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Variation in the senescence of carnation (*Dianthus caryophyllus* L.) cultivars. II. Comparison of sensitivity to exogenous ethylene and of ethylene binding

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ABSTRACT

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Flowers of the 'Sandra' and 'Chinera' cultivars of carnation (*Dianthus caryophyllus* L.) last about twice as long (14 days) as those of 'White Sim' cultivar (7 days). Application of C_2H_4 shortened the vase life of all three cultivars. 'Chinera' flowers were the least sensitive to C_2H_4, and 'Sandra' flowers were more than 10 times as sensitive. Although 'Sandra' flowers normally produce very little C_2H_4, C_2H_4 treatment induced a climacteric in C_2H_4 production by this cultivar. The differences in C_2H_4 sensitivity could only partly be explained by differences in ethylene-binding activity (number of binding sites and K_d) in petal tissues from the different cultivars.

Keywords: carnation; cultivars; ethylene; ethylene binding; senescence.

Abbreviations: K_d = hormone-binding site dissociation constant; K_m = half-maximal hormone concentrations; STS = silver thiosulfate.

INTRODUCTION

The long vase lives of cultivars 'Sandra' and 'Chinera' carnation flowers are associated with the absence, or reduction and delay, of the climacteric rises in respiration and C_2H_4 production found in most carnation flowers (Wu et al., 1991). Long vase life may be due solely to delayed or inhibited C_2H_4 production, but might also reflect a reduction in sensitivity to C_2H_4. In ripening mutants of tomato, where C_2H_4 production is absent or reduced, sen-

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sitivity to C_2H_4 is also reduced. Since C_2H_4 is a common air pollutant, reduced C_2H_4 sensitivity is of more practical value than reduced C_2H_4 production, much as treatment with silver thiosulfate (STS) (to inhibit C_2H_4 action) is of more value, commercially, than treatment with aminooxyacetic acid (AOA) (to inhibit C_2H_4 biosynthesis).

In the present study, we examined the sensitivity of different carnation cultivars to C_2H_4, and we also examined the basis for the differences in sensitivity we encountered.

MATERIALS AND METHODS

Unless otherwise noted, the experiments used the materials and methods described previously (Wu et al., 1991). Exogenous C_2H_4 was applied by placing flowers in large glass tanks ventilated with flowing streams of air (ca. 30 l h^{-1}) containing C_2H_4 at the desired concentrations. Ethylene concentrations in the tanks were monitored daily. Each treatment had at least four replicates, and every experiment was repeated at least once.

The binding of C_2H_4 to carnation petals was measured following the procedure of Sisler (1979). Carnations were harvested and placed at 2°C overnight to allow wound C_2H_4 to subside before exposing them to $^{14}C_2H_4$. Petals were placed in 1 mm mesh metal screen baskets then sealed in 3.4 l jars fitted with a small vial containing 38 kBq of $^{14}C_2H_4$ (935 MBq $mmol^{-1}$, Amersham/Searle) bound as the $HgClO_4$ complex (Young et al., 1952). The jar was fitted with a trap containing 2 ml 40% NaOH to absorb CO_2, and a magnetic stirring bar at the bottom of the jar. The $^{14}C_2H_4$ was released into the jar from the complex by injecting 0.5 ml saturated LiCl into the vial (this gave about 0.1 $\mu l\ l^{-1}$ $^{14}C_2H_4$ in the jar). The labeling was carried out in the presence or absence of 1000 $\mu l\ l^{-1}$ unlabeled C_2H_4 to determine total binding sites. Petals were also labeled with $^{14}C_2H_4$ in the presence of different concentrations of unlabeled C_2H_4 to obtain a Scatchard plot from which the K_d for displacement of C_2H_4 was obtained (Sisler, 1979). After a 3 h exposure to $^{14}C_2H_4$, petals were removed from the jar in a fume hood and ventilated for 3 min, then resealed in 1 l jars. Released C_2H_4 was trapped in scintillation vials containing 0.3 ml of 0.25 M $HgClO_4$. The jars were placed in the dark for 15–20 h, then the scintillation vials were removed and 20 ml of scintillation fluid (RPI 3a70B) was added. The radioactivity of the trapped C_2H_4 was determined by liquid scintillation spectrometry (Beckman LS 100C).

RESULTS

Sensitivity to continuous C_2H_4 applications. — Continuous application of C_2H_4, even at very low concentrations, shortened the vase life of all three cultivars (Fig. 1). In response to C_2H_4 application, flowers of all three culti-

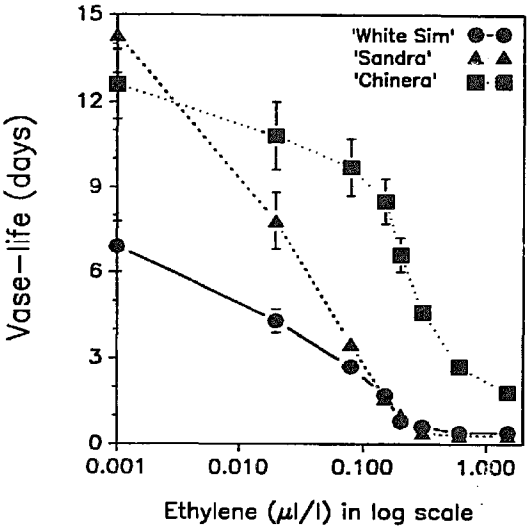

Fig. 1. Vase life of 'White Sim', 'Sandra' and 'Chinera' carnation flowers treated with different concentrations of C_2H_4. Flowers were harvested at commercial maturity and placed in large tanks ventilated with flowing streams of air containing different concentrations of C_2H_4. Data are the means ± SE of two experiments (five replicates per treatment).

vars, including 'Sandra', showed the in-rolling and wilting of petals which occurs during natural senescence of cultivar 'White Sim' flowers.

Sensitivity to short exposures of C_2H_4. — The reduction in vase life effected by C_2H_4 was a function of concentration and exposure time in all three cultivars tested (Fig. 2). The response surfaces indicate that, as in continuous exposure, the vase life of 'Sandra' flowers was reduced more than that of flowers of the other cultivars when they were exposed to C_2H_4 for short periods of time.

Ethylene production by C_2H_4-treated 'Sandra' flowers. — Although 'Sandra' flowers do not produce C_2H_4 during their senescence in air, a 16 h treatment with 0.6 μl l^{-1} C_2H_4 was sufficient to shorten the vase life and induce some C_2H_4 production (Fig. 3). As the C_2H_4 treatment time increased, the peak of C_2H_4 production by 'Sandra' carnations was earlier and higher. Climacteric C_2H_4 production in C_2H_4-treated 'Sandra' flowers commenced after petal in-rolling was visible. Treatment with 0.6 μl l^{-1} C_2H_4 for 12 h shortened the vase life of 'Sandra' flowers without inducing climacteric C_2H_4 production (Figs. 2 and 3), but petals on these flowers also in-rolled.

Measurement of C_2H_4 binding. — Carnation flowers incubated with saturating concentrations of unlabeled C_2H_4 in the presence of $^{14}C_2H_4$ retained much less radioactivity than those incubated in $^{14}C_2H_4$ alone. The difference, a

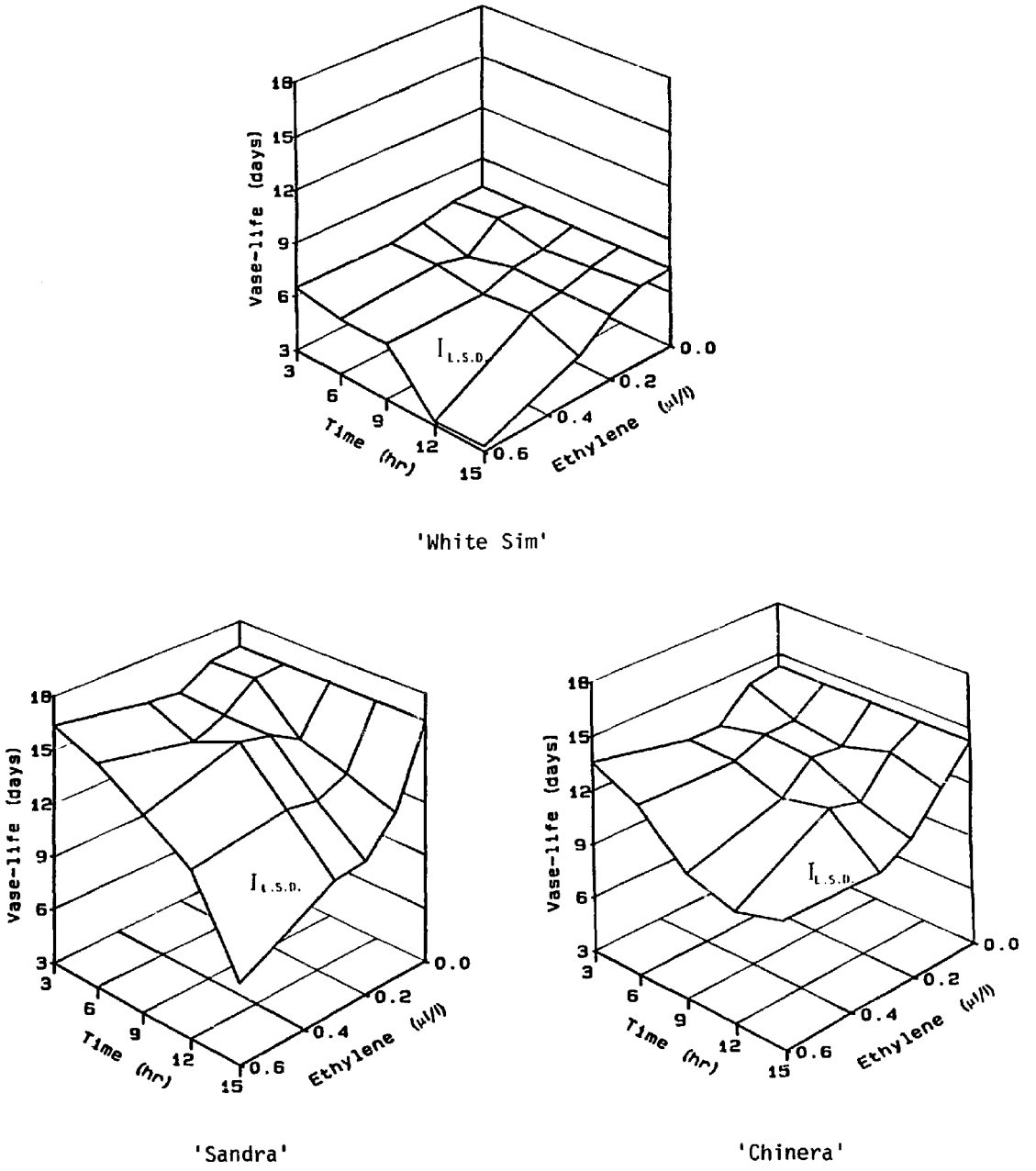

Fig. 2. Vase life of 'White Sim', 'Sandra' and 'Chinera' carnation flowers treated with different concentrations of C_2H_4 for different periods. Flowers harvested at commercial maturity were placed for different lengths of time in large tanks ventilated with flowing streams of air containing different concentrations of C_2H_4. At the end of the C_2H_4 exposure, the flowers were placed in a similar tank ventilated with C_2H_4-free air for determination of vase life. Data are the means of two experiments (five replicates per treatment). The LSD ($P = 0.05$) is represented by the vertical bar in each panel.

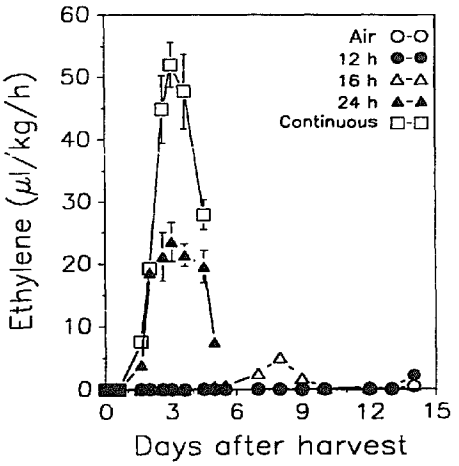

Fig. 3. Ethylene production of 'Sandra' carnation flowers treated with 0.6 ppm C_2H_4 for different periods. Flowers harvested at commercial maturity were placed for different lengths of time in large tanks ventilated with flowing streams of air containing different concentrations of C_2H_4. At the end of the C_2H_4 exposure, the flowers were placed individually in 0.5 l jars, which were sealed at intervals for determination of C_2H_4 production by the flowers. Data are the means ± SE of two experiments (five replicates per treatment).

TABLE 1

Ethylene binding activity assayed by $^{14}C_2H_4$ competition with unlabeled C_2H_4 from 'White Sim', 'Sandra' and 'Chinera' carnation petals. Data are the means of two experiments (four replicates per treatment)

	Unlabeled C_2H_4 ($1000\ \mu l\ l^{-1}$)	Binding (Bq kg^{-1} fresh wt.)	No. of binding sites/cell	K_d ($\mu l\ l^{-1}$)
'White Sim'	–	3633		
	+	98	26045	0.020
'Sandra'	–	4645		
	+	145	38831	0.035
'Chinera'	–	3030		
	+	97	25608	0.043

measure of specific C_2H_4 binding, was similar for flowers of 'White Sim' and 'Chinera' (Table 1). Specific binding (Sisler, 1979) was somewhat higher in 'Sandra' flowers. A Scatchard plot (Fig. 4) of the data obtained by incubating flowers with $^{14}C_2H_4$ and different concentrations of unlabeled C_2H_4 was used to estimate the K_d for the binding sites and the number of binding sites per cell.

'Sandra' carnation petals had 50% more C_2H_4 binding sites with a substantially lower affinity for C_2H_4 (higher K_d) than those of 'White Sim' (Table 1). The binding sites in 'Chinera' petals had an even lower affinity for C_2H_4.

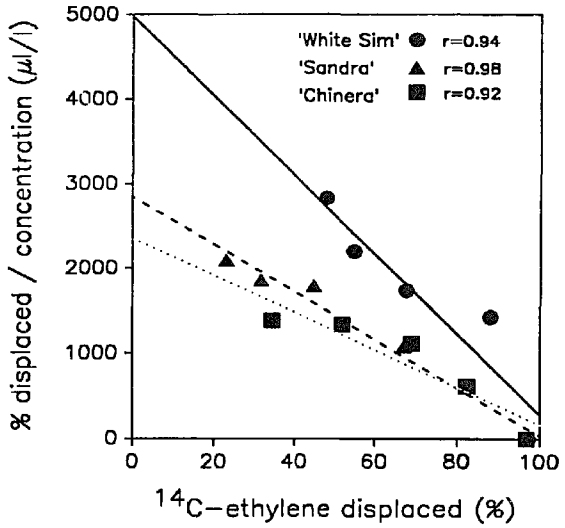

Fig. 4. Scatchard plots of $^{14}C_2H_4$ displacement from petals of different carnation cultivars equilibrated with $^{14}C_2H_4$ in the presence of different concentrations of unlabeled C_2H_4. Data are the means of two experiments (four replicates per treatment).

DISCUSSION

The data reported here indicate that the long vase life of 'Sandra' flowers is due to their inability, under normal conditions, to produce C_2H_4. This cultivar proved to be more sensitive than the others to exogenous C_2H_4, whether applied continuously, or for short periods of time (Figs. 1 and 2). In this respect 'Sandra' is different from the *rin* tomato mutant, which lacks C_2H_4 production but is also insensitive to applied C_2H_4, and more like the non-climacteric fruits, which do not produce C_2H_4, but respond to exogenous applications (Biale and Young, 1962). Unlike non-climacteric fruits, however, the response of 'Sandra' flowers to C_2H_4 includes the climacteric production of C_2H_4 (Fig. 3). These features of 'Sandra' make this cultivar less useful in efforts to improve the life of commercial carnations, since its vase life will be drastically reduced at concentrations of C_2H_4 commonly found in areas where flowers are handled and sold.

In marked contrast to 'Sandra', flowers of 'Chinera' not only produce less C_2H_4 during natural senescence, but are also much less sensitive to C_2H_4 (Figs. 1 and 2). This is analogous to the *Nr* tomato ripening mutant which has similar characteristics (Roberts et al., 1987). 'Chinera' and its relatives, particularly '8367', which has been suggested to confer long vase life (W.G. van Doorn, personal communication, 1988), may be very useful in efforts to improve the vase life and C_2H_4 resistance of carnations using conventional breeding techniques.

The effects of different C_2H_4 concentrations on the vase life of the three

cultivars examined may be depicted in a double reciprocal plot (Fig. 5). The intercepts of these linear regressions (calculated without the results of the lowest concentrations in the C_2H_4-producing cultivars 'White Sim' and 'Chinera') indicate the lowest K_m for 'Sandra' flowers, followed by 'White Sim' and 'Chinera'. This derived K_m should reflect the affinity of C_2H_4 for its binding site in the hypothetical scheme for C_2H_4 induction of flower senescence (Sisler et al., 1983). This scheme proposes that C_2H_4 binds to a specific binding site, an interaction which results in release of a "second message", a molecule which itself, or through the mediation of subsequent messenger molecules, results in transcription of new mRNA species from the genome.

Sisler (1979) reported an isotope competition technique for assaying specific C_2H_4 binding, but there is still no direct evidence that the binding activity assayed in this way reflects binding to an active receptor (Sisler and Goren, 1981). A number of authors have examined the relationships between C_2H_4 sensitivity and binding activity measured as proposed by Sisler and have not found a consistently close relationship. Although Sisler et al. (1986) showed that STS treatment of carnations reduced binding activity, and Bleeker et al. (1988) showed reduced binding activity in a C_2H_4-insensitive mutant of *Arabidopsis thaliana*, Blankenship and Sisler (1989) found no change in activity in flowers of *Ipomoea* (morning glory) during a period when the flowers' sensitivity to C_2H_4 is known to increase dramatically. The different sensitivities of the carnations studied here provide another means of assessing the physiological significance of binding activity measured by the isotopic

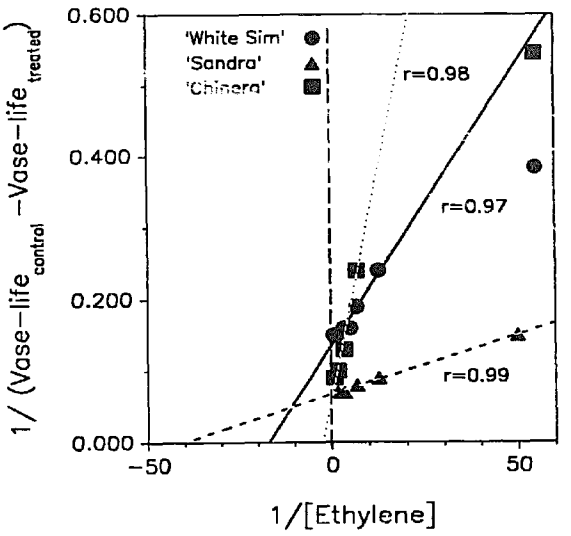

Fig. 5. Double reciprocal representation of the effects of C_2H_4 concentration on vase life of flowers of 'White Sim', 'Sandra' and 'Chinera' carnations. Flowers were harvested at commercial maturity, and placed in large tanks ventilated with flowing streams of air containing different concentrations of C_2H_4. Data are the means of two experiments (five replicates per treatment).

competition technique. Here, too, the results are not completely consistent. The relatively low binding affinity in 'Chinera' petals is consistent with the high K_m (half-saturating C_2H_4 concentration, determined from the abscissa intercept) for C_2H_4 action in this cultivar (Fig. 5), but the K_d for 'Sandra' is much higher than that anticipated from the extreme sensitivity of this cultivar. The higher number of binding sites calculated for 'Sandra' petals might offset their reduced affinity.

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