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Ethylene Evolution from Detached Apple Spurs in Response to Chemical Thinners

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***Abstract*. Ethylene evolution from detached fruiting apple spurs was measured after application of various bloom and post-bloom thinning agents. Ethylene evolution from fresh detached spurs of 'Splendor' apple trees increased one day after application of a bloom thinning spray of ethephon or NAA, and remained higher than rates of ethylene evolution by detached spurs from unsprayed control trees for 6 (NAA) or 10 (ethephon) days. Both ethylene evolution and fruit abscission during the initial drop period were higher on trees treated with ethephon compared to NAA, however final fruit set was similar for these two treatments. Ethylene evolution was significantly higher following NAA application onto 'Fuji' trees compared with NAAm, but final fruit set was reduced by a similar amount (≈20%) for both of these materials. Application of BA to 'Pacific Rose™' apple trees when the average diameter of spur fruit was either 4 mm (6 days after full bloom) or 7 mm (12 days after full bloom) resulted in a significant increase in the rate of ethylene evolution and also reduced final fruit set. When application of BA was delayed until the average diameter of spur fruit was 14 mm (24 days after full bloom) neither the rate of ethylene evolution or final fruit set was affected. Although an increase in the rate of ethylene evolution was a prerequisite for thinning in the present experiments, the magnitude of this increase was not related to the final thinning efficacy. Chemical names used: benzyladenine (BA); 2-chloroethyl phosphonic acid (ethephon); naphthaleneacetic acid (NAA); naphthalene acetamide (NAAm).**

Natural abscission of immature apple fruit occurs in three waves, the first two of which may overlap. According to Murneek (1933), the first wave consists largely of abnormal and unpollinated flowers, but may also include pollinated flowers in which fertilization has failed due to pollen incompatibility. The second wave of abscission involves flowers in which fertilization has taken place but is followed almost immediately by abortion of the embryo. The third wave of abscission involves fruit either with or without developing seeds, and typically occurs 7–9 weeks after bloom. Final fruit set is determined by the cumulative effect of each of these waves of fruit abscission. In spite of extensive natural fruit abscission, apple trees will typically set more fruit than can develop to the size demanded by the market. Chemical thinning agents are used to further reduce fruit set, although the response to some thinning materials is not always predictable (Looney, 1986). Although NAA is used as a post-bloom thinner in most other apple growing regions, it is used exclusively as a bloom thinner in New Zealand. NAA sprays are routinely followed by applications of a post-bloom thinner such as carbaryl or BA. These secondary thinners are applied before any visual assessment of the efficacy of primary bloom thinners is available. Thus, in many cases, application of secondary thinners in the post-bloom period may in fact be unnecessary. Earlier indication of the efficacy of bloom thinners would provide additional information on which to base post-bloom thinning strategies.

In some cases, endogenous ethylene production increases before fruit abscission (Brown, 1997). Teubner and Murneek (1955) suggested that increased fruit abscission following sprays of NAA as a post-bloom thinner may be an indirect effect through the production of ethylene. Natural ethylene, 1-aminocyclopropane 1-carboxylic acid (ACC) levels, or both, are often higher during periods of abscission in fruit and this rise in ethylene production is most likely responsible for accelerating abscission (Brown, 1997). Ethylene evolution from fruit has been measured following the application of various post-bloom thinning sprays to apple trees. Chiba and Kubota (1979) concluded that the thinning effect of carbaryl sprays appeared to be related to ethylene evolution by fruitlets, measured 2 d after treatment. Walsh et al., (1979) demonstrated a rapid increase in ethylene evolution from detached fruiting spurs in response to post-bloom thinning sprays of NAA, NAAm, or ethephon. Flore et al., (1990) measured a 4-fold increase in ethylene evolution from bourse shoot leaves of apple 24–48 h after application of NAA, concluding this response was directly related to the amount of NAA absorbed. In a study involving five different apple cultivars, abscission at the end of the drop period was correlated with ethylene evolution from leaves 24–48 h after application of a post-bloom NAA thinning spray (Flore et al., 1994). However, Lakso et al. (1991) reported a poor relationship between ethylene induced in the leaves and effectiveness of post-bloom NAA thinning sprays. Greene et al. (1992) reported that while post-bloom thinning sprays of BA and NAA increased ethylene production by both spur leaves and fruit, the effect of BA was more pronounced in the fruit. However, Greene et al. (1992) considered that the magnitude of this ethylene response was not large enough to be the primary cause for thinning.

The objectives of the current work were to: 1) determine if application of NAA or NAAm thinning sprays during the bloom period also result in an increased rate of ethylene evolution; and 2) test the hypothesis that the efficacy of a range of bloom and post-bloom chemical thinning treatments can be related in a quantitative manner to an ethylene response following treatment.

Materials and Methods

Ethephon or NAA bloom thinning treatments (1998). Ethephon or NAA was applied to mature 'Splendor'/MM.106 apple trees on the HortResearch Crosses Road Research Orchard in Hawke's Bay, New Zealand at full bloom in Oct. 1998 as a whole-tree thinning spray and compared to an unsprayed control. Ethephon (200 ppm) and NAA (7.5 ppm) were applied to the point of leaf wetness (2.5 L spray per tree) with a motorized airblast sprayer and included a nonionic surfactant (Regulaid, 250 mL/100 L; Kalo, Overland Park, Kans.). Each treatment was applied to a two-tree plot arranged in a randomized complete-block design with three replications. Four typical fruiting spurs, each with an actively growing bourse shoot of about equivalent length, were removed from 2-year-old wood from each plot daily until 12 d after application. The sampled spurs did not necessarily have the same number of flowers/fruit. The four detached spurs were enclosed together in a gas-tight 1 L white plastic container at 20 °C for 4 h. After this time, a 1-mL headspace sample was taken from the container and injected into a gas chromatograph (model GC-8A; Shimadzu, Columbia, Md.) fitted with a flame ionization detector (150 °C) and a glass column containing alumina at 100 °C. The rate of ethylene evolution was expressed as nL ethylene per gram fresh weight of spur tissue per hour ($nL \cdot g^{-1} \cdot h^{-1}$). The number of fruit per spur was assessed twice weekly from 6 d until 20 d after treatment and weekly thereafter until fruit drop ceased 40 d after treatment on a random sample of 40 spurs per plot (20 spurs per tree). A separate sample of 40 spurs per plot were selected on each assessment date.

Time of application of NAA vs. NAAm (1999). NAA (7.5 ppm) or NAAm (40 ppm) were applied to mature 'Fuji'/MM.106 apple trees on the HortResearch Lawn Road Research Orchard in Hawke's Bay, New Zealand at three different times after full bloom in Oct. 1999 with an air blast sprayer in 2000 L

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water·ha^{-1}. Each product was applied, together with a nonionic surfactant (Regulaid, 250 mL/100 L water) either at petal fall, one or two weeks after petal fall in Oct. 1999. In addition there was an unsprayed control treatment. The seven treatments were applied to fully guarded single-tree plots, arranged in a randomized complete-block design with five replicates. A sample of four fruiting spurs per tree was removed from 2-year-old wood on treatment and control trees, as in the previous experiment, 2 d after application of each thinning treatment. The rate of ethylene evolution (nL·g^{-1}·h^{-1}) from detached spurs was measured as described previously. The efficacy of each thinning treatment was assessed as fruit number per 100 spur flower clusters on three limbs per tree from counts made 10 weeks after full bloom. Two of the sample limbs were chosen in the lower section of the tree and the third in the middle section of the tree, each selected limb carrying at least 100 total flower clusters.

Time of application of benzyladenine (1999). Benzyladenine (150 ppm) was applied to mature 'Pacific Rose™'/MM.106 trees on the HortResearch Crosses Road Research Orchard in Hawke's Bay, New Zealand at full bloom in Oct. 1999. Benzyladenine was applied either 6, 12, or 24 d after full bloom, corresponding to a mean diameter of the king fruit on spur clusters of either 3.8, 6.9, or 14.4 mm, respectively. The treatments were applied together with a nonionic surfactant (Regulaid, 250 mL/100 L water) to fully guarded single tree plots as a dilute spray with an air blast sprayer in 2000 L water per hectare. In addition, there was an unsprayed control treatment. The four treatments were arranged in a randomized complete-block design with five replicates. The rate of ethylene evolution (nL·g^{-1}·h^{-1}) from detached spurs was measured as described previously. The efficacy of each thinning treatment was assessed as in the previous experiment.

Statistical analyses. For the two studies in 1999 (time of application of NAA vs. NAAm, time of application of BA), ethylene evolution and final fruit set were calculated as a proportion of the unsprayed control trees and analyzed after log transformation to make the error variances more nearly homogeneous (Fernandez, 1992). All statistical analyses were carried out using SAS software (SAS Institute, Cary, N.C.).

Results and Discussion

There was a general trend for ethylene evolution to decrease with time from full bloom on unsprayed control trees in the 1998 study (Fig. 1), from ≈2 nL·g^{-1}·h^{-1} on the day of application to ≈1 nL·g^{-1}·h^{-1} 12 d later. Application of ethephon or NAA as a bloom thinning spray increased the rate of ethylene evolution from detached spurs. The peak of ethylene evolution occurred much sooner after a bloom thinning spray of ethephon (1 d after treatment) and remained higher for a longer period compared with ethylene evolution following the bloom thinning spray of NAA which increased slowly until 4 d after treatment and declined rapidly thereafter (Fig. 1). Walsh et al., (1979) found that the ethylene peak following application of ethephon or NAA as a post-bloom thinner occurred at the first time of measurement, 1 d or 2.5 d after treatment on 'Golden Delicious' and 'Northern Spy' respectively. Differences in these responses may be due either to the different cultivars used in each study, or to differences in sensitivity related to the different times of treatment. However, even 1 d after application of an NAA or ethephon bloom thinning spray, there were significant differences in the rate of ethylene evolution between each of the three treatments. At this time, the rate of ethylene evolution by spurs sampled from trees sprayed with ethephon was over twice that of spurs sprayed with NAA. The higher rates of ethylene evolution compared to the controls continued for 5 d following a bloom thinning spray of NAA, and for 10 d following a bloom thinning spray of ethephon. Since the present study with bloom thinners and previous studies with post-bloom thinners (Walsh et al., 1979) both show that differences in ethylene evolution may occur rapidly after treatment but these effects can disappear in a matter of days, it was decided to sample spurs for determination of the rate of ethylene evolution in the subsequent studies at a single time, 2 d after treatment.

Bloom thinning sprays of ethephon or NAA reduced the mean number of fruit per spur on 'Splendor' trees compared to the unsprayed control (Fig. 2). At the final time of measurement (40 d after full bloom) there was an average of 1.7 fruit per spur on the control trees, and only 1.0 or 0.9 fruit per spur on the ethephon and NAA treated trees, respectively. Thus, whereas application of ethephon at full bloom resulted in a 2.5-fold higher rate of ethylene evolution compared to NAA application 3 d after treatment, both chemicals had a similar final thinning efficacy. It has been suggested the ethylene evolved following NAA and ethephon treatments may not be physiologically equivalent; higher levels of ethylene released from ethephon might not be localized, compartmentalized, or as active in stimulating fruit abscission as NAA-induced ethylene (Walsh et al., 1979). This suggestion may explain the results of the ethephon/NAA (1998) study, where a reduced ethylene response following NAA sprays had an equivalent final thinning response to an ethephon treatment that resulted in a much larger initial ethylene response. Alternatively, increased rates of fruit abscission may be triggered above a threshold level of ethylene; any additional ethylene generated above this threshold having no additional effect on fruit abscission.

Although there was no difference in the final fruit set on spur clusters between NAA and ethephon thinning treatments, the initial rate of fruit abscission appeared to be higher on trees thinned with ethephon compared to trees thinned with NAA (Fig. 2). Thus, differ-

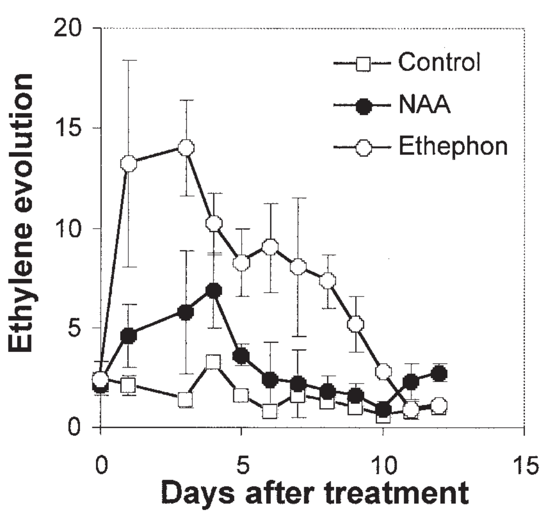

Fig. 1. Effect of bloom thinning sprays of ethephon (200 ppm) or NAA (7.5 ppm) on the rate of ethylene evolution (nL·g^{-1}·h^{-1}) from detached spurs of 'Splendor' apple during the 12 d after treatment. Vertical bars ± SD (n=3).

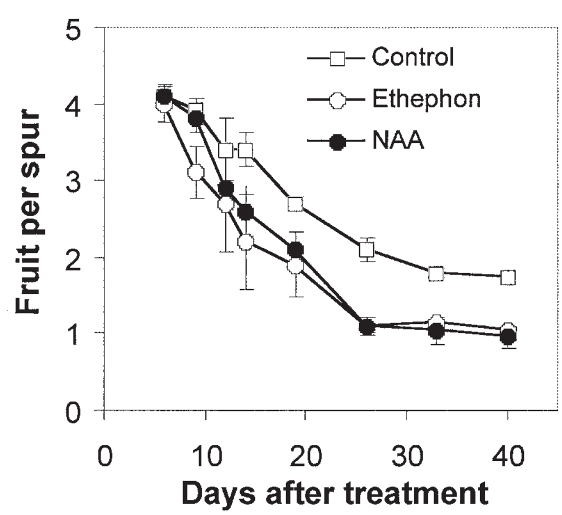

Fig. 2. Effect of bloom thinning sprays of ethephon (200 ppm) or NAA (7.5 ppm) on fruit number per spur of 'Splendor' apple during the period of fruit abscission. Vertical bars ± SD (n=3).

ences in the rate of ethylene evolution following application of ethephon or NAA thinning sprays did not appear to be related to final fruit set.

Cultivar may have a significant effect on the pattern of ethylene evolution over time on unthinned trees, as well as on the ethylene response following application of various post-bloom thinners (Walsh et al., 1979). Ethylene and thinning responses were expressed as a proportion of the unsprayed control treatment within each of the two trials carried out in 1999 in order to account for cultivar differences in the natural pattern of ethylene evolution and differences in the time of application of thinning agents.

Spur fruit set on unsprayed control trees in the NAA/NAAm timing experiment was high, at 198 fruit per 100 flower clusters (Table 1). NAA and NAAm reduced final spur fruit set by a similar amount (20%) compared to the unsprayed control trees (Table 2). However the rate of ethylene evolution, measured 2 d after treatment, was significantly higher following application of NAA compared to NAAm (Table 2). Thus, differences in the rate of ethylene evolution in response to these two thinning agents, measured two days after application, were not related to final spur fruit set. Walsh et al. (1979) showed that the rate of ethylene evolution following a post-bloom NAA thinning treatment was highest at the first time of sampling, either 1 d ('Golden Delicious') or 2.5 d ('Northern Spy') after treatment. However, ethylene evolution from detached spurs following a post-bloom NAAm spray did not peak until 5 d after treatment. Differences in the pattern of ethylene evolution following NAA or NAAm thinning sprays may account for the lack of any relationship between ethylene evolution, measured 2 d after treatment, and final thinning response in the present study.

There was a significant increase in the proportion of pygmy fruit (fruit <90 g fresh weight) following delayed application of NAA or NAAm to 'Fuji' (Table 1). An average of 3.6% of the fruit on unsprayed control trees were <90 g fresh weight, whereas 17.8% and 12.6% of the fruit on trees sprayed with NAA or NAAm, respectively, 14 d after petal fall were <90 g. Application of NAA to 'Hi-Early red Delicious' at 10 or 15 d after full bloom (Bound et al., 1991), or to 'Fuji' at 14 d after petal fall (Reginato et al., 1998) also increased the number of pygmy fruit compared to earlier applications. Marini (1996) reported that NAA applications to spur 'Delicious' apples at a mean fruit diameter >9 mm caused excessive development of pygmy fruit.

Application of BA either 6 or 12 d after full bloom increased the rate of ethylene evolution from detached spurs by 1.8 or 1.6 times, respectively, compared to the unsprayed control (Table 4) and reduced fruit set (Table 3). Application of BA 24 d after full bloom, when the mean diameter of spur fruit was 14.4 mm, had no effect on either ethylene evolution or final fruit set (Table 4). Thus, thinning sprays of BA that resulted in an increase in the rate of ethylene evolution also reduced final fruit set,

Table 1. Effects of time of application of NAA (7.5 ppm) or NAAm (40 ppm) thinning sprays on ethylene evolution from detached spurs, measured 2 d after application, final fruit set of spurs, and pigmy fruit formation on mature 'Fuji'/MM.106 apple trees. Each value is the mean of five observations.

Treatment	Time of spur sampling (days from PF[z])	Ethylene evolution ($nL \cdot g^{-1} \cdot h^{-1}$)	Fruit set (%)	Pigmy fruit[y] (no./tree)
Unsprayed control	+2	1.96 bc	198 a	32.6 a
	+9	0.81 d	---	---
	+16	1.23 cd	---	---
NAA @ PF	+2	3.81 a	192 a	48.2 a
NAA @ PF + 7 d	+9	2.16 bc	170 ab	87.2 ab
NAA @ PF + 14 d	+16	2.23 b	134 c	183.8 c
NAAm @ PF	+2	2.24 b	177 ab	68.6 a
NAAm @ PF + 7 d	+9	0.95 d	150 bc	84.4 ab
NAAm @ PF + 14 d	+16	1.55 bcd	184 ab	133.4 b

[z]Petal fall.
[y]Fruit <90 grams.
Mean separation within columns by Duncan's new multiple range test at $P = 0.05$.

Table 2. Main effects of time of application of NAA (7.5 ppm) or NAAm (40 ppm) thinning sprays on ethylene evolution, measured 2d after application, and final fruit set of spurs on mature 'Fuji'/MM.106 apple trees. Data, expressed as a proportion of the unsprayed control values, were analysed as a 3 × 2 factorial design after log transformation to make the error variances more nearly homogeneous. Data in brackets are back-transformed means.

Treatment	Ethylene evolution (proportion of control)	Fruit set (proportion of control)
	Thinning chemical	
NAA	0.34 (2.19)	–0.087 (0.82)
NAAm	0.06 (1.16)	–0.092 (0.81)
	Time of application	
PF[z]	0.25 (1.78)	–0.049 (0.89)
PF + 7 d	0.19 (1.54)	–0.109 (0.77)
PF + 14 d	0.17 (1.46)	–0.110 (0.78)
	P value	
Thinning chemical	0.0054	0.91
Time of application	0.73	0.52
Interaction	0.85	0.33

[z]Petal fall.

Table 3. Effects of time of application of benzyladenine (BA) thinning sprays (150 ppm) on ethylene evolution from detached spurs, measured two days after application, and final fruit set of spurs on mature 'Pacific Rose™'/MM.106 apple trees. Each value is the mean of five observations.

Treatment	Time of spur sampling (DAFB[z])	Ethylene evolution ($nL \cdot g^{-1}/h$)	Fruit set (%)
Unsprayed control	8	1.57 b	78.4 a
	14	0.97 b	---
	26	0.91 b	---
BA @ 6 DAFB	8	2.77 a	68.1 b
BA @ 12 DAFB	14	1.35 b	53.1 c
BA @ 24 DAFB	26	0.97 b	80.3 a

[z]Days after full bloom.
Mean separation within columns by Duncan's new multiple range test at $P = 0.05$.

Table 4. Effects of time of application of benzyladenine (BA) thinning sprays (150 ppm) on ethylene evolution, measured 2d after application, and final fruit set of 'Pacific Rose™'/MM.106 apple spurs. Data, expressed as a proportion of the unsprayed control values, were analysed after log transformation to make the error variances more nearly homogeneous. Data in brackets are back-transformed means.

Time of BA application		Ethylene evolution	Final fruit set
DAFB[z]	Mean spur fruit diam (mm)	(proportion of control)	(proportion of control)
6	3.8	0.25 (1.76)	–0.061 (0.87)
12	6.9	0.20 (1.60)	–0.177 (0.66)
24	14.4	–0.01 (0.98)	0.003 (1.01)
P value		0.41	0.006

[z]Days after full bloom.

whereas those that had no effect on ethylene evolution had no thinning activity.

Dennis (1987) reported that high ethylene levels could occur without stimulating abscission. In the present studies, an increase in the rate of ethylene evolution immediately following application of a chemical thinner was a prerequisite for thinning activity. However, the relationship between ethylene evolution and fruit abscission was not quantitative in nature; the magnitude of the ethylene response did not appear to be related to final thinning efficacy. Ethylene and fruit retention data presented in Figs. 1 and 2 suggest that differences in the rate of ethylene evolution may be positively related to short term differences in the rate of fruit abscission, but are not necessarily related to final fruit set. Thus, while ethylene evolution from detached apple spurs may provide an early indication of thinning activity for some chemical thinners, the increase in ethylene evolution measured in these studies could not be used to predict final fruit set. It appears that for bloom thinning sprays of ethephon and NAA at least, both the onset and intensity of what was presumably the second wave of fruit drop were different. The increase in ethylene evolution might be related to short-term changes in the rate of fruit abscission, but clearly other factors are involved in the ultimate thinning response.

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