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Variation in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars. I. Comparison of flower life, respiration and ethylene biosynthesis

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ABSTRACT

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Cut flowers of the 'Sandra' and 'Chinera' cultivars of carnation (*Dianthus caryophyllus* L.) lasted about twice as long (approx. 14 days) as those of 'White Sim'. Vase life of 'White Sim' was unaffected by the age of the flower at harvest, but the life of 'Sandra' and 'Chinera' fell as flower age at harvest increased. The senescence of 'White Sim' and 'Chinera' carnations, indicated by petal in-rolling and wilting, was accompanied by a marked increase in respiration, 1-aminocyclopropane-1-carboxylic acid (ACC) content, ethylene-forming enzyme (EFE) activity, and C_2H_4 production. In contrast, 'Sandra' flowers showed neither petal in-rolling nor climacteric respiration and C_2H_4 production during their eventual senescence. There was negligible ACC and very low EFE activity in petals of 'Sandra' throughout the post-harvest period.

Keywords: ACC; carnation; EFE; ethylene; senescence.

Abbreviations: ACC = 1-aminocyclopropane-1-carboxylic acid; EFE = ethylene-forming enzyme; STS = silver thiosulfate; DI = deionized water.

INTRODUCTION

The senescence of carnation flowers is normally studied using the cultivar 'White Sim' (*Dianthus caryophyllus* L.) (Bufler et al., 1980; Whitehead et al.,

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1984). In 'White Sim' flowers the end of vase life is indicated by in-rolling of the petals, accompanied by a marked increase in respiration and C_2H_4 production (Nichols, 1968; Mayak and Dilley, 1976). The ACC content and EFE activity of the petals rise co-ordinately with the increase in C_2H_4 production (Bufler et al., 1980; Whitehead et al., 1984). Treatment of flowers either with inhibitors of C_2H_4 biosynthesis such as aminoethoxyvinylglycine (AVG) (Baker et al., 1977) and amino-oxyacetic acid (AOA) (Borochov et al., 1982; Fujino et al., 1981) or with inhibitors of C_2H_4 action such as STS (silver thiosulfate) (Veen, 1979) not only delays the onset of flower senescence and inhibits petal in-rolling, but also suppresses climacteric respiration and C_2H_4 production. Pretreatment with STS is now widely used commercially to inhibit the acceleration of carnation senescence by endogenous or exogenous C_2H_4 (Reid et al., 1980). The agricultural use of silver has been criticized because of its negative environmental implications and its cost. Alternative techniques for extending the life of cut carnation flowers are therefore of commercial interest. One possible strategy is the development of lines of carnations that produce no C_2H_4, or are insensitive to C_2H_4. The potential for this approach is indicated by the fact that C_2H_4 production and sensitivity varies greatly among flowers of different taxa (Woltering and van Doorn, 1988), even some that are closely related.

Mutants with impaired C_2H_4 biosynthesis or sensitivity are of great value in the study of C_2H_4-mediated processes in plants. For example, tomato fruit ripening has been explored in detail using the ripening mutants *rin, nor, alc,* and *Nr* (Roberts et al., 1987). These mutants show a range of natural ripening behavior and responses to C_2H_4. The *rin* cultivar produces no C_2H_4, and ripens very slowly even when treated with C_2H_4. *Nr*, in contrast, displays the normal ripening behavior of tomatoes, but much attenuated and delayed.

Recent molecular studies indicate that, like ripening of tomatoes (Biggs et al., 1986), the senescence of carnation petals is not a stochastic destructive process, but an ordered series of events that may be controlled, at least partially, at the level of gene expression (Woodson and Lawton, 1988; Lawton et al., 1989). Carnation mutants varying in C_2H_4 production or response would be very useful in further understanding the control of flower senescence. In a preliminary report (Wu et al., 1989), we showed that cultivar 'Sandra' flowers senesce without the in-rolling typical of carnations. They showed neither the normal increase in C_2H_4 production, nor a marked respiration climacteric during their eventual senescence. This suggests that the production of C_2H_4 or response to it might vary among carnation cultivars. We therefore sought other carnation cultivars with a longer than normal vase life. Cultivar 'Chinera' carnations were also suggested to last much longer than those of 'White Sim' (H. Fukutome, personal communication, 1986).

We describe here the longevity of flowers of these different cultivars and their senescence pattern. The relationship among their vase life, C_2H_4 biosynthesis and respiration is also described.

MATERIALS AND METHODS

Carnations (*Dianthus caryophyllus* L.) were grown in the greenhouse at 21°C/16°C day/night temperature using standard production methods, or obtained from a commercial grower and transported dry under cool conditions to University of California at Davis on the day of harvest. Three carnation cultivars were used in this study. 'Sandra', a raspberry pink cultivar cultivated commercially in California, was bred by an Italian company, Sapia, from non-commercial breeding lines; it is a fourth generation descendant of the old Italian commercial cultivars 'Silvia', 'Poher' and 'Adele'. 'Chinera', a commercial cultivar whose salmon flowers have strongly dentate petals, was bred by an Italian company, Nobbio, from a non-commercial pink selection, '8367', and 'Faust', a German cultivar with red flowers. Flowers of the 'White Sim' cultivar served as controls. Flowers were tagged with a date label on the day (1D) that they reached commercial maturity (outer petals horizontal).

The longevity of intact flowers was determined by tagging flowers growing in the greenhouse, and examining them daily for symptoms of senescence. For experiments with cut flowers, stems of freshly harvested flowers were trimmed to a length of 40 cm, then placed in test solutions or in deionized water (DI) containing 200 ppm Physan-20 (Consan Pacific Inc., Whittier, CA, USA). The life of the flowers was evaluated under standard conditions (20°C, 12 h cool white fluorescent light, 55% RH) (Reid and Kofranek, 1981). The flowers were examined twice a day; vase life was considered terminated when the corolla was noticeably wilted, dried or necrotic. In experiments determining post-harvest changes in fresh weight, flowers were removed from the vases daily and weighed. Each treatment was replicated at least five times, and experiments were carried out at least twice.

To measure C_2H_4 production and respiration, the flowers (stems trimmed to 5 cm and placed in a small vial) were sealed in a 450 ml jar for a short time. Ethylene and CO_2 production were determined by measuring the concentrations of these gases in a 1 ml sample of headspace gas using photoionization gas chromatography and infrared absorption (ADC type 225-2B-SS), respectively.

The ACC content of the flowers was determined, using petals from the outermost whorl, by the technique of Bufler et al. (1980). EFE activity in petals was determined as described by Whitehead et al. (1984).

RESULTS

Vase life of different cultivars. – 'Sandra' cut flowers had the longest vase life among the three selected cultivars, followed by 'Chinera' flowers (Table 1). The vase life of 'White Sim' flowers was about half that of 'Sandra' flowers. Flowers of 'Chinera' showed the in-rolling (sleepiness) and wilting character-

TABLE 1

Comparison of flower longevity and vase life of 'White Sim', 'Sandra', and 'Chinera' carnations. Flowers were tagged at commercial maturity. Replicate flowers were left on the plant (intact) or harvested (cut) and placed in DI water containing 200 ppm Physan for measurement of vase life. Data are the means ± SE of three experiments (five replications per treatment)

	Flower life (days)		
	'White Sim'	'Sandra'	'Chinera'
Intact	19.3 ± 1.3	16.1 ± 1.2	18.8 ± 1.6
Cut	7.0 ± 0.6	14.3 ± 1.0	13.1 ± 1.2

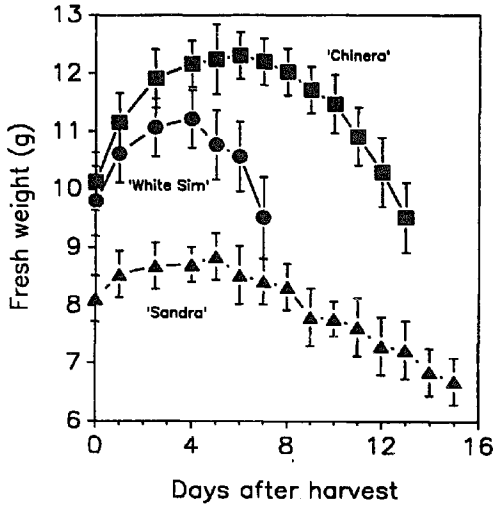

Fig. 1. Fresh weight changes in 'White Sim', 'Sandra' and 'Chinera' carnations during senescence. Flowers were harvested at commercial maturity, and placed in DI water containing 200 ppm Physan. Their fresh weight was then determined at intervals. Data are the means ± SE of three experiments (five replications per treatment).

istic of senescence in 'White Sim'. In contrast, 'Sandra' flowers did not wilt, but faded and turned brown as the petals dehydrated from the tips. There was no clearly defined end point in the vase life of 'Sandra' flowers.

Effects of removal from the plant. – On the plant, the flowers of all three cultivars lasted for 17–19 days (Table 1). The lives of 'Chinera', and particularly 'White Sim' were substantially reduced by removal from the plant.

Changes in fresh weight. – The patterns of change in fresh weight in the different carnation flowers reflected the differences in their vase lives. In all cultivars, flower fresh weight increased during the first few days in the vase (Fig. 1). For 'White Sim', fresh weight was greatest 4 days after harvest, and fell

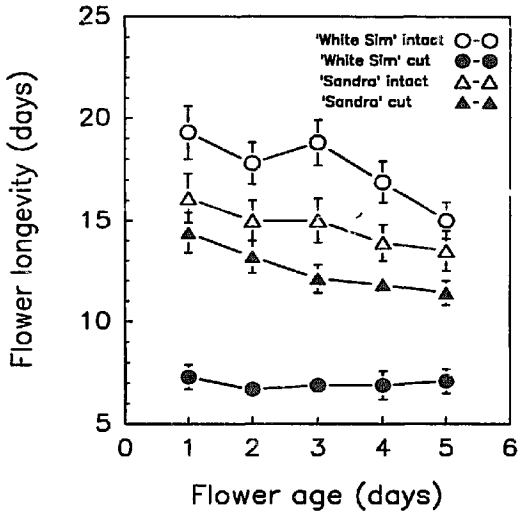

Fig. 2. Effect of flower age on vase life and flower longevity of 'Sandra' and 'White Sim' carnations. Flowers were tagged at commercial maturity, then harvested at intervals and placed in DI water containing 200 ppm Physan for evaluation of vase life. Data are the means ±SE of two experiments (five replications per treatment).

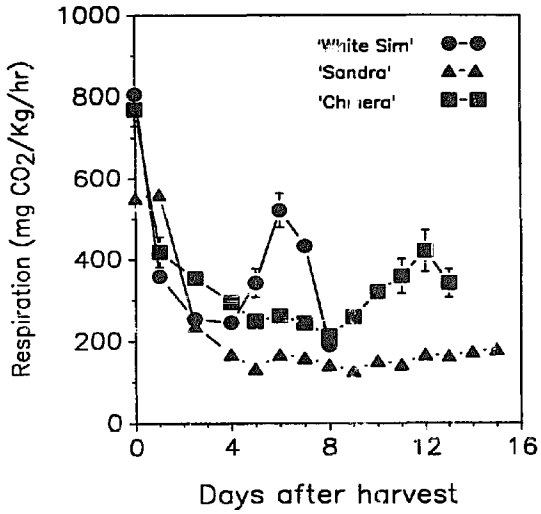

Fig. 3. Respiration of 'White Sim', 'Sandra' and 'Chinera' carnations. Flowers were harvested at commercial maturity, and placed individually in DI water containing 200 ppm Physan in jars ventilated with flowing air. Data are the means ±SE of four experiments (five replications per treatment).

rapidly thereafter. 'Chinera' flowers gained weight until 6 days after harvest then wilted relatively rapidly. Changes in fresh weight of cut 'Sandra' flowers followed a markedly different pattern, rising slightly after harvest, remaining steady for 4 days, then falling gradually during the post-harvest period (Fig. 1).

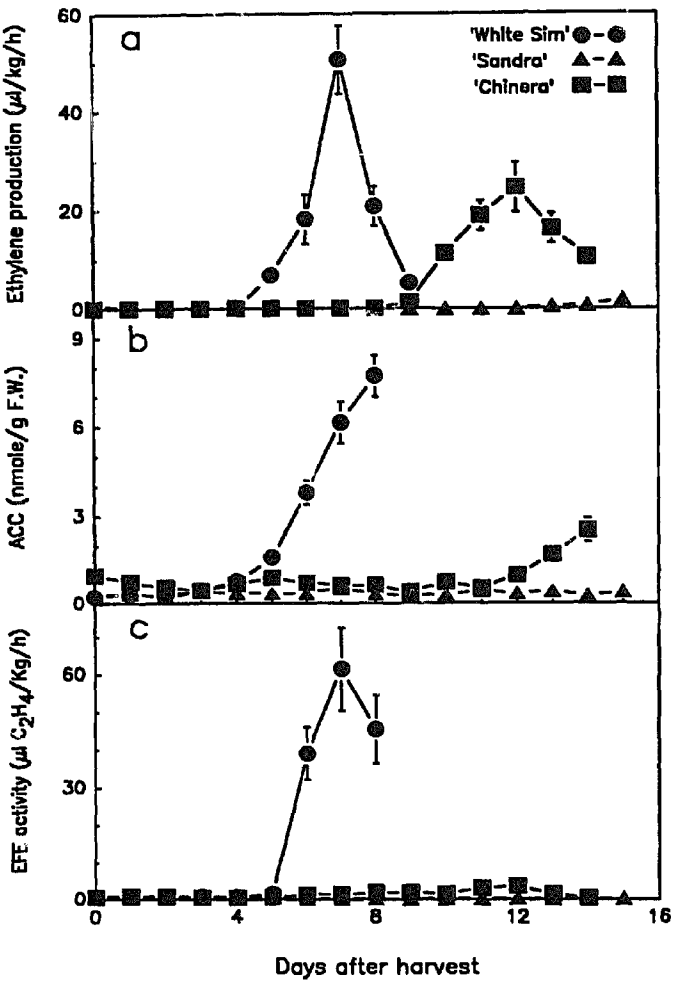

Fig. 4. Ethylene production (a), ACC content (b) and EFE activity (c) of 'White Sim', 'Sandra and 'Chinera' carnation flowers. Flowers were harvested at commercial maturity, and placed in DI water containing 200 ppm Physan. Ethylene production was measured on one group of flowers; replicate flowers from the other group were harvested at intervals for determination of ACC content and EFE activity. Data are the means ± SE of two experiments. Where not visible, error bars are smaller than the data symbol.

Effects of flower age on vase life. – The vase life of 1D 'Sandra' flowers was about twice that of 'White Sim' flowers (Table 1 and Fig. 2), but vase life of 'Sandra' flowers fell as their age at harvest increased. In contrast, the longevity of 'White Sim' flowers appeared unaffected by flower age at harvest. Young (1D) 'Chinera' flowers lasted 6 days longer than old (10D) flowers (data not shown).

Respiration rate. – As in 'White Sim', the senescence of 'Chinera' flowers was associated with a climacteric peak of respiration. In contrast, 'Sandra' flowers showed a non-climacteric respiratory pattern (Fig. 3), with an initial asymp-

totic decline to a low steady respiration rate during the last 10 days of their vase life.

Ethylene production. – In 'White Sim' and 'Chinera' carnations, a climacteric increase in C_2H_4 production coincided with the respiratory peak (Fig. 4a). 'Sandra' flowers produced only trace amounts of C_2H_4 until the end of their vase life, when a slight increase in C_2H_4 production occurred after the petals had started to desiccate.

Changes in petal ACC content. – The petal ACC content of 'White Sim' and 'Chinera' flowers rose along with their increase in C_2H_4 production (Fig. 4b). Unlike C_2H_4 production, the petal ACC continued to increase after the corollas were senescent. The ACC content of 'White Sim' petals increased more than 10-fold during senescence. 'Chinera' petals had a higher ACC content than those of 'White Sim' at harvest, but their ACC content was only one-third of that of 'White Sim' during senescence, and the major increase in ACC occurred as the petals wilted and C_2H_4 production fell. The ACC content of petals from 'Sandra' flowers was similar to that of 'White Sim' petals at harvest, and remained low throughout their vase life.

Changes in petal EFE activity. – Petal EFE activity increased co-ordinately with the increases in C_2H_4 production and ACC content of senescing carnations (Fig. 4c). By Day 7, EFE activity in 'White Sim' was more than 100 times that at harvest. In contrast, the EFE activity of 'Chinera' petals increased only slightly during senescence to about 6% of that in senescing 'White Sim' petals. The EFE activity of 'Sandra' petals was one-third that of 'White Sim' flowers at harvest, and remained unchanged throughout their post-harvest life.

DISCUSSION

The role of C_2H_4 in senescence varies among different flowers. The most extensively studied, as models, are flowers of carnation, morning glory and trandescantia (Halevy and Mayak, 1979), where a climacteric increase in C_2H_4 production and respiration accompanies senescence of the flowers. They resemble the climacteric fruits such as bananas, apples, peaches, tomatoes and avocados (Biale and Young, 1962). Many flowers, however, do not show this "typical" pattern of changes during senescence. Cyclamen flowers neither produce nor are sensitive to C_2H_4 in natural senescence. They do, however, show a climacteric peak of C_2H_4 evolution and become sensitive to C_2H_4 after pollination (Halevy et al., 1984). Other flowers, such as chrysanthemum, produce very low levels of C_2H_4 through their life and are unaffected by exposure to C_2H_4 (Woltering and van Doorn, 1988). Our data indicate inter-

esting variations in the pattern of senescence amongst cultivars of carnation and a close correlation between prolonged vase life and inhibition and/or retardation of the normal climacteric of respiration and C_2H_4 production. Changes in fresh weight of the different cultivars reflected their different senescence patterns. The rapid loss of weight in 'White Sim' and 'Chinera' reflects the wilting that occurs immediately after the start of in-rolling of their petals. The long, slow loss of weight in 'Sandra' reflects the gradual petal desiccation, without in-rolling, that characterizes the senescence of this variety.

The asymptotic decline in the respiration of cut 'Sandra' flowers resembles that of harvested non-climacteric fruits. Like such fruits too, there is no increase in C_2H_4 production, nor of ACC or EFE activity during 'Sandra' senescence. The lack of petal in-rolling, shown by Wang and Woodson (1989) to be directly associated with the action of C_2H_4, suggests that normal senescence in 'Sandra' does not involve the action of C_2H_4. It thus seems clear that the long vase life and unique senescence pattern of 'Sandra', like the tomato mutant *rin*, is due to the low activity of the pathway for C_2H_4 biosynthesis, and a non-ethylene senescence.

'Chinera' flowers, although long-lived, eventually senesced in a typical carnation fashion, with in-rolling and wilting of the petals. The production of C_2H_4 by 'Chinera' is not only delayed (sufficiently to explain the long vase life of these flowers), but also considerably less than that of 'White Sim'. This low production is associated with low activity of the EFE, which is, however, sufficient to oxidize all the ACC generated by ACC synthase, since ACC levels in the petals rise only when C_2H_4 production falls and the petals are senescent. Indeed, most of this latter rise is probably due to desiccation of the petals, and consequent reduction in their fresh weight (the basis on which the ACC content is reported). The *Nr* mutant of tomato has reduced C_2H_4 and respiratory climacterics, like 'Chinera', and is somewhat affected by C_2H_4 exposure.

The observation that 'White Sim' and 'Chinera' flowers lived longer on the plant than when cut indicates that harvesting accelerates the processes that lead to C_2H_4 biosynthesis and eventual petal in-rolling. In a companion study (Wu et al., 1991), we report on the C_2H_4 sensitivity of these interesting carnation cultivars.

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