Naphthaleneacetic Acid and Ethephon Are Florigenic in the Biennial Apple Cultivars Golden Delicious and York Imperial

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Abstract. ‘Golden Delicious’ and ‘York Imperial’ are apple cultivars that are prone to develop a biennial bearing habit. A successful chemical thinning program with carbaryl plus 6-benzyladenine applied at the 10-mm fruit diameter stage reduced cropland and increased return bloom of ‘York Imperial’, although the improvement in return bloom resulting from chemical thinning was insufficient to ensure a commercial cropland in the year after treatment (fewer than 10% of spurs developing flowers). A chemical thinning program with multiple applications of a naphthaleneacetic acid (NAA) and ethephon mixture during the period from 36 to 73 days after bloom increased return bloom of ‘York Imperial’ trees to commercially acceptable levels (25% or greater of spurs flowering). NAA applied during the period from 50 to 100 days after bloom (summer NAA program) or from 110 to 140 days after bloom (preharvest NAA program) increased return bloom of ‘Golden Delicious’. When aminoethoxyvinylglycine (AVG) was included with the first NAA spray in a summer program, the efficacy was reduced, indicating that ethylene may be partly involved in the florigenic activity of NAA. Dissection of ‘Golden Delicious’ buds sampled from three locations (Asheville, NC; Amherst, MA; Wenatchee, WA) at ≈14-day intervals beginning 50 days after bloom indicated that the time of floral transition (doming of the meristem apex) occurred during the period from 65 to 105 days after bloom at each location. Thus, NAA applications in a summer program for return bloom coincided with the period when floral determination normally occurred. Preharvest NAA programs effectively promoted return bloom in the experiments where a summer NAA program was also effective. These responses indicate that NAA can trigger floral development within vegetative buds relatively late in the summer and outside of the time period when it is generally believed possible to influence flower bud formation.

Many important apple (*Malus × domestica* Borkh.) varieties develop a biennial bearing habit, in which there is a repeating cycle of

a heavy crop 1 year followed by a light crop or no crop in the next year. Regulating the flower bud formation process to restore a more equal balance between vegetative and reproductive spurs is one strategy for restoring trees from a biennial bearing habit to consistent cropping. Early removal of fruit from apple spurs can promote return bloom (Aldrich, 1932; Aldrich and Fletcher, 1932; Harley and Masure, 1937; Harley et al., 1934). An effective chemical fruit thinning program reduces the number of fruit per tree or per spur, thereby increasing the probability that the terminal axillary meristem on a flowering or fruiting spur will develop

reproductive structures. However, cultivars with a strong natural tendency for biennial bearing remain predisposed to an alternating habit, even after a successful chemical thinning program has reduced the fruit number to a commercially acceptable level (personal observation by authors). In such situations, additional strategies are needed to restore consistent cropping.

Application of growth regulators GA₃, GA₄₊₇, or GA₇ to apple trees in the non-fruiting year of the biennial bearing cycle can inhibit flower bud formation (Fulford, 1973; Marino and Greene, 1981; McArtney, 1994; McArtney and Li, 1998; Meador and Taylor, 1987; Schmidt et al., 2010), whereas application of ethephon (Williams, 1972) or NAA (Harley et al., 1958; McArtney et al., 2007) in the heavy cropping year of a biennial bearing cycle can stimulate flower bud formation. Williams (1972) reported that postharvest application of ethephon to young vigorous ‘Wellspur Delicious’ apple trees stimulated flower bud formation. Thus, the transition from vegetative to floral development may be triggered by a chemical stimuli relatively late in the season.

‘York Imperial’ is an important processing apple in Virginia and Pennsylvania that can develop a biennial bearing habit. Hand-thinning ‘York Imperial’ trees 8 d after bloom resulted in abundant fruit bud formation, whereas thinning later than 25 d after bloom did not effectively increase return bloom on trees with a heavy fruit set (Aldrich and Fletcher, 1932), suggesting that a signal inhibiting flower bud formation emanated from the fruit of this cultivar very early in the growing season. The combination of limb-ringing and fruit removal to establish 100 leaves per fruit stimulated fruit bud formation on ‘York Imperial’ apple trees when it was imposed before 60 d after full bloom but was less effective after this time (Magness et al., 1934). It was proposed that buds on high-vigor trees remain in a meristematic state later into the season compared with buds on low-vigor trees and presumably therefore respond to florigenic stimuli later than buds on less vigorous trees (Magness et al., 1934).

NAA directly stimulates flower development in apple (Harley et al., 1958). Applications of NAA in the heavy cropping year of a biennial bearing cycle increased the proportion of flowering spurs in the next year (McArtney et al., 2007). Applications of NAA to ‘Golden Delicious’ at weekly intervals during the month leading up to harvest (120 to 150 d after bloom) increased return bloom just as effectively as four biweekly summer applications during the period 60 to 100 d after bloom (McArtney et al., 2007). The florigenic activity of foliar NAA sprays applied during the month before harvest (McArtney et al., 2007) and of a postharvest ethephon application (Williams, 1972) is unexpected given these growth regulators were applied after the period when apple buds are believed to transition from vegetative to reproductive development.

Broadening of the meristem apex within the buds developing on 1-year-old wood of

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apple occurs during the period 21 to 42 d after bloom (Buban and Faust, 1982; Foster et al., 2003; Pratt et al., 1959) and this change provides the earliest physical indication of the transition to floral development. Doming of the apex follows soon after broadening, providing the first easily observable indication of the floral transition. Doming is typically observed 70 to 100 d after full bloom (Foster et al., 2003; Hirst and Ferree, 1995; Hoover et al., 2004; McArtney et al., 2001) and is relatively synchronized within buds sampled from wood of the same age (Foster et al., 2003; Hoover et al., 2004; McArtney et al., 2001). Doming of the meristem apex has not been observed later than ≈ 110 d after bloom, i.e., well before harvest of most apple cultivars. Thus, the timing of summer NAA sprays for return bloom coincides with the period during which buds are normally doming. In contrast, the application of NAA sprays during the month before harvest occurs well after buds have normally made this transition. The florigenic activity of preharvest NAA sprays for return bloom provides evidence to suggest that NAA can trigger floral development within vegetative buds relatively late in the season. Because ethephon sprays during the postharvest period are also florigenic (Williams, 1972), it may be possible that the positive effect of NAA on return bloom is mediated by an auxin-induced ethylene response (Curry, 1991).

The objectives of the present studies were to 1) evaluate the efficacy of summer NAA sprays for promotion of return bloom of 'York Imperial' and 'Golden Delicious' apple; 2) verify that weekly NAA applications during the month leading up to harvest are florigenic; 3) establish if ethylene is involved in the florigenic activity of NAA sprays; and 4) determine the time of floral transition in different growing environments and confirm that preharvest NAA sprays are applied after the developing buds have normally made the transition from vegetative to floral development.

Materials and Methods

Efficacy of summer NAA and ethephon sprays for promoting return bloom of 'York Imperial'. An experiment was initiated at the Alson H. Smith, Jr. Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, in Winchester, VA, in 2007 to evaluate the effect of chemical thinning and different NAA and ethephon treatments on return bloom of 'York Imperial' apples. The six treatments in this study were 1) an unthinned control; 2) a chemically thinned control with 1200 mg·L⁻¹ carbaryl (Sevin XLR; Bayer CropScience, Research Triangle Park, NC) plus 75 mg·L⁻¹ 6-benzyladenine (6-BA, MaxCel; Valent USA, Walnut Creek, CA); 3) four applications (31 May, 11 June, 21 June, and 2 July) of 5 mg·L⁻¹ NAA (Fruitone N; Amvac Chemical Corp., Los Angeles, CA) plus 225 mg·L⁻¹ ethephon (Ethrel; Bayer CropScience); 4) three applications (31 May, 11 June, and 21 June) of 6.75 mg·L⁻¹ NAA plus 300 mg·L⁻¹ ethephon; 5) two applications (31 May and

11 June) of 10 mg·L⁻¹ NAA plus 450 mg·L⁻¹ ethephon; and 6) a single application (31 May) of 10 mg·L⁻¹ NAA plus 900 mg·L⁻¹ ethephon. The treatments were arranged in a randomized complete block design experiment with six blocks. The treatments were applied to single-tree plots with a handgun to the point of drip. Full bloom in 2007 occurred on 25 Apr. All of the treatments except the unthinned control were chemically thinned on 10 May as in (2). Treatment effects on crop density in 2007 were evaluated from three sample limbs per tree by calculating the number of fruit on each limb after the completion of fruit drop. Crop density was expressed as the number of fruit per square centimeter limb cross-sectional area. Treatment effects on return bloom were measured as the percent of flowering spurs on each sample limb in 2008.

Effects of aminoethoxyvinylglycine on the efficacy of summer NAA and preharvest NAA programs for promoting return bloom. Experiments were conducted on 'Golden Delicious'/M.7 apples in 2006 (commercial orchard in Henderson County, NC) and 'York Imperial'/M.9 apples in 2008 (Alson H. Smith, Jr. Agricultural Research and Extension Center, Virginia Polytechnic Institute and State University, in Winchester, VA) to investigate the involvement of ethylene in NAA-induced stimulation of flower bud formation. The 'Golden Delicious' study included seven treatments: 1) control; 2) a summer NAA program of four biweekly applications of 5 mg·L⁻¹ NAA (7 June, 19 June, 7 July, 20 July) (Fruitone N; Amvac Chemical Corp.); 3) summer NAA sprays as in (2) except the first application included 125 mg·L⁻¹ AVG; 4) a preharvest NAA program of four weekly sprays of 5 mg·L⁻¹ NAA beginning 4 weeks before harvest (9 Aug., 16 Aug., 24 Aug., 29 Aug.); 5) preharvest NAA sprays as in (4) except the first application included 125 mg·L⁻¹ AVG; 6) a summer ethephon program of two applications of 562 mg·L⁻¹ ethephon (Ethrel; Bayer CropScience) (7 June and 7 July); and 7) a summer ethephon program as in (6) except AVG was included with the first application at a concentration of 125 mg·L⁻¹. Full bloom of the 'Golden Delicious' trees in 2006 occurred on 18 Apr. The treatments were arranged in a randomized complete block design experiment with six blocks. The spray treatments in the 'Golden Delicious' study were applied to fully guarded single-tree plots with an airblast sprayer calibrated to deliver 1496 L·ha⁻¹ (160 gal/acre). All of the trees received a standard thinning spray of 20 mg·L⁻¹ NAA when the fruit were 10 mm in diameter. Return bloom in 2007 was assessed by counting the number of vegetative and floral spurs on two representative limbs per tree, each limb carrying a minimum of 100 spurs. From these data the percent of floral spurs on each limb was calculated.

The 'York Imperial'/M.9 study included seven treatments arranged in a randomized complete block design experiment with seven blocks. The treatments were 1) an unthinned control; 2) a chemically thinned control,

500 mg·L⁻¹ carbaryl (Sevin XLR) plus 50 mg·L⁻¹ 6-BA (MaxCel; Valent USA) applied when the mean fruit diameter was 10 mm; 3) four applications (5 June, 17 June, 26 June, 7 July) of 5 mg·L⁻¹ NAA (Fruitone L; Amvac Chemical Corp.) plus 225 mg·L⁻¹ ethephon (Ethrel; Bayer CropScience); 4) three applications (5 June, 17 June, 26 June) of 6.75 mg·L⁻¹ NAA plus 300 mg·L⁻¹ ethephon; 5) two applications (5 June, 17 June) of 10 mg·L⁻¹ NAA plus 450 mg·L⁻¹ ethephon; 6) a single application (5 June) of 10 mg·L⁻¹ NAA plus 900 mg·L⁻¹ ethephon; and 7) four applications of NAA plus ethephon as in treatment (3) except that 250 mg·L⁻¹ AVG was included in the first and third sprays. All of the treatments except the unthinned control were chemically thinned on 6 May as in treatment (2). The treatments were applied to fully guarded single-tree plots with seven replications arranged in a randomized complete block design experiment. The treatments were applied to single tree plots with a handgun to the point of drip. Full bloom of the 'York Imperial' trees in 2008 occurred on 25 Apr. Treatment effects on croplod (fruit/cm² limb cross-sectional area) were determined in two sample limbs per tree in 2008, and return bloom was assessed on the same two limbs in 2009 as the number of flower clusters per cm² limb cross-sectional area and as the percent of floral spurs.

In a block of mature 'Golden Delicious'/M.7 apple trees in Massachusetts 18 trees were selected that exhibited a uniformly heavy bloom in 2008. The trees were separated into six groups (replications) of three trees each. Within each group, trees were randomly assigned to receive one of following three treatments: Control, Summer NAA sprays, or Preharvest NAA sprays. Trees were in full bloom on May 14. Summer NAA sprays consisted of four biweekly applications (8 July, 27 July, 5 Aug., 19 Aug.) of 5 mg·L⁻¹ NAA. Preharvest NAA sprays consisted of four weekly sprays of 5 mg·L⁻¹ NAA beginning four weeks before harvest (9 Aug., 16 Aug., 24 Aug., 29 Aug.). The treatments were applied as a dilute spray with a handgun. Return bloom was measured in 2009 on two sample limbs in each tree by calculating the flower cluster density (clusters/cm² limb cross sectional area) and the proportion of floral buds separately for spurs and one-year old wood.

Time of transition to flowering in relation to NAA programs for promoting return bloom of 'Golden Delicious' at three different locations. Buds were sampled from mature 'Golden Delicious'/M.7 apple trees growing in North Carolina, Massachusetts, and Washington State during the 2008 growing season. One hundred non-fruiting buds were removed from 1-year-old wood on five (North Carolina, Massachusetts) or ten (Washington) trees at ≈ 14 -d intervals beginning 50 d after full bloom until harvest at each location. The buds were stored in fixative (HistoChoice; Amresco Inc., Solon, OH) until dissection under a stereo microscopic dissection (Leica Stereo Zoom 6 Photo; Cambridge Instruments, Buffalo, NY) at $\times 40$ magnification. The stage of bud

development was rated using the scale developed by Hoover et al. (2004): Stage 1, apical meristem flat (vegetative); Stage 2, apical meristem domed (floral commitment); Stage 3, bractlets developing on the terminal floral meristem; Stage 4, sepals clearly differentiated on the terminal floral meristem and bractlets differentiated on the lateral floral meristems; and Stage 5, sepals clearly differentiated on the lateral floral meristems. The proportion of buds rated at Stage 2 (doming) was calculated at each sampling date to determine the time of floral commitment at each location.

Statistical analyses were performed using Statistical Analysis Software (SAS Inst., Cary, NC). Return bloom data (percent floral spurs) was arcsine-transformed to correct deficiencies in normality and homogeneity of variance before being submitted to an analysis of variance using the generalized linear model procedure in SAS. Means separations were performed using Duncan's multiple range test ($P \leq 0.05$).

Results and Discussion

Return bloom of 'York Imperial' trees that were neither chemically thinned nor received summer NAA and ethephon sprays was low with fewer than 5% of spurs floral in the next year (Tables 1 and 2). These responses illustrate the severity of biennial bearing in this cultivar. Although chemical thinning significantly reduced crop density and increased return bloom of 'York Imperial' compared with unthinned control, the level of return bloom was still not more than 10% (Tables 1 and 2), which in our opinion is insufficient to ensure a commercially acceptable cropload. However, return bloom was higher when the chemical thinning spray was followed by summer NAA + ethephon sprays compared with chemical thinning alone. The summer NAA plus ethephon treatments resulted in more than 25% of spurs floral in the year after treatment, providing additional evidence for a direct stimulatory effect of NAA and/or ethephon on flower bud formation that is independent of, and additive to, a cropload effect. We estimate that a minimum of 25% of the spurs must be floral to produce a full commercial crop. These data demonstrate that a successful chemical thinning program was not enough to guarantee sufficient return bloom of this strongly biennial cultivar. In contrast, a successful chemical thinning program combined with summer NAA + ethephon sprays resulted in sufficient return bloom.

The efficacy of summer NAA plus ethephon sprays applied to 'York Imperial' in 2008 was unaffected by the ethylene biosynthesis inhibitor AVG (Table 2); however, AVG did reduce the efficacy of summer NAA sprays applied to 'Golden Delicious' in 2007 (Table 3). AVG was without effect on the efficacy of the NAA plus ethephon combination sprays applied to 'York Imperial', which was not unexpected because AVG would not have inhibited ethylene released from

Table 1. Effects of early summer naphthaleneacetic acid (NAA) and ethephon sprays applied in 2007 on return bloom of 'York Imperial'/M.9 apples in 2008 in Virginia.^z

Treatment	Application (DAFB)	Crop density in 2008 (fruit/cm ² BCSA)	Return bloom in 2009 (% floral spurs)
Control (not thinned)		10.3 a ^y	0.3 a (2.7)
Chemically thinned		2.7 b	14.9 b (6.6)
NAA ^y (5.0 mg·L ⁻¹) + ethephon (225 mg·L ⁻¹)	36, 47, 57, 68	3.2 b	29.5 c (24.2)
NAA (6.75 mg·L ⁻¹) + ethephon (300 mg·L ⁻¹)	36, 47, 57	2.8 b	27.2 c (20.9)
NAA (10.0 mg·L ⁻¹) + ethephon (450 mg·L ⁻¹)	36, 47	1.7 b	33.7 c (30.8)
NAA (10.0 mg·L ⁻¹) + ethephon (900 mg·L ⁻¹)	36	2.3 b	34.9 c (32.7)

^zReturn bloom data were arcsine square root transformed before analysis. Data in parentheses are the back-transformed means. Full bloom occurred on 25 Apr. All trees except for the control were chemically thinned with 1200 mg·L⁻¹ carbaryl + 75 mg·L⁻¹ 6-BA on 10 May, when the fruit were 10 mm in diameter.

^yMeans with different letters within each column are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test.

BCSA = branch cross-sectional area; 6-BA = 6-benzyladenine; DAFB = days after full bloom.

Table 2. Effects of AVG on the efficacy of early summer NAA and ethephon sprays applied in 2008 on return bloom of 'York Imperial'/M.9 apples in 2009 in Virginia.^z

Treatment	Application (DAFB)	Crop density in 2008 (fruit/cm ² BCSA)	Return bloom in 2009 (% floral spurs)
Control (not thinned)		11.9 a ^y	1.2 a (0.0)
Chemically thinned		3.4 b	19.2 b (10.8)
NAA (5.0 mg·L ⁻¹) + ethephon (225 mg·L ⁻¹)	41, 53, 62, 73	3.5 b	22.5 bc (14.6)
NAA (6.75 mg·L ⁻¹) + ethephon (300 mg·L ⁻¹)	41, 53, 62	3.4 b	34.0 c (31.3)
NAA (10.0 mg·L ⁻¹) + ethephon (450 mg·L ⁻¹)	41, 53	3.2 b	33.9 c (31.1)
NAA (10.0 mg·L ⁻¹) + ethephon (900 mg·L ⁻¹)	41	3.2 b	34.9 c (33.0)
NAA (5.0 mg·L ⁻¹) + ethephon (225 mg·L ⁻¹) + AVG ^x (250 mg·L ⁻¹)	41, 53, 62, 73	3.5 b	18.7 b (10.3)

^zReturn bloom data were arcsine square root transformed before analysis. Data in parentheses are the back-transformed means. Full bloom occurred on 25 Apr. All trees except for the control were chemically thinned with 500 mg·L⁻¹ carbaryl + 50 mg·L⁻¹ 6-BA on 6 May.

^yMeans with different letters within each column are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test.

^xAVG was included with the first and third sprays only (41 DAFB and 62 DAFB).

AVG = aminoethoxyvinylglycine; NAA = naphthaleneacetic acid; DAFB = days after full bloom; BCSA = branch cross-sectional area; 6-BA = 6-benzyladenine.

Table 3. Effects of NAA, ethephon, and AVG treatments in 2006 on return bloom of 'Golden Delicious'/M.7 apples in 2007.^z

Treatment	Application (DAFB)	Return bloom in 2007 (% floral spurs)
Control		19.2 a ^y (10.9)
Summer NAA (5 mg·L ⁻¹)	50, 62, 80, 93	54.3 c (66.0)
AVG ^x (125 mg·L ⁻¹) + summer NAA (5 mg·L ⁻¹)	50, 62, 80, 93	41.0 b (43.0)
Preharvest NAA (5 mg·L ⁻¹)	113, 120, 128, 133	56.1 c (68.8)
AVG ^y (125 mg·L ⁻¹) + preharvest NAA (5 mg·L ⁻¹)	113, 120, 128, 133	54.8 c (66.8)
Summer ethephon (560 mg·L ⁻¹)	50, 80	41.1 b (43.3)
AVG ^y (125 mg·L ⁻¹) + summer ethephon (560 mg·L ⁻¹)	50, 80	38.5 b (38.8)

^zData were arcsine square root transformed before analysis. Data in parentheses are the back-transformed means. Full bloom occurred on 18 Apr. All trees were chemically thinned with 20 mg·L⁻¹ NAA on 28 May.

^yMeans with different letters within each column are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test.

^xAVG was only included with the first NAA or ethephon spray.

NAA = naphthaleneacetic acid; AVG = aminoethoxyvinylglycine; DAFB = days after full bloom.

ethephon, which occurs through a physicochemical rather than a biochemical reaction. However, a partial reduction in efficacy of the summer NAA program for return bloom that occurred when AVG was applied with the first spray to 'Golden Delicious' implies that NAA-induced ethylene is involved in the return bloom response to NAA.

Preharvest NAA sprays applied to 'Golden Delicious' in 2007 increased return bloom in 2008 just as effectively as a summer NAA program (Table 3). Although only 11% of the spurs on control trees were floral, the

summer NAA and preharvest NAA programs applied in 2006 increased the proportion of floral spurs in 2007 to 66% and 69%, respectively. The efficacy of preharvest NAA sprays for promoting return bloom in the current study is consistent with previously reports (McArtney et al., 2007). Application of summer ethephon sprays to 'Golden Delicious' also effectively increased return bloom with 43% of the spurs floral in the year after treatment. Although summer ethephon sprays were less effective than NAA treatments in this study, they still provided

a level of return bloom that was sufficient to produce a commercial crop. Combining AVG with the first spray in a preharvest NAA program or a summer ethephon program did not reduce their efficacy.

The timing of transition to floral development in 'Golden Delicious' buds, as determined by doming of the axillary meristem in non-fruiting buds, was relatively consistent among the three different growing regions (Fig. 1). Doming was first observed in buds that were sampled 64 d after bloom in North Carolina and Washington State; however, fewer than 5% of buds collected at these two locations exhibited doming at that time. Doming peaked at 78 to 92 d after bloom and relatively few buds exhibited doming later than 106 d after bloom. Thus, the transition from vegetative to floral development in 'Golden Delicious' buds was completed by 106 d after bloom in all three locations. Hoover et al. (2004) reported that different apple cultivars exhibited unique patterns of floral development with doming occurring earlier in 'Fuji' (86 d after bloom) compared with 'Braeburn', 'Gala', or 'Pacific Rose' (104 to 112 d after bloom). Data from the present study suggest that the timing of floral development in 'Golden Delicious' buds is similar to 'Fuji' and earlier than 'Gala', 'Braeburn', or 'Pacific Rose'.

Application of summer NAA sprays to 'Golden Delicious' in 2008 increased the percentage of floral buds on spur buds and buds on 1-year-old wood in Massachusetts (Table 4). Preharvest NAA sprays were

Fig. 1. Changes in the proportion of 'Golden Delicious' buds exhibiting floral morphogenesis, i.e., with a domed meristem apex, over time in three U.S. states. Each data point is derived from a sample of 100 buds removed at ≈ 14 -d intervals starting 50 d after bloom in 2008.

without effect on return bloom of spurs but did result in a significant increase in total flower cluster density compared with the control. Summer and preharvest NAA programs for return bloom had no effect in the North Carolina or Washington State experiments (data not shown).

Summer NAA + ethephon sprays were applied to 'York Imperial' during the period from 36 to 68 d after full bloom in 2007 and 41 to 73 d after full bloom in 2008. Summer NAA sprays were applied to 'Golden Delicious' in the 2007 and 2008 experiments during the period from 50 to 100 d after bloom, whereas the summer ethephon applications to this cultivar in 2007 were made 50 d and 80 d after bloom. Thus, the first application of summer NAA and ethephon in a program of multiple sprays for return bloom were made just before when the first buds are transitioning from vegetative to floral development, and the application of subsequent sprays coincides with the period when buds are normally making this transition. Weekly NAA sprays during the month before harvest stimulated flower bud formation in 'Golden Delicious' as effectively as a summer NAA program in the same year (Tables 3 and 4). The efficacy of preharvest NAA sprays for return bloom in the current study is consistent with previously published research (McArtney et al., 2007). Vegetative apple buds typically produce six to eight true leaf primordia before floral commitment, and the number of true leaf primordia within vegetative buds tends to stay at this number for the remainder of the season (McArtney, personal observation). Thus, these data suggest that NAA can trigger a florigenic response in these quiescent vegetative buds. Floral development proceeds rapidly after doming of the meristem apex with sepals clearly differentiated on lateral floral meristems ≈ 30 to 35 d after doming (Hoover et al., 2004). In the case of 'Golden Delicious', there was sufficient time in both North Carolina (typically 60 d between harvest and leaf fall) and Massachusetts (typically 30 d between harvest and leaf fall) for floral organ differentiation triggered by the preharvest NAA sprays to be completed before leaf fall.

Meristem fate in apple buds is regulated by genetic, physiological, and chemical signals. Exogenous application of compounds including GA₄ (Looney et al., 1985), NAA (Harley et al., 1958; McArtney et al., 2007),

ethylene (McArtney et al., 2007; Williams, 1972), and an analog of 9,10-ketol-octadecadienoic acid (Kittikorn et al., 2013) exhibits florigenic activity in apple. In this work, we have shown that applying NAA during the period when meristems are normally doming promotes flower bud formation. We also report that NAA applications during the month before harvest promote flowering, indicating that NAA triggers floral development in quiescent vegetative buds. Chailakhyan (1936) first proposed the concept of a mobile floral stimulus in plants. Recent research has revealed that the proteins encoded by FLOWERING LOCUS T (FT) in Arabidopsis and its orthologs in other plant species is a key component of florigen (Corbesier et al., 2007; Lin et al., 2007; Tamaki et al., 2007; Zeevart, 2006). Kittikorn et al. (2013) reported that application of 9,10-ketol-octadecadienoic acid increased MdFT1 expression and flower bud formation in apple. Because NAA sprays at different times during the growing season are florigenic, analysis of gene expression after NAA treatments may provide an alternative approach to studying the endogenous control of flower bud formation in apple.

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Table 4. Effects of summer NAA and preharvest NAA sprays applied in 2008 on return bloom of 'Golden Delicious'/M.7 apple trees in Massachusetts in 2009.^z

Treatment	Application (DAFB)	Return bloom in 2009				
		Flower cluster density (clusters/cm ² LCSA)			Percent floral buds	
		Spurs	One year old	Total	Spurs	One year old
Control		6.8 a ^z	3.8 a	10.6 a	78 a	36 a
Summer NAA	56, 70, 84, 98	8.5 a	6.2 b	14.7 ab	90 b	57 b
Preharvest NAA	121, 128, 135, 142	9.5 a	6.9 b	16.4 b	86 ab	50 ab

^zSummer NAA sprays (5 mg·L⁻¹) were applied 56 d, 70 d, 84 d, and 98 d after bloom. Preharvest NAA sprays (5 mg·L⁻¹) were applied at weekly intervals during the month before harvest (121 d, 128 d, 135 d, and 142 d after bloom).

^yMeans with different letters within each column are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test.

NAA = naphthaleneacetic acid; DAFB = days after full bloom; LCSA = limb cross-sectional area.

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