Changes in Ripening Physiology of ‘Delicious’ and ‘Fuji’ Apples Treated Preharvest with NAA

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Abstract

I-Naphthaleneacetic acid (NAA) is used in apple crop management to promote fruitlet abscission when applied during the first few weeks after anthesis. In contrast, when applied to maturing fruit several weeks before harvest, NAA delays abscission. Because the physiological responses are opposed, it is reasonable to investigate whether the mechanisms of action of NAA applied in the spring vs. the fall, are also different. A number of experiments were conducted over several years, the results of which indicate, on late-season apple cultivars, NAA applied preharvest suppresses ethylene production and delays loss of firmness after harvest.

INTRODUCTION

NAA was first used to delay early abscission of mature ‘Bartlett’ (Pyrus communis) pears in 1939 (Gardner, et al.). When evaluated the following spring to increase set on apples NAA, instead, induced fruitlet abscission, and thus began the practice of post-bloom thinning of young fruitlets (Burkholder and McCown, 1941). Because NAA promoted apple fruit abscission in the early spring, arguably by an ethylene-induction mechanism (Curry, 1991), it has been tempting to assume NAA may stimulate ripening of mature apple fruit by a similar mechanism. Some have intimated that NAA applied preharvest advances ripening in ‘Gala’ (Brackmann and Waclawovsky, 2001), or that it may stimulate ethylene production in ‘Macoun’ (Watkins and Nock, 2004); however, for later maturing varieties such as ‘Delicious’ or ‘Fuji’ (Malus × domestica), data supporting NAA-induced ripening is lacking. This series of experiments was conducted over several years to examine whether NAA affects ripening physiology of later maturing apple cultivars.

MATERIALS AND METHODS

I. ‘Bartlett’ Pear - Effect of Increasing Rates of NAA on Ripening

On 10 Aug 2002, 160 ‘Bartlett’ pears of similar size and color were harvested from a single mature tree in a commercial orchard located in Cashmere, WA. The fruit were allowed to equilibrate in the laboratory for 4 h at 23°C before treatment. To ensure consistency and uniformity of coverage, individual fruit were dipped by holding the pedicel and submerging the fruit for 1 minute in an aqueous solution, also held at 23°C, containing NAA (potassium salt) at 0, 10, 100 or 1000 µg·l⁻¹. There were 40 pears in each treatment. After drying for 30 minutes, fruit were transferred to dry fiber trays. Half the fruit from each treatment was allowed to ripen in the dark at 23°C. Internal ethylene concentration (IEC) was monitored in each ripening fruit by inserting a sterile 5 cm, 18-gauge needle with a side-port tip, fitted with a rubber septum cap, through the calyx approximately to the center of the seed cavity. The fruit/needle junction was sealed using about 5 g of inert mastic (Quabitac, Qubit Systems Inc., Kingston, Ontario). About every three days for two weeks, 1 ml of gas was withdrawn through the septum cap using a 1 ml syringe and analyzed for ethylene gas using a gas chromatograph (GC) equipped with a flame ionization detector (FID) according to standard methods. The other 20 fruit were placed in perforated liners in closed cardboard boxes and kept in the dark at -1°C.
Treatments were boxed separately. After 30 days, remaining fruit were ripened in the dark at 23°C. IEC was monitored in each fruit as described previously.

II. ‘Delicious’ Apple - Preharvest Treatment with NAA or ACC

NAA (20 µl·l⁻¹) or 1-aminocyclopropane-1-carboxylic acid (ACC) (100 µl·l⁻¹) were applied to tree limbs of mature ‘Oregon Spur Delicious’/seedling 2 weeks before harvest, on September 9, 1995. At 0, 6, 24 and 48 h after treatment, whole leaves or fruit were taken to the laboratory and placed in glass vessels at least 3 times the volume of the tissue. The vessels were sealed for 1 h after which a 1 ml sample of headspace gas withdrawn for ethylene analysis as described above.

III. ‘Delicious’ Apple - Preharvest Treatment with NAA at 4 x

NAA (80 µl·l⁻¹) was applied to whole ‘Oregon Spur Delicious’/seedling trees 2 weeks before harvest, on September 1, 1990. At 7 and 17 days after treatment, five fruit of similar size and color from each of eight trees were taken to the laboratory for evaluation of fruit quality and IEC. Fruit flesh firmness, total soluble solids, juice acidity, starch rating, and IEC were measured according standard methods.

IV. ‘Delicious’ and ‘Fuji’ Apple Tissue - Preclimacteric Treatment NAA In Vitro

In 2004, 20 large ‘Delicious’ and ‘Fuji’ apples were sampled on September 10 and October 15, respectively, and taken to the laboratory where they were allowed to equilibrate at 23°C for 4 h. IEC was measured from each fruit as described above; only apples with IEC <0.5 µl·l⁻¹ were used for the experiment. Fruit were dipped in 0.003% sodium hypochlorite for 10 s and rinsed briefly in distilled H₂O. Subsequent steps were performed aseptically in a laminar flow hood. From each apple, one tissue plug about 25 mm thick was taken from each quadrant at the equatorial axis using a 20 mm diameter cork borer. The tissue plug was cut into three measured sections, two of which were used for further analysis. The peel section was 3 mm thick. The deep flesh section consisted of 4 mm cortex tissue at a depth of 16-20 mm from the fruit surface. Sections from each fruit were placed in separate Petri dishes containing either H₂O or NAA (20 µl·l⁻¹) for 2 minutes. All peel or cortex sections from a single fruit were placed in a 50 ml vial containing 1 ml H₂O and kept in the dark at 23°C. At 0, 12, 24, 48 and 72 h after treatment, vials were sealed with a rubber septum cap for 1 hour and sampled for ethylene by withdrawing 1 ml of the headspace gas. After sampling, the septum caps were removed. Water was added to the vials as needed to keep about 1 ml in the vial. Ethylene was analyzed by GC/FID. There were 4 replications per treatment and the experiment was repeated twice.

RESULTS AND DISCUSSION

I. ‘Bartlett’ Pear - Effect of Increasing Rates of NAA on Ripening

The purpose of this trial was to verify previous data reporting NAA stimulated ripening of ‘Bartlett’ pears when applied preharvest. Although fruit were treated immediately after harvest instead of 2 weeks before harvest, fruit were still preclimacteric and exhibited little IEC at the time of treatment (data not shown). Fruit treated with NAA at 0 µl·l⁻¹ showed a typical initiation of autocatalytic ethylene production at 5-7 days after treatment, whereas fruit treated with 1000 µl·l⁻¹ exhibited this rise about 3 days sooner (Fig. 1A). After two weeks, there was no difference in IEC of fruit treated with 0 or 10 µl·l⁻¹ NAA. Fruit treated with 100 µl·l⁻¹ showed greater IEC than the control, and those treated with 1000 µl·l⁻¹ had the highest IEC. After 1 month at -1°C, IEC was directly related to concentration of NAA (Fig. 1B). Thus, ‘Bartlett’ pears treated with NAA before initiation of the climacteric showed a dose-dependent stimulation of ripening, especially after 30 days at -1°C.
II. ‘Delicious’ Apple - Preharvest Treatment with NAA or ACC

Treating whole leaves or fruit with 100 µl·l⁻¹ ACC resulted in ethylene production within several hours, with leaves showing the greater response (Fig. 2A,B). Whereas there was no difference in ethylene production in whole leaves treated with 20 µl·l⁻¹ NAA compared with untreated leaves (Fig. 2A), whole fruit showed a marked decrease in ethylene over controls, beginning about 24 h after treatment (Fig. 2B). These data indicate whereas older leaves lose the ability for NAA-induced ethylene production as reported previously by Curry (1991), they retain the constitutive enzymes necessary to convert ACC to ethylene. Furthermore, at 48 h after treatment, fruit treated with NAA was producing less than 20% the ethylene from untreated controls.

III. ‘Delicious’ Apple - Preharvest Treatment with NAA at 4x

Table 1 shows fruit maturity values for ‘Delicious’ fruit treated with 80 µl·l⁻¹ NAA compared with untreated controls, at 7 and 17 days after treatment. Within a sampling date, there was no difference in percentage soluble solids among treatments. In contrast, values for juice acidity, starch rating and flesh firmness were higher in NAA-treated fruit. Perhaps of greater significance was the percentage change in the different maturity indices from day 7 to day 17 after treatment. Whereas there was no difference in the rate of change among treatments for soluble solids, starch rating or firmness, juice acidity and IEC were markedly different. Compared with about 11% in controls, juice acidity in treated fruit decreased only 2%. Similarly, IEC in untreated fruit increased by about 1500%, whereas in NAA-treated fruit the increase was only about 300%. Thus, even though the fruit treated with NAA appeared more mature when starch rating was compared within a sampling date, the rate of change in starch clearing and IEC between sampling dates suggested ripening had been suppressed. Indeed, the increase in mean starch rating of fruit treated with NAA may simply indicate that treatment delayed abscission of riper fruit compared with those on untreated trees. That is, riper fruit on untreated trees would have fallen, thereby eliminating the contribution of these fruit to the mean starch rating.

In another related experiment, where all fruit were harvested from NAA-treated or untreated trees and evaluated individually for soluble solids and starch rating, there was a wider distribution of these maturity index values in fruit from treated trees and the greater the concentration of NAA applied (0, 10 or 100 µl·l⁻¹) the wider the distribution (data not shown). These data suggest that ‘NAA-induced fruit ripening’ may be a perception based on retention of riper fruit on treated trees. In fact, ripening is slowed by treatment with NAA.

IV. ‘Delicious’ and ‘Fuji’ Apple Tissue - Preclimacteric Treatment NAA In Vitro

Other than the absolute amount of ethylene (higher in ‘Delicious’), data from experiments using preclimacteric ‘Delicious’ and ‘Fuji’ apple were similar; thus, only data from ‘Fuji’ fruit will be presented. NAA reduced ethylene in both fruit peel and deep cortical tissue disks. In fruit peel sections, the difference in ethylene production between treatments was apparent at 48 h; however, after 72 h, there was no difference. More striking was the difference in ethylene production between treatments in deep cortical sections. At 24 h after treatment, control sections began showing an increase in ethylene production. At 48 and 72 h after treatment, mean ethylene production in NAA-treated tissue was about 10% and 5%, respectively, that of untreated fruit.

CONCLUSIONS

In deciduous tree fruit production, one of the first uses of NAA was to delay abscission of ‘Bartlett’ pears during fruit maturation (Gardner et al., 1939). Often accompanying this effect was a stimulation of fruit ripening. Data herein verified this. Moreover, increase in IEC was directly related to concentration of NAA, especially in fruit that was stored at -1°C for 30 days (Fig. 1). Initiation of ripening in ‘Bartlett’ pear is preceded by an accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC), whereas
in apple this is generally not the case (Lelièvre et al., 1997). Indeed, in immature climacteric fruit, inhibitors of ethylene action enhance ethylene production (Atta-Aly et al., 1987), whereas in mature climacteric fruit the opposite is true (Dupille and Sisler, 1995). As a synthetic auxin, NAA may well fall into this ambidextrous category. Other plant bio-regulators exhibit such bell-shaped dose/response curves. In apple, NAA (10 μM) applied 10 days after anthesis induces ethylene production in ‘Delicious’ fruitlets, whereas during early fruit maturation the same treatment induces no positive response (Curry, 1991). Indeed, in ‘Delicious’ apple, NAA applied preharvest at higher rates suppressed ethylene production (Fig. 2, Table 1). Previous work in ‘Golden Delicious’ showed ethylene production from tissue of fruit ripening in situ was delayed by treatment with NAA; moreover, activity of IAA oxidase, peroxidases and polyphenoloxidases was reduced (Masia et al., 1998).

In other species, NAA altered physiological maturity depending on fruit stage at which treatment was applied. NAA applied when loquat fruit were at 50% of their final size caused a significant delay in maturity, whereas when applied to fruit at 30% of their final size 3% of the total production was harvested 10 days earlier than that of untreated trees (Amorós et al., 2004). Others have reported a change in sensitivity to NAA with age of fruit or leaf tissue. In immature peach fruit, NAA treatment caused a 3-fold enlargement of excised mesocarp discs in vitro (Ohmiya, 2000). Moreover, in tissue discs obtained at 36 days after anthesis a higher concentration of NAA was needed to elicit maximum response compared with tissue harvested from fruit at 57 days after anthesis, suggesting an alteration in the rate of uptake and/or sensitivity during fruit development. Changes in sensitivity to auxin during cell differentiation have also been reported in wheat leaf cells in vitro (Wernicke et al., 1986). Stimulation of cell division in more mature regions of the leaf required higher concentrations of auxin; however, neither alterations in uptake rate nor alterations in metabolism accounted for the loss of auxin responsiveness.

Possibilities for the difference in NAA-induced suppression of ethylene between ‘Fuji’ peel and deep cortex sections reported here (Fig. 3) might include: 1) variability in rate of uptake or mobility of NAA within tissue, 2) unique ethylene metabolic systems or 3) differential capacity of the tissue to respond metabolically. Others have suggested response variation to applied auxin in climacteric fruit tissue may be due to uneven penetration (Vendrell, 1970).

These data suggest species, cultivar, tissue type and physiological age are important factors in understanding the physiological mode of action of NAA and its effects on fruit ripening.

**Literature Cited**


Tables

Table 1. Effect on fruit maturity of NAA (80 µl l⁻¹) applied to mature ‘Delicious’ trees on 9/1/90. At 7 and 17 days after treatment, 40 fruit per treatment were evaluated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sol. Solids (%)</th>
<th>Acidity (% Malic Acid)</th>
<th>Starch Rating (1-6)</th>
<th>Firmness (N)</th>
<th>IEC (µl-g⁻¹·h⁻¹)</th>
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<td>Control</td>
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<td>0.308b</td>
<td>3.1b</td>
<td>78.3b</td>
<td>1.6b</td>
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<tr>
<td>Evaluated 9/18/90</td>
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<td>0.240a</td>
<td>2.9a</td>
<td>67.6a</td>
<td>16.5b</td>
</tr>
<tr>
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<td>0.302b</td>
<td>4.1b</td>
<td>73.4b</td>
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<td>% Change from 9/8/90 to 9/18/90</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
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<td>31.8</td>
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<td>9.4</td>
<td>-1.9</td>
<td>32.3</td>
<td>-6.3</td>
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</table>

¹ Mean values within a sampling date followed by the same letter, not significant by LSD at p > 0.05
Fig. 1. Effect of concentration of NAA applied at harvest, on internal ethylene production of "Bartlett" pears held at 23°C. Fruit ripened without (A) cold storage and after 1 month (B) at -1°C. bars indicate LSD at p >0.05.
Fig. 2. Effect of NAA or ACC applied to limbs of mature 'Delicious' trees 2 weeks before harvest, on ethylene production of leaves (A) and (B). Tissue was excised at various times after treatment and held in sealed containers for 1 hour at 23°C. Bars indicate LSD at p > 0.05
Fig. 3. Effect on ethylene production of NAA applied in vitro to tissue sections of preclimacteric ‘Fuji’ apples. Peel sections (A) were 20 mm in diameter, were 4 mm thick and were sampled at a depth of 16-20 mm from the fruit surface. Vials containing the tissue were held at 23 °C under sterile conditions. Headspace was sampled after periodically sealing the vials for 1 hour. Bars indicate LSD at p >0.05.