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Postharvest petal senescence of two cultivars of carnation flowers with different vase lives

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Abstract

This work studies the influence of variations in free polyamines, 1-aminocyclopropane-1-carboxylic acid and ethylene, and their possible relationship, during the different development stages of two carnation cultivars (*Dianthus caryophyllus* L. cultivars Domingo and Famosa) with noticeable differences in vase life. A pre-treatment with ethylene or silver thiosulphate helped to verify the possible link between polyamines, ethylene biosynthesis and carnation vase life. The senescence periods in carnation petals, both untreated and treated with ethylene (1 ppm for 8 h) and silver thiosulphate (1 mM for 2 h), were studied at 21^{o}C and 60-70% relative humidity during eight different stages of senescence. ´Famosa´ (long-life cultivar) was associated with lower ethylene production and 1-aminocyclopropane-1-carboxylic acid concentration in petals compared with ´Domingo´ (short vase-life cultivar). The effects of pre-treatments with exogenous ethylene or silver thiosulphate were only evident in the short-life cultivar Domingo, in which ethylene production were increased or reduced, respectively. Silver thiosulphate reduced total ACC content during senescence in Domingo. The total polyamine content in Famosa (128-235 nmol g^{-1} FW) was lower than in Domingo (220-372 nmol g^{-1} FW). High free Putrescine and Spermidine concentrations were detected in earlier stages of flower senescence in the short vase-life cultivar. The high percentage of spermine in the petal tissue of 'Famosa' may inhibit ACC accumulation and ethylene production, resulting in increased flower longevity.

Keywords: *Dianthus caryophyllus*, ethylene production, exogenous ethylene, silver thiosulphate, putrescine, spermidine, spermine
Abbreviations: ACC_1-aminocyclopropane-1-carboxylic acid, EP_ethylene production, ACC synthase_1-aminocyclopropane-1-carboxylic acid synthase, ACC_oxidase-1-aminocyclopropane-1-carboxylic acid oxidase, SAM_*S*-adenosyl methionine, Pas_polyamines

Introduction

Senescence in carnation flowers is associated with a climacteric-like increase in EP, although a degree of ethylene-independent flower senescence has also been reported (Shibuya, 2012). In petal tissue, ethylene is responsible for inducing many of the biochemical processes leading to programmed cell death, including the activation of senescence-related gene transcription (Lawton et al., 1990; Woodson et al., 1992; Shibuya, 2012). Before the ethylene burst, flowers produce a low constant rate of ethylene, but this is followed by a coordinated increase in the activities of ACC synthase and ACC oxidase (Peiser, 1986; Serrano et al., 1991; Tanase et al., 2008), which converts SAM into ACC which, in turn, is converted into ethylene. A long vase life in some carnation cultivars has been correlated with low expression, in the gynoecium and petals, of some genes of the *Dianthus caryophyllus* (DC) ACS and ACC oxidase (ACO) multigenic families (DC-ACS1, DC-ACS2 and DC-ACO1) (Tanase et al., 2008). Low sensitivity to ethylene is a common target to select carnation for breeding purposes (Onozaki et al., 2008). Endogenous ACC levels in different flower parts increase during senescence (Nichols et al., 1983). Exposure of isolated carnation petals, separated into upper and basal parts, to ethylene shows that most ethylene evolves from the basal part of the petals. The endogenous ACC content of the basal portion of senescing carnation petals is 3 to 5 times higher than that of the upper parts. During flower senescence, ACC is translocated from the basal part, where it is synthesized, to the upper part, where it is converted into ethylene (Overbeek and Woltering, 1990). It is reported that exogenous ACC treatments advance carnation senescence (Pun et al., 2001a). A degree of competition between PAs and ethylene, through their common precursor SAM (Valero et al., 2002), has been demonstrated, the balance between these two growth regulators being critical for retarding or accelerating the senescence process (Bouchereau et al. 1999; Valero, 2010). However, the effects of PAs on ethylene production vary greatly in different plant species and tissues and in the face of different treatments (Li

et al., 2004). A lower amount of total PAs has been associated with greater longevity (Serrano et al., 1991). PAs, especially spermine, delayed the senescence of cut carnation flowers and reduced ethylene production, endogenous ACC content and the activities and transcript amounts of ACC synthase and/ ACC oxidase in petals (Lee et al., 1997). An increase in tomato fruit shelf life and reduced senescence has been associated with an enhanced PA content, particularly spermidine (Nambeesan et al., 2010). However, other treatments which enhance carnation vase life, such as the ethanol vapor treatment, fail to control the production of spermidine, indicating that several factors may induce the delay in the senescence process (Pun et al., 2001b). This work studies the influence of variations in free polyamines, ACC and ethylene, and their possible relationship, during the different development stages of two carnation cultivars with noticeable differences in vase life. A pre-treatment with ethylene or silver thiosulphate helped to verify the possible link between polyamines, ethylene biosynthesis and carnation vase life.

Results

Flower longevity

The mean vase life of Domingo was 13.8 days but only 10.3 days when exposed to ethylene, whereas Famosa flowers showed a 26 day vase life, and 23.8 days when exposed to ethylene (Fig 1). STS pre-treatment prolonged flower longevity in the Domingo cultivar (Fig 1) and was ineffective in extending the vase life of Famosa, while exogenous ethylene treatment did not produce a significantly lower vase life than seen for the control (Fig 1).

Ethylene production during flower senescence and senescence symptoms

During the first 4 stages after harvest, ethylene production was not detected in the flowers of either cultivar, except in Domingo carnations previously exposed to ethylene (Fig 2A). The burst in EP was earlier and 7-fold higher in the petals of Domingo compared with Famosa (Fig 2C). Maximum ethylene production in Domingo coincided with the appearance of senescence symptoms, such as petal in-rolling and wilting. However, Famosa did not show either type of behaviour. The first visible symptom of senescence in this flower was necrosis of the petal tips, which is consistent with the findings of Brandt and Woodson et al. (1992). Ethylene treatment brought the EP peak forward in both cultivars, but only stimulated higher ethylene production in Domingo, while in Famosa greater variability in ethylene production was found (Fig. 2A). STS delayed the peak of ethylene production in Domingo (Fig 2B).

Total and free ACC levels during flower senescence

Total and free ACC levels were very low in the early stages of flower senescence after harvest in both cultivars and slowly increased after the fourth or fifth day after harvest (in stage 3 in Domingo and in stage 4 in Famosa) (Fig 3). In cv. Domingo, the upsurge of ACC (total or free) preceded the peak of ethylene production in stage 6 (Fig 2). Total ACC levels in the Domingo cultivar (0.50-26.12 nmol g^{-1} FW) were greater than those found in Famosa (0.10-7.72 nmol g^{-1} FW). In Famosa carnation, no difference in free and total ACC between treatments was observed (Fig 3B-3D). However, total ACC increased in Domingo carnation

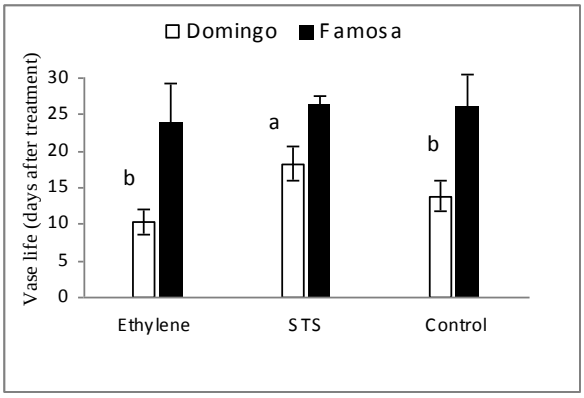

Fig 1. Vase life of Famosa (■) and Domingo (□) flowers submitted to different treatments (mean ± SE, *n*=10). Ethylene=Treatment with 1 ppm ethylene for 8 h. STS=1 mM silver-tiosulphate for 2 h. Control=Control in air. Different letters above the column indicate significant differences between the treatments in ´Domingo´at p≤0.05 while differences in ´Famosa´ were non-significant.

cultivars subjected to exogenous ethylene pre-treatment (Fig 3C). Overall, STS pre-treatment in Domingo carnation cultivars reduced the total ACC concentration (Fig 3C).

Polyamine levels

The main significant effects (P≤0.05 or below) on polyamine levels were associated with the senescence stage and, to a lesser extent, with the effect of the treatments (always being significant in both cultivars, except for Spermine or Spermidine in Famosa), or the treatment x stage interaction significant for the three polyamines in both cultivars (except for Spermine in Domingo) (data not shown). Putrescine, and especially Spermidine, were the predominant polyamines in both cultivars, while Spermine was detected in low levels (14-21 nmol g^{-1} FW in Domingo and 16-26 nmol g^{-1} FW in Famosa) during the petal senescence period (Fig 4). During the first 4 stages of flower senescence there was no significant difference in the trend of the total PA content, although total PA levels in Domingo were higher than those found in Famosa (Fig 4G- 4H). In Domingo, maximum total PA levels were found in stage 6 (386 nmol g^{-1} FW), and in Famosa in stage 5 (maximum concentration 230 nmolg^{-1} FW) (Fig 4G-4H). In Domingo, the Putrescine concentration increased sharply from stage 3 to a maximum in stage 6 (Fig 4A). Famosa carnations showed a similar pattern of change in Putrescine, although its concentration was lower than in Domingo (Fig 4A-4B). In Domingo, the Spermidine content increased rapidly from stage 5 and then remained constant until the end of the study, while in Famosa, Spermidine only increased in stage 5 and then decreased (Fig 4C-4D). The maximum Spermidine concentration in Domingo (222 nmolg^{-1} FW in stage 6) was about 2-fold higher than in Famosa (117 nmol g^{-1} FW in stage 5) (Fig 4D).

Discussion

The postharvest behavior of Domingo was very different from that of Famosa, as evidenced by its shorter vase life, which was concomitant with higher ACC and ethylene production levels, and was in agreement with the findings of Brandt and Woodson et al. (1992). In contrast, the longer shelf life found in Famosa was associated with low EP and a low rate of increases in total ACC. This low ethylene

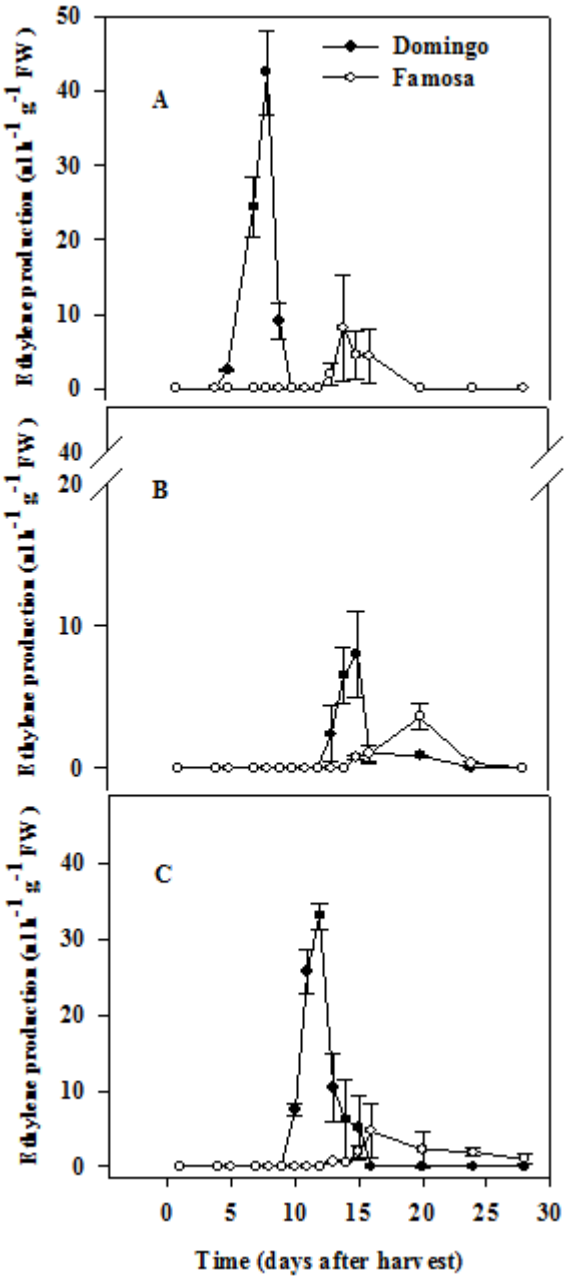

Fig 2. Ethylene production (mean ± SE, *n*=4) in petals of two carnation cultivars (Domingo and Famosa). A. Treatment with 1 ppm ethylene for 8 h. B. Treatment with 1 mM silver-tiosulphate for 2 h. C. Control in air. Senescence stages are defined in Fig 5.

production was due to a combination of two factors: limited available ACC, probably as a result of low ACC synthase activity and a failure to convert ACC into ethylene because of restricted ACC oxidase activity (Serrano et al., 2001; Serrano et al., 1991). Another possibility could be the presence of different variants of carnation ACC synthase genes, leading to differences in ACC synthase activity (Satoh et al., 2011). Exogenous ethylene and STS were only effective in Domingo carnation (short vase life), while in Famosa (long vase life) neither treatment was effective and this latter cultivar can be considered ethylene-insensitive. These results suggest that the responses to treatments differ not only between species but also within carnation cultivars, which agree with the findings of Pun et al. (2001a) and with results for other flowers (Shahri and Tahir, 2011; Shibuya, 2012). The effectiveness of STS in preventing the increase in ACC in the short vase life cultivar Domingo and the subsequent steps of ethylene biosynthesis (Fig 3) were consistent with the findings of Bufler et al. (1980) in cv. White Sim., but treatment of cut carnation with STS had no effect on the levels of Pas, reflecting the findings of Serrano et al., (1999). The relationship between ethylene and polyamine content is not clear and contradictory results have been found not only in different species, but also between various studied carnation cultivars. Pas and ethylene depend on a common precursor, SAM, for their biosynthesis. But these two molecules have opposite effects in relation to senescence (Pandey et al., 2000). When d-arginine, difluoromethylarginine and methylglyoxal bis were used to inhibit specific steps in polyamine synthesis, ethylene production and the onset of senescence were promoted (Roberts et al., 1984). If a high concentration of polyamines contributes to suppressing flower senescence, a long vase life cultivar would be expected to contain a high polyamine concentration. In our case, the cultivar with a high polyamine concentration, Domingo, showed high ethylene production and a short vase life. Related with this, Lee et al. (1997) found that the application of spermine delayed the senescence of cut carnation flowers and reduced ethylene production, the endogenous ACC content and the activity and transcript amounts of ACC synthase and ACC oxidase in petals. However, also in carnation, Pandey et al. (2000) found that treatment with PA did not always increase flower longevity and may even result in an accelerated senescence. On the other hand, the intracellular free polyamine pool not only depends on ethylene synthesis, but also on several processes such as polyamine degradation, polyamine transport and polyamine conjugation (Galston and Kaur-Sawhney, 1990; Martin-Tanguy, 2001), processes which also have some influence on polyamine concentration. Additionally, differences between cultivars may be associated with differences in metabolic processes due to different genetic backgrounds. If we consider the total amount of polyamides, the percentage of Spermidine in both cultivars was similar, 56 % in Domingo and 60 % in Famosa. However Famosa had a high percentage of Spermine (6 - 20%) while Domingo showed a low percentage of between 4 and 6%. Also, Famosa presented a high concentration of Spermine in the first stage of senescence, which could be responsible for its greater longevity. Spermine and Spermidine seem to be more active in retarding senescence according to Pandey et al (2000).

Materials and Methods

Plant material

Carnations (*Dianthus caryophyllus* L. cvs. Domingo and Famosa) were obtained from a local greenhouse in La Mojonera (Almeria, Spain). Flowers were harvested at the early stage of flower opening and immediately transferred to the laboratory at the University of Almeria (Spain). Both cultivars were selected according to the differences they showed in previous studies on carnation vase life (Ebrahimzadeh et al., 2011): Domingo showed a short vase-life while Famosa showed a long vase-life behavior. In the laboratory, the flowers were trimmed to a 30-cm stem length, and randomly divided into three groups, which were assigned to three different treatments. The first pre-treatment was exposure to 1 ppm ethylene for 8 h, with the stems kept in distilled water. In the second treatment, flowers were kept in

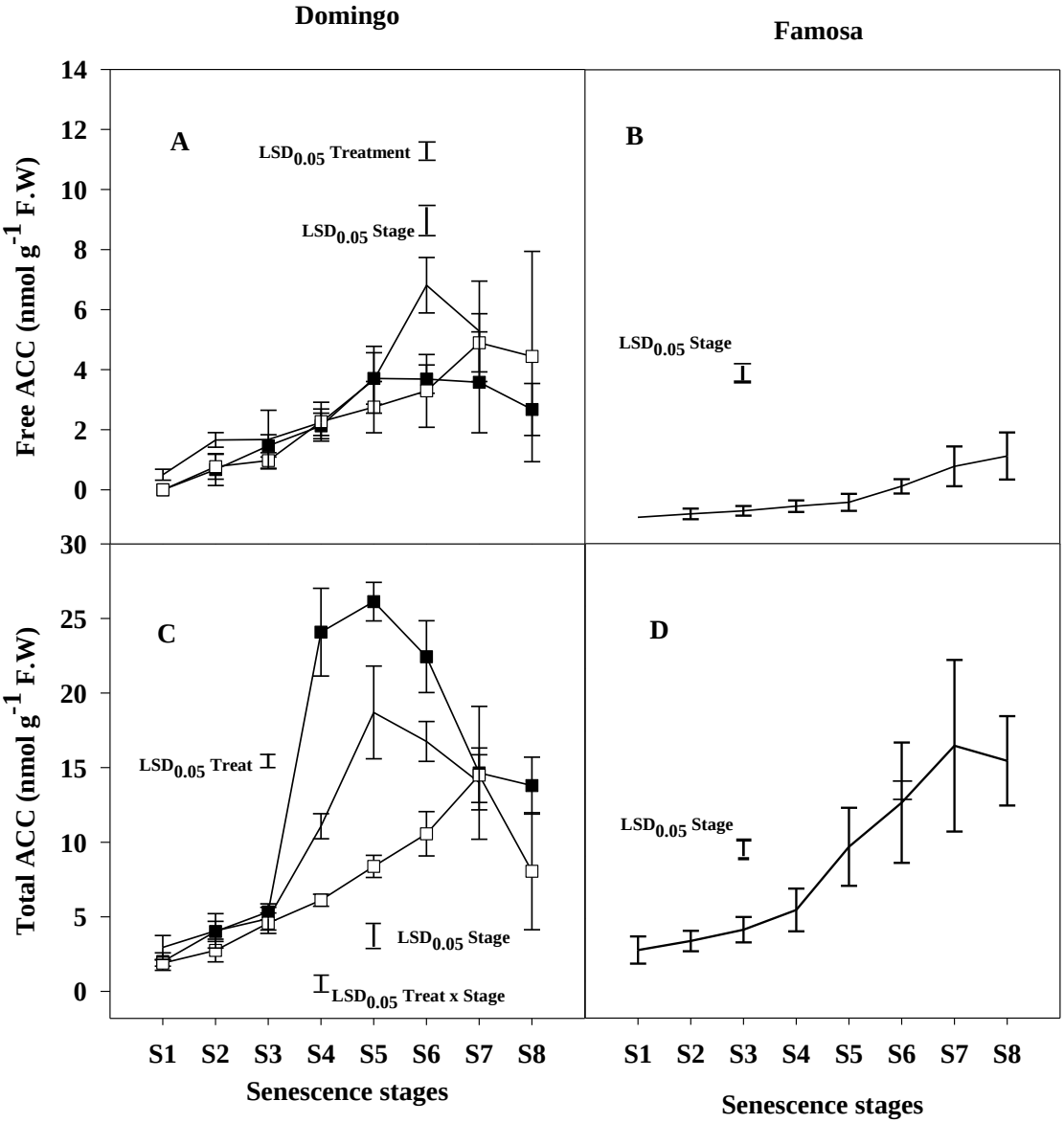

Fig 3. Free (A, B) and Total ACC levels (C, D) (mean ± SE, *n*=4) in petals of two carnation cultivars (Domingo and Famosa, left and right, respectively) after different treatments: 1 ppm ethylene for 8 h (■), treatment with 1 mM silver-tiosulphate for 2 h (□), control in air ( ). Flowers were analyzed during eight senescing stages after harvest. Bold bars are the least significant difference (LSD) at p≤0.05 calculated for the interaction senescence stage x treatment reported in Fig 5.

a solution of silver thiosulphate (STS) at 1 mM for 2 h, and then another 6 h in distilled water (all in an air atmosphere). Control flowers were kept in distilled water and in an air atmosphere for 8 h. After the treatments (start of the measurements), flower stems in individual test tubes were maintained in distilled water and the volume was kept constant by replenishing the test tubes every two days. The environmental conditions maintained throughout the experiment were 12 h light, 12 h darkness at 21 ±1ºC and 60-70% relative humidity. At eight different stages of senescence after harvest (Fig 5), petals from ten flowers from each treatment were removed from the external whorls of the flower and strictly randomized into four groups of two to obtain 1-2 g of petals, in which ACC content, ethylene production and polyamine concentrations were analyzed.

Carnation vase life

The vase life of each flower was defined as the number of days elapsing after cutting until the petals showed in-rolling, or browning, and had no decorative value (Nukui et al., 2004). Flowers were evaluated daily. Vase life values are the mean of 10 flowers.

Ethylene production

Ethylene production was measured in four replicates of all senescence stages throughout the experiments. Firstly, petals were separated by hand from other parts of the flowers, and then the outer petals of each flower were enclosed in 20-mL

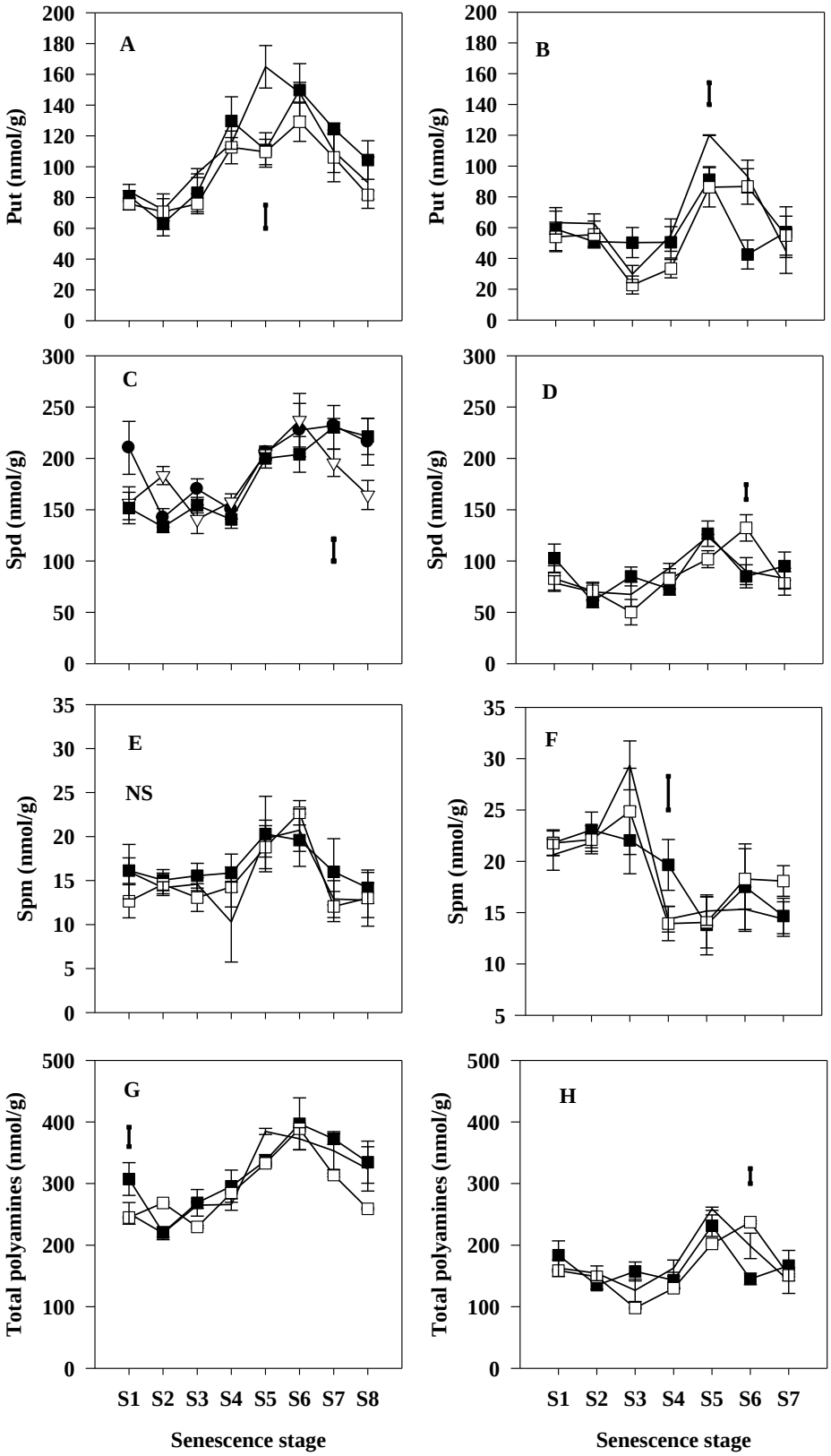

Fig 4. Changes in the levels of polyamines: (A, B) Putrescine (Put); (C, D) Spermidine (Spd); (E, F) Spermine (Spm); (G, H) total free polyamines (mean ± SE, *n*=4) in two carnation cultivars: Domingo (left) and Famosa (right) during flower senescence. Bold bars are the least significant difference (LSD) at $p \leq 0.05$ calculated for the interaction senescence stage x treatment reported in Fig 1. Treatments were: 1 ppm ethylene for 8 h (■), treatment with 1 mM silver-tiosulphate for 2 h (□) and control in air ( ).

	Senescence stage	Features
Pre-senescence	Stage 1	Flowers open to 1/3 of the final size
	Stage 2	Flowers open to 2/3 of the final size
	Stage 3	Fully open flowers
	Stage 4	most of petals horizontally oriented
Climacteric	Stage 5	Flowers start to show slight wilting
	Stage 6	Flowers start to show senescence typical symptoms such in-rolling , wilting or form necrotic spots on petal tips
Post -senescence	Stage 7	Flowers wilt clearly and formed necrotic points on petals
	Stage 8	Completely wilted petals; dry petal tips and show considerable fading and sometime decay

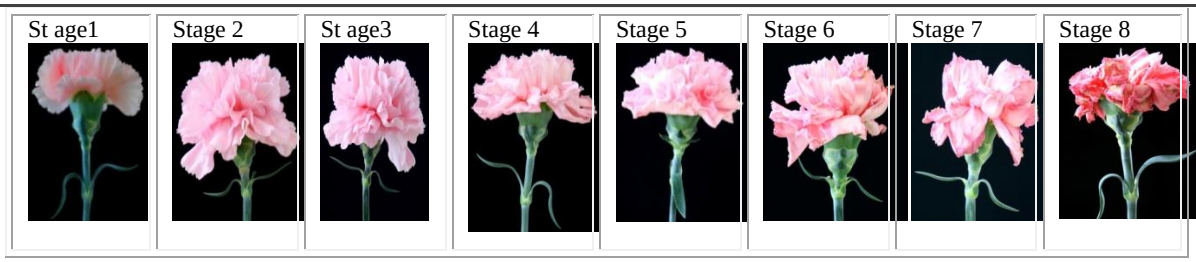

Fig 5. Flower senescence process and days after harvest classified in eight stages in carnation flowers.

glass jars and incubated at 21°C (Lee et al., 1997). After 60 min incubation, a 1-ml gas sample was injected into a gas chromatograph (Varian 3900) fitted with a flame ionisation detector and a GS-Q 30 m x 0.53 mm ID column (Agilent J&W, USA), according to the chromatographic conditions reported by Ebrahimzadeh et al. (2011).

ACC extraction and assay

Total ACC (free and conjugated) was extracted as described by Serrano et al. (2001) by using 2 g of petal tissues. The ACC content was determined according to Lizada and Yang (1979). The efficiency of ACC conversion to ethylene was determined by comparison with the production of ethylene from an internal ACC standard. Conjugated ACC was hydrolyzed to free ACC with 6 N HCl at 100°C for 1 h according to Hoffman et al. (1983). The hydrolyzed sample was neutralized with NaOH (13.25 N) and used for quantification of total ACC. A relative calibration procedure was used to determine the amount of ACC in samples using a standard curve of ACC from Sigma-Aldrich (Germany). The results were expressed as nmol per gram fresh weight (nmol g^{-1} FW), and are the mean ± S.E of 4 replicates.

Polyamine (putrescine, spermidine, spermine) extraction and quantification

Polyamines were extracted with $HClO_4$ and analyzed by the benzoylation method. Extracts for polyamine analysis were prepared in duplicate by homogenizing 10 carnation petals as previously reported by Serrano et al. (1991). Benzoyl-polyamines were analyzed by HPLC using a Hewlett–Packard system, series 1100 (Waldbrom, Germany). The elution system consisted of methanol: water (64:36, v/v as solvent), run isocratically with a flow rate of 0.8 mL min^{-1}. The benzoyl-polyamines were eluted through a reverse-phase column (LiChroCart 250-4, 5 µm, Merck, Darmstadt, Germany) and detected by absorbance at 254 nm. A relative calibration procedure was used to determine the amounts of polyamines in the samples using standard curves of putrescine, spermidine and spermine and adding hexanediamine as the internal standard. Results (mean ± S.E) were expressed as nmol per gram fresh weight (nmol g^{-1} F.W.).

Statistical analysis

Statistical analysis was carried out individually in each cultivar because the stage considered did not correspond to the same temporal scale (Fig 5). The experimental design consisted in a randomized complete block design per cultivar. For every cultivar, a two-way analysis of variance was performed for each of the traits measured (ethylene production, ACC or polyamines), using pre-treatments (exogenous ethylene, STS and control), 4 replications and the stages of senescence (a qualitative variable, 1 to 8) as factors (Fig 5). If significant effects were detected in the ANOVA, a least significant difference (LSD) test at $p \leq 0.05$ was applied. Otherwise, only means of the significant effects were plotted in the figures.

Conclusions

Petal senescence in the short-lived cultivar Domingo was associated with a high polyamine concentration, coinciding with higher ethylene and ACC production. However, petal senescence in Famosa was accompanied by reduced total ACC and ethylene production (concomitant with lower polyamine accumulation). Exogenous STS and ethylene pre-

treatments were much more effective in the cultivar with a shorter vase life (Domingo) than in Famosa carnations, which showed a longer vase life and limited ethylene sensitivity.

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