initiation of apple. If one presumes that a tree with a light crop has a different hormone profile than one with a heavy crop, then it is reasonable to expect that the response to exogenous bioregulators might be different between those trees. This paper reports on a series of experiments which further consider the influence of initial crop load on the efficacy of GA and ethephon applications in apple cultivars prone to biennial bearing.

Materials and Methods

Experimental design. All field trials for this study were conducted in commercial apple orchards in several growing districts of Washington State. Aside from elimination of bioregulator programs which affect flower initiation, standard orchard management strategies were followed by grower-cooperators. Each trial employed a randomized complete block design with six replicates, except for two 2004 trials on 'Cameo' in Tonasket which used five replicates due to field limitations. In all cases, whole individual trees served both as experimental and sampling units. To isolate treatments, at least one buffer row was maintained between rows receiving treatment. In addition, a minimum of three meters (2-5 trees) separation between treated trees was maintained within the row for all trials.

Data were analyzed with the Statistical Analysis System (SAS) of the SAS Institute, Cary, NC. Means were separated with the general linear model using Tukey's Studentized Range Test at 0.05 by one-way or factorial analysis of variance (Proc GLM). Where fixed-effects variables allowed regression analysis, the General Linear Models (GLM) procedure of SAS was used to evaluate the homogeneity of slopes, curvatures, and intercepts of the regressions on crop load, bioregulator concentration, or application timing, as appropriate. Only significant findings are included in this report.

Treatments. For each of the six trials in this study, three levels of crop load were established prior to application of growth regulators. At the late pink or "balloon" stage of blossom development, all flowers were pruned from every cluster in one-third of treatment trees (0% crop load), all flowers were pruned from alternating clusters in one-third of treatment trees (50% crop load), or flowers were left untouched (100% crop load) in the remaining one-third of treatment trees. Flower pruning was achieved by clipping

flower pedicels with hand blossom shears, while preserving spur buds and leaves (Figure 2-1).

The commercial GA_{4+7} formulation 'ProVide' (Valent Biosciences, Libertyville, IL) was used in four trials, and in the remaining two trials, ethephon was sprayed as 'Ethrel' (Bayer CropScience, Research Triangle Park, NC). Applications were timed according to phenologic fruit development, as determined by the mean diameter of king apples of 30 randomly selected fruit clusters measured with digital calipers (Mitutoyo Corp., Japan) in the respective trial blocks. Applications were made by handgun with a 25 gallon 'Nifty' power sprayer (Rears Manufacturing, Eugene, OR) adjusted to a fine mist at 200 lbs/in^2 pressure. Whole trees were sprayed until all visible foliage was wet, but not to the point of dripping from more than 10% of all leaves. No adjuvants were used for any spray.

2004 trials. Two trials were established in a severely alternating seven year old 'Cameo'/Bud.9 orchard near Tonasket, WA (48.8º N, 119.4º W). Trees were planted 1 m x 3.5 m and trained to a three wire vertical trellis in a spindle system. In one trial, 400 mg/L GA_{4+7} was sprayed on each crop level (0%, 50%, 100%) at petal fall, 10 mm fruitlet size, or 20 mm fruitlet size. Unsprayed control treatments were maintained at each crop level. In an adjacent trial, 300, 600, or 900 mg/L ethephon was sprayed on trees of each crop level at 45 days after full bloom (DAFB). Again, unsprayed controls were preserved for each crop level.

In a third 2004 trial, five year old 'Honeycrisp'/EMLA.9 were likewise adjusted for crop level near Wiley City, WA (46.5º N, 120.7º W). Trees in the trial block were planted 2 m x 3.5 m and trained to a vertical axis system on a two wire vertical trellis.

Trees at each crop level were either left unsprayed or sprayed with 300 mg/L GA_{4+7} at 10 mm fruitlet size.

2005 trials. Protocols identical to the Tonasket 'Cameo' trials described above were carried out in a relatively annually bearing nine year old 'Cameo'/Nic.29 orchard near Quincy, WA (47.3º N, 119.7º W). Trees in this block were spaced 1.5 m x 3.5 m and trained to a five wire V-trellis.

A third trial was conducted on twelve year old 'Fuji'/M.26 near Othello, WA (46.8º N, 119.5º W) using a protocol identical to the Wiley City 'Honeycrisp' trial described above. Trees in this block were trained to a Lincoln canopy system with 1 m x 4 m spacing.

Data collection. After manual crop adjustment, trunk circumferences and whole tree bloom counts were recorded. Fruit set was evaluated after June drop and whole tree fruit counts and yields were recorded at commercial harvest in September and October. Twenty fruit samples were taken from each bearing tree for standard harvest quality analyses including indices of fruit weight, length, diameter, color, firmness, sugars, and acid content. Fruit finish was graded visually for sunburn and six classes of russet indicating location and size of surface blemishes. Following completion of commercial harvest, ten vertical shoots per tree were measured for the current season's growth, long after terminal buds had set. In the spring after treatment, trunk circumferences were again measured and flower clusters were counted on whole trees at the pink stage of blossom development. As in the year of treatment, fruit counts and yields were again recorded during commercial harvest in autumn.

Results and Discussion

2004 'Cameo' GA trial. This trial was initiated in the on year of a block in a severe biennial cycle. According to the grower, yields swung by a factor of 3-4x between light and heavy years. Relatively low flower (0-6 clusters/cm^2) and fruit (0-9 fruits/cm^2) density values (Table 2-1a) for all treatments in the trial reflect the block's predisposition to produce a light crop in 2005. Occasional trees in the block were in the off year of their cycles in 2004, but were excluded from the trial. Regression analyses revealed that crop load had powerful linear and quadratic reductions of bloom density, fruit density, and yield in the year after treatment. The application of GA_{4+7} did significantly decrease those same parameters, with 10 mm and 20 mm timings showing the greatest treatment effects. Interactivity between crop load and application timing was largely insignificant, except in the case of 2005 yields, where petal fall, 10 mm, and 20 mm sprays all showed significant curvilinear effects (Table 2-1b). Shoot growth was not impacted by any treatment and analyses of fruit quality at harvest were unremarkable (data not shown).

Unsprayed trees, which were allowed to carry full crops in 2004, generated only 0.4 flower clusters/cm^2 trunk cross-sectional area (TCSA) in the following spring; application of GA_{4+7} to trees of that same crop level essentially eliminated the entire 2005 crop. At the opposite end of the spectrum, unsprayed trees whose entire crop was manually eliminated in 2004 produced 5.8 flower clusters/cm^2 TCSA in 2005; application of GA_{4+7} in 2004 to trees of that crop level diminished return bloom by 5-30%. Unsprayed trees with 2004 crops were reduced by half generated 1.6 flower clusters/cm^2 TCSA in 2005. Trees of this moderate 50% 2004 crop load showed the greatest relative response to GA_{4+7}, as 2005 cropping was virtually eliminated, regardless of timing. Results from this trial imply that application of exogenous GA can do little to affect

flowering of 'Cameo' with extremely heavy or extremely light crop loads, but that it is able to inhibit floral initiation in more moderate cropping situations; before drawing firm conclusions, however, these results should be viewed in light of data from the following trial, which followed the same protocol, but in annually cropping 'Cameo'.

2005 'Cameo' GA trial. 'Cameo' trials were relocated in 2005 to another orchard with relatively no background alternation. The grower reported year-to-year swings in yields of no greater than 10-15%. To coin a popular industry phrase, trees in this block were "settled down," as indicated by the 9 cm of mean terminal growth in untreated control trees (Table 2-2), as compared to 32 cm growth in the 2004 'Cameo' trial block (Table 2-1a). As in the earlier 'Cameo' GA trial, crop load produced significant linear and quadratic reductions on all measures of cropping in the season after treatment (Table 2-2). Application of GA_{4+7} significantly diminished those same parameters at all timings, including petal fall, which had yielded results slightly inferior to those of the later timings in the 2004 trial. No interactive effects between initial crop load and spray timing were detected in this trial. Unlike the previous year, shoot growth was increased 29-138% by GA_{4+7}, with the strongest effects resulting from earlier timings. Harvest fruit quality analysis revealed no significant treatment effects (data not shown).

While the inhibitory effects of GA_{4+7} were most clear at the 50% crop level in the 2004 'Cameo' GA trial, all three crop levels demonstrated clear reductions in flowering, fruit density, and yield in the 2005 trial. Unsprayed trees at the 100%, 50%, and 0% crop levels produced 6.1, 6.5, and 13.7 flower clusters/cm^2 TCSA in 2006, respectively. Application of GA_{4+7} reduced 2006 flowering 78-89% in trees with full crops in 2005, 92-97% in trees with 50% crops in 2005, and 30-61% in trees with no crop in 2005. As

with shoot growth, the strongest treatment effects were consistently produced by petal fall sprays. Like the 2004 trial, the greatest efficacy of GA_{4+7} was seen at the 50% crop level, but in this more annual 'Cameo' block, GA_{4+7} also powerfully inhibited flowering at the two extreme crop levels.

Upon removal of all flowers prior to anthesis, it is reasonable to assume that a major source of endogenous gibberellins, i.e. juvenile seeds, has been eliminated, which should, in turn, make an apple tree more susceptible to the effects of exogenously applied GA. In other words, with no significant internal hormonal restraint, de-cropped trees should produce a massive return bloom, and be relatively sensitive to the effects of an external floral inhibitor. In both 'Cameo' GA trials, however, the greatest proportional responses were manifested in trees with only half of their flowers removed, implying the presence/absence of seed-derived gibberellins does not exclusively account for the fate of undifferentiated apple buds. Further, regularly cropping 'Cameo' showed greater proportional sensitivity to GA than did severely biennial 'Cameo', suggesting that some phenomenon associated with alternation within the latter had, to some degree, predetermined the 2005 fate of nascent buds prior to the spring of 2004, when their exposure to endogenous and exogenous gibberellins was experimentally manipulated.

2004 'Honeycrisp' GA trial. Trial designs for this and the 2005 'Fuji' GA trials were simplified versions of the 'Cameo' GA trials; crop loads were still manually adjusted to 100%, 50%, or 0% levels, but only one timing of GA application, i.e. 10 mm, was imposed in factorial fashion on those crop levels. Background alternation in this block was moderate, with approximately 30% swings in annual yields. Linear and quadratic reductions of crop load were observed for 2005 floral density, fruit density, and

yield (Table 2-3). Application of GA_{4+7} did not significantly affect 2004 shoot growth or 2005 flower density, but did decrease fruit density and yield by 30%. De-cropped trees demonstrated greater 2004 shoot growth than fully cropped trees, presumably due to increased carbohydrates and nutrients partitioned to shoot apices. Interactive effects of initial crop level and GA had linear and quadratic significance relative to 2005 floral density, fruit density, and yield for sprayed fruit. 2004 fruit quality was unaffected by any treatment (data not shown).

The relative efficacy of GA_{4+7} in this moderately biennial block mirrors that of the severely alternative 2004 'Cameo' GA trial; namely, application of GA_{4+7} to trees with 100% or 0% crop levels did little to influence return bloom, but reduced 2005 floral density in trees with 50% crop levels in 2004 by 65%. Again, the relative lack of GA sensitivity in de-cropped trees suggests that undifferentiated buds in those trees are compelled to flower by signals aside from the influence of seed-derived or exogenous GA.

2005 'Fuji' GA trial. Consistent annual production in this block indicates no significant biennial bearing habits, with no greater than 10% swings in year-to-year yields. Adjusted crop level in 2005 produced linear decreases in flowering density, fruit density, and yield in 2006 (Table 2-4). Application of GA_{4+7} increased shoot extension 32%, and decreased 2006 return bloom, fruit density, and yield by 88%, 80%, and 67%, respectively. Overall values for the latter two parameters were low due to poor pollination and fruit set in the entire block. As in the relatively annual 2005 'Cameo' GA trial, no significant interaction between adjusted crop level and GA_{4+7} was observed. This lack of interactivity again suggests that annually cropping apple trees are more

responsive to GA than are trees in alternation. Fruit quality analyses yielded no significant results (data not shown).

2004 'Cameo' ethephon trial. This trial was established in the same severely alternating block used for the 2004 GA trial. Trial designs were identical with respect to crop load adjustment, but in contrast to attempting to inhibit floral initiation with exogenous GA, ethephon was applied at four concentrations in an effort to promote flowering of trees at each crop level. Multiple regression analyses revealed that 2004 crop load was negatively correlated with 2005 flower density, fruit density, and yield, in linear fashion, as well as in curvilinear fashion for 2005 yield (Table 2-5). Increasing ethephon concentration inhibited 2004 shoot extension and promoted 2005 return bloom, flower density, and yield; no second order effects of ethephon were observed, and interactive effects of crop load and ethephon were generally insignificant. Harvest fruit quality analyses were unremarkable (data not shown).

Unsprayed trees carrying a full crop in 2004 produced only 0.2 flower clusters/cm^2 TCSA in 2005; flowering density was increased to 0.7, 1.7, and 1.8 flower clusters/cm^2 TCSA with application of 300, 600, and 900 mg/L ethephon, respectively. Unsprayed trees whose 2004 crop was entirely removed generated 7.1 flower clusters/cm^2 TCSA in 2005, but no concentration of ethephon sprayed to de-cropped trees substantially increased flowering. As with all four GA trials, the greatest relative response to ethephon was found in trees at the 50% crop level. Unsprayed trees of this category produced 1.7 flower clusters/cm^2 TCSA, and application of 300, 600, and 900 mg/L ethephon increased 2005 floral density 65%, 159%, and 212%, respectively.

2005 'Cameo' ethephon trial. The same annually bearing block used in the 2005 'Cameo' GA trial was utilized for this experiment. Crop load exerted linear diminishment of 2006 flower density, fruit density, and yield, but not 2005 shoot extension (Table 2-6). Ethephon showed no direct or interactive treatment effects. 2005 harvest fruit quality analyses revealed no differences between treatments (data not shown).

Baseline levels of flowering were much higher in this trial than the 2004 'Cameo' ethephon trial (6.3 vs. 0.2 flower clusters/cm^2 TCSA), sharpening the contrast between the two trial blocks. More pronounced treatment effects due to ethephon and manual crop reduction in the biennial block suggest greater receptivity to horticultural interventions which might encourage flowering, and mitigate the amplitude of alternation.

Throughout all six trials, the strongest proportional floral initiation effects from bioregulators were seen at the 50% crop level. In biennial trial sites, i.e. 2004 'Cameo' and 'Honeycrisp', the baseline levels for return bloom were extremely low, leaving little margin to observe additional floral inhibition from application of GA. Conversely, where baseline levels of return bloom were relatively high, i.e. de-cropped 2005 'Cameo', little margin remained to observe increased return bloom due to ethephon application. In simple terms, it is difficult to further reduce flowering on blank trees or to increase flowering if virtually all spurs are already reproductive. An alternative approach to imposing treatments on trees with artificially manipulated crop levels would be to select trees with marked natural variation, and attempt to develop regression curves which describe the influence of initial crop level on bioregulator efficacy. Aggressive

concentrations of GA_{4+7} were used in these studies to ensure treatment effects, but future studies with more modest concentrations (50-200 mg/L) would likely add increased resolution to those regression curves, and reflect more realistic grower strategies from a financial perspective (see Appendix B).

In the 2004 'Cameo' trial, GA_{4+7} showed greater effects at 10 mm and 20 mm than at petal fall. Results from the 2005 'Cameo' trial were more similar to other work with GA_4 in 'Fuji' (Chapter 3), which suggested that earlier application timing, i.e. petal fall, provided greater floral inhibition. Results at 10 mm fruitlet size were good in all trials, and those seeking to reduce spray concentrations while maintaining efficacy might be well served to "aim for the middle" and apply GA at that timing.

Ethephon increased flowering in cases where poor return bloom was expected (< 2 flower clusters/cm^2 TCSA), such as trees carrying full or half crops in the 2004 trial; floral initiation was increasingly promoted by higher concentrations of ethephon. Spray effects were largely nonexistent, however, in de-cropped trees from 2004 or all trees in the 2005 trial, where baseline levels of return bloom were relatively high (> 6 flower clusters/cm^2 TCSA). These trends imply that commercial applications of ethephon to improve return bloom might be relatively ineffective in orchards with reasonable balance between vegetative and reproductive growth.

In all trials, partial or complete removal of flowers in one season dramatically promoted flower bud differentiation, as evidenced by increased cropping in the following season. The diminution of endogenous gibberellins produced in developing fruits is likely a primary contributor to these effects, but alone, does not explain all of our results. The relative lack of sensitivity to exogenous GA in artificially de-cropped trees in the two

sites with greatest background levels of alternation (2004 ‘Cameo’ and ‘Honeycrisp’) either suggests a more complex model of floral initiation or lack of spray material efficacy. The latter explanation is unlikely, however, because the same spray programs showed clear efficacy in trees with 50% crops. Overall, the biennial ‘Cameo’ orchard, entering its off year, was more sensitive to the floral promotion effects of ethephon, and less sensitive to the floral inhibition effects of GA, than was the more annual ‘Cameo’ site. In simple terms, it was relatively easier to increase flowering in trees destined for light bloom, and to decrease flowering in trees destined for moderate bloom, even though experimental interventions were identical in each case. These tendencies suggest a predilection of presumably undifferentiated buds to a certain fate before the effects of developing fruits or applications of exogenous bioregulators could be imposed upon them. In short, our results support a complex model of floral initiation in apple, influenced, but not controlled, by the presence of seed-derived gibberellins.

Horticulturists will need to account for crop load and cultivar when developing appropriate spray concentrations and timings for potential commercial adoption of these programs, but we are confident that bioregulators may be successfully used to strategically promote or inhibit flowering in apple to help mitigate biennial bearing.

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Table 2-1a. Effects of spray timing and crop load on response to 400 mg/L GA_{4+7} application in 2004 in severely alternating 'Cameo'/Bud.9 apple trees. Treatments are listed by phenological application timing (unsprayed controls, petal fall, 10mm fruit size, or 20mm fruit size) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2004 shoot extension (cm)[z]	2004 yield/ TCSA[y] (kg/cm^2)	2005 bloom/ TCSA[y] (flower clusters/cm^2)	2005 harvested fruits/TCSA[y] (fruit/cm^2)	2005 yield/ TCSA[y] (kg/cm^2)
Control, 100	32.1	1.6	0.4	0.9	0.2
Control, 50	33.0	1.4	1.6	4.1	0.8
Control, 0	35.3	0.0	5.8	7.4	1.0
PF, 100	34.8	1.1	0.1	0.4	0.1
PF, 50	33.0	1.0	0.2	0.8	0.2
PF, 0	33.5	0.0	5.5	8.6	1.1
10mm, 100	38.5	1.1	0.0	0.0	0.0
10mm, 50	34.4	1.1	0.1	0.4	0.1
10mm, 0	39.2	0.0	4.2	8.3	1.0
20mm, 100	30.9	1.2	0.0	0.0	0.0
20mm, 50	31.1	1.3	0.1	0.0	0.0
20mm, 0	35.6	0.0	4.1	6.2	0.8
Significance					
GA_{4+7} timing[x]					
None	33.5[NS]	1.0 a	2.6 a	4.1 a	0.7 a
Petal Fall	34.6	0.7 b	2.0 ab	3.5 ab	0.5 b
10mm	37.4	0.7 b	1.5 b	2.9 ab	0.4 bc
20mm	33.5	0.8 ab	1.5 b	2.2 b	0.3 c
p values	0.32	0.002	0.02	0.0009	<0.0001
Crop load[w]					
L	NS	****	****	****	****
Q	NS	****	****	****	****
Crop load*GA_{4+7} timing Interaction[w]					
L	NS	NS	NS	NS	see Table 2.1b
Q	NS	NS	NS	**	see Table 2.1b
Model r^2	0.33	0.92	0.86	0.93	0.93

[z]Mean of 10 terminal shoots per tree

[y]Trunk cross-sectional area

[x]Analysis by factorial ANOVA and means separation by Tukey's Studentized Range Test (p=0.05). Means followed by the same letter are not statistically significant within a column.

[w]L = linear effect, Q = second-order (quadratic) effect.

NS, **, ****Nonsignificant or significant at p = 0.01 or 0.0001, respectively.

Table 2-1b. Interactive effects of spray timing and crop load related to 2005 yield/ TCSA[z] (kg/cm^2) response to 400 mg/L GA_{4+7} application in 2004 in severely alternating 'Cameo'/Bud.9 apple trees.

GA_{4+7} timing[y]	Regression significance
Control	
L	****
Q	NS
Model r^2	0.90
Petal Fall	
L	****
Q	***
Model r^2	0.97
10mm	
L	***
Q	**
Model r^2	0.93
20mm	
L	***
Q	**
Model r^2	0.94

[z]Trunk cross-sectional area
[y]L = linear effect, Q = second-order (quadratic) effect.
NS, **, ***, ****Nonsignificant or significant at p = 0.01, 0.001, or 0.0001, respectively.

Table 2-2. Effects of spray timing and crop load on response to 400 mg/L GA_{4+7} application in 2005 in mildly alternating 'Cameo'/Nic.29 apple trees. Treatments are listed by phenological application timing (unsprayed controls, petal fall, 10mm fruit size, or 20mm fruit size) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2005 shoot extension (cm)[z]	2005 yield/ TCSA[y] (kg/cm^2)	2006 bloom/ TCSA[y] (flower clusters/cm^2)	2006 harvested fruits/TCSA[y] (fruit/cm^2)	2006 yield/ TCSA[y] (kg/cm^2)
Control, 100	8.8	2.4	6.1	4.8	1.0
Control, 50	8.5	1.2	6.5	4.9	1.1
Control, 0	8.0	0.0	13.7	8.6	1.7
PF, 100	18.3	1.0	0.7	0.8	0.2
PF, 50	20.8	0.9	0.2	0.3	0.1
PF, 0	21.1	0.0	5.3	5.8	1.2
10mm, 100	13.5	1.1	1.4	1.2	0.3
10mm, 50	14.8	0.8	0.5	0.7	0.2
10mm, 0	15.5	0.0	7.5	5.9	1.3
20mm, 100	10.2	1.1	1.4	1.7	0.4
20mm, 50	11.6	1.1	0.5	0.6	0.1
20mm, 0	10.6	0.0	9.6	8.2	1.6
Significance					
GA_{4+7} timing[x]					
None	8.4 c	1.2[NS]	8.8 a	6.1 a	1.3 a
Petal Fall	20.0 a	0.6	2.1 b	2.3 b	0.5 b
10mm	14.6 b	0.6	3.1 b	2.6 b	0.6 b
20mm	10.8 c	0.7	3.8 b	3.5 b	0.7 b
p values	<0.0001	0.06	<0.0001	<0.0001	<0.0001
Crop load[w]					
L	NS	****	****	****	****
Q	NS	0.08	****	****	****
Crop load*GA_{4+7} timing Interaction[w]					
L	NS	NS	NS	NS	NS
Q	NS	NS	NS	NS	0.06
Model r^2	0.78	0.58	0.84	0.84	0.82

[z]Mean of 10 terminal shoots per tree

[y]Trunk cross-sectional area

[x]Analysis by factorial ANOVA and means separation by Tukey's Studentized Range Test (p=0.05). Means followed by the same letter are not statistically significant within a column.

[w]L = linear effect, Q = second-order (quadratic) effect.

NS, ****Nonsignificant or significant at p = 0.0001, respectively.

Table 2-3. Effects of spray and crop load on response to 300 mg/L GA_{4+7} application at 10 mm fruitlet size in 2004 on 'Honeycrisp'/EMLA.9 apple trees. Treatments are listed by application status (unsprayed or sprayed) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2004 shoot extension (cm)[z]	2004 yield/ TCSA[y] (kg/cm^2)	2005 bloom/ TCSA[y] (flower clusters/cm^2)	2005 harvested fruits/TCSA[y] (fruit/cm^2)	2005 yield/ TCSA[y] (kg/cm^2)
Unsprayed, 100	20.6	1.5	0.4	1.2	0.3
Unsprayed, 50	19.6	1.3	4.9	6.6	1.5
Unsprayed, 0	29.1	0.0	12.7	14.6	2.3
Sprayed, 100	21.3	1.4	0.1	0.4	0.1
Sprayed, 50	23.7	1.4	0.7	1.9	0.5
Sprayed, 0	29.6	0.0	13.2	14.3	2.1
Significance					
GA_{4+7}[x]					
Unsprayed	23.1 NS	0.9 NS	6.0 NS	2.9 a	1.3 a
Sprayed	24.9	0.9	4.7	2.0 b	0.9 b
p values	0.29	0.68	0.14	0.0002	<0.0001
Crop load[w]					
L	**	****	****	****	****
Q	0.06	****	***	****	*
Crop load*GA_{4+7} Interaction[w]					
L	NS	NS	*	****	***
Q	NS	NS	*	****	***
Model r^2	0.54	0.93	0.88	0.97	0.95

[z]Mean of 10 terminal shoots per tree

[y]Trunk cross-sectional area

[x]Analysis by factorial ANOVA and means separation by Tukey's Studentized Range Test (p=0.05). Means followed by the same letter are not statistically significant within a column.

[w]L = linear effect, Q = second-order (quadratic) effect.

NS, *, **, ***, ****Nonsignificant or significant at p = 0.05, 0.01, 0.001, or 0.0001, respectively.

Table 2-4. Effects of spray and crop load on response to 300 mg/L GA_{4+7} application at 10 mm fruitlet size in 2005 on 'Fuji'/M.26 apple trees. Treatments are listed by application status (unsprayed or sprayed) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2005 shoot extension (cm) [z]	2005 yield/ TCSA[y] (kg/cm^2)	2006 bloom/ TCSA[y] (flower clusters/cm^2)	2006 harvested fruits/TCSA[y] (fruit/cm^2)	2006 yield/ TCSA[y] (kg/cm^2)
Unsprayed, 100	21.7	0.8	1.6	0.9	0.2
Unsprayed, 50	22.2	0.6	2.7	1.7	0.3
Unsprayed, 0	21.5	0.0	3.9	2.9	0.5
Sprayed, 100	26.3	0.5	0.2	0.1	0.0
Sprayed, 50	27.5	0.6	0.1	0.2	0.0
Sprayed, 0	26.9	0.0	0.7	0.8	0.2
Significance					
GA_{4+7}[x]					
Unsprayed	21.8 b	0.4[NS]	2.7 a	1.9 a	0.3 a
Sprayed	26.9 a	0.4	0.3 b	0.4 b	0.1 b
p values	<0.0001	0.09	<0.0001	0.0001	0.0001
Crop load[w]					
L	NS	****	**	**	**
Q	NS	****	NS	NS	NS
Crop load*GA_{4+7} Interaction[w]					
L	NS	NS	0.07	NS	NS
Q	NS	NS	NS	NS	NS
Model r^2	0.53	0.86	0.71	0.63	0.65

[z]Mean of 10 terminal shoots per tree

[y]Trunk cross-sectional area

[x]Analysis by factorial ANOVA and means separation by Tukey's Studentized Range Test (p=0.05). Means followed by the same letter are not statistically significant within a column.

[w]L = linear effect, Q = second-order (quadratic) effect.

NS, **, ****Nonsignificant or significant at p = 0.01 or 0.0001, respectively.

Table 2-5. Effects of spray concentration and crop load on response to ethephon application at 45 days after full bloom in 2004 in severely alternating 'Cameo'/Bud.9 apple trees. Treatments are listed by spray concentration (mg/L) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2004 shoot extension (cm)[z]	2004 yield/ TCSA[y] (kg/cm^2)	2005 bloom/ TCSA[y] (flower clusters/cm^2)	2005 harvested fruits/TCSA[y] (fruit/cm^2)	2005 yield/ TCSA[y] (kg/cm^2)
0, 100	30.0	1.2	0.2	0.7	0.1
0, 50	31.7	1.0	1.7	3.6	0.8
0, 0	32.1	0.0	7.1	5.9	0.9
300, 100	28.7	1.1	0.7	1.2	0.2
300, 50	27.3	1.1	2.8	4.7	0.8
300, 0	30.2	0.0	7.7	6.1	0.9
600, 100	28.4	1.2	1.7	3.8	0.6
600, 50	28.1	1.1	4.4	5.7	0.9
600, 0	23.5	0.0	7.8	8.6	1.1
900, 100	24.6	0.9	1.8	3.5	0.6
900, 50	24.2	0.9	5.3	7.0	1.1
900, 0	27.0	0.0	6.3	7.7	1.0
Significance					
Crop load[x]					
L	NS	****	****	****	****
Q	NS	****	NS	NS	**
Ethephon concentration[x]					
L	**	NS	*	****	***
Q	NS	NS	NS	NS	NS
Interaction[x]					
C*E	NS	NS	NS	NS	0.07
C*C*E	NS	NS	*	NS	NS
C*E*E	NS	NS	NS	NS	NS
Model r^2	0.33	0.87	0.71	0.71	0.67

[z]Mean of 10 terminal shoots per tree
[y]Trunk cross-sectional area
[x]L = linear effect, Q = second-order (quadratic) effect, C = crop load, E = ethephon.
NS, *, **, ***, ****Nonsignificant or significant at p = 0.05, 0.01, 0.001, or 0.0001, respectively.

Table 2-6. Effects of spray concentration and crop load on response to ethephon application at 45 days after full bloom in 2005 in annually cropping 'Cameo'/Nic.29 apple trees. Treatments are listed by spray concentration (mg/L) and manually adjusted crop load (100, 50, or 0% crop).

Treatment	2005 shoot extension (cm)[z]	2005 yield/ TCSA[y] (kg/cm^2)	2006 bloom/ TCSA[y] (flower clusters/cm^2)	2006 harvested fruits/TCSA[y] (fruit/cm^2)	2006 yield/ TCSA[y] (kg/cm^2)
0, 100	10.4	1.3	6.3	5.1	1.0
0, 50	11.7	1.1	8.8	6.3	1.3
0, 0	10.5	0.0	11.6	7.3	1.5
300, 100	11.3	1.4	7.9	6.4	1.4
300, 50	11.7	1.2	7.6	5.1	1.1
300, 0	10.1	0.0	14.6	8.2	1.6
600, 100	10.2	1.1	8.5	5.5	1.2
600, 50	10.3	1.1	10.2	5.8	1.2
600, 0	11.7	0.0	12.7	7.2	1.4
900, 100	10.1	1.0	8.7	5.9	1.2
900, 50	9.7	1.1	9.9	6.5	1.3
900, 0	9.6	0.0	14.8	7.9	1.5
Significance					
Crop load[x]					
L	NS	****	*	*	**
Q	NS	****	NS	NS	NS
Ethephon concentration[x]					
L	NS	NS	NS	NS	NS
Q	NS	NS	NS	NS	NS
Interaction[x]					
C*E	NS	NS	NS	NS	NS
C*C*E	NS	NS	NS	NS	NS
C*E*E	NS	NS	NS	NS	NS
Model r^2	0.21	0.90	0.61	0.47	0.42

[z]Mean of 10 terminal shoots per tree
[y]Trunk cross-sectional area
[x]L = linear effect, Q = second-order (quadratic) effect, C = crop load, E = ethephon.
NS, *, **, ***, ****Nonsignificant or significant at p = 0.05, 0.01, 0.001, or 0.0001, respectively.

Figure 2-1. Manual adjustment of crop load in ‘Cameo’, Tonasket, WA, 2004. Pedicels were clipped while sparing spur leaves and buds.

CHAPTER 3

Practical applications of gibberellic acids to inhibit floral initiation in apple

Keywords: biennial bearing, alternate bearing, flowering, GA isomers, timing, concentration, return bloom, shoot growth, Fuji, Honeycrisp, organic

Abstract

Several isomers of gibberellic acid (GA) influence floral initiation of apple (*Malus domestica* Borkh.), but overall, research results have shown inconsistent, if not contradictory effects. This series of trials explores practicable GA programs, including optimum GA formulations, timings, and concentrations, as potential tools for remediation of alternate bearing. In 2004, 'Fuji' apple trees were sprayed with 0, 150, 300, or 450 mg/L GA_4 at one of three phenologic timings: petal fall, 11 mm, or 20 mm fruitlet size. All rates at all timings inhibited 2005 floral density 30-75%, with the two later timings showing greater effect than petal fall. Floral inhibition generally increased in correlation with rising material concentration. In 2005, 'Fuji' apple trees were sprayed with 0, 100, or 200 mg/L of GA_4, GA_7, or GA_{4+7}. All treatments inhibited 2006 floral density 53-87%; GA_4 showed the greatest suppression at 100 mg/L, as did GA_7 at 200 mg/L. A second 2005 trial in 'Honeycrisp' found that 400 mg/L GA_4 and GA_{4+7} reduced 2006 flowering 82-90% and increased 2005 shoot extension 10%. Results from all trials reveal consistent floral inhibition from GA_4, in contrast to earlier reports. Implications for practical GA programs to mitigate alternate bearing are discussed.

Introduction

The phenomenon of biennial or alternate bearing is a significant impediment to profitable commercial apple production. In "on" years, trees produce high yields of

small, poor quality fruit which are undesirable in most markets; in "off" years, low yields from those same trees return little income to the farm. Judicious pruning, effective nutrient management, and aggressive chemical thinning can help promote annual bearing, but many growers would benefit from further remediation via bioregulators applied outside of a standard thinning program. Summer applications of ethephon (Walsh and Kender, 1982), naphthaleneacetic acid (Harley et al., 1958), and naphthylacetamide (Harley and Regeimbal, 1959) have been proven to bolster return bloom following moderate to heavy crops both in research and commercial practice. Field trials have also suggested the potential for suppressing return bloom after small crops with gibberellic acid (GA) (Unrath, 1974), but inconsistent and sometimes conflicting research results, coupled with high material costs, have discouraged many apple growers from adopting this approach as a tool for crop load management.

While apples contain dozens of gibberellins (Hedden et al., 1993; Ramirez, 1995), research with exogenous forms of GA has largely been limited to three isomers: GA_3, GA_4, and GA_7. Trial results have generally indicated that GA_7 is the strongest inhibitor of flowering, whether applied as a pure material or blended with GA_4 (Bertelsen and Tustin, 2002; Greene, 1993; Marino and Greene, 1981; McArtney and Li, 1998; Tromp, 1982). Luckwill (reviewed in Meilan, 1997) showed that 50 mg/L of GA_3 inhibits apple flower bud formation, while 500 mg/L enhanced it. Other work has shown GA_3 to be a moderate inhibitor of apple flowering at rates ranging from 100-500 mg/L (Bertelsen and Tustin, 2002; Karaszewska et al., 1986; McArtney, 1994; Prang et al., 1998; Tromp, 1982), while GA_4 seems to have weak and inconsistent effects (Prang et al., 1998;

Tromp, 1982), sometimes increasing flowering in 'Golden Delicious' (Greene, 1993; Looney et al., 1985).

Research trials with various gibberellins have typically shown dose responses. Greene (1989) demonstrated increasing flowering inhibition on 'Empire' with 0, 50, 100, and 150 mg/L applications of GA_{4+7}, respectively. McArtney (1994) observed similar patterns with 10, 33, 100, and 300 mg/L of GA_3 applied to 'Braeburn'. Later work by McArtney and Li (1998) found that 100, 200, and 400 mg/L of GA_3 or GA_{4+7} had little effect on 'Braeburn' spur flowering, but that the higher concentrations increasingly inhibited bloom on one year-old wood. Bertelsen et al. (2002) reported modest rate responses on return flowering in 'Pacific Rose' from GA_3 and GA_{4+7}.

Studies on application timing effects of GA on floral initiation in apple have yielded variable results. Taylor (1978) observed stronger treatment responses at early timings, reporting reduced return bloom with petal fall applications of GA_{4+7} at rates as low as 10 mg/L. Tromp (1982) showed the greatest suppression of return bloom in 'Cox's Orange Pippin' when GA was applied at full bloom (FB), followed by FB+14 days, and FB+28 days showing the weakest response. Conversely, Bertelsen and Tustin (2002) found the strongest effects on 'Pacific Rose' at FB+14 days, as opposed to earlier or later timings. Greene (1989) reported strong return bloom suppression in 'McIntosh' from GA applied at many timings ranging from FB-6 days to FB+35 days, but that 'Empire' showed clearest effects at FB+10 days. McArtney and Li (1998) significantly reduced 'Braeburn' lateral flowering with GA applications made at six, nine, and twelve weeks after full bloom. Unrath and Whitworth (1991) had inconsistent results in two studies applying various rates of GA at monthly intervals to 'Red Chief' throughout the

growing season, but in a third trial, return bloom was virtually eliminated by serial applications of 250 or 500 mg/L of GA_{4+7} at one, two, three, and four months after full bloom.

With such divergent information from published reports, it would be daunting for horticulturists to select commercially appropriate GA formulations, rates, and timings to help mitigate alternate bearing. Further, little work has been conducted in the Pacific Northwest or on cultivars important to contemporary industry. This study explores effective GA application timings, concentrations, and isomers on two biennial commercial cultivars, 'Fuji' and 'Honeycrisp.'

Materials and Methods

Experimental design. Three field trials were conducted in commercial apple orchards in Washington State. Aside from elimination of bioregulator programs which affect flower initiation, standard orchard management strategies were followed by grower-cooperators. Each trial employed a randomized complete block design with six replicates. In the 2005 'Fuji' trial, whole individual trees served both as experimental and sampling units. Whole trees were also treated in the 2004 'Fuji' and 2005 'Honeycrisp' trials, but sampling units were restricted to eastern- and western-oriented scaffold limbs due to large tree size. To isolate treatments, at least one buffer row was maintained between rows receiving treatment. In addition, a minimum of three meters (1-3 trees) separation between treated trees was maintained within the row for all trials.

Data were analyzed with the Statistical Analysis System (SAS) of the SAS Institute of Cary, NC. Means were separated with the general linear model using Tukey's Studentized Range Test at 0.05 by analysis of variance (Proc GLM). Radiating

regression analyses were conducted for data from both 'Fuji' trials according to procedures described by Elfving and Allen (1987). Only significant findings are included in this report.

Trial blocks. A six year old organic 'Fuji'/MM.106 block near Royal City, WA (46.8º N, 119.6º W) was utilized for a study of optimal spray concentrations and timings of GA in 2004. Freestanding trees were planted 2.5 m x 5 m and trained to a low input central leader system. Treated trees were sprayed with 150, 300, or 450 mg/L GA_4; each concentration was applied at petal fall (5 days after full bloom), 11 mm (20 DAFB), or 20 mm (33 DAFB) fruitlet size and control trees were left altogether unsprayed.

In 2005, a new trial was established to evaluate various concentrations of four GA formulations in a conventional eleven year old 'Fuji'/M.26 block also near Royal City, WA (46.8º N, 119.5º W). Trees in this block were spaced 1 m x 4 m and trained to a Lincoln canopy system, aided by routine summer pruning and aggressive Apogee (prohexadione-Ca) programs to help check vegetative growth. 100 and 200 mg/L GA_4, GA_7, and GA_{4+7} were applied at 10 mm fruitlet size (11 DAFB); GA_3 was sprayed at 1000, 2000, and 3000 mg/L due to mathematical error (rather than 100, 200, and 300 mg/L) and control trees were not treated with any spray.

A second 2005 trial investigated effects of three GA formulations on fourteen year old 'Honeycrisp'/P.18 near Brewster, WA (48.2º N, 119.7º W). These freestanding trees were 2.5 m x 5 m and trained to a vertical axis system. Treatments in this trial featured 400 mg/L of GA_4 and GA_{4+7} sprayed at 10 mm fruitlet size (15 DAFB) and untreated control; again, due to mathematical error, 4000 mg/L (rather than 400 mg/L) GA_3 was also applied at the same timing.

Sprays. The commercial GA_4 formulation 'Novagib 10L' (Fine Agrochemicals, Worcester, UK) was used in all three trials. Both 2005 trials also included commercial formulations of GA_3, 'Falgro 4L' (Fine Agrochemicals, Worcester, UK) and GA_{4+7}, 'ProVide' (Valent Biosciences, Libertyville, IL). The 2005 'Fuji' trial additionally featured an experimental formulation of pure GA_7 (Lot # SAF5C062LAB) provided by Fine Agrochemicals. All applications were sprayed by handgun with a 25 gallon 'Nifty' power sprayer (Rears Manufacturing, Eugene, OR) adjusted to a fine mist at 200 lbs/in^2 pressure. Whole trees were sprayed until all foliage was visibly wet, but not to the point of dripping from more than 10% of all leaves. No adjuvants were used for any spray. Fruitlet size with respect to spray timing was determined by calculating the mean diameter of 30 randomly selected fruitlets in the king position of fruit clusters measured with digital calipers (Mitutoyo Corp., Japan).

Data collection. Initial flower cluster counts were recorded for each sampling unit during the late pink stage of bloom development in the year of spray treatment. After terminal bud set, final shoot length was measured on ten upright, one year old shoots in each tree. Return bloom was assessed in the spring following treatment during the late pink stage of blossom development by counting flower clusters in the same sampling units used for initial counts. Trunk and/or branch circumferences were measured at the time of both bloom counts.

In both 2005 trials, thirty fruit were randomly collected for harvest quality analyses from each tree within 2 days of harvest by grower-cooperators; crop load was too light in the 2004 'Fuji' trial to allow meaningful harvest analysis. Fruit were held in 34º F regular atmosphere storage until they could be processed, typically within 48 hours.

All fruit were weighed, and measured for length and diameter before running over a single lane color grader (Aweta-Falcon grading system) programmed to replicate a commercial packing line with standard color grades. A twenty fruit subsample from each set of thirty fruit was rated for visual defects including sunburn, bitter pit, and splitting. Fruit russet incidence and severity was recorded in categories of stem bowl, fruit shoulder, smooth solid, and net-type on fruit flanks. Fruit firmness was measured by punching two opposite sides of each peeled apple with a standard 7/16 inch penetrometer (Model EPT-1 Pressure Tester) used for Magness-Taylor tests. Tissue pieces from each fruit were mechanically juiced to produce a bulk sample for evaluation of soluble solids (Sper Scientific 0-35% Digital Refractometer) and titratable acidity (Mettler Toledo DL50 Graphix Titrator). For all parameters, statistical analyses were conducted using mean values for each tree, rather than values for individual subsamples.

Results and Discussion

2004 'Fuji' GA concentration x timing trial. This trial block was forced into a pronounced biennial bearing pattern by a severe late spring frost in 2002, which thinned nearly the entire crop for that season. In turn, the 2003 crop was relatively large, and the 2004 crop, in the year of GA application, quite light. The grower reported a 2x swing in yields between those "off" and "on" years. 'Novagib,' being certified by the Organic Materials Review Institute (OMRI), was the only commercial GA formulation registered for apple available at the time for use in this certified organic block.

All concentrations of GA_4 at all timings tested reduced floral density in the season following treatment (Table 3-1); spray concentration effects showed linear and quadratic significance for the petal fall timing, as well as linear significance at the 11 mm timing.

Flowering was increasingly inhibited in correlation with spray concentration at the two later timings, but 300 mg/L at petal fall reduced bloom density more than 450 mg/L. Treatment effects on shoot extension and fruit quality parameters (data not shown) were not significant.

Contrary to earlier published results, GA_4 treatments in this trial consistently inhibited flowering. Applications made between petal fall and 11 mm fruit size showed greater efficacy than those applied at 20 mm, suggesting that optimal spray timings for reduction of return bloom are within three weeks of full bloom.

2005'Fuji' GA isomer x concentration trial. All GA treatments significantly reduced floral density in the year after treatment (Table 3-2). Concentration effects on return bloom for each GA formulation were significant in linear regressions, although 2006 floral density was higher for 200 mg/L GA_4 than 100 mg/L. Quadratic regressions were also significant for both of the pure isomers, but not GA_{4+7}. Shoot growth was increased in linear fashion with respect to concentration of GA_{4+7}; no other formulations showed significant effects. Harvest fruit quality analyses produced no remarkable results (data not shown).

Interestingly, GA_7, often presumed to be the most powerful floral inhibitor of the isomers here tested, showed no greater affect than pure GA_4 at 100 mg/L, the presumed weakest inhibitor, whether applied alone or formulated with GA_4. Applied at mistakenly high concentrations, 1000, 2000, and 3000 mg/L GA_3 all reduced return bloom more dramatically than any other treatments, but showed no differences between the three concentrations. This lack of dose response suggests that 0.3 blossom clusters/cm^2 of trunk cross-sectional area (TCSA) may have been the practical minimum level of

flowering attainable with exogenous gibberellins in these particular trees. Production records for this trial block reveal consistently annual cropping, with no more than 10% swings in yields from one season to the next; as a result, biennial trends are unlikely to have significantly influenced trial results.

2005 'Honeycrisp' GA isomer trial. An aggressive concentration of each GA formulation (400 mg/L) was used in this trial to bolster chances of observing treatment effects. Pure GA_7 was not included in this trial due to limited availability of product. Crops from this block were reportedly consistent with approximately 10% swings in yields from year to year. GA treatment effects on 2006 floral density were highly significant. In fact, all products tested reduced return bloom to only 10-20% of control levels (Table 3-3), well beyond desirable levels of inhibition; trees treated in this trial were thrown into alternation for the coming seasons. As before, GA_4 reduced flowering to levels similar to those of GA_{4+7}. 4000 mg/L GA_3 was as inhibitory to flowering as 400 mg/L of GA_4 or GA_7. In contrast to 'Fuji', each GA treatment significantly increased shoot extension. The relative sensitivity of 'Honeycrisp' to GA is further corroborated by other trial results described in Chapters 2 and 4.

Because it is the least expensive retail source of gibberellin (Appendix B), GA_3 may well be the most viable commercial GA option for apple growers; it is roughly one-tenth the price of GA_4 or GA_{4+7} on an equivalent active ingredient basis. The extremely high concentrations of GA_3 evaluated in these trials produced excessive results, suggesting that 100-500 mg/L might well provide more desirable reductions in return bloom. Since these studies were initiated, a new formulation of GA_{4+7} with certification from OMRI, ProVide 10 SG, has replaced the liquid formulation of ProVide in the tree

fruit market. As such, organic growers may potentially use all four major GA products registered for tree fruits. It should be noted, however, that neither GA_3 product (ProGibb or Falgro) is currently registered for use in apple and the labels for GA_4 (Novagib) and GA_{4+7} (ProVide) only allow use for russet control and suppression of cracking in 'Stayman' apples. Legal use of any of these products to manipulate flowering in apple would require changes in each of their labels.

The relative performance of GA_4 at all sites is instructive; the 400 mg/L concentration used on 'Honeycrisp' was too strong to produce a reasonable result and the more modest 100 and 200 mg/L concentrations used on 'Fuji' in 2005 still reduced flowering by two-thirds. Yet when applied to the highly biennial 'Fuji' site in 2004, 150 and 300 mg/L GA_4 reduced bloom to roughly half of the levels of control. The results of these three trials further confirm that initial cropping patterns strongly influence the efficacy of GA programs to inhibit flowering (see also Chapter 2), suggesting that trees trapped in more severe alternation have greater "inertia" to overcome with respect to manipulating flowering behavior. In contrast to earlier studies, GA_4 clearly and consistently inhibited floral initiation in all three trials, encompassing two growing seasons and unique cultivars. Even though pure GA_7 may be the most powerful isomer for bloom suppression, it did not clearly distinguish itself from alternative formulations in these trials. Our distinct results are likely due to differences between these modern cultivars and those traditionally used in GA research, as well as the particular traits of Washington orchard conditions.

Future research investigating lower concentrations (<100 mg/L) of GA should provide more moderate results and help fine tune potential programs for industry

adoption. Commercial use of GA will likely need to be customized relative to cropping history (see also Chapter 2) and cultivar (see also Chapter 4). Results from these trials indicate that the most cost effective use of gibberellins in commercial settings to manipulate vegetative/reproductive growth balance could be GA_3 applied near 10 mm fruitlet size, and future research into appropriate material concentrations are strongly indicated.

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Table 3-1. Effects of GA_4 spray timing and concentration in 2004 on lightly cropped 'Fuji'/MM.106 apple trees. Treatments are listed by phenological application timing (petal fall, 11mm fruit size, or 20mm fruit size) and spray concentration (150, 300, or 450 mg/L a.i.).

Timing	Concentration (mg/L)	2004 shoot extension (cm)[z]	2005 bloom/ LCSA[y] (flower clusters/cm^2)
Control	0	38.4	6.8
Petal Fall	150	34.5	3.9
	300	37.8	2.6
	450	38.9	2.9
11mm	150	40.1	3.5
	300	39.3	2.8
	450	42.2	1.7
20mm	150	37.1	4.8
	300	37.6	4.5
	450	34.3	3.9

2005 bloom density (Model r^2=0.52)

	Concentration	
Significance	Linear	Quadratic
Petal Fall	***	*
11mm	**	0.09
20mm	0.08	NS

[z]Mean of 10 terminal shoots per tree; effects were not significant.
[y]LCSA = limb cross-sectional area
NS, *, **, ***,Nonsignificant or significant at p = 0.05, 0.01, or 0.001, respectively.

Table 3-2. Effects of GA formulation and concentration in 2005 on 'Fuji'/M.26 apple trees. Treatments are listed by GA formulation (GA_4, GA_7, GA_{4+7}) and spray concentration (100, 200, 300 mg/L a.i.).

Formulation	Concentration (mg/L)	2005 shoot extension (cm)[z]	2006 bloom/ TCSA[y] (flower clusters/cm^2)
Control	0	21.0	3.0
GA_4	100	25.4	0.7
	200	28.2	1.0
GA_7	100	24.1	0.8
	200	24.2	0.4
GA_{4+7}	100	27.1	1.4
	200	27.5	0.6

2005 shoot extension (Model r^2=0.64)

	Concentration	
Significance[x]	Linear	Quadratic
GA_4	NS	NS
GA_7	NS	NS
GA_{4+7}	**	0.09

2006 bloom density (Model r^2=0.74)

	Concentration	
Significance[x]	Linear	Quadratic
GA_4	****	**
GA_7	***	*
GA_{4+7}	*	NS

GA_3[x]	1000	25.2	0.3
	2000	25.2	0.3
	3000	28.0	0.3

[z]Mean of 10 terminal shoots per tree

[y]TCSA = trunk cross-sectional area

[x]Concentrations of GA_3 were not proportionate to other treatments; data were excluded from radiating regression analyses.

NS, *, **, ***, ****Nonsignificant or significant at p = 0.05, 0.01, 0.001, or 0.0001, respectively.

Table 3-3. Effects of GA formulation in 2005 on 'Honeycrisp'/P.18 apple trees. All treatments were applied at 400 mg/L a.i. at 10 mm fruitlet size (15 DAFB) in 2005. Means followed by the same letter are not significantly different within a column (n = 6, $p \leq 0.05$).

Formulation	2005 shoot extension (cm)[z]	2006 bloom/ LCSA[y] (flower clusters/cm^2)
Control	18.8 b	2.8 a
GA_4	21.8 a	0.5 b
GA_{4+7}	21.7 a	0.3 b
p values	0.006	0.0002
4000 mg/L GA_3[x]	21.2	0.3

[z]Mean of 10 terminal shoots per tree

[y]LCSA = limb cross-sectional area

[x]Concentration of GA_3 was not proportionate to other treatments; data were excluded from ANOVA and means separation.

CHAPTER 4

Gibberellic acid accelerates 'Honeycrisp,' but not 'Cameo' apple fruit maturation

Keywords: biennial bearing, floral initiation, GA isomers, return bloom, shoot growth, starch, ripening, storage

Abstract

Gibberellins show potential to inhibit flowering in apple (*Malus domestica* Borkh.) to promote annual bearing. This study examines collateral tree and fruit effects of using high rates of gibberellic acids (GA), with particular focus on in-season fruit maturity. In 2004, GA_{4+7} was sprayed on the biennial cultivars 'Cameo' and 'Honeycrisp' at 0, 200, 400, or 600 mg/L. Treated 'Honeycrisp' fruit demonstrated advanced maturity in terms of starch levels, flesh firmness, and titratable acidity, while 'Cameo' showed no treatment effects. 0, 300, 600, 900, or 1200 mg/L GA_{4+7} was applied to 'Cameo' in 2005, and fruit maturity was once again unaffected. Three commercial GA products (GA_3, GA_4, GA_{4+7}) were applied in 2005 to 'Honeycrisp' at 400 mg/L. All formulations caused fruit to have less flesh firmness and acidity and increased levels of starch conversion compared to the untreated control at harvest and after 140 days of common storage, with GA_3 showing the strongest effects. All GA treatments in all four trials significantly diminished flowering in the season after treatment. Results demonstrate differences in sensitivity to GA between the two cultivars.

Introduction

Biennial bearing is a major problem for apple producers, who would benefit from new options to manage cropping and ensure consistent yields of high quality fruit. Flowering promoters such as ethephon or naphthaleneacetic acid (NAA) are widely used

in the United States to improve return bloom after moderate to heavy crops. Floral initiation inhibitors, namely GAs, show potential as crop load management tools by reducing return bloom after light crops. There is an abundance of literature reporting effects of various GA isomers on flowering in apple in the season following application (Bertelsen and Tustin, 2002; Marino and Greene, 1981; McArtney, 1994; Meador and Taylor, 1987; Tromp, 1982). However, to date, little has been reported regarding the effects of GA on the fruit present during treatment (i.e. current season fruit).

In sweet cherry (*Prunus avium* L.), GA_3 can delay fruit maturation (Proebsting, 1972), and the industry widely uses 10-20 mg/L to increase fruit size and quality, and to extend commercial harvest. GA_3 has also been shown to delay maturity and improve fruit quality of prunes and plums (*P. domestica*) (Looney, 1996). Impacts of GA on apple maturity are not widely reported, but Greene (1989) found decreased flesh firmness at harvest and increased storage breakdown of GA-treated 'Empire' apples, suggesting that 50-150 mg/L GA_{4+7} might accelerate ripening. Looney et al. (1992) saw no effect from 7.5 or 15 mg/L GA_4 or GA_{4+7} on firmness of 'Golden Delicious', but did report higher sugar levels and decreased russeting. If growers are to use GA to help manage cropping in apple, the secondary effects of those programs on the current season's crop must be better understood.

The capacity of gibberellins to improve fruit finish is well-documented (Looney, 1996). Taylor (1978) found GA_{4+7} to be more effective than similar rates of GA_3 to reduce russet in 'Golden Delicious.' The ability of GA_{4+7} to reduce russet in 'Golden Delicious' was later confirmed by Meador and Taylor (1987) and Elfving and Allen (1987). Reuveni et al. (2001) reported similar reductions in fruit russet from three

different commercial bioregulator formulations containing GA_{4+7}. In addition to improving fruit finish, GA can affect other quality parameters. Unrath (1974) and Looney et al. (1992) both observed increased fruit length and length/diameter ratio in apples treated with GA_{4+7}. Spray concentrations employed in these studies were 10-20x less than those typically used to manipulate flowering, making extrapolation their results to significantly higher rates tenuous.

The trials reported here explore the collateral effects on in-season apple fruit maturity in two notoriously biennial varieties from GA programs designed to inhibit return bloom as part of a comprehensive crop load management program.

Materials and Methods

Experimental design. Two field trials each in 2004 and 2005 were conducted in commercial apple orchards in three distinct growing districts of Washington State. Aside from elimination of bioregulator programs which affect flower initiation, standard orchard management strategies were followed by grower-cooperators. Each trial employed a randomized complete block design with six replicates. In two 'Cameo' trials, whole individual trees served both as experimental and sampling units. Whole trees were also treated in two 'Honeycrisp' trials, but sampling units for bloom counts were restricted to an eastern- and western-oriented scaffold limb due to large tree size; fruit for harvest analysis were randomly selected from entire trees. The 2005 'Honeycrisp' trial was located near the 2004 trial in the same orchard block. To isolate treatments, at least one buffer row was maintained between rows receiving treatment. In addition, a minimum of three meters (1-3 trees) separation between treated trees was maintained within the row for all trials.

Data were analyzed with the Statistical Analysis System (SAS) of the SAS Institute, Cary, NC. Means were separated with the general linear model using Tukey's Studentized Range Test at 0.05 by analysis of variance (Proc GLM). Where fixed-effects variables allowed regression analysis, the General Linear Models (GLM) procedure of SAS was used to evaluate the homogeneity of slopes, curvatures and intercepts of the regressions on bioregulator concentration. Only significant findings are included in this report.

2004 trials. We established a trial in a seven year old 'Cameo'/Bud.9 orchard near Tonasket, WA (48.8º N, 119.4º W). Trees were planted 1 m x 3.5 m and trained to a three-wire vertical trellis in a spindle system. Treated trees were sprayed with 200, 400, or 600 mg/L GA_{4+7}; control trees were left unsprayed.

A second trial was conducted near Brewster, WA (48.2º N, 119.7º W) on six year old 'Honeycrisp' grafts on fourteen year old 'Regent' interstems on P.18 rootstocks. These free-standing central leader trees were spaced 3 m x 5 m. Spray applications were identical to those in of the 'Cameo' trial.

2005 trials. A trial was established in nine year old 'Cameo'/M.9 Nic.29 near Quincy, WA (47.3º N, 119.7º W). Trees were spaced 1.5 m x 4 m and trained to a five-wire V-trellis. Due to modest response from treatments in the 2004 'Cameo' trial, more aggressive concentrations of 300, 600, 900, or 1200 mg/L of GA_{4+7} were applied.

Fifteen year old 'Honeycrisp'/P.18 near Brewster, WA (48.2º N, 119.7º W) were selected for a study of fruit maturity effects of fruit untreated or sprayed with 400 mg/L GA_4, or GA_{4+7}; due to mathematical error, GA_3 was applied at 4000 mg/L. Unlike in 2004, these trees were not grafted and had no interstem.

Sprays. The commercial GA_{4+7} formulation 'ProVide' (Valent Biosciences, Libertyville, IL) was used in all four trials. The 2005 'Honeycrisp' trial also included the commercial formulations of GA_3, 'Falgro 4L' (Fine Agrochemicals, Worcester, UK) and GA_4, 'Novagib 10L' (Fine Agrochemicals, Worcester, UK). All applications were sprayed at 10 mm fruitlet size, determined by the mean diameter of king apples of 30 randomly selected fruit clusters measured with digital calipers (Mitutoyo Corp., Japan) in the respective trial blocks (13-15 days after full bloom). Applications were made by handgun with a 25 gallon 'Nifty' power sprayer (Rears Manufacturing, Eugene, OR) adjusted to a fine mist at 200 lbs/in^2 pressure. Whole trees were sprayed until all visible foliage was wet, but not to the point of dripping from more than 10% of all leaves. No adjuvants were used for any spray.

Data collection. Initial flower cluster counts were recorded for each sampling unit during the late pink stage of bloom development in the season of treatment. After terminal bud set, final shoot length was measured on ten upright, one year old shoots in each tree. Return bloom was assessed in the subsequent spring by counting flower clusters in the same sampling units used for initial counts. Trunk and/or branch circumferences were measured at the time of both bloom counts.

In all trials, thirty fruit were randomly collected for harvest quality analyses from each tree 1-3 days before harvest by grower-cooperators. A second random sample of thirty fruit was also taken for medium term storage (90-140 days) and subsequent quality analyses in all cases except for the 2004 'Honeycrisp' trial. Fruit were held in 34º F common storage until they could be processed, typically within 48 hours unless they were intended for storage.

All fruit were weighed and measured for length and diameter before running across a single lane color grader (Aweta-Falcon grading system) programmed to replicate a commercial packing line with standard color grades. A twenty fruit subsample from the thirty fruit sample from each tree was rated for visual defects, including sunburn, bitter pit, and splitting. Fruit russet incidence and severity was recorded in categories of stem bowl, fruit shoulder, smooth solid, and net-type on fruit flanks. Fruit firmness was measured by punching two opposite sides of each peeled apple with a standard 7/16 inch penetrometer (Model EPT-1 Pressure Tester) used for Magness-Taylor tests. All twenty fruit were bisected laterally at the equator; calyx halves were treated with 10% iodine solution for standard starch readings and tissue pieces from the stem halves of each fruit were mechanically juiced to produce a bulk sample for evaluation of soluble solids (Sper Scientific 0-35% Digital Refractometer) and titratable acidity (Mettler Toledo DL50 Graphix Titrator). For all parameters, statistical analyses were conducted using mean values for each tree, rather than values for individual subsamples.

After 90-140 days in 34º F regular atmosphere storage, the second sets of fruit samples were analyzed similarly except for starch readings, which were omitted due to nearly complete loss of starch reserves during storage.

Results and Discussion

2004 'Cameo' GA concentration trial. The effects of GA_{4+7} on 'Cameo' fruit maturity were unclear. Analyses of fruit quality/maturity parameters at harvest were inconclusive. Control fruit exhibited lower flesh firmness (Table 4-1), suggesting that untreated fruit were more mature than treated fruit. In contrast, control fruit had higher acidity, which would indicate less-advanced maturity (Mattheis, 1996). Fruit size, shape,

and finish were unaffected by any treatment (data not shown). In addition, no significant treatment effects for any maturity parameter were observed in fruit analyzed after 90 days of common cold storage.

Return bloom was profoundly diminished by GA_{4+7} in linear and to a lesser degree, curvilinear fashion. GA_{4+7} at 400 mg/L and higher concentrations completely eliminated flowering in the subsequent season (Table 4-1). This trial was conducted in the on year of a severe biennial bearing cycle. According to grower records, yields from this block in "on" years were approximately 400% of yields in "off" seasons. This extreme alternation accounts for the relatively poor 2005 return bloom in control trees. Vegetative extension growth was 10-25% greater in all sprayed trees; while not statistically significant (p=0.05), this resulting increased shoot length could have meaningful implications to the grower in terms of increased pruning costs and shading to lower parts of the canopy, potentially inhibiting fruit color development and floral initiation.

2004 'Honeycrisp' GA concentration trial. Treated fruit in this trial showed advanced maturity across several indices (Table 4-3). Strong linear effects of elevated starch conversion, decreased flesh firmness, and reduced titratable acidity suggest that maturity of treated fruit was 2-5 days more advanced than control fruit. Soluble solids content was not affected by any treatment.

The experimental design included collection of fruit samples for maturity evaluation at three timings: commercial harvest minus seven days, commercial harvest, and commercial harvest plus seven days. Unfortunately, only the first sample was secured before the grower strip-picked the entire trial block five days ahead of schedule,

including trial trees. Bitter pit and fruit russet were to be assessed along with standard maturity parameters in fruit sampled at commercial harvest, but were not because the first set of fruit had already been destructively evaluated before the problem was discovered. Although intended to reflect maturity one week before projected harvest, the fruit sample analyzed was actually collected within 48 hours of commercial harvest. While unfortunate from the research perspective, these experiences are not uncommon for 'Honeycrisp' growers and underscore the challenges of determining appropriate commercial harvest maturity with standard starch and penetrometer readings in this cultivar.

2005 flowering was significantly diminished in direct linear relation to spray concentration. No effect on shoot growth was observed.

2005 'Cameo' GA concentration trial. As in 2004, maturity effects of GA_{4+7} on maturity of 'Cameo' were inconclusive. While not statistically significant, increased levels of starch conversion (Table 4-2) at high spray concentrations would likely be sufficient to drive commercial decisions regarding harvest timing and storage regimes. Clear trends could not be discerned from firmness, sugar, acidity, or fruit finish data in either fruit analyzed at harvest or after 120 days of storage. Overall, the data suggested little effect of the postbloom GA treatments on fruit physiological behavior at and following harvest.

Diminished fruit diameter and weight were consistently associated with higher concentrations of GA_{4+7} in both 'Cameo' trials (data not shown), but the effects were not significant (p=0.05). This trend is corroborated by a series of trials by the Washington Tree Fruit Research Commission which found that benzyladenine (BA)+GA_{4+7}

formulations had a tendency to reduce fruit diameter in numerous strains of 'Delicious' (McFerson, 2003). Since 'Cameo' is believed to be a chance seedling of 'Delicious,' it is reasonable to expect GA_{4+7} to act similarly on both cultivars.

Trees in this orchard demonstrated good balance between vegetative and reproductive growth, and consistent harvest yields indicated no biennial bearing habit. Upright shoot growth was rather modest in control plots (approximately 10 cm). Final shoot length was generally 20-40% longer in trees treated with GA_{4+7} (data not shown). Return bloom was significantly inhibited in both linear and curvilinear fashion with respect to concentration, with little difference between results for 600, 900, or 1200 mg/L (Table 5-2).

2005 'Honeycrisp' GA isomer trial. Fruit maturity was not as clearly accelerated by GA in 2005 as in the 2004 'Honeycrisp' trial. Both GA_4 and GA_{4+7} produced fruit at harvest with decreased titratable acidity (Table 4-4), but effects on fruit firmness, starch conversion, and soluble solids content were not significant. Fruit treated with the mistakenly high concentration of GA_3 accelerated maturity in terms of each harvest parameter measured, but these data were excluded from statistical analysis due to their irrelevance to other treatments. Interestingly, fruit firmness and acidity were still elevated in control fruit and relatively low in GA_3 treated fruit after 140 days of 34º regular atmosphere storage.

Fruit finish was improved by GA_4, which reduced overall incidence of fruit russet by approximately 50%. Most russet was observed in the stem bowl or on fruit shoulders, with few blemishes appearing on the flanks of fruit. Assessment of russet on fruit stored for 140 days was confounded by decay and other postharvest disorders and results are

excluded from this report. All three isomers caused a 12-15% increase in shoot extension and a 5-10% increase in harvest fruit weight. GA_4 and GA_{4+7} significantly increased fruit length and length:diameter ratio, but fruit diameter was unaffected. Incidence of bitter pit may have trended slightly higher in all GA treatments, but sample size was inadequate to draw clear conclusions (data not shown).

All three GA treatments reduced return bloom by more than 80%, which is likely an excessive correction for most commercial circumstances. We chose an aggressive concentration of 400 mg/L to increase our odds of producing clear results. Future studies examining more modest concentrations (50-200 mg/L) of these materials would likely provide more practical information to growers trying to decide how to manage alternate bearing blocks.

Gibberellins are often the hormonal antithesis of ethylene, producing opposite effects with respect to shoot growth and floral initiation. However, the ethylene-inducing growth regulator, ethephon, accelerates ripening of apple (Greene, 1996) and many other fruits. The advanced maturity of GA treated 'Honeycrisp' suggests upregulation of their ethylene synthesis pathways, perhaps as part of a wounding response from damaging levels of GA early in the growing season. In future studies of this type, regular analysis for the presence of ethylene or its metabolic precursors such as s-adenosyl methionine (SAM) or aminocyclopropane carboxylic acid (ACC) might provide insight as to how application of high levels of exogenous GA accelerates maturity. The maturity response of GA-treated fruit could also be explored with field applications of 1-methylcyclopropene (1-MCP), theoretically inhibiting ethylene perception.

Apple cultivars responding differently to various bioregulators is a common phenomenon; published reports document cultivar-specific responses to diaminozide (Crowe, 1968; Walsh and Kender, 1982; McLaughlin and Greene, 1991), prohexadione-Ca (Buban et al., 2004), 'Atonik' (Koupil, 1997), benzyladenine (McLaughlin and Greene, 1991), ethephon (Walsh and Kender, 1982), naphthaleneacetic acid (Krzewinska et al., 1992; Elezaby and Hasseeb, 1995) and two triazoles, paclobutrazol and uniconazole (Zimmerman and Steffens, 1995). Elezaby and Hasseeb (1995) also reported that GA_3 increased pollen germination in 'Anna' and 'Bericher,' but not 'Dorsett Golden'; Promalin ($BA+GA_{4+7}$) produced an opposite effect, increasing pollen germination of 'Dorsett Golden,' but not 'Anna' or 'Bericher.' Based on unique responses to an unspecified gibberellic acid applied to root collars of 'Shampion,' 'Paulared,' and 'Lobo', Grochowska et al. (1995) proposed that individual cultivars have cultivar-specific patterns of endogenous hormones and/or gibberellin metabolic pathways.

'Honeycrisp' crop load is relatively easy to moderate with blossom and postbloom chemical thinners. In contrast, 'Cameo' requires more aggressive thinning programs. Phenotypic differences between these two cultivars are numerous and the relative sensitivity of 'Honeycrisp' to GA and insensitivity of 'Cameo' to GA and ethephon (Chapter 2) we observed support the hypothesis of cultivar-specific hormone profiles and unique metabolic pathways.

In conclusion, concentrations of GA designed to influence flowering advanced fruit maturity in 'Honeycrisp,' but not 'Cameo'. Results suggest all formulations of GA tested induced early ripening of 'Honeycrisp.' Gibberellins show promise as floral

inhibitors for crop load management purposes, but cultivar-specific responses in our trials highlight the need for GA programs to be customized for individual varieties; other factors to consider may include rootstock, cropping history, and bloom and postbloom chemical thinning programs. Future research in apple genomics likely holds the ultimate answers regarding cultivar-specific responses to bioregulators. Until those metabolic pathways are elucidated, further exploration of primary and collateral effects of using GA to promote annual flowering would be useful to assist growers in making more informed management decisions.

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Table 4-1. Effects of concentration of GA_{4+7} (applied in 2004) on fruit quality/maturity parameters and return bloom of 'Cameo'/Bud.9 apple. Fruit were analyzed at harvest or after 90 days of regular atmosphere cold storage.

Concentration (mg/L)	Starch index (1-6)	Flesh firmness (N)	Soluble solids (%)	Titratable acidity (%)	Russeted fruit (%)	2005 return bloom (flower clusters / cm^2 TCSA[z])
Harvest						
0	4.4	62.0	11.8	0.39	11	0.6
200	4.3	64.3	12.2	0.35	8	0.1
400	4.1	63.5	12.1	0.34	10	0.0
600	4.1	63.3	12.2	0.36	14	0.0
Significance						
Concentration						
Linear	NS	*	NS	**	NS	****
Quadratic	NS	NS	NS	*	NS	**
Model r^2	0.20	0.32	0.15	0.64	0.42	0.77
Harvest+90 d						
0	--	54.4	11.7	0.42	13	--
200	--	53.5	11.9	0.43	23	--
400	--	55.2	11.9	0.43	9	--
600	--	55.6	12.0	0.43	16	--
Significance						
Concentration						
Linear	--	NS	NS	NS	**	--
Quadratic	--	NS	NS	NS	**	--
Model r^2	--	0.55	0.68	0.28	0.52	--

[z]Trunk cross-sectional area

NS, *, **, ****Nonsignificant or significant at P = 0.05, 0.01, or 0.0001, respectively.

Table 4-2. Effects of concentration of GA_{4+7} (applied in 2005) on fruit quality/maturity parameters and return bloom of 'Cameo'/Nic.29 apple. Fruit were analyzed at harvest or after 120 days of regular atmosphere cold storage.

Concentration (mg/L)	Starch index (1-6)	Flesh firmness (N)	Soluble solids (%)	Titratable acidity (%)	Russeted fruit (%)	2005 return bloom (flower clusters / cm^2 TCSA[z])
Harvest						
0	4.8	69.5	12.3	0.24	13	9.2
300	4.9	69.4	13.5	0.25	13	2.3
600	5.1	71.1	12.9	0.22	12	0.4
900	5.3	69.3	12.7	0.22	13	0.3
1200	5.5	68.2	12.3	0.22	12	0.3
Significance						
Concentration						
Linear	NS	NS	*	NS	NS	****
Quadratic	NS	NS	**	NS	NS	****
Model r^2	0.50	0.32	0.42	0.59	0.28	0.82
Harvest+120 d						
0	--	55.6	12.3	0.27	20	--
300	--	51.2	13.3	0.26	15	--
600	--	54.5	12.4	0.28	33	--
900	--	53.0	12.7	0.25	14	--
1200	--	55.3	12.0	0.24	23	--
Significance						
Concentration						
Linear	--	NS	NS	NS	NS	--
Quadratic	--	NS	NS	NS	NS	--
Model r^2	--	0.35	0.24	0.55	0.09	--

[z]Trunk cross-sectional area

NS, *, **, ****Nonsignificant or significant at P = 0.05, 0.01, or 0.0001, respectively.

Table 4-3. Effects of concentration of GA_{4+7} (applied in 2004) on fruit quality/maturity parameters and return bloom of 'Honeycrisp'/P.18 apple.

Concentration (mg/L)	Starch index (1-6)	Flesh firmness (N)	Soluble solids (%)	Titratable acidity (%)	2005 return bloom (flower clusters / cm^2 LCSA[z])
0	4.4	65.5	12.6	0.45	4.9
200	4.7	62.8	12.7	0.42	3.2
400	4.9	60.4	12.3	0.36	1.3
600	5.2	59.2	12.7	0.38	0.7
Significance					
Concentration					
Linear	****	****	NS	*	****
Model r^2	0.74	0.79	0.27	0.52	0.73

[z]Limb cross-sectional area
NS, *, ****Nonsignificant or significant at P = 0.05, or 0.0001, respectively.

Table 4-4. Effects of 400 mg/L a.i. GA4 and GA4+7 (applied in 2005) on fruit quality/maturity parameters and return bloom of 'Honeycrisp'/P.18 apple. Means followed by the same letter are not significantly different within a column (n = 6, p ≤0.05).

Isomer	Starch index (1-6)	Flesh firmness (N)	Soluble solids (%)	Titratable acidity (%)	Russeted fruit[z] (%)	2005 return bloom (flower clusters / cm^2 LCSA[y])
Harvest						
Control	5.2[NS]	67.7 [NS]	12.9 [NS]	0.25 a	40 a	2.8 a
GA_4	5.4	64.2	13.1	0.21 b	20 b	0.5 b
GA_{4+7}	5.3	62.7	13.2	0.21 b	35 ab	0.3 b
p values	0.71	0.06	0.79	0.01	0.02	0.0002
4000 mg/L GA_3[x]	5.8	59.7	12.4	0.18	23	0.3
Harvest+140 d						
Control	--	63.4 a	12.6 [NS]	0.32 a	--	--
GA_4	--	57.2 b	12.1	0.26 b	--	--
GA_{4+7}	--	56.3 b	12.4	0.27 b	--	--
p values	--	0.002	0.45	0.001	--	--
4000 mg/L GA_3[x]	--	53.4	12.0	0.24	--	--

[z]n=120 fruit per treatment

[y]Limb cross-sectional area

[x]Concentration of GA_3 was not proportionate to other treatments; data were excluded from means separations.

APPENDIX A

ROSTER OF THESIS PROJECT TRIALS

SITE	CULTIVAR	ROOT	YEAR PLANTED	TREATMENTS	REPLICATIONS	TRIAL TYPE
2004-2005						
Tonasket, WA	Cameo	Bud.9	1998	12	5	GA timing vs. crop load
Wiley City, WA	Honeycrisp	EMLA.9	2000	6	6	GA vs. crop load
Royal City, WA	Fuji	MM.106	1999	10	6	GA rate vs. timing
Brewster, WA	Honeycrisp	P.18	1991[z]	4	6	GA rate effects on fruit maturity
Tonasket, WA	Cameo	Bud.9	1998	4	6	GA rate effects on fruit maturity
Tonasket, WA	Cameo	Bud.9	1998	12	5	Ethephon rate vs. crop load
2005-2006						
Quincy, WA	Cameo	Nic.29	1997	12	6	GA timing vs. crop load
Royal Slope, WA	Fuji	M.26	1994	6	6	GA vs. crop load
Royal Slope, WA	Fuji	M.26	1994	10	6	GA isomer vs. rate
Brewster, WA	Honeycrisp	P.18	1991	4	6	GA isomer effects on fruit maturity
Quincy, WA	Cameo	Nic.29	1997	5	6	GA rate effects on fruit maturity
Quincy, WA	Cameo	Nic.29	1997	12	6	Ethephon rate vs. crop load

[z]Honeycrisp scion grafted to Regent interstem in 1998

APPENDIX B

ESTIMATED GROWER COSTS FOR BIOREGULATOR PROGRAMS

NOTE: products may not be labeled for commercial use as described in these studies; this report is shared for research purposes and neither constitutes recommended uses nor condones inappropriate application of these materials.

BIOREGULATOR	TRADE NAME	MANUFACTERER	OMRI [z] CERTIFIED	RELATIVE UNIT COST [y]
GA_3	Falgro® 4L	Fine Agrochemicals, Ltd.	yes	$30 / acre
GA_3	ProGibb®	Valent Biosciences Corp.	yes	$30 / acre
GA_4	Novagib™ 10L	Fine Agrochemicals, Ltd.	yes	$400 / acre
GA_7	na	Fine Agrochemicals, Ltd.	no	not commercially available
GA_{4+7}	ProVide® 10 SG	Valent Biosciences Corp.	no	$300 / acre
Ethephon	Ethrel®	Bayer CropScience LP	no	$2.25 / acre

[z]Organic Materials Review Institute, Eugene, OR

[y]100 mg/L a.i. applied at 100 gallons water/acre. Based on retail prices quoted by Wenatchee, WA, area chemical distributors, October 2006.