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The Effect of a Substituted Amino Acid on Ethylene Biosynthesis, Respiration, Ripening and Preharvest Drop of Apple Fruits[1]

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Abstract. Spraying trees of 'King of the Pippin' and 'Golden Delicious' (*Malus domestica* Borkh.) with the substituted amino acid aminoethoxyvinylglycine hydrochloride (AVG), within 1 month of harvest delayed fruit ripening, reduced preharvest drop, and increased fruit removal force (FRF). Five sprays of 5 × 10^{-4}M AVG inhibited the development of the climacteric of 'Golden Delicious' apple fruits. Reduced biosynthesis of ethylene was probably responsible for these effects. Naphthaleneacetic acid and AVG synergistically reduced ethylene biosynthesis and warrant further investigation, especially in connection with preharvest drop.

The predominant hormones in the regulation of preharvest drop of apples are C_2H_4 and auxins (3, 5, 8, 11, 17). Therefore, reducing C_2H_4 synthesis might diminish losses caused by premature fruit abscission. Moreover, C_2H_4 not only participates in fruit abscission, but is also one of the principal ripening hormones in climacteric fruits (8, 12, 14). The control of ethylene synthesis is the key to controlling ripening, and thus, the storage life of these fruits. Compared with controlled atmosphere (CA) or hypobaric storage, which also check or prevent C_2H_4 synthesis and/or action, chemical methods require less sophisticated equipment and are less expensive. Until now, only aminoethoxyvinylglycine (13) or analogues of it have been reported to reduce C_2H_4 production in apple fruits (9). However Lieberman (9) infiltrated fruits under partial vacuum, which is not a practical method of application. To determine if the chemical were effective as a tree spray the rhizobitoxine analogue AVG was sprayed on leaves and fruits on the tree. Preliminary results with 'King of the Pippin' in 1975 showed that 2 applications of AVG (5 × 10^{-4}M) reduced preharvest drop from 15 to 5.5% and internal C_2H_4 concn during storage from 316 to 6 ppm. Therefore more detailed studies were conducted in 1976 to test the reproducibility of these results, as well as the effects of the chemical on ethylene synthesis and ripening.

Material and Methods

Uniform 11-year-old 'King of the Pippin' and 'Golden Delicious' trees on Malling 9 rootstock were selected at the Hohenheim Horticultural Experiment Station. Whole trees were sprayed to runoff with 5 × 10^{-4}M AVG, leaving every other tree in the same row unsprayed. Each treatment was applied to 3 trees. Time and frequency of application are indicated in the tables and figures.

Because 'Golden Delicious' does not exhibit preharvest abscission under our conditions, preharvest drop was investigated solely with 'King of the Pippin' fruits treated with AVG or 20 ppm 2-(2,4,5-trichlorophenoxy) propionic acid (fenoprop). Fenoprop was applied 10 days before harvest to 3 trees. Fruits which dropped before harvest were counted every 4th day. Because only 2/3 of the fruits from this cultivar were harvested at the normal date (Sept. 9), the "portharvest drop" from the remaining 1/3 was followed in the same manner.

Use of auxin sprays to control preharvest drop sometimes stimulates C_2H_4 synthesis and ripening during subsequent storage (2, 15, 16, 17). A combination of AVG and NAA was therefore used to determine whether AVG could prevent this effect. For this experiment 1 limb of each 'Golden Delicious" tree sprayed either 2 or 3 times with AVG was treated 10 days before harvest with 25 ppm NAA.

Fruit removal force (FRF) was measured at harvest with a pull-spring gauge with special tongs for attaching to apple fruits.

Fruits were harvested on Sept. 9th ('King of the Pippin') and Oct. 5th ('Golden Delicious') and held at either 20°C or 2°C. Immediately after harvest 2.5 kg samples of fruits were placed in respirometer chambers at 20° and flushed continuously with air (30 liters/hr). CO_2 and C_2H_4 in the outgoing air were monitored daily using gas chromatography. Samples of 8 fruits were removed from storage at intervals for determination of firmness and internal C_2H_4 concn. Firmness was measured with an effe gi penetrometer and internal gas samples for C_2H_4 determinations were taken with a syringe while the fruit was submerged in water.

Results and Discussion

C_2H_4 production of treated 'King of the Pippin' fruits sampled one day after harvest was about 1/4 that of untreated fruits (Fig. 1A). Despite the comparatively early harvest on Sept. 9, C_2H_4 production in untreated fruits was high and increased rapidly, indicating that these fruits had already reached the state of autocatalytic C_2H_4 synthesis. In treated fruits the increase was much slower, but production had reached almost 1/3 that of the controls after 16 days.

Respiration (CO_2 production) of treated fruits was also remarkably reduced (Fig. 1B), presumably as a result of reduced C_2H_4 synthesis. Although a respiratory climacteric occured in both treated and untreated fruits, that in the former appeared later and its maximum was lower. In contrast with 'King of the Pippin' fruits, treated 'Golden Delicious' fruits showed only a slight rise in C_2H_4 evolution, which later decreased (Fig. 1A). Consequently, a climacteric rise in CO_2-production was not observed (Fig. 1B).

Treated fruits of both cultivars were already firmer than untreated ones at the time of harvest, and this difference increased during subsequent storage at 20°C (Table 1 and Fig. 2). The effect was particularly marked in AVG-treated 'Golden Delicious' fruits (Fig. 2). No softening occurred in these fruits during the 13 day storage period, indicating that not only the respiratory climacteric, but the whole complex of ripening was considerably inhibited. Loss of chlorophyll and titratable acidity was also delayed (data not shown).

C_2H_4 production does not necessarily reflect the internal concn (3). Because internal C_2H_4 concn is more important physiologically than C_2H_4 evolution, samples of the internal atmosphere were analysed. The internal C_2H_4 concn of control fruits of both cultivars at harvest considerably exceeded

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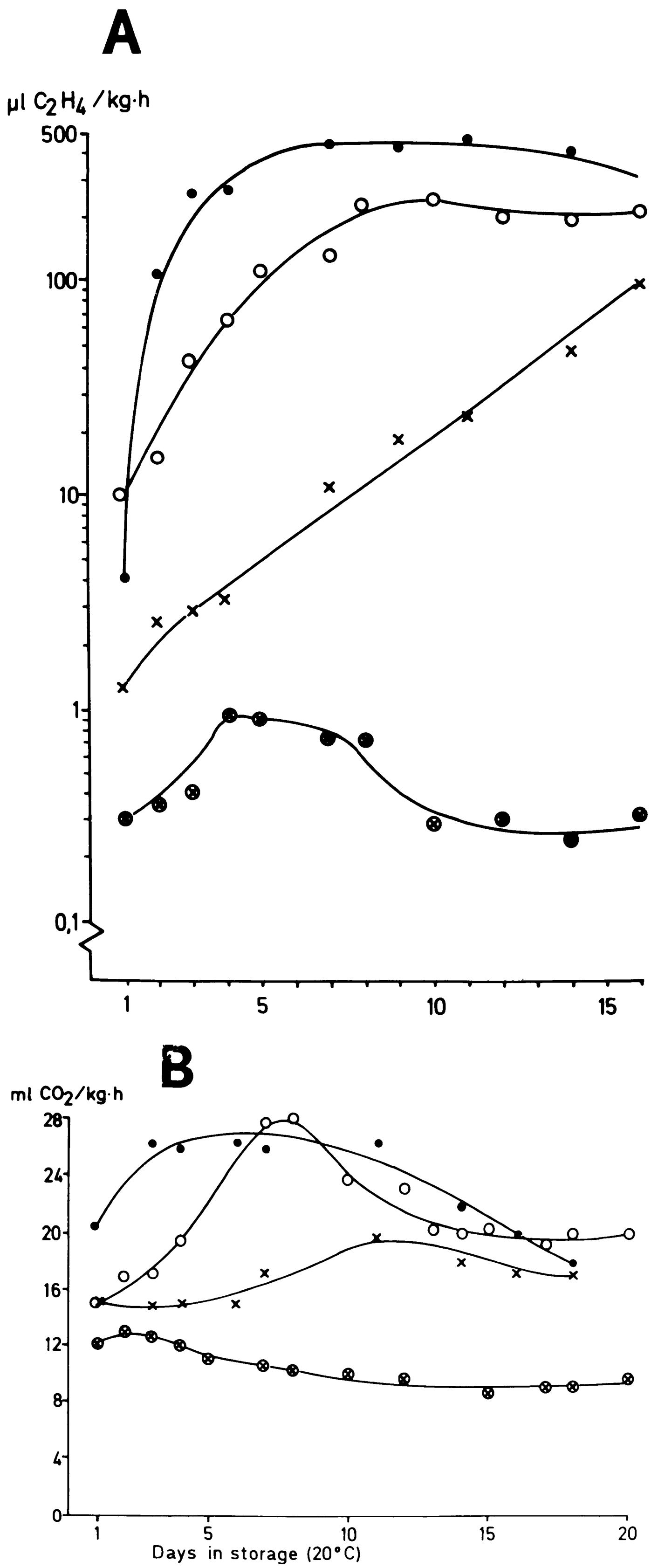

Fig. 1. Effects of AVG (5×10^{-4}M) on (A) C_2H_4 production and (B) respiration of 'King of the Pippin' and Golden Delicious' fruits during storage at 20°C. •——• 'King of the Pippin' control; x——x 'King of the Pippin' fruits treated 27, 18, 7 and 1 day before harvest with AVG. o——o 'Golden Delicious' control; ⊗——⊗ 'Golden Delicious' fruits treated 39, 27, 18, 7 and 1 day before harvest with AVG.

0.1 ppm (Table 1 and 2), which is considered to be the threshold value for the induction of ripening of many fruits (1, 6, 13). Therefore, most of the control fruits had entered the climacteric stage in spite of the comparatively early harvest. This explains the rapid rise in internal C_2H_4 concn after harvest in untreated 'King of the Pippin' fruits (Fig. 1A). Even in fruits of this cultivar treated 4 times with AVG the endogenous level of C_2H_4 exceeded 50 ppm after 11 days in storage. Thus these fruits showed a delayed but nevertheless rapid ripening during the second half of the storage period. In contrast C_2H_4 concn in 'Golden Delicious' fruits treated 5 and 3 times with AVG was comparatively high only at harvest and dropped rapidly thereafter (Fig. 3). 'Golden Delicious' fruits treated only twice behaved like treated 'King of the Pippin' fruits in this respect.

All AVG-treated 'Golden Delicious' fruits stored at 2°C were firmer than untreated fruits after 35 or 65 days storage (Table 2), the greatest difference occurring in the most frequently sprayed fruits. Although C_2H_4 concn of these fruits increased continuously, the internal level was low, never exceeding 2,5 ppm, which is far below the 1/2 max concn at 2°C. Single sprays of fenoprop reduced fruit fiirmness, and both NAA and fenoprop increased internal etylene concn (Table 2). Surprisingly, the combination of AVG and NAA not only prevented the increase in internal C_2H_4 caused by NAA but significantly reduced the C_2H_4 concn below the corresponding value for AVG alone (Table 2). When auxin-induced C_2H_4 stimulation is prevented, NAA appears to act as a fruit ripening inhibitor (see also 6, 14). NAA did not significantly affect softening during storage when applied either alone or in combination with AVG (Table 2).

AVG might be expected to increase FRF and reduce preharvest drop because of its effect on fruit ripening. The data (Table 3) indicate that AVG treatment was as effective as fenoprop in reducing drop of 'King of the Pippin' fruits. AVG also increased FRF, but the differences were not significant. With 'Golden Delicious' both AVG and fenoprop increased FRF significantly ($P < 5\%$).

Conclusions

Ripening, especially of the late cultivar 'Golden Delicious' was remarkably retarded by AVG treatment. Earlier treatments may be required with early ripening cultivars like 'King of the Pippin'. In preliminary experiments with 'James Grieve' and 'Cox's Orange Pippin' retardation of ripening was intermediate between 'King of the Pippin' and 'Golden Delicious'.

AVG, however, was less effective in retarding ripening than was CA storage (data not shown). Additional experiments are required to investigate whether more frequent treatments and/or higher concn of AVG can further improve its effects. A single application at 2×10^{-3}M seven days before harvest was

Table 1. Effects of AVG (5×10^{-4}M) and fenoprop on fruit firmness and internal C_2H_4 concn of 'King of the Pippin' fruits at harvest and during storage at 20° C.

Time of AVG application (days before harvest)	Fenoprop (ppm)	Firmness (kg/cm^2) Days in storage 0	6	11	C_2H_4 concn (ppm) Days in storage 0	6	11
Control (no treatment)	0	8,0a	6,1a	4,2a[z]	70c	304c	298a
27, 18, 7, and 1	0	8,4a	7,8b	5,2b	0,5b	4a	51a
7 and 1	0	8,4a	7,3b	5,3b	32b	105b	218b
–	20	8,1a	6,5a	4,0a	166d	–	301c

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 2. Effects of AVG (5 x 10^{-4}M), NAA, and fenoprop on firmness and internal C_2H_4 concn of 'Golden Delicious' fruits at harvest and after storage at 2°C.

Time of AVG application (days before harvest)	NAA (ppm)	Fenoprop (ppm)	Firmness (kg/cm^2) Time of sampling (days after harvest) 0	35	65	C_2H_4 concn (ppm) Time of sampling (days after harvest) 0	35	65
Control (no treatment)	0	0	7.4b[z]	5.6b	4.2a	0.9b	102d	95d
39, 27, 18, 7 and 1	0	0	8.0c	6.6c	5.6b	0.8b	1a	2a
18, 7 and 1	0	0	7.6b	5.8b	5.0b	0.9b	3b	2a
18, 7 and 1	25	0	7.7b	5.9b	4.9b	0.1b	1a	1a
18 and 7	0	0	7.9bc	5.9b	5.1b	0.8b	4b	5b
18 and 7	25	0	7.7b	6.0b	5.2b	0.1a	1a	3b
–	25	0	7.5b	5.3ab	4.0a	3.0c	118d	128e
0	0	20	6.9a	4.8a	4.3a	2.1c	54c	70c

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

not completely satisfactory, although timing of the application may not have been optimal.

Considerable reduction in internal C_2H_4 concn occurred even where direct treatment of the fruits was prevented by covering the fruits with plastic bags when the tree was sprayed (data not shown). This indicates considerable foliar uptake and subsequent translocation to the fruits.

AVG treatment reduced preharvest drop and increased FRF to an extent comparable to the widely used auxin treatments, without the danger of stimulating C_2H_4 synthesis and advancing ripening. Therefore, AVG treatment may allow fruits to be left longer on the tree, permitting greater flexibility in harvesting.

A combination of AVG with auxins may have a synergistic effect on reducing C_2H_4 biosynthesis and hence preharvest drop. The latter effect is especially worth investigation because AVG, in contrast to succinic acid–2,2-dimethylhydrazide, the only other chemical with a similar effect, does not appear to have adverse effects on fruit size, fruit shape, internal breakdown and vetative growth of less vigorous trees.

AVG may be particularly suited for aerial applications because at higher concn than those used here no phytotoxic effects were seen on pears, wheat, barley, tomato, grapevine and chrysanthemum (personal communication, W.H. de Silva of

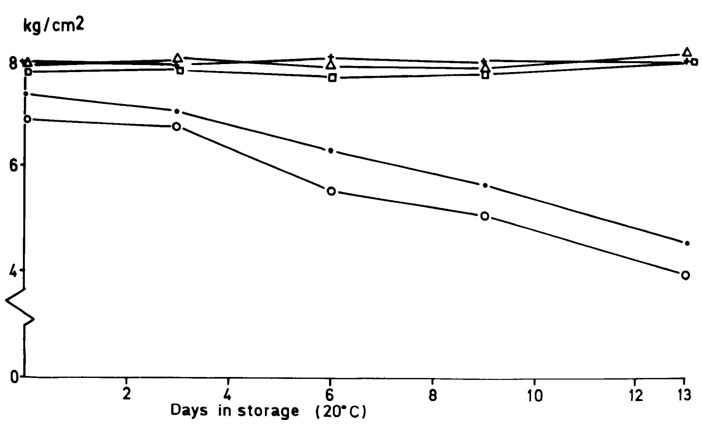

Fig. 2. Effects of AVG (5 x 10^{-4}M) and fenoprop (20 ppm) on fruit firmness of 'Golden Delicious' fruits. •——• Control; x——x fruits sprayed 39, 27, 18, 7 and 1 day before harvest with AVG: □——□ sprayed 18, 7 and 1 day before harvest; △——△ sprayed 18 and 17 days before harvest; ○——○ sprayed 10 days before harvest with fenoprop.

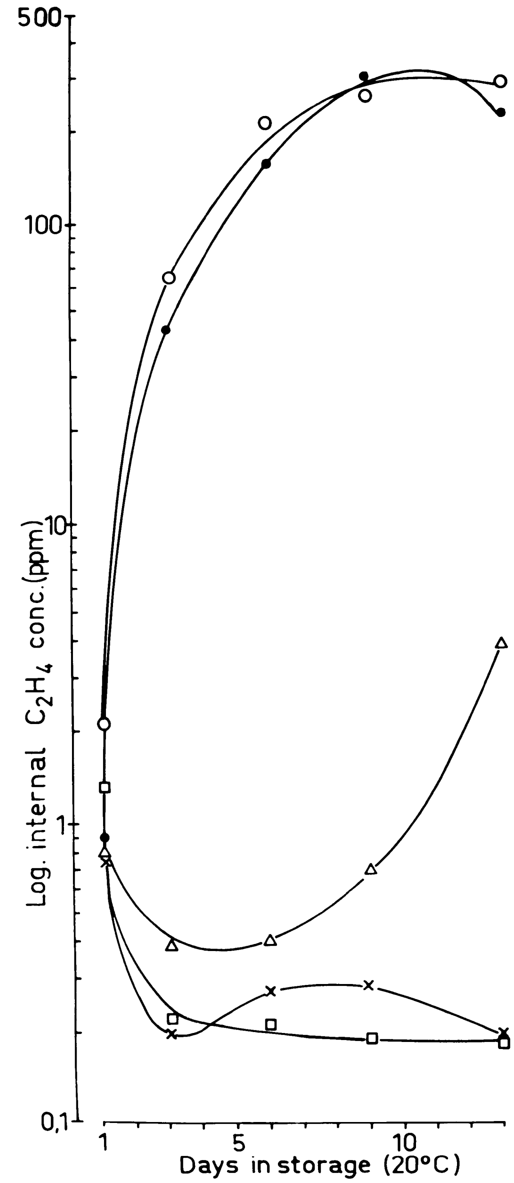

Fig. 3. Effects of AVG (5x10^{-4}M) and fenoprop (20 ppm) on internal C_2H_4 concn of 'Golden Delicious' fruits, •——• control; x——x fruits sprayed 39, 27, 18, 7 and 1 day before harvest with AVG; □——□ sprayed 18, 7 and 1 day before harvest; ○——○ sprayed 10 days before harvest with fenoprop.

Table 3. Effects of AVG (5×10^{-4}M) and 2,4,5-TP on fruit drop and fruit removal force (FRF).

Time of AVG application (days before harvest)	Fenoprop (ppm)	Fruit drop (%) Normal harvest	Fruit drop (%) 2 weeks after harvest	FRF at normal harvest (kg/fruit)
King of the Pippin				
Control	0	15.8c[z]/	26.0c	1.5a
27, 18, 7 and 1	0	5.9a	10.2a	1.8a
7 and 1	0	9.7b	17.9b	1.8a
–	20	8.5b	22.1bc	1.8a
Golden Delicious				
Control	0	–	–	1.9a
39, 27, 18, 7 and 1	0	–	–	2.2b
18, 7 and 1	0	–	–	2.2b
18 and 7	0	–	–	2.3b
–	20	–	–	2.4b

[z]Mean separation within columns and cultivars by Duncan's multiple range test, 5% level.

Dr. R. Maag Ltd.). Therefore there is no danger of damage through spray drift, at least on these particular crops.

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The Structure of Processed Pine Bark[1]

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Abstract. **The external surfaces and internal structures of particles of milled pine bark (*Pinus taeda* L. and *P. elliottii* Engelm.) were examined with scanning electron microscopy. Numerous external openings, cracked cell walls and internal cellular connections, that might allow water penetration were observed. Periderm surfaces were without pores, and contained rough surfaces and apparently waxy substances that might resist water penetration or absorption.**

Satisfactory growth can be obtained with a wide range of woody ornamental plants (8) and with selected herbaceous pot plants (9) when grown in a milled pine bark medium or one containing milled pine bark as an organic amendment. One problem encountered has been early growth delay which may be serious when growing short-term container crops such as bedding plants (4). However, similar plant growth delay has been encountered with a perlite-vermiculite potting mixture containing a high percentage of perlite. Marshall et al. (6) postulated the growth response resulted from a water deficiency in the perlite-vermiculite substrate.

It has been observed that when plants are established in a medium containing milled pine bark, they require less frequent irrigation when compared to plants grown in a medium containing peat moss. Apparently the utilization of either perlite or milled pine bark in a growing medium results in greater H_2O availability, not to be confused with retention, to the plant (10). A recent study of K distribution and retention in pine bark and sand media suggests that capillary pores exist within

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