Development and senescence of *Grevillea* 'Sylvia' inforescences, flowers and flower parts

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Abstract: To characterise the physiology of development and senescence for *Grevillea* 'Sylvia' floral organs, respiration, ethylene production and ACC concentrations in harvested flowers and flower parts were measured. The respiration rate of harvested inflorescences decreased over time during senescence. In contrast, both ethylene production and ACC concentration increased. Individual flowers, either detached from cut inflorescences held in vases at 20^{o}C or detached from *in planta* inflorescences at various stages of development, had similar patterns of change in ACC concentration and rates of respiration and ethylene production as whole inflorescences. The correlation between ACC concentration and ethylene production by individual flowers detached from cut inflorescences held in vases was poor (r^2 = 0.03). The isolated complete gynoecium (inclusive of the pedicel) produced increasing amounts of ethylene during development. Further sub-division of flower parts and measurement of their ethylene production at various stages of development revealed that the distal part of the gynoecium (inclusive of the stigma) had the highest rate of ethylene production. In turn, anthers had higher rates of ethylene production and also higher ACC concentrations than the proximal part of the gynoecium (inclusive of the ovary). Rates of ethylene production and ACC concentrations for tepal abscission zone tissue and adjacent central tepal zone tissue were similar. ACC concentration in pollen was similar to that in senescing perianth tissue. Overall, respiration, ethylene and ACC content measurements suggest that senescence of *G*. 'Sylvia' is non-climacteric in character. Nonetheless, the phytohormone ethylene is produced and evidently mediates normal flower development and non-climacteric senescence processes.

Key words: ACC (1-aminocyclopropane-1-carboxylic acid), Ethylene, Flower, *Grevillea*, Respiration, Senescence

Introduction

Grevillea 'Sylvia' is a native Australian plant with cut flower production potential (Costin and Costin 1989). It is an F_2 hybrid from *Grevillea* 'Pink Surprise' (F_1 hybrid of *G. banksii* and *G. whiteana*; Costin and Costin 1989). Its inflorescences are comprised of many small flowers or florets (60 to 120 flowers per inflorescence), large (ca. 25 cm long) and bright pink in colour. *Grevillea* flowers are composed of the pedicel that supports the torus, which in turn supports the pistal, nectary gland and perianth tubes (Olde and Marriot 1994). The pistal consists of the ovary, style and pollen presenter with stigma. The perianth tube has four tepals. *G*. 'Sylvia' flowers have several abscission zones that have been classified as Z1 (tepal to tepal), Z2 (perianth to torus) and Z3 (pedicel to stem) (Joyce and Beal 1999).

The use of *G*. 'Sylvia' as a cut flower, particularly in the export trade, is limited by its relatively short vase life (Joyce *et al.* 1995, 1996). In preliminary studies on *Grevillea* physiology, Joyce *et al*. (1995) showed that rates of ethylene production by whole *G*. 'Sylvia' inflorescences and individual flowers taken from cut inflorescences, increased during senescence. No associated peak in respiration was apparent.

Ethylene plays a central role in the senescence of many cut flowers (Reid 1989). Cut flowers that show distinct peaks in respiration and ethylene production during senescence are classified as climacteric. Flowers that do not manifest this distinct physiological process are non-climacteric (Halevy and Mayak 1979; Wu *et al.* 1991a). Ethylene production by cut flowers is also associated with abscission (Sexton *et al*. 1985), pollination (Halevy 1986) and wounding, including wounding by pathogens (Edney 1967; Whitehead *et al*. 1984; Leshem *et al*. 1986; Piskornik 1986; Woltering 1990).

The involvement of increased ethylene production during senescence of *G*. 'Sylvia' is unclear. Studies to help clarify the role of ethylene production are needed, as such information may underpin development of postharvest technologies to improve vase life. The present series of experiments were undertaken to more fully characterise respiration and ethylene production during development and senescence of *G*. 'Sylvia' flowers.

Materials and methods

Plant material and flower assessments

G. 'Sylvia' inflorescences were harvested either from potted plants growing at The University of Queensland, Gatton (152°20′E, 27°33′S) or from in-ground plants at Greenhouses Holdings Pty Ltd, Redland Bay (153°18′E, 27°37′S) in south east Queensland. Vases contained an anti-microbial solution of 10 mg l^{-1} available chlorine provided as DICA (dichloroisocyanurate; Joyce *et al*. 1995). Rating scales were used to classify the cut flower conditions of flower opening, abscission, wilting and perianth discolouration. The rating scales were: 1 = <5%, 2 = ≥5 to <25%, 3 = ≥25% to <75%, 4 = ≥75% to <90%, and, 5 = ≥90% for flower opening and abscission; and, 1 = none, 2 = slight, and, 3 = severe for wilting and perianth discolouration.

Respiration, ethylene and ACC quantification

Head space samples were withdrawn with a 5 ml or a 1 ml syringe for 2.2 l (intact inflorescences) and 15 ml (separated flowers) incubation vessels, respectively. CO_2 accumulation was measured after 1 hour incubation at 20 ^oC for an inflorescence, whilst flowers required 30 min. The gas samples were injected into a portable carbon dioxide Indicator^{TM} model RI 411 (Gastech) infra-red gas analyser. A CO_2 standard (β-grade BOC Gases) was 0.575 % CO_2 in nitrogen, and a calibration curve was prepared by injecting 2, 1, 0.5 and 0.25 ml aliquots of the standard gas mixture, diluted as necessary, to 2 ml with air. The electrical output signal from the CO_2 analyser was amplified and recorded on a BBC Goerz Metrawatt strip chart recorder (Clegg *et al.* 1978).

Ethylene in headspace samples was quantified with a Shimadzu model GC-8AIT gas chromatograph fitted with a flame ionisation detector. Ethylene concentrations were recorded with a Shimadzu model CR-6A integrator. Temperatures of the injection port, column and detector were 120, 90 and 120^oC, respectively. The 900 mm-long and 5 mm i.d. glass gas chromatograph column was packed with activated alumina mesh size 80/100. The concentration of the ethylene standard gas was 0.09 ± 0.02 μl l^{-1} (BOC Gases β-grade) and the balance gas was nitrogen. The carrier gas (1 kg cm^{-2} pressure) was high purity nitrogen (BOC Gases). Oxygen (0.3 kg cm^{-2}) was supplied as medical grade air and hydrogen (0.45 kg cm^{-2}) as high purity grade from BOC Gases.

To prepare for ACC extraction, ca. 1 g of fresh frozen floral tissue was finely chopped. A 5 ml aliquot of acidified methanol (0.1 M HCl in 80 % (v/v) methanol) was added to the tissue and left to extract for 4 h at 20^oC. Following mixing, a 500 μl aliquot of the sample extract was pipetted into a 15 ml Venoject^{TM} plain blood serum collection tube, and made up to 800 μl with distilled water. Duplicates were prepared for each sample extract. One of each duplicate was spiked with ca. 100 nmol ACC (1-aminocyclopropane-1-carboxylic acid, Sigma Chemical Co.) to determine recovery. The extracts were then neutralised to the phenolphthalein endpoint (pH >8.5 - <9, pink) by drop-wise addition of 10% (w/v) KOH. A 200 μl aliquot of 0.1 M mercuric (II) chloride was added, and the tubes were sealed with a rubber septum. A 0.1 ml aliquot of a mixture of liquid chlorine (White King^{TM}; ca. 40 g l^{-1} available chlorine, as sodium hypochlorite) and saturated NaOH (2:1, v/v) was injected into the tube. Following vortex mixing, the mixture was left for 15 min at 20^oC. A 1 ml sample of headspace gas was then injected into the gas chromatograph for quantification of the amount of ethylene liberated. This procedure (Jobling *et al*. 1991) gave a recovery of ACC of ca. 70 %. ACC concentrations are presented as nmol g^{-1} fresh weight.

Inflorescences in vases

This experiment aimed to characterise the physiology of inflorescences held in a vase life room [20^oC, 70% RH, 12 h photoperiod (12 μmol m^{-2} s^{-1})] after harvest at a recommended commercial maturity stage. Individual inflorescences were comprised of flowers at different development stages. Eighty-four *G.* 'Sylvia' inflorescences were harvested in April (mid-autumn) at the late looping maturity stage (Fig. 1)

from Redland Bay. Ten of these inflorescences were used for measurement of ethylene production during vase life. Another 10 inflorescences were kept for measurement of respiration rate. These measurements were made daily from days 0 to 7. Ethylene production and respiration rates were measured for individual inflorescences each sealed singly into 2.2 l glass jars. Of the remaining 64 inflorescences, eight replicates were used each day for determination of ACC concentration. Inflorescence fresh weight and condition ratings were recorded daily for the 20 inflorescences kept for respiration and ethylene production. Treatment means and standard errors of the means (n = 8) are presented. The experiment was repeated once, and results obtained in both experiments were similar.

Harvested flowers from inflorescences in vases

This experiment was to characterize ethylene production by individual flowers as compared with whole inflorescences. Development stage 3 flowers (Fig. 1) were chosen since this stage is close to anthesis, and subsequent flower development and senescence could be monitored. Seventy two *G*. 'Sylvia' inflorescences were harvested in March (early autumn) from Redland Bay. They were held at 20^{o}C and 70% RH under a 12 h photoperiod (12 μmol m^{-2} s^{-1}). Individual flowers at maturity stage 3 were tagged on day 0. Then, daily thereafter until day 7, nine replicate inflorescences bearing tagged flowers were sampled. Thirty tagged flowers from the inflorescences were excised, pooled and then randomly divided into three sets of 10. These sets were used for the measurement of respiration rate, ethylene production, and for determination of ACC concentration. Individual flowers from the three sets were all weighed to determine average fresh weight (n = 30). Respiration rate and ethylene production were measured using a static system (250 ml glass jars). Treatment means and standard errors of the means are presented. The experiment was repeated once.

Flowers at six different development stages harvested from in planta *inflorescences*

The experiment was conducted to characterise ethylene production by individual flowers at different development stages harvested from inflorescences on the plant. Inflorescences were sampled in February (late summer) from potted *G*. 'Sylvia' plants at The University of Queensland, Gatton. Flowers at each of six development stages were studied (Fig. 1). Approximately 30 g of flowers at each of the six maturity stages were detached and placed into three separate 250 ml jars within 1 h. A 1.7 l h^{-1} flow of ethylene-free air was passed through each jar. They were kept at 20^{o}C and 70% RH under a 12 h (20 μmol m^{-2} s^{-1}) light regime. Ethylene production was measured every hour over the first 12 hours and, thereafter, at 6 h intervals over the following 1 to 5 days. Treatment means and standard errors of the means are presented.

Further characterisation of flowers from in planta *inflorescences*

This experiment was to more thoroughly characterise flower development on the plant. Individual flowers at each of six stages of development (Fig. 1) were harvested in February (late summer) from *G*. 'Sylvia' plants growing at Redland Bay. Ethylene production and respiration rate by three individual replicate flowers were measured in turn in a static system (15 ml glass tubes). The flowers were then frozen for determination of ACC concentration. Treatment means and standard errors of the means are presented.

Ethylene production by excised flowers parts at different development stages

The experiment was to determine ethylene production by gynoecium and perianth parts. Individual flowers were harvested in June (early winter) from inflorescences on potted plants at The University of Queensland, Gatton. Three types of excised parts were used: whole flowers, isolated perianth tubes (i.e. four tepals and fused androecium), and, isolated gynoecium parts (i.e. pedicel, gynoecium, style, and pollen presenter/stigma). Immediately upon arrival in the laboratory, perianth and gynoecium parts were separated by hand from individual flowers for each of development stages 2, 3, 4, 5, and 6 (Fig. 1). Within 1 h, three replicates of approximately 25 g excised tissue were sealed into 50 ml jars and connected to a flow-through system for the measurement of ethylene production. The experiment was analysed by ANOVA, and means were compared using Fischer's Protected LSD test (Petersen 1985).

Further characterization of flower parts at three development stages

This experiment was to examine ethylene production by gynoecium and perianth parts in more detail. Individual flowers at each of development stages 2, 4 and 6 (Fig. 1) were harvested from potted plants at The University of Queensland, Gatton and divided into five different parts: the proximal 1/3 of the gynoecium (pedicel, gynoecium, and bottom of style), middle 1/3 (style), distal 1/3 (top of style, pollen presenter, and stigma), perianth segments, and anthers. The flower parts were excised with a scalpel blade. Sample preparation time ranged from 10 to 30 min. Ethylene production was measured on 5 replicates of ca. 1 g tissue using a static system (15 ml glass tubes). Samples were frozen after measurement for later determination of ACC concentration. Means and standard errors of means are presented. This experiment was repeated once.

Senescence and the perianth abscission zone

This experiment compared ethylene production by perianth abscission zones and perianth tissues. *G*. 'Sylvia' flowers at development stages 2, 4 and 6 (Fig. 1) were harvested in June (early winter) from potted plants at The University of Queensland, Gatton. Their perianth tubes consisting of four tepals were divided longitudinally into two types of parts with a scalpel blade and small scissors. One type was the middle part of each tepal. The other was the abscission zone joining adjacent tepals (Z1, see Introduction). Time for sample preparation ranged from 32 to 75 min. Ethylene production was measured on three replicates of 0.5 g of tissue using a static system (15 ml glass tubes). Samples were frozen after measurement for later determination of ACC concentrations. Means and standard errors of the means are presented. The experiment was repeated once.

ACC concentration in pollen

This experiment was to investigate pollen as a potential source of ACC. *G*. 'Sylvia' flower pollen was collected in June (early winter) from The University of Queensland, Gatton and in July (midwinter) from Redland Bay. Collection was from flowers at the style looped to length of perianth tube stage (Fig. 1), which is just prior to anthesis. The pollen was sealed into a glass tube, frozen in liquid nitrogen and stored at -80^{o}C. To extract ACC, three replicates of 20 mg of pollen were each mixed with 1.5 ml of

acidified methanol (0.1 M HCl in 80% (v/v) methanol) in a 1.5 ml microfuge tube. The tube was then closed, sonicated (Soniclean 120 water bath) for 1 h at 20°C, left for 1 h at 20°C, and then sonicated for another hour. The tubes were centrifuged at 7500 x g for 45 min at 20°C. Finally, 500 µl of supernatant was analysed for ACC as described above.

Results and discussion

The relative fresh weight of cut *G*. 'Sylvia' inflorescences held in vases increased from day 0 to day 2 (Fig. 2a). It was constant on days 2 and 3. Thereafter, relative fresh weight decreased from day 3 to day 7. On day 7, inflorescences were less than half of their initial fresh weight. Flowers opened mostly during the first 4 days after the inflorescences were harvested (Fig. 2b). Flower abscission steadily increased from day 0 to day 7 (Fig. 2c). Discolouration increased dramatically after day 4 (Fig. 2d). The symptom of bluing that characterized discolouration is possibly due to pH-mediated changes in co-pigmentation associated with loss of cellular compartmentation during senescence (Mayak and Halevy 1980; Ligawa *et al*. 1997). There was very little wilting of inflorescences between days 0 and 5 (data not shown).

Ethylene production, respiration and ACC concentration in harvested inflorescences during senescence in vases

Ethylene production by inflorescences was low from day 0 to day 3, and increased from day 3 until day 6 (Fig. 3a). Respiration rate decreased initially and then tended to plateau from day 3 onwards (Fig. 3b). Thus, the trend in respiration was virtually opposite to that observed for ethylene production. ACC concentrations in inflorescences were low and constant from day 0 to day 5, and then increased on days 6 and 7 (Fig. 3c). Thus, trends in ethylene production and ACC concentration were similar.

Considered collectively, the data described above (Figs. 2 and 3) suggest that there are two phases of senescence in cut *G*. 'Sylvia' inflorescences. During the first phase at 0 to 3 days after harvest neither loss of fresh weight (Fig. 2a) nor discolouration (Fig. 2d) were evident. Ethylene production (Fig. 3a) and ACC concentrations (Fig. 3c) were low. Respiration rate decreased dramatically (Fig. 3b), but flower growth and development, including flower opening, still occurred. This initial phase may be prolonged by application of exogenous sucrose (Ligawa *et al*. 1997), which helps maintain a positive water balance and elevated respirable substrate levels in cut flowers (Halevy and Mayak 1979). When normal water relations and growth processes cannot be maintained (Spikman 1986), the second phase begins. This second phase (days 3 to 7) is characterised by decreases in inflorescence fresh weight (Fig. 2*a*), cessation of flower opening (Fig. 2b), perianth discolouration (Fig. 2d), increases in tissue ACC concentrations (Fig. 3b), and increases in ethylene production (Fig. 3a). However, rates of ethylene production are low, and there is no ethylene peak. During the time of ethylene increase in phase 2, respiration rate remains constant (Fig. 3b). Water stress and/or depletion of respirable substrates may promote senescence (Halevy and Mayak 1979, Spikman 1986), but ethylene may only have a role in the second phase of senescence. Wound ethylene production, due to detachment of the inflorescences, could be observed as higher rates of ethylene production on day 0 as compared with lower production levels on day 1 (Fig. 3a). The trend in ethylene production reported here is different to the trend suggested by

Joyce *et al.* (1995) who found a peak in ethylene production on day 5 in a similar experiment. The vase life room temperature in the present work was 20°C, whilst Joyce *et al.* (1995) used 22°C, and a peak may have been discerned in the current experiment if the inflorescences were left longer. Ethylene production was highest at the end of the experiment (Fig. 3a).

Ethylene production, respiration and ACC concentration in flowers detached from inflorescences held in vases

Trends in loss in flower weight (Fig. 4a), ethylene production (Fig. 4b), respiration rate (Fig. 4c), and ACC concentration (Fig. 4d) for isolated flowers were very similar to those observed in whole inflorescences held in vases (Figs. 2 and 3). Overall, the data in Fig. 4 show that detached stage 3 flowers, which are more uniform than whole inflorescences, show the same two phases of deterioration that charcterised the whole inflorescences. That is, in phase one, fresh weight increases (Fig. 4a), respiration rate decreases markedly (Fig. 4c) and ACC concentration and ethylene production are low (Fig. 4b,d). In contrast, during the second phase, there is a progressive decrease in fresh weight (Fig. 4a) and increases in ACC concentration and ethylene production (Figs. 4d,b). Nevertheless, ethylene production is still low compared with climacteric flowers (Brandt and Woodson 1992), and there is no transient peak in ethylene production. Thus, the individual flowers on *G*. 'Sylvia' inflorescences are evidently non-climacteric (Joyce *et al*. 1995). Joyce *et al*. (1995) found that ethylene production by stage 3 flowers was at low levels from day 0 to day 3, and the results of the current experiments show a similar trend.

The coefficient of determination (r^2) for the correlation between ACC concentration and ethylene production was only 0.033 (Fig. 4e), indicating no close relationship between ACC concentration and ethylene production in *G*. 'Sylvia' flowers. This is not necessarily unexpected, since wounding, water stress and carbohydrate depletion (Halevy and Mayak 1979; Spikman 1986; Joyce *et al*. 1995) may each enhance ethylene production from flowers, and ethylene production is not always tightly correlated with ACC concentrations in other flowers (Nichols *et al*. 1983).

Ethylene production by detached flowers at different development stages

Ethylene production was low between 0 and 48 h after commencement of measurements on flowers harvested between the young (stage 1) to mature (stage 3) stages (Fig. 5). By comparison, production of ethylene by flowers harvested at the open flower stage (stage 4) was moderate, and production by senescent flowers (stage 5) and those flowers with abscised perianths (stage 6) was high. A small peak in ethylene production was measured within the first 24 h of harvest in flowers at development stages 4, 5, and 6 (Fig. 5, inset). This may be a wound response. A similar burst of ethylene from wounded flowers was also found in *Petunia hybrida* (Whitehead *et al.* 1984) and *Narcissus pseudonarcissus* (Piskornik 1986). However, there was no peak of wound ethylene from stage 1, 2 and 3 flowers. From 48 h onwards, ethylene production by flowers at development stages 1 to 3 increased progressively until the experiment ended at 126 h (Fig. 5). At this time, stage 2 flowers had lower rates of ethylene production than stage 1 flowers. Flowers are not fully formed by stage 1 (Fig. 1), and immature tissues may contain different levels of plant growth regulators (such as cytokinin) as compared to mature tissues. For flowers

harvested at development stage 4, ethylene production peaked just after 72 h from the beginning of the experiment. Flowers at stages 5 and 6 showed relatively steady ethylene production. The peak of ethylene production by stage 4 flowers may suggest that *G*. 'Sylvia' flowers are climacteric, but this peak may be due to a lack of resources to continue producing ethylene after 3 days, when the tissues were close to death as flowers bearing senesced perianths that had already split (stage 5) or perianths that had abscised (stage 6) did not show elevated rates of ethylene production (Fig 5). The increase in ethylene production observed in flowers at stages 1 to 4 at around 48 h (Fig. 5) may, therefore, be associated with senescence of perianth parts.

Ethylene production, respiration and ACC concentration in flowers harvested at different development stages

Ethylene production by individual flowers harvested from the plant at successive development stages increased in an exponential fashion from extremely low (stage 1, young flower) to around 5.7 $\mu l\ h^{-1}\ kg^{-1}$ fresh weight (stage 6, perianth abscised, Fig. 6a). Respiration rates were only slightly lower as flowers developed through stages 1 to 6 (Fig. 6b), but the respiration rate of development stage 3 flowers was distinctly lower than those of flowers at development stages 2 and 4. ACC concentration was relatively constant during stages 1 to 4, but rose markedly in stage 5 (senescent) and stage 6 (perianth abscised) flowers (Fig. 6c). Ethylene production and ACC concentrations were correlated ($r^2 = 0.66$, n = 18, Fig. 6d). This correlation was stronger than that for individual flowers harvested from inflorescences in vases (Fig 4e). The difference in strength of correlation might be due to wound ethylene production and depletion of carbohydrate in flowers excised from the plant (Nichols *et al*. 1983; Whitehead *et al*. 1984; Sacalis and Lee 1987). In flowers harvested from inflorescences on plants, only the perianth senesced. In contrast, in flowers from inflorescences held in vases, the gynoecium also senesced.

Rapid respiration by physiologically young flower tissue is needed to fulfill energy demands for growth and development (Halevy and Mayak, 1979). Relatively constant respiration rates were recorded in this experiment, where flowers were detached from the plant (Fig. 6b). Respiration rate is variable in flowers from cut inflorescences (Fig. 3*b*) and in flowers detached from cut inflorescences (Fig. 4*c*). The difference may reflect the constant supply of respirable substrate available to inflorescences left on the plant.

Ethylene production by intact flowers and isolated gynoecium and perianth tissues

Ethylene production by whole flowers held at 20 °C tended to increase, at least initially, over successive development stages (Fig. 7a to e), as found earlier (Fig. 6). Up to the end of the experiment (72 h), elevated ethylene production was evident at all developmental stages, except stage 2 flowers. Similar results were found in a previous experiment. Although there were no significant differences in ethylene production, detachment of the perianths tended to result in elevated ethylene production by the perianths themselves and also by the gynoecium tissues from which they were separated (Fig. 7a to d). This effect was most pronounced in less fully developed flowers, namely stage 2 immature flowers (Fig. 7a) to stage 5 senescent flowers (Fig. 7d). At development stages 2 and 3, ethylene production by the isolated gynoecium tissues tended to increase more than that by the perianths for the first 48 h (Fig. 7a,b).

However, at development stages 4 and 5, the opposite trend was evident (Fig. 7c,d). Transient peaks in wound ethylene production were evident upon detachment of the perianths at all development stages (Fig. 7, between 0 and 10 h), but most clearly at stage 4 (Fig. 7c). This transient wound ethylene response by *G*. 'Sylvia' is neither as large as, nor as prolonged, as that in *Petunia hybrida* (Nichols and Frost 1985; Hoekstra and Weges 1986). Ethylene production after the wound transient was apparently associated with active perianth abscission and senescence, since flowers at development stage 6 did not produce any such peak (Fig. 7e).

A second burst in ethylene was observed that was far greater in both magnitude and duration than the wound response (Fig. **7**c,d), and was mainly contributed by the perianth parts. Only development stage 5 flowers showed an ethylene production peak, and only the perianth parts produced this peak (Fig. 7d).

Ethylene production by five different flower parts at four development stages

Ethylene production by five different excised flower parts (viz. proximal 1/3, middle 1/3, and, distal 1/3 of the gynoecium; proximal perianth tissues, and distal perianth tissues including anthers) of individual flowers at development stage 2 (immature), was low (Fig. 8a). With the exception of the middle 1/3 of the gynoecium, ethylene production by flower parts tended to increase over development stages 4 (open flower) and 6 (perianths abscised; Fig. 8a). Ethylene production by each of the three gynoecium sections subsequently fell (stage 7, post-perianth abscission; Fig. 8a). The ranking in relative rates of ethylene production was: distal 1/3 of gynoecium (the style including stigma and pollen presenter) > proximal perianth tissue > distal perianth tissue (including anthers) > proximal 1/3 of gynoecium (including ovary, nectary, torus, pedicel) > middle 1/3 of gynoecium (style). The perianth is always fated to senescence, whereas the gynoecium will continue growing if the ovary is fertilised (Richardson *et al.* 2000).

ACC concentrations in all flower parts at development stages 2 and 4, and in those derived from gynoecium tissues at development stage 7, were low (Fig. 8b). However, while ACC levels were similarly low for all parts derived from the gynoecium at development stage 6, those in the proximal and distal perianth parts were high (Fig. 8b). Thus, while high ACC concentrations were correlated with high ethylene production by perianth-derived parts, elevated ethylene production by gynoecium-derived tissues (Fig. 8a) was not accompanied by elevated ACC levels (Fig. 8b). This observation is consistent with the generally poor correlations obtained between ACC concentration and ethylene production for individual flowers harvested from inflorescences held in vases, as noted earlier. Accumulation of ACC in the perianth tissues at development stage 6 indicates that high concentrations of ACC failed to be converted to ethylene. This observation suggests that the activity of ACC oxidase, as opposed to ACC synthase, may have been limiting the rate of ethylene production in the senescing perianth tissues of *G*. 'Sylvia'. By contrast, ACC oxidase activity increased in the stigma of petunia flowers upon pollination (Borochov and Woodson 1989; John 1997).

Increased ACC concentration associated with the increase of ethylene production in the *G*. 'Sylvia' perianths during late senescence may be associated with mobilisation of sugars from the

senescing petals to the developing ovary. In carnation cv. 'White Sim', which lacks functional anthers (i.e. male sterile), sugars were translocated from petals to the ovary when the petals began wilting (Nichols and Ho 1975; Larsen *et al*. 1995). During normal cut carnation senescence, petal wilting starts after both respiration rate and ethylene production have begun to increase (Maxie *et al*. 1973). The onset of petal wilting in carnation cv. 'White Sim' was hastened by application of exogenous ethylene (Nichols and Ho 1975; Nichols 1976) and by pollination with carnation cv. 'Exquisite' pollen (Nichols *et al*. 1983). Treatment of carnation cv. 'White Sim' with ethylene also induced enlargement of the ovary and an increase in the ratio of sucrose to reducing sugars. The pollinated flower parts of carnation cv. 'White Sim' sequentially produced ethylene in the stigma, ovary, receptacle and petal (Nichols *et al*. 1983). ACC accumulated mainly in the stigma, and on the second day, the concentration was highest in the petals and ovary (Nichols *et al*. 1983). However, in *G*. 'Sylvia', ACC concentration was very low in the stigma even though its ethylene production was the highest of any of the flower parts.

Ethylene production by perianth segments from flowers harvested at three stages of development

Ethylene production and ACC concentrations in perianth tissue, either including the inter-tepal abscission zones (Z1, see Introduction) or not including them, were low for flowers at development stages 2 (immature flowers) and 4 (open flowers), but increased markedly for stage 6 (perianth abscising) flowers (Fig. 9a,b). Rates of ethylene production by perianth parts with or without the inter-tepal abscission zones were not different for any of the three development stages (Fig. 9a). Similarly, ACC concentrations within these different parts also did not differ (Fig. 9b). The absence of any difference between the two parts suggests that relatively high ethylene production by perianth tissues is not solely associated with tepal separation, but can be attributable to overall senescence of the perianth.

ACC concentration in pollen

ACC concentrations measured in two samples of *G*. 'Sylvia' pollen were 2.31 ± 0.62 nmol g^{-1}, fresh weight (n = 3) and 3.88 ± 0.43 nmol g^{-1}, fresh weight (n=3). These concentrations are similar to ACC concentrations in *Cucurbita pepo* pollen (zucchini squash; 4.6 nmol g^{-1}), but are considerably lower than concentrations in pollen of *Petunia hybrida* (296.0 nmol g^{-1}), *Lathrytus odoratus* (sweet pea; 50.0 nmol g^{-1}), and *Dianthus caryophyllus* (carnation; 22.8 nmol g^{-1}) (Whitehead *et al*. 1983). *G*. 'Sylvia' pollen contained the same concentration of ACC as the perianth (Fig. 8b). These observations suggest that the high ethylene production by *G*. 'Sylvia' stigmas (Fig. 8) is not a direct result of the ACC contained in the pollen.

Conclusion

Overall, the results show that *G*. 'Sylvia' respiration rate does not change markedly despite increases in ethylene production by whole inflorescences (Fig. 3), by individual flowers harvested from inflorescences in vases (Fig. 4), and by individual flowers of different development stages (Fig. 6). The pattern of respiration rate during the later phase of senescence suggests that *G*. 'Sylvia is a non-climacteric flower. The rate of ethylene production by *G*. 'Sylvia' is low when compared with carnations (Wu *et al*. 1991b). Moreover, as opposed to respiration, the timing of ethylene production is late in senescence. These

observations suggest that ethylene production does not have an initiating role in senescence of *G*. 'Sylvia' flowers. Ethylene production by *G.* 'Sylvia' flowers is principally associated with senescence of perianth and other flower parts.

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List of figures

Figure 1. Late looping stage of *G*. 'Sylvia' inflorescence maturity (left side), and sequential stages of development of individual flowers (right side). The development stages are: 1 = young flower, perianth starting to split, style visible, 2 = immature flower, small style loop, 3 = mature flower, style looped to length of perianth tube, 4 = open flower, style reflexed, 5 = senescent flower, perianth segment separating, and, 6 = perianth abscised.

Figure 2. Relative fresh weight (a) and scores for flower opening (b), abscission (c), and discolouration (d) of harvested *G*. 'Sylvia' inflorescences held in vases at 20°C for 7 days. In each panel, means and standard errors are given (*n* = 20). Where error bars are not evident, they are contained within the symbols.

Figure 3. Ethylene production (*n* = 10) (a), respiration rate (n = 10) (b), and ACC concentration (*n* = 8) (c) of *G*. 'Sylvia' inflorescences held in vases at 20°C for 7 days. Means and standard errors are given. Units are expressed on a fresh weight basis.

Figure 4. Fresh weight (*n* = 30) (a), ethylene production (*n* = 10) (b), respiration rate (*n* = 10) (c), ACC concentration (*n* = 10) (d), and correlation between ACC concentration and ethylene production (e) for individual flowers sampled from harvested *G*. 'Sylvia' inflorescences held in vases at 20 °C for 7 days. Means and standard errors are given. Units are expressed on a fresh weight basis.

Figure 5. Ethylene production at 20 °C by detached *G*. 'Sylvia' flowers sampled from the plant at different development stages: stage 1, style visible (●); stage 2, small style loop (■); stage 3, style looped to length of perianth tube (▲); stage 4, style reflexed (▼); stage 5, perianth segment separating (◆); and stage 6, perianth abscised (○).Means and standard errors are given (*n* = 3). Ethylene production is expressed on a fresh weight basis. The insert shows an expanded first 24 h.

Figure 6. Ethylene production (a), respiration rate (b), ACC concentrations (c), and correlation between ACC concentration and ethylene production (d) for *G*. 'Sylvia' flowers harvested from the plant at 6 different development stages. In *a* to *c*, means and standard errors are given (n = 3). Where errors are not evident, they are contained within the symbol. Units are expressed on a fresh weight basis. First measurement was ca. 2 h after harvest.

Figure 7. Ethylene production by flowers and parts of *G*. 'Sylvia' flowers at 20 °C taken at five development stages: stage 2, small style loop (a); stage 3, style looped to length of perianth tube (b); stage

4, style reflexed (c); stage 5, perianth separating (d); stage 6, perianth abscised (e). For flower parts: whole flowers (●), gynoecium (■), and perianths (▲). FPLSD = Fischer's protected LSD (n = 3). Units of ethylene production are on a fresh weight basis.

Figure 8. Ethylene production (a) and ACC concentrations (b) in five *G*. 'Sylvia' flower parts at 4 stages of development; proximal 1/3 of the gynoecium (●), middle 1/3 of the gynoecium (■), distal 1/3 of the gynoecium (▲), perianths (▼), and anthers (◆). Means and standard errors are given (*n* = 5). Units are on a fresh weight basis.

Figure 9. Ethylene production (a), and ACC concentration (b) in perianth abscission zones (●) and in neighbouring perianth tissue (■) of *G*. 'Sylvia' flowers at 3 stages of development. Means and standard errors are given (*n* = 3). Units are on a fresh weight basis.

Figure 1

Figure 2

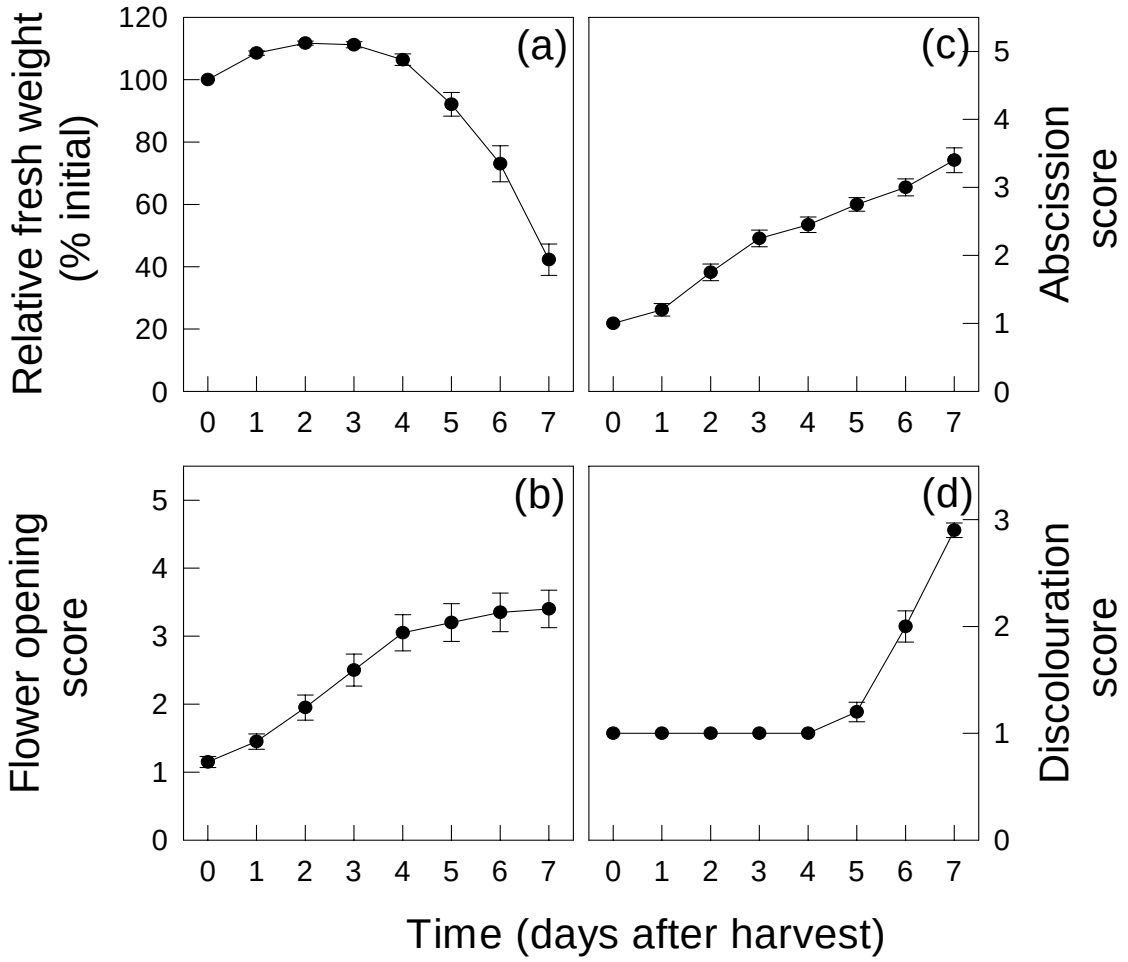

Relative fresh weight (% initial)
(a)
(c)
Abscission score
Flower opening score
(b)
(d)
Discolouration score
Time (days after harvest)

Figure 3

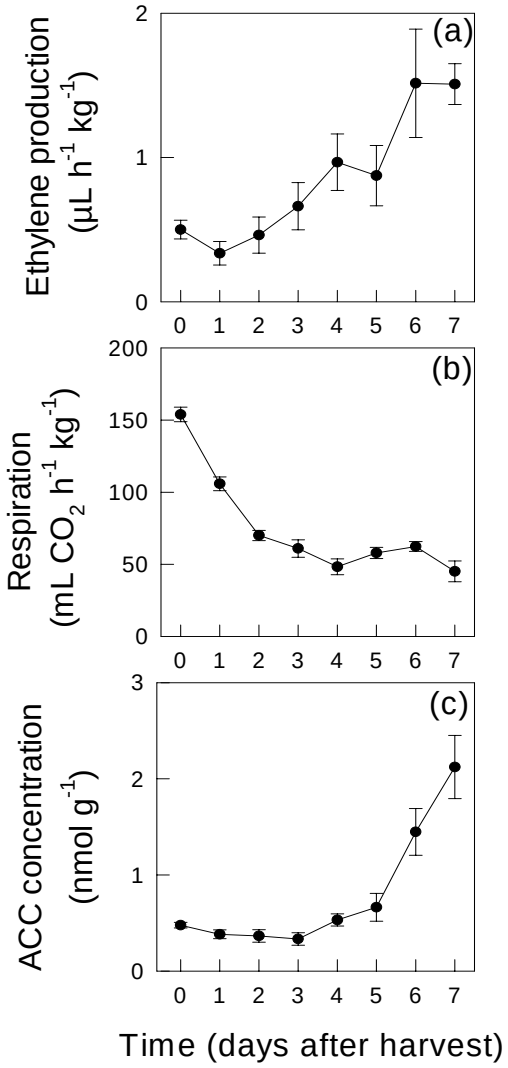

Ethylene production
(µL h-1 kg-1)
(a)
Respiration
(mL CO2 h-1 kg-1)
(b)
ACC concentration
(nmol g-1)
(c)
Time (days after harvest)

Figure 4

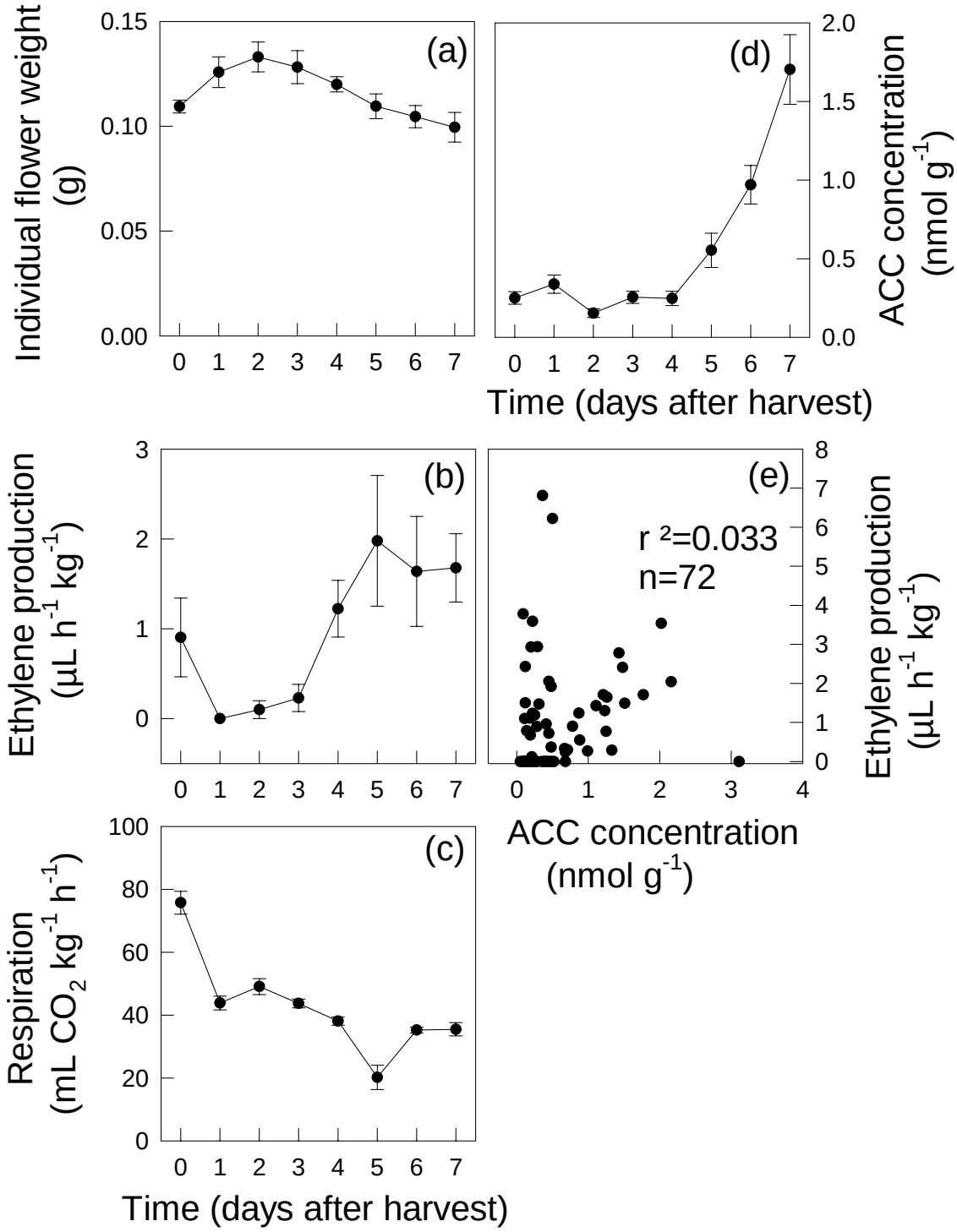

(a)
(d)
(b)
(e)
(c)
Individual flower weight (g)
ACC concentration (nmol g-1)
Ethylene production (µL h-1 kg-1)
Respiration (mL CO2 kg-1 h-1)
Time (days after harvest)
ACC concentration (nmol g-1)
r 2=0.033
n=72

Figure 5

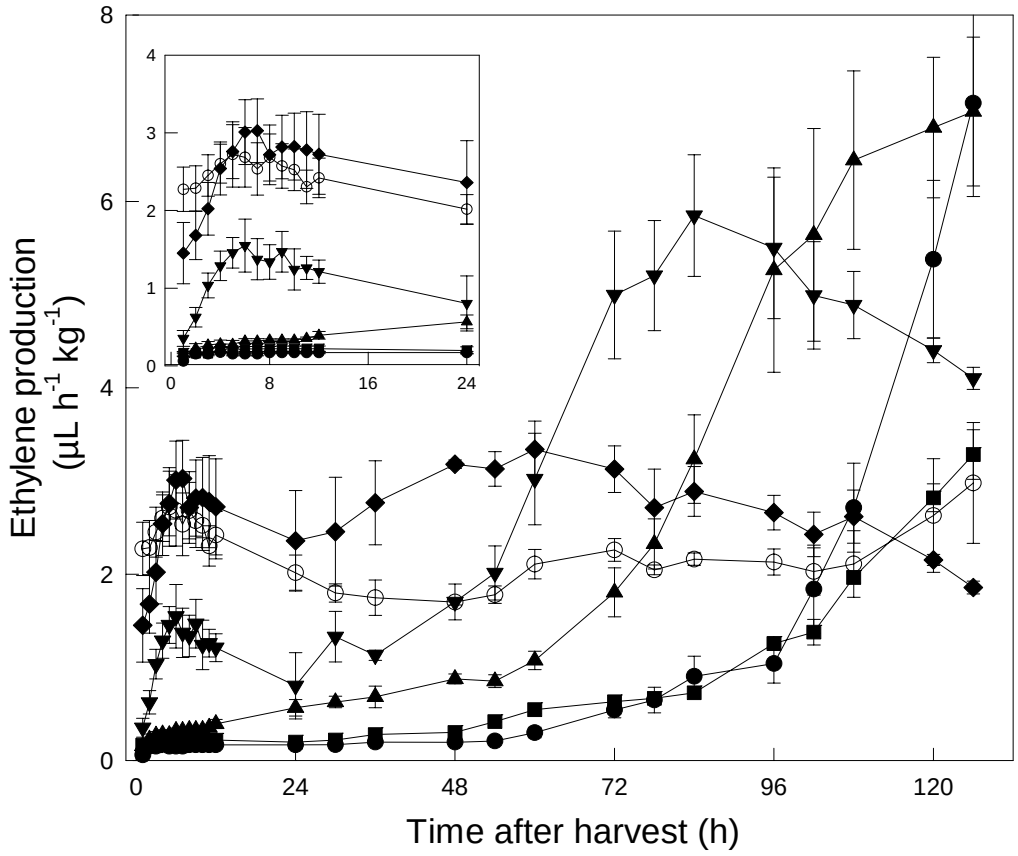

Ethylene production
(µL h-1 kg-1)
Time after harvest (h)
0
2
4
6
8
0
24
48
72
96
120
0
1
2
3
4
0
8
16
24

Figure 6

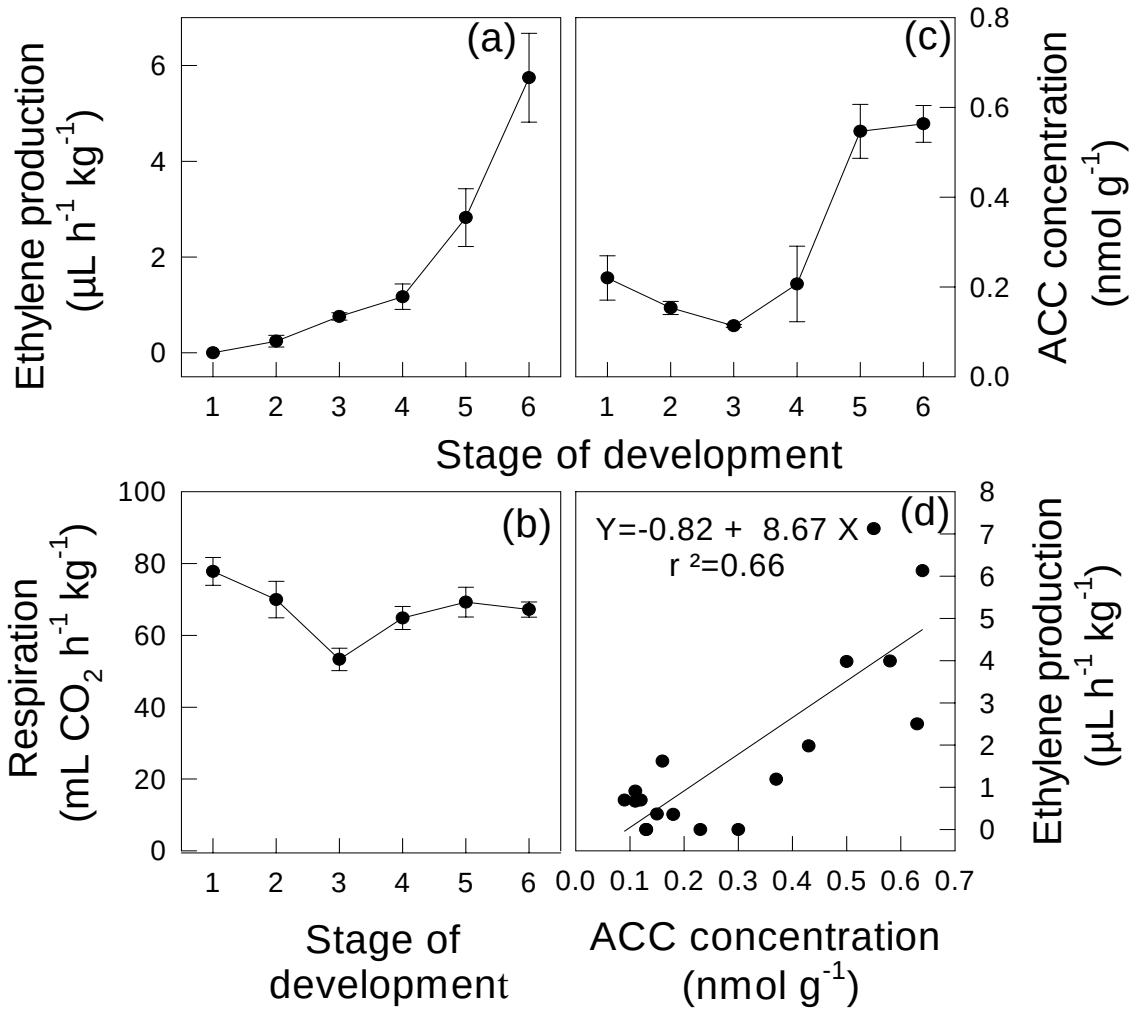

(a)
(c)
(b)
(d)
Ethylene production (µL h-1 kg-1)
ACC concentration (nmol g-1)
Respiration (mL CO2 h-1 kg-1)
Ethylene production (µL h-1 kg-1)
Stage of development
Stage of development
ACC concentration (nmol g-1)
Y=-0.82 + 8.67 X
r ²=0.66

Figure 7

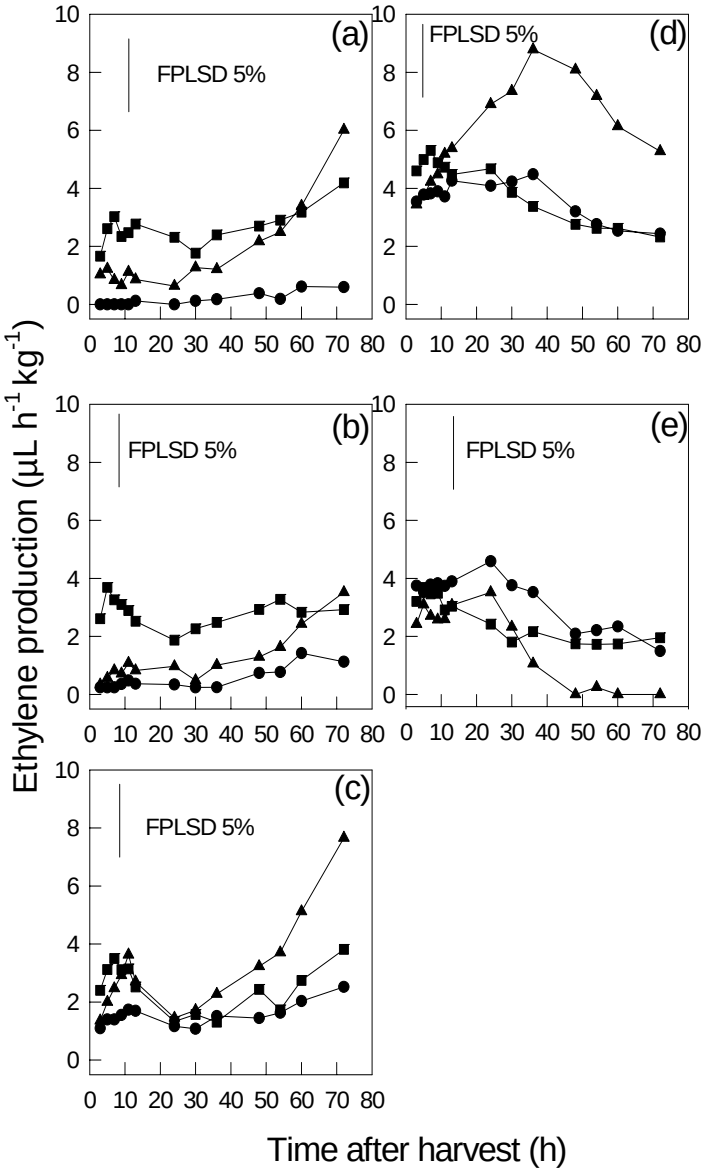

(a)
(b)
(c)
(d)
(e)
FPLSD 5%
Ethylene production (µL h-1 kg-1)
Time after harvest (h)
0 10 20 30 40 50 60 70 80
0 2 4 6 8 10

Figure 8

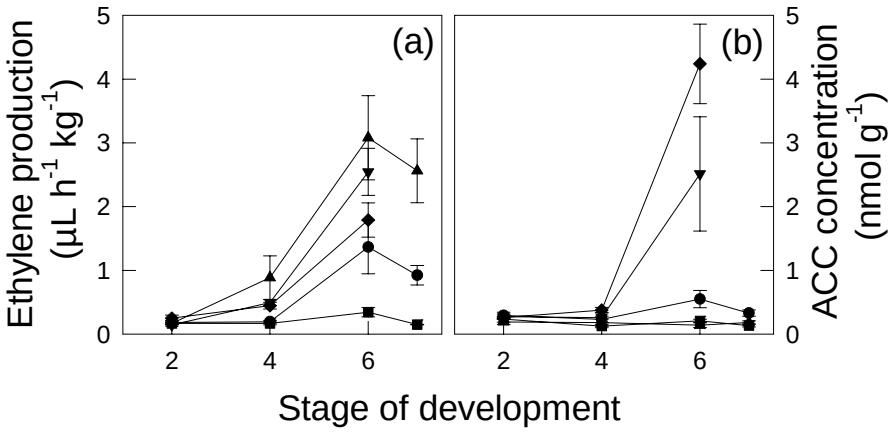
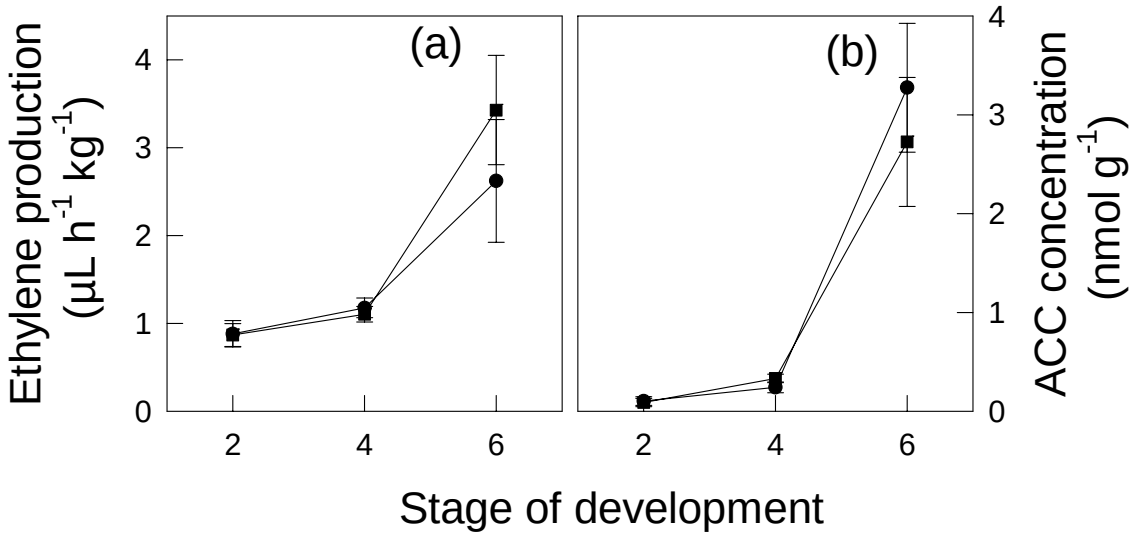

Ethylene production
(µL h-1 kg-1)
(a)
(b)
ACC concentration
(nmol g-1)
0
1
2
3
4
5
2
4
6
Stage of development

Figure 9


Ethylene production
(µL h-1 kg-1)
(a)
(b)
ACC concentration
(nmol g-1)
0
1
2
3
4
2
4
6
Stage of development