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Hormonal regulation of ripening in the strawberry, a non-climacteric fruit

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Abstract. Anthocyanin accumulation is one measure of ripening in the strawberry (*Fragaria ananassa* Duch.), a non-climacteric fruit. Neither aminoethoxyvinylglycine, an inhibitor of 1-aminocyclopropane carboxylic acid synthase, nor inhibitors of ethylene action (silver, norbornadiene) affected anthocyanin accumulation in ripening fruit. When the achenes were removed from one half of an unripe fruit there was an accelerated accumulation of anthocyanin and induction of phenylalanine ammonia lyase on the de-achened portion of the ripening fruit. These effects of achene removal could be prevented by the application of the synthetic auxins 1-naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid to the de-achened surface. The introduction of 1-naphthalene acetic acid into intact unripe strawberry fruit through the peduncle delayed their subsequent ripening, as measured by the accumulation of anthocyanin, loss of chlorophyll and decrease in firmness. These findings suggest that the decline in the concentration of auxin in the achenes as strawberry fruit mature modulates the rate of fruit ripening.

Key words: Auxin (fruit ripening) – *Fragaria* – Fruit ripening.

Introduction

Ethylene is thought to play an essential role in the regulation of ripening of climacteric fruit. For example in the tomato the application of ethylene speeds up the onset of ripening as measured by lycopene synthesis and the induction of polygalacturonase (Grierson and Tucker 1983), while the application of silver as the thiosulphate, which is believed to inhibit ethylene perception (Beyer 1976), inhibits lycopene accumulation and the increase in polygalacturonase activity (Hobson et al. 1984). In contrast, the role of ethylene in ripening of the strawberry, a non-climacteric fruit, is unknown. Although a low level of ethylene is produced throughout the development of the fruit (Knee et al. 1977), the application of ethylene to green strawberries apparently does not affect their rate of ripening (Hoad et al. 1971). Ethylene is synthesised from S-adenosyl methionine which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase and thence to ethylene by ACC oxidase. One aim of the present study was to examine the effect of inhibitors of ACC synthase and ethylene action on ripening of strawberry fruit.

Although the presence of auxin produced by the achenes is essential for expansion of the receptacle during strawberry fruit development (Nitsch 1950) it is not known whether this auxin also plays a role in strawberry fruit ripening. Thus a second aim of the present study was to investigate this question by examining the effect of removing the achenes and of applying synthetic auxins.

We present evidence to indicate that ethylene does not play an essential role in ripening of strawberry fruit but that the declining production of auxin in the achenes as the fruit mature (Dreher and Poovaiah 1982) may modulate the rate of ripening of the fruit.

Material and methods

Plant material. Plants of the strawberry (*Fragaria ananassa* Duch.) cv. Brighton were grown as described by Given et al. (1988a).

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Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = aminoethoxyvinylglycine; NAA = 1-naphthaleneacetic acid; PAL = phenylalanine ammonia-lyase; POA = phenoxyacetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid

Chemicals. All chemicals used were of analytical grade. Double distilled water was used throughout.

Assays. Measurement of anthocyanin, chlorophyll, protein, fruit firmness and extraction and assay of phenylalanine ammonia-lyase (PAL) were as described by Given et al. (1988a).

Chemical treatments. Aminoethoxyvinylglycine (AVG) and 1-naphthaleneacetic acid (NAA), each 1 mM in 10 mM potassium phosphate pH 6.5, and silver thiosulphate (80 mM sodium thiosulphate, 20 mM silver nitrate) were introduced into intact strawberry fruit through the peduncle as described previously for L-α-aminooxy-β-phenylpropionic acid (Given et al. 1988a). The auxins, 2,4-dichlorophenoxyacetic acid (2,4-D) and NAA and the inactive auxin analogue phenoxyacetic acid (POA), each at 1 mM in 10 mM potassium phosphate pH 6.5 containing 2% dimethylsulphoxide (DMSO), were applied to the surface of de-achened strawberry fruit with cotton-wool swabs. Control fruit received only DMSO in buffer.

Norbornadiene, calculated to give a concentration of 5000 $mg \cdot l^{-1}$ in the vapour phase, was applied to a small piece of filter paper which was enclosed in a double-layered polythene bag. Each bag was sealed around the peduncle of a large green fruit. Control fruit were treated in a similar fashion but with no norbornadiene on the filter-paper wick. The bags were removed after 48 h. Because of the possible hazards associated with the use of norbornadiene, this chemical was administered in a fume cupbord, while wearing gloves, onto filter paper which was placed in a double-layered polythene bag which was sealed immediately. The neck of the bag was opened briefly to place the bag around the strawberry fruit and immediately re-sealed. At the end of the experiment norbornadiene-treated fruit were discarded.

Results

The accumulation of anthocyanin and induction of PAL activity in ripening strawberry fruit are useful markers of ripening in the strawberry (Given et al. 1988a). To investigate the possible role of ethylene in regulating ripening of strawberry fruit, inhibitors of ethylene action (silver, norbornadiene) and of the ethylene biosynthetic enzyme ACC synthase (AVG) were administered to strawberry fruit at the large green stage. There was no significant difference between the anthocyanin content or PAL activity of control fruit and fruit treated with AVG, silver thiosulphate or norbornadiene (Table 1). There was some browning of the peduncle in both silver-thiosulphate-treated and control sodium-thiosulphate-treated fruit.

In the absence of any effect of inhibitors of ACC synthase or of ethylene action on strawberry fruit ripening, the possible role of the achenes in regulating ripening was examined by following the effect of their removal on the subsequent development of unripe fruit. The achenes were removed from one complete side of each of twelve green strawberry fruit. At various times after the removal of achenes the fruit were harvested and the firm-

Table 1. The effect of AVG (1 mM), silver thiosulphate (20 mM) and norbornadiene (5000 $mg \cdot l^{-1}$) on the anthocyanin content and PAL activity of ripening strawberry fruit. In separate experiments AVG (1 mM in 10 mM potassium phosphate pH 6.5, 1 ml per fruit) and silver thiosulphate (20 mM, 1 ml per fruit) were administered through the peduncle to large green strawberry fruit for 2 d. Control fruit were treated either with buffer (AVG experiment) or with sodium thiosulphate (silver-thiosulphate experiment). Norbornadiene was administered in sealed polythene bags to similar fruit for two days. Within each experiment all the fruit (four per treatment) were harvested when the control fruit showed an even red colour. Values are means ± SD

Treatment	Anthocyanin ($nmol \cdot g^{-1}$ FW)	PAL activity ($pkat \cdot mg^{-1}$ protein)
Control	280 ± 46	47.2 ± 9.4
AVG	345 ± 109	41.0 ± 10.2
Control	228 ± 241	34.4 ± 16.0
Silver	246 ± 148	48.5 ± 34.0
Control	399 ± 85	61.3 ± 14.3
Norbornadiene	467 ± 89	61.0 ± 7.6

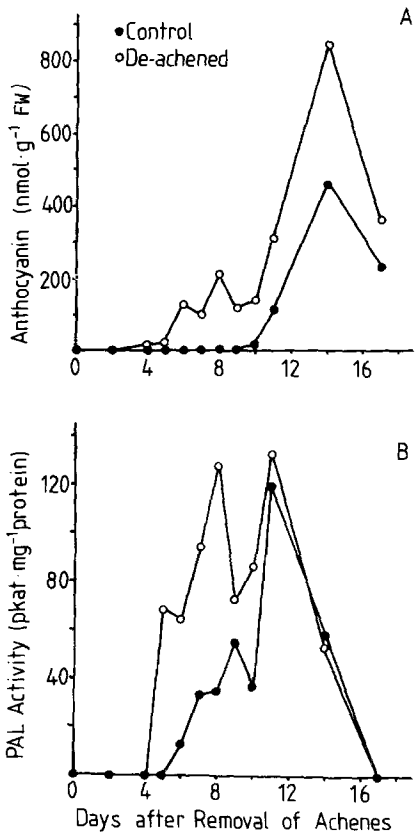

Fig. 1 A, B. Effect of removal of the achenes on the anthocyanin content (**A**) and PAL activity (**B**) of ripening strawberry fruit. The achenes were removed from one longitudinal half of each of twelve strawberries at the large green stage. At various times after removal of the achenes, PAL activity and anthocyanin content were measured on each half-fruit

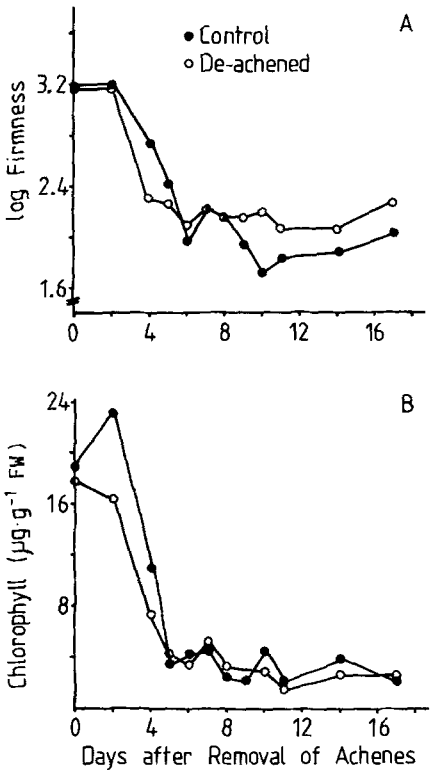

Fig. 2A, B. Effect of removal of the achenes on the firmness (**A**) and chlorophyll content (**B**) of ripening strawberry fruit. The firmness and chlorophyll content of strawberry fruit were measured at various times after removal of the achenes from half of each fruit, as described in the legend to **Fig. 1**

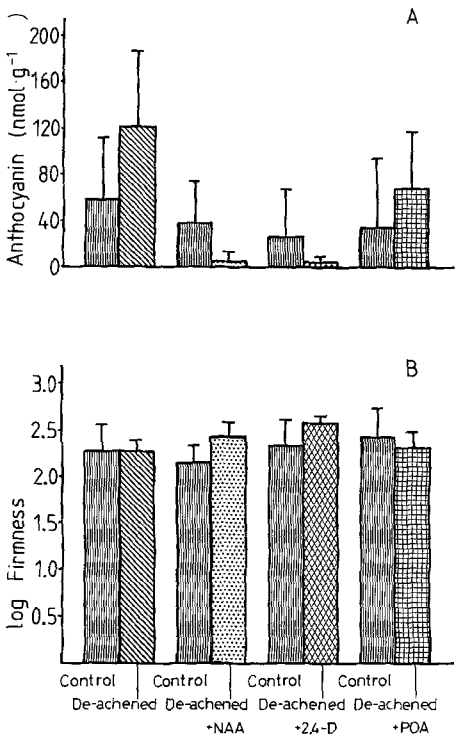

Fig. 3A, B. The effect of removal of the achenes and the application of the synthetic auxins NAA and 2,4-D and the inactive auxin analogue POA on the anthocyanin content (**A**) and firmness (**B**) of strawberry fruit. The achenes were removed from one half of each of twenty strawberry fruit. 1-Naphthalene acetic acid, 2,4-D and POA (each at 1 mM) were applied to the de-achened surfaces of groups of five fruit. Seven days after the removal of the achenes the firmness and anthocyanin content were measured on each half-fruit separately. Values represent mean ± SD

ness, chlorophyll, anthocyanin content and PAL activity were measured.

As the fruit ripened their anthocyanin content increased (Fig. 1A), following a somewhat earlier increase in PAL activity (Fig. 1B), while the firmness (Fig. 2A) and chlorophyll content (Fig. 2B) of the fruit declined. However, there were differences in the timing of these events between the de-achened and control sides of the fruit. Between 5 and 12 d after the removal of the achenes the anthocyanin content (Fig. 1A) and PAL activity (Fig. 1B) were higher on the de-achened side compared to the control side of the fruit. Although the anthocyanin content of the de-achened side remained higher between 12 d after the removal of the achenes and the end of the experiment (16 d) (Fig. 1A), the PAL activity was similar on both sides of the fruit over this period, and had declined to an undetectable level on both sides by the end of the experiment (Fig. 1B).

Early in the experiment the firmness (4–5 d) (Fig. 2A) and chlorophyll content (2–4 d) of the de-achened side of the fruit appeared to be lower than the control side (Fig. 2B), though this tendency was not maintained at later times. Since auxin secreted by the achenes is necessary for strawberry fruit development (Nitsch 1950) it was decided to examine the effect of applying the synthetic auxins 1-naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) to de-achened half-fruits. Seven days after the removal of the achenes from half of each of five fruit, the anthocyanin content on the de-achened side was significantly higher than that on the control side (Fig. 3, paired *t*-test, $P<0.01$). However, when de-achened half-fruits were treated either with NAA or 2,4-D though not with the inactive auxin analogue, POA, anthocyanin concentration was greatly reduced compared to de-achened half-fruits treated with buffer alone (*t*-test, $P<0.05$, Fig. 3). The firmness of de-achened half-fruits treated with either NAA or 2,4-D (Fig. 3B) was higher than that of the control half-fruit although only in the case of NAA was this effect significant (paired t-

Table 2. Effect of NAA on the anthocyanin content and PAL activity of de-achened strawberries. Achenes were removed completely from five strawberry fruit and NAA (1 mM) was applied with a cotton-wool swab to one half of each. Nine days after achene removal, the anthocyanin content and PAL activity of each fruit half was measured. Values represent mean ± SD

	De-achened	De-achened +1 mM NAA
Anthocyanin ($nmol \cdot g^{-1}$ FW)	116 ± 56	33 ± 28
PAL activity ($pkat \cdot mg^{-1}$ protein)	111 ± 28	75 ± 21

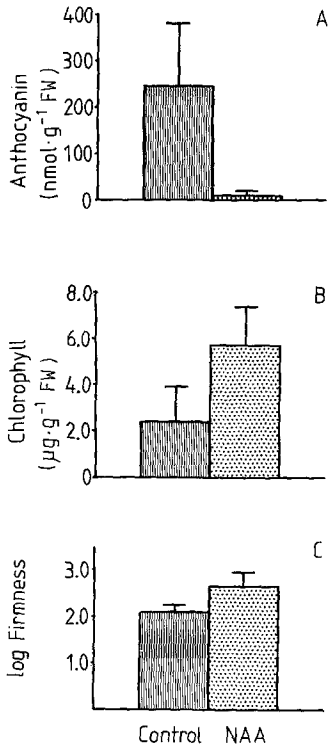

Fig. 4A–C. The effect of NAA administered through the peduncle on the anthocyanin content (**A**), chlorophyll content (**B**) and firmness (**C**) of ripening strawberry fruit. Large green strawberry fruit were treated with NAA (1 mM, 1 ml per fruit) administered for 3 d through the peduncle. Control fruit were treated with buffer alone. The firmness, anthocyanin and chlorophyll content of the fruit were measured 13 d after the start of the treatment. Values represent means ± SD of five replicates

test, $P<0.05$). When all the achenes were removed from large green fruit and NAA (1 mM) was applied to one half of each fruit, the anthocyanin content and PAL activity of the NAA-treated halves were lower than those of the control side treated with buffer alone (Table 2, paired *t*-test, $P<0.05$).

To determine whether the effect of removing the achenes on anthocyanin content was a consequence of the wounding that inevitably accompanies achene removal, large green fruit were wounded with fine forceps between the achenes. However, this treatment led to partial or complete shedding of the achenes and accelerated accumulation of anthocyanin at the wounded site (data not shown) and was therefore not a useful control. The application of 1 mM NAA to one half of the surface of intact large green strawberry fruit had no significant effect on the chlorophyll content, firmness or anthocyanin content of the fruit as they ripened (data not shown). However, when 1 mM NAA was applied to large green strawberry fruit through the peduncle, the anthocyanin content of the auxin treated fruit was significantly lower, 13 d later, while the chlorophyll content and firmness were significantly higher than the values for equivalent control fruit treated with buffer alone (Fig. 4, *t*-test, $P<0.05$).

Discussion

The administration of AVG, silver thiosulphate or norbornadiene (Table 1) to large green strawberry fruit did not significantly affect either the accumulation of anthocyanin or the induction of PAL activity as the fruit ripened, indicating that, unlike the situation in climacteric fruit, ethylene does not play an essential role in the regulation of ripening of the strawberry.

Nitsch (1950) showed that the presence of the achenes was necessary for receptacle expansion, and that this effect could be reproduced by the administration of auxin. The achenes are a rich source of indole-3-acetic acid (IAA) (Dreher and Poovaiah 1982). Free and esterified IAA reach a high concentration 14 and 8 d after anthesis, respectively, (Archbold and Dennis 1984) and subsequently decline. Whilst it is clear that expansion of the developing strawberry receptacle is dependent on auxin supplied by the achenes, information on the role of auxin and the achenes in strawberry fruit ripening remains scarce. Previous reports suggested that removal of the achenes from unripe fruit of the cultivars Marshall (Nitsch 1950) or Rabunda (Guttridge and Nunns 1974) did not affect the subsequent rate of ripening of the de-achened receptacle. However, we find that when achenes were removed from one half of large green fruit of the cultivar Brighton, anthocyanin accumulated more rapidly on the de-achened side of the ripening fruit (Fig. 1A). The accumulation of anthocyanin in de-achened fruit was associated with an accelerated reduction of chlorophyll and

firmness (Fig. 2) indicating that this is a general effect on ripening rather than a specific effect on anthocyanin synthesis.

One explanation for the observation that removal of the achenes from unripe strawberry fruit can accelerate ripening of the fruit is that auxin produced by the achenes inhibits ripening, and that the decline in auxin levels as the fruit mature modulates the rate of ripening. In support of this hypothesis we observed that the accelerated accumulation of anthocyanin which accompanies the removal of achenes from strawberry was prevented by the administration of either of two synthetic auxins (Fig. 3), but was not affected by the inactive analogue, POA, indicating that this is an auxin-specific effect.

In ripening strawberries the accumulation of anthocyanin is associated with the induction of PAL activity (Hyodo 1971; Given et al. 1988a) and de-novo synthesis of the enzyme (Given et al. 1988b). The observations that induction of PAL activity in the de-achened half of ripening strawberries is followed by the accumulation of anthocyanin (Fig. 1), while the application of NAA after removal of the achenes results in delayed rises in anthocyanin and PAL activity (Table 2), provide confirmatory evidence that the increase in PAL activity in ripening strawberries is necessary for anthocyanin synthesis. It is not yet known whether the effect of NAA on the induction of PAL activity in de-achened strawberries is associated with a changed rate of enzyme synthesis.

One possible interpretation of the effect on PAL activity and anthocyanin synthesis of removing the achenes from strawberry fruit is that this represents a specific response of the fruit to wounding. Although surface application of NAA to intact strawberry fruit did not affect their rate of ripening, probably because of poor penetration, when NAA was applied to large green strawberry fruit through the peduncle, ripening of the auxin-treated fruit was delayed, (Fig. 4). This indicates that the accelerated accumulation of anthocyanin following the removal of the achenes from strawberry fruit is a consequence of removing a source of auxin rather than wounding per se, and therefore that the declining auxin concentration in the achenes of strawberry fruit as they mature may modulate the rate of ripening of this fruit. This is the first demonstration that auxin may regulate strawberry development at other than the expansion phase and indicates that for non-climacteric fruit auxin, rather than ethylene may be the hormone controlling ripening.

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