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Effect of Methyl Jasmonate and Sucrose on Endogenous Non-structural Carbohydrates in Petals and Leaves of Cut 'First Red' Roses (*Rosa hybrida* L.)

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Abstract

Effects of exogenously applied methyl jasmonate (MeJA) and sucrose on vase life and non-structural carbohydrate concentration in petals and leaves of cut 'First Red' roses were investigated. Roses were placed in sealed plastic containers and received MeJA vapour treatment (0.1 µL MeJA L^{-1}) for 24 h at 20°C. Flowers were then placed in individual bottles containing 0 or 2% (v/v) sucrose solution. Flower petals and the two uppermost five-leaflet leaves were detached on days 0, d 5 and d 10 of vase life. Samples were individually snap-frozen in liquid nitrogen and freeze-dried. Non-structural carbohydrates were extracted and quantified using standard HPLC coupled to evaporative light scattering detection. The MeJA vapour treatment enhanced vase life of flower and foliage of 'First Red' roses. Significant differences were observed between foliage life of cut 'First Red' roses that were treated with MeJA and sucrose, but not for flower life. Rose stems treated with MeJA in the absence of sucrose had an extended vase life compared to roses treated with 2% sucrose alone (14.0 vs. 12.8 days of vase life). Sucrose and myo-inositol, and to a lesser extent glucose concentrations in petals of cut roses decreased during vase life, even when flowers were supplied with 2% sucrose. Concomitant to this, fructose levels in petals increased during vase life. Neither sucrose nor MeJA had a significant effect on any of the sugars measured in petals of cut roses. In contrast, significant differences were apparent for all sugars measured in leaves that were treated with MeJA and sucrose solutions. The combination of MeJA and 2% sucrose solution sharply increased endogenous sucrose concentration in leaves, but the opposite was shown in the absence of 2% sucrose. Sucrose treatment alone did not consistently alter endogenous sucrose concentration. Interactions between MeJA and sucrose on sugar metabolism are discussed.

Keywords: Fructose, Glucose, Myo-inositol, *Rosa hybrida* L., Sucrose.

INTRODUCTION

Many authors have demonstrated the positive effect of sucrose in enhancing vase life of cut flowers (Halevy and Mayak, 1979; Pompodakis *et al*., 2004; Ranwala and Miller, 2009). Methyl jasmonate has also been reported to promote vase life of several cut flowers, including freesias (*Freesia hybrida* L.) (Darras *et al*., 2005; 2006; 2007), roses (*Rosa hybrida* L.) (Meir *et al.*, 1998; Meir *et al.*, 2005) and peonies (*Paeonia lactiflora* L.) (Gast, 2001) by suppressing Botrytis cinerea or by closing the stomata in kalanchoe (*Kalanchoe blossfeldiana* L.) (Engelmann *et al.,* 1997) and nicotiana (*Nicotiana glauca* L.) flowers (Suhita *et al.,* 2003). Studies have also determined that sugar solutions affect longevity of cut flower viz. carnations (*Dianthus caryophyllus* L.) (Dilley and Carpenter, 1975; Sacalis and Lee, 1987), limonium (*Limonium hybrid*) (Doi and Reid, 1995), snapdragons (*Antirrhinum majus* L.) (Ichimura and Hisamatsu, 1999), delphinium (*Delphinium hybrid*) (Ichimura *et al.*, 2000) and roses (*Rosa hybrida* L.) (Mayak *et al.*, 2001; Pompodakis *et al.*, 2004). Soluble carbohydrate concentrations in petals of sweet pea (*Lathyrus odoratus* L.) flowers continuously treated with sucrose 10% (w/w) for 24 h at 23°C increased during vase life (Ichimura and Suto, 1999). However, the concentration of glucose and fructose in sepals of cut delphinium (*Delphinium hybrid*) flowers that received 0.55 M glucose, increased during vase life, yet sucrose concentration was not affected (Ichimura *et al.,* 2000). This study aimed to examine the possible interaction between MeJA and sucrose treatment in extending vase life of cut roses.

MATERIALS AND METHODS

Plant materials and experimental design

Flower stems of *Rosa hybrida* L. 'First Red' roses were grown to the tight bud stage in a commercial glasshouse in Ierapetra, on the south-eastern coast of Crete (Greece). Flower stems were placed in individual bottles containing 300 mL of 10 mg mL^{-1} dichloroisocyanuric acid (DICA) solution. The vase life experiment was carried out in a vase life room at 20 ± 2 °C and 60



Fig. 1. Successive stages of petals and leaves used to record vase life of flower and foliage of cut 'First Red' roses.

± 10% relative humidity (RH). Light intensity of 35 µmol m^{-2} s^{-1} photon flux was calculated with a photometer (Macam Photometrics Ltd., UK) and administered at a 12 h on-off cycle by lamps throughout the experiment (Pompodakis *et al*., 2004). After cutting, roses were treated with MeJA (0 and 0.1 µL MeJA L^{-1}) and stood in vases containing sucrose (0 and 2% (v/v)) plus 10 mg mL^{-1} DICA (n = 20). All chemicals used were of analytical grade and were obtained from Sigma (WI) unless otherwise indicated.

Vase life evaluation

Longevity was recorded as days of vase life from the time that each flower was placed into an individual bottle (d 0). Flowers were considered to have ended their vase life when the whole flower lost its decorative value; showing signs of wilting or fading in most petals (Mayak *et al.,* 1974). Leaves were considered to have ended their vase life when more than 50 % of the leaflets had shriveled, faded or fallen. Flower opening was monitored by measuring the maximum diameter of flower head and by using an arbitrary scale. The following stages were used to record flower opening: 1: tight bud stage, the half sepals are released, 2: bud begins to open, some petals are released, 3: the half petals are released, but stamens still not visible, 4: flower almost open, stamens are just visible, 5: flowers fully open with advanced signs of color fading (Fig. 1, left). The following stages were also adopted to record foliage life: 1: foliage green with good turgor, 2: foliage with the first signs of fading, 3: loss of turgor in some leaflets, 4: foliage with advanced signs of shriveling and fading, 5: almost all leaflets have shriveled and fallen (Mayak *et al*., 1974) (Fig. 1, right).

MeJA postharvest vapour treatment

MeJA vapour treatment was performed in sealed plastic containers (60 cm x 90 cm x 60 cm) for 24 h at 20 °C, in which flowers stems were stood in bottles containing deionised water. MeJA was left to evaporate from a cotton pad soaked with 1% (v/v) liquid MeJA in ethanol (Darras *et al.*, 2005) placed inside the plastic container. The concentration of MeJA vapour was 0.1 µL L^{-1} assuming that complete evaporation was achieved (Darras *et al.*, 2005). Control rose stems were treated in the same way, but with pure ethanol not MeJA The cotton pad dried completely after 24 h.

Sample preparation and extraction of non-structural carbohydrates

Flower petals and the two uppermost five-leaflet leaves were detached on days 0, 5 and 10 of vase life, individually snap-frozen in liquid nitrogen, and freeze-dried at -50 ºC for 24 h (Heto-Holten A/S., FD 8.0, Denmark). Samples were subsequently stored at -40 °C until required. Flower tissues were extracted according to Davis *et al*. (2007) with slight modifications. Freeze-dried powder (150 mg) from each sample was combined with 3 mL of 62.5:37.5 methanol: water (v/v) and mixed well. Vials (7 ml polystyrene bijou vials; Sterilin, Staffs., UK) of the slurry were placed in a shaking water bath (Fisons, Suffolk, UK) at 55 ºC for 15 min. Samples were removed briefly and vortexed (Vortex Genie 2, Scientific Industries, NY) for 20 s every 5 min to prevent layering, and then left to cool. The cooled samples were filtered through a 0.2 µm Millex-GV syringe driven filter unit (Millipore Corp., MA) and stored at -40 ºC until required.

Quantification of non-structural carbohydrates

Non-structural carbohydrates were quantified according to Davis *et al.* (2007) using a HPLC system comprising a P580 pump and GINA 50 autosampler (Dionex, CA). Extracts were diluted 1:9 (v/v) with HPLC grade water immediately before analysis. The diluted extract (20 µL) was injected into a Waters Carbohydrate Analysis Column (NH_2 size exclusion column of 300 mm x 3.9 mm diameter, 10 µm particle size; Waters, Herts., UK; Part no. WAT 084038, with a µBondapak NH2 security guard cartridge of 4 mm x 3 mm diameter (Part no. WAT 046865). The mobile phase was 80% (v/v) acetonitrile (Fischer Scientific, Dorset, UK) at a flow rate of 2.0 mL min^{-1}. Column

temperature was held at 30 °C using a Dionex STH column thermostat. Eluted carbohydrates were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the Dionex system using a Dionex UCI-50 universal chromatography interface. The presence and abundance of fructose, glucose, sucrose and myo-inositol (Yamada *et al.*, 2007) were automatically calculated against external standards using Chromeleon Version 4.6 software (Dionex). Assays (n = 72) were performed in triplicate.

Statistical analysis

A completely randomized design was adopted. Data was analysed by Analysis of Variance using SPSS Release 12.0 (Statistical Package for the Social Sciences, IL). A probability test level of $P<0.05$ was used. Least significant difference (LSD; $P<0.05$) values were calculated for comparison of individual treatment means.

RESULTS AND DISCUSSION

Treatment of 'First Red' rose stems with MeJA alone significantly extended vase life for both flower and foliage (14 and 12.8 days, respectively), compared to roses treated with 2% sucrose after MeJA treatment (12.8 and 10.6 days, respectively) (Table 1). However, treatment of flowers with 2% sucrose alone also extended vase life compared to control flowers (Table 1). Meir et al. (1998; 2005) previously demonstrated that MeJA positively affected vase life of different varieties of cut roses by suppressing *B. cinerea*. Similarly, Darras *et al*. (2005) and Gast (2001) have also used MeJA as a vapour treatment to control *B. cinerea* and therefore extend vase life of cut freesias and peonies, respectively. Since no disease symptoms were observed in the control flowers in the experiments presented herein, it is suggested that the extension of vase life of cut 'First Red' roses by MeJA may have occurred by a mechanism other than disease suppression.

Fructose has been reported as the predominant carbohydrate during opening of rose flowers (Ichimura *et al.*, 1999). The levels of fructose and glucose increased rapidly in petals at the time of flower opening and continued to increase until petal fall (van Doorn, 2001). The concentrations of sucrose, fructose, glucose and myo-inositol (mg g^{-1} DW) reported here are in general agreements with those previously reported in petals of roses (Yamada *et al*., 2007). Sucrose concentration in petals declined during vase life for all treatments, even with exogenous supply of sucrose (Fig. 2, 3A & 3B). Significant differences were apparent for MeJA and sucrose solutions on sucrose concentration in petals on day 10 ($P = 0.035$). Fructose concentration in petals of 'First Red' roses increased during vase life (from 78.07 to 266.52 mg g^{-1}; Fig. 2, 1A & 1B). These results suggest that

Table1. Levene's test for equality of variances for *Cyclamen* species.

Treatments		Vase life (days)	
		Flower	Foliage
+MeJA	Suc 0% + DICA	14.0(±0.0)	12.8(±0.5)
	Suc 2% + DICA	12.8(±0.4)	10.6(±0.2)
- MeJA	Suc 0% + DICA	10.4(±1.3)	11.0(±0.6)
	Suc 2% + DICA	12.0(±1.3)	11.6(±0.6)
Main factors			
MeJA (A)		P=0.034	P=0.048
Sucrose (B)		P=0.836	P=0.140
Interaction			
A x B		P=0.159	P=0.015

Individual treatment data are $\bar{X}$ ± S.E; n = 5.

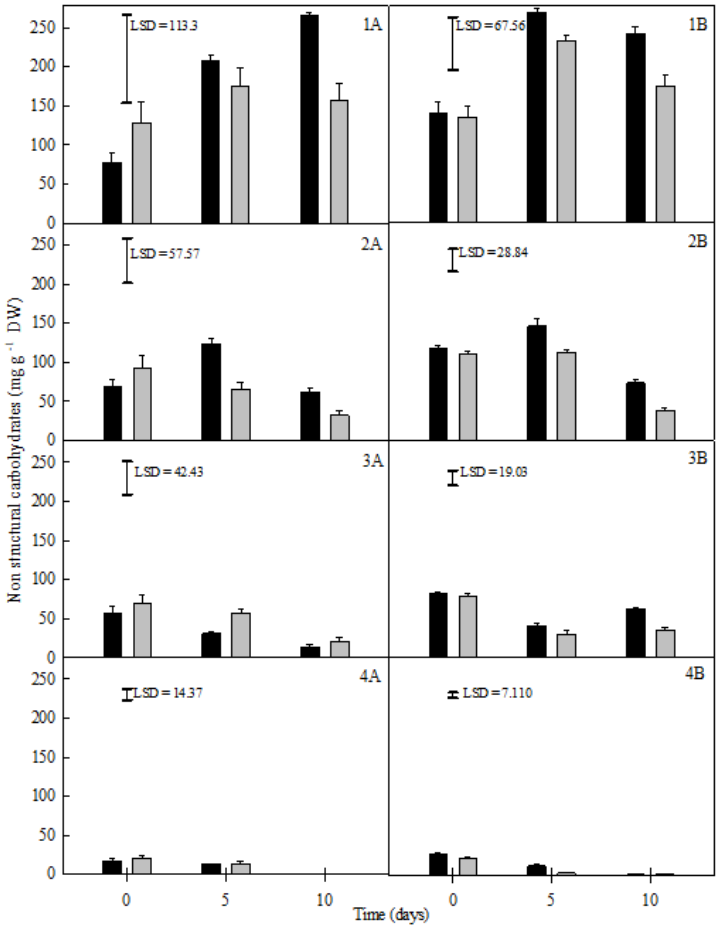


Fig. 2. Changes in non-structural carbohydrate content (mg g^{-1} DW) in petals of 'First Red' roses at different sucrose concentrations in the presence and absence of MeJA treatment (0.1 µL MeJA L^{-1}). Key for graphs: 2 and 0% sucrose (v/v); (**1**) Fructose, (**2**) Glucose, (**3**) Sucrose and (**4**) Myo-inositol; (**A**) 'First Red'+MeJA and (**B**) 'First Red'-MeJA; Individual treatment data are $\bar{x}$ ± S.E.; n = 3. LSD ($P < 0.05$) values are for comparison of individual treatment means.

sucrose was hydrolysed to glucose and fructose during vase life (Kaltaler and Steponkus, 1974). Glucose concentration in petals generally decreased during vase life (Fig. 2, 2A & 2B). Myo-inositol concentration fell sharply in all treatments (Fig. 2, 4A & 4B), but neither MeJA nor sucrose treatment had a significant effect on any of the four sugars measured in petals of 'First Red' roses.

In contrast, significant differences were shown for sugar concentration in foliage of roses treated with MeJA and sucrose solutions after harvest (Fig. 3). Sucrose concentration in leaves sharply increased during vase life (from 190.17 to 329.26 mg g^{-1} DW) after exogenous supply of sucrose (Fig. 3, 3A). MeJA vapour treatment and sucrose solutions had a significant effect on sucrose concentration in leaves of roses during vase life ($P \leq 0.001$). Fructose and glucose concentration in leaves increased during vase life especially when treated with MeJA and 2% sucrose, while significant differences were also shown for both fructose and glucose ($P = 0.021$ and $P = 0.009$, respectively) (Fig. 3, 1A, 2A). However, MeJA vapour and sucrose solutions had no significant effect on myo-inositol levels in leaves of roses (Fig. 3, 4A & 4B). Ichimura *et al.* (1999) hypothesized that myo-inositol in leaves is metabolized to sucrose; however the role of myo-inositol in plants is still unclear. In addition, Ichimura *et al.* (1999) demonstrated that sucrose concentration in leaves of 'Sonia' roses treated with 3% sucrose increased during vase life, but myo-inositol concentration remained stable.

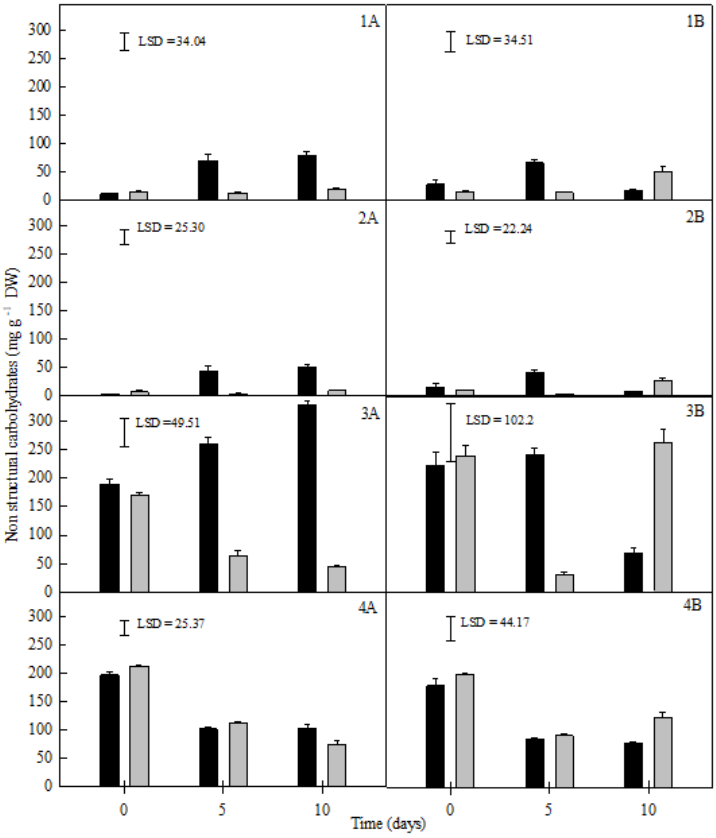


Fig. 3. Changes in non-structural carbohydrate content (mg g^{-1} DW) in leaves of 'First Red' roses at different sucrose concentrations in the presence and absence of MeJA treatment (0.1 µL MeJA L^{-1}). Key for graphs: 2 and 0% sucrose (v/v); (1) Fructose, (2) Glucose, (3) Sucrose and (4) Myo-inositol; (A) 'First Red'+MeJA and (B) 'First Red'-MeJA; Individual treatment data are $\bar{x}$ ± S.E.; n = 3. LSD ($P < 0.05$) values are for comparison of individual treatment means.

Ichimura and Korenaga (1998) reported that the addition of sugars to vase water promoted bud opening and vase life longevity of *Eustoma* cut flowers and could therefore increase the amount of endogenous sugars. Kaltaler and Steponkus (1974) reported that the addition of sucrose to the vase solution resulted in an increase of glucose and fructose concentration, but had little effect on sucrose content in petals of cut roses. They hypothesized that the sucrose translocated to the petals was probably catabolized to glucose and fructose in petal cells. The results presented herein support the findings of Kaltaler and Steponkus (1974), since fructose concentration in petals of cut 'First Red' roses supplied with 2% sucrose increased during vase life (Fig. 2, 1A). However, MeJA and sucrose solutions had no significant effect on fructose concentration in petals during vase life. The level of sucrose in petals gradually declined during vase life, whilst myo-inositol abruptly decreased (Fig. 2, 3A & 4A). Yamada *et al.* (2007) demonstrated that myo-inositol concentration in petals of cut 'Febesa' roses treated with 90 mM sucrose in vase solution also markedly decreased during vase life. Sucrose and myo-inositol concentration in petals of cut 'Sonia' roses treated with 3% sucrose significantly decreased during vase life. However, the concentration of glucose and fructose were up to 2.7-fold higher, respectively, at the stage of full bud opening (Yamada *et al.*, 2007). It is suggested that effect of MeJA on vase life may be related to altered sugar metabolism and may explain the increased vase life discussed by others (Meir *et al.*, 1998; 2005).

CONCLUSION

MeJA applied as vapour treatment tended to increase vase life of 'First Red' roses. The addition of sucrose as a vase solution extended vase life, but without MeJA vapour treatment, vase life was limited. Sucrose supplied as vase solution, enhanced fructose concentration in petals and sucrose concentration in leaves of roses that received MeJA vapour treatment. However, more research is needed to improve knowledge of the relationship between MeJA and sucrose in vase life of cut roses and other cut flowers.

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