Ethylene and anti-ethylene treatment effects on cut 'First Red' rose

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Abstract
Studies were conducted to determine the effects of ethylene and anti-ethylene treatments on postharvest life of cut 'First Red' rose flowers. Effects of exogenous ethylene at 1, 10 and 100 µl l^{-1} for 48 h at 22 °C on cut 'First Red' rose flowers were investigated. Ethylene at different concentrations reduced postharvest life, with 100 µl l^{-1} having the greatest effect. Ethylene production measurements suggested that 'First Red' rose is climacteric during senescence. Pre-treatment of 'First Red' rose flowers with 0.5 mM silver thiosulfate (STS) for 2 h at 22 °C increased vase life, but pre-treatment with 1 µl l^{-1} 1- methylcyclopropene (1-MCP) did not. Pre-treatment of 'First Red' rose with 0.5 mM STS and, to a lesser extent, 1 µl l^{-1} 1-MCP for 2 h at 22 °C, protected flowers from subsequent exposure to 10 µl l^{-1} ethylene. Maximum vase life in both ethylene-treated and non-ethylene-treated 'First Red' rose flowers was obtained with 0.5 mM STS.

Key words: Ethylene, 1-methylcyclopropene (1-MCP), postharvest life, relative fresh weight, *Rosa hybrida* cv. 'First Red', senescence, silver thiosulfate, vase life.

Introduction
Ethylene is an important factor in the postharvest life of cut flowers (Joyce and Poole, 1993; Elgar *et al.,* 1999; Han *et al.,* 2003), as it accelerates flower abscission and leaf yellowing (Joyce and Poole, 1993; Cameron and Reid, 2001; Celikel *et al.,* 2002). Reid *et al.* (1989) found that various cut rose cultivars show a range of responses to ethylene treatment, such as inhibition of opening, acceleration of opening, abnormal opening, petal and leaf abscission, and loss of petal gloss.

Treatments with anti-ethylene compounds, such as STS (silver thiosulfate) and 1-MCP (1-methylcyclopropene), can effectively protect flowers against exogenous ethylene (Serek *et al*., 1995; Serek and Trolle, 2000; Redman *et al.,* 2002; Hunter *et al.,* 2004). Pre-treatment of *Pelargonium peltatum* plants with 1 µl l^{-1} 1- MCP for 2 h completely inhibited ethylene-induced petal abscission (Cameron and Reid, 2001). Celikel *et al.* (2002) reported that pre-treatment of oriental lilies (*Lilium* cvs 'Monalisa' and 'Stargazer') with 500 nl l^{-1} 1-MCP for 18 h at 25 °C completely inhibited the ethylene response, but did not prevent normal senescence, wilting and abscission of open flowers. Application of 1-MCP at 25 nl l^{-1} for 6 h to cut phlox flowers reduced flower abscission from 100% to 22% (Porat *et al.,* 1995). Similarly, flower abscission was reduced to 24% in flowers pre-treated with 1 mM STS for 2 h. *Grevillea* 'Sylvia' inflorescences treated with 10 nl l^{-1} 1-MCP for 12 h at 20 °C were afforded protection against ethylene treatment for 2 days after 1-MCP treatment (Macnish *et al.,* 2000).

Application of 1-MCP at 10 µl l^{-1} for 12 h at 20 °C to Geraldton waxflower cvs. 'Lollypop', 'Alba' and 'Mid Pink' largely protected flowers from ethylene for 6, 4 and 3 days, respectively (Macnish *et al.,* 2000), but they remained insensitive to ethylene after treatment with 0.5 mM STS for 12 h at 20 °C. Serek *et al.* (1995) reported that pre-treatment of *Rosa hybrida* 'Victory Parade', *Begonoa elatior* and *Kalanchoe blossfeldiana* with 5-20 nl l^{-1} 1- MCP provided as much protection against ethylene-induced bud and flower abscission and flower senescence as application of STS.

'First Red' rose is among the most commercially important cut rose cultivars. The present study was undertaken to ascertain the possible role of ethylene in postharvest life on 'First Red' rose. The study also investigated the potential use of 1-MCP and STS to inhibit ethylene effects.

Materials and methods

Plant material: *Rosa hybrida* cv. 'First Red' flowers at the commercial stage of bud opening (petals starting to reflex) were harvested early in the morning during winter from plants grown in a commercial greenhouse at Karalee in South East Queensland, Australia. Flowers were held in water in a cold room (4 °C) and then transported within 1 hour to the laboratory. Stem ends were recut under water to remove air emboli and then placed into vases containing 10 mg l^{-1} free chlorine from dichloroisocyanurate.

Ethylene treatment: Flowers were treated with ethylene at 1, 10 and 100 µl l^{-1} for 48 h at 22 °C (Reid *et al.,* 1989). For treatment, the flowers were sealed in 38.8 × 39.0 × 38.8 cm (*ca.* 59 l volume) glass chambers. A 25 ml solution of 1M KOH was also placed inside the chambers to maintain low CO_2 concentrations from respiration. Aliquots of pure ethylene gas were injected by syringe into the different chambers to achieve treatment concentrations. Ethylene concentrations inside the chambers were checked by gas chromatography. Ten stems were used for each concentration plus 10 stems for the control (treated with air for 48 h at 22 °C).

Anti-ethylene treatments: Flowers were pulse-treated with 0.5 mM STS for 2 h at 22 °C (Reid *et al.,* 1980). For preparation of STS, 1.36 g $AgNO_3$ was dissolved in 100 ml of deionized water and 7.94 g sodium thiosulphate penthahydrate ($Na_2S_2O_3.5H_2O$) was dissolved in 100 ml of deionized water. The two solutions were then combined by slowly pouring $AgNO_3$ solution into the vigorously stirred $Na_2S_2O_3$ solution to obtain 200 ml of 4 mM STS. This stock was diluted with water to obtain the working concentration (0.5 mM). Flowers were treated with 1-MCP at 1 µl l^{-1} for 2 h at 22 °C (Cameron and Reid, 2001). Plants were sealed in 38.8 × 39.0 × 38.8 cm glass chambers with 0.01 mg of EthylBloc® (Biotechnologies for Horticulture Ltd.) in a beaker taped to the inside wall of the chamber. Since a significant proportion of 1-MCP is released immediately after hydration, the chamber lid was sealed and 10 ml hot water was injected into the beaker using a hypodermic needle to pierce a rubber septum in the lid. 1-MCP concentration was monitored by gas chromatography, and after 20 min the concentration was 1 µl l^{-1}. Flowers were removed from the chambers after 2 h exposure.

Half the flowers treated with STS (0.5 mM) or 1-MCP (1 µl l^{-1}) were then treated with ethylene at 10 µl l^{-1} for 48 h at 22 °C (Macnish *et al.* 2000) in the glass chambers. Ethylene concentration in the chamber was quantified by gas chromatography. Non-treated flowers were used as the control.

Assessments of vase life: Vase life was recorded as the number of days after harvest (day 0) that flowers reached the end of their longevity due to bent neck or advanced signs of fading on all petals (Mayak and Halevy, 1974; Liao *et al.,* 2000). The vase life room was maintained at 22 °C. Relative fresh weight of stems was calculated using the formula: RFW (%) = $(W_t/W_{t=0}) \times 100$; where W_t = weight of stems (g) at t = days 0, 2, 4, 6, etc. and $W_{t=0}$ = weight of the same stem (g) on day 0. Vase solution usage was determined using the formula: Solution uptake (ml day^{-1} g^{-1} fresh weight) = $(S_{t-1}-S_t)/W_{t=0}$; where, S_t = solution weight (g) at t = days 1, 2, 3, etc. S_{t-1} = solution weight (g) on the preceding day, and $W_{t=0}$ = fresh weight of the stem (g) on day 0. Ethylene was quantified with a Shimadzu model GC- 8AIT gas chromatograph fitted with a flame ionisation detector (Liao *et al.,* 2000). The concentration of the ethylene standard gas was 0.09 ± 0.02 ml 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). To measure rates of ethylene production using a closed system, single stems were placed in 2.2 l glass jars in the vase life room. Each jar contained a 100 ml beaker and a fluted Whatman No. 1 filter paper wetted with 5 ml of 1M KOH solution to maintain CO_2 concentration below 0.1% (v/v). The glass jar was sealed with a metal lid for 21 ± 1 h, and 1 ml samples of the headspace were removed, using a needle and syringe, and injected into the gas chromatograph. Five replicate stems were used for ethylene production measurements. Flowers were aerated for 2 hours per day before the jars were sealed in preparation for the next ethylene production measurements.

Statistical analysis: In all experiments, flowers were arranged using a completely randomized design (CRD) in a controlled environment vase life room. For the experiment with ethylene, 10 replications were used for each treatment and data were analysed by one way ANOVA using Minitab® Release 13.2 (Minitab Inc.). Following ANOVA, the least significant difference (LSD) test at $P = 0.05$ was used to separate treatment means. For the experiment with STS and 1-MCP, nine replications were used for each treatment and all data were analysed by the general linear model ANOVA of Minitab® Release 13.2 (Minitab Inc.). Following ANOVA, treatment means were compared using the LSD test at $P = 0.05$.

Results

Ethylene treatment: Treatment of cut 'First Red' rose with increasing concentrations of ethylene (1, 10, and 100 µl l^{-1}) progressively reduced postharvest life (Table 1). Exposure to ethylene at 100 µl l^{-1} reduced vase life by about 30%. Relative fresh weight of 'First Red' rose declined in a linear manner for the controls and the treated flowers (Fig. 1A). Actual relative fresh weight loss was increased by the ethylene treatments and the differences became apparent on the first day of measurement after the ethylene treatment period. Flowers continued to absorb vase solution faster than it was lost until day 3 (Fig.

1B). After that time, loss was faster than uptake for all flowers. From day 3, significant ethylene concentration effects on vase solution uptake were discerned (Fig. 1B), and solution uptake was most reduced following the 100 $\mu l\ l^{-1}$ treatment.

Ethylene production: Ethylene measurements suggest a climacteric-type behaviour of cut 'First Red' rose flowers during their development and senescence in the vase (Fig. 2). Ethylene production peaked at day 6.

Anti-ethylene treatments: Vase life of non-ethylene-treated flowers was around 13 days (Table 2). Pre-treatment with 1 $\mu l\ l^{-1}$ 1- MCP did not affect vase life, but the STS pre-treatment gave a significant extension of vase life of about 8 days. When flowers were challenged with 10 $\mu l\ l^{-1}$ ethylene, STS still provided a 7 day extension of vase life, but 1 $\mu l\ l^{-1}$ 1-MCP provided no further extension over that for the non-ethylene-treated flowers. However, the 1-MCP pre-treatment provided 13 days of vase life when flowers were challenged with ethylene, but vase life of unprotected flowers was reduced to 11 days (Table 2).

The relative fresh weight of all non-ethylene-treated flowers declined steadily, and noticeable differences only became apparent after 6 days (Fig. 3A). At this time, STS-treated flowers maintained a higher relative fresh weight. Ethylene treatment changed the pattern of relative fresh weight loss, such that the loss was much reduced by STS pre-treatment, and reduced by 1-MCP (Fig. 3B).

All flowers absorbed more solution than they lost over the first 3 days (Fig. 3C, D). In the absence of applied ethylene (Fig. 3C), solution uptake declined rapidly after day 3 in all pre-treated flowers except in those pre-treated with STS. When challenged with ethylene (Fig. 3D), solution uptake declined most dramatically in those flowers not pre-treated, but by contrast, 1-MCP permitted continued net uptake, and STS greatly assisted in maintaining solution uptake throughout the 9 days of measurements. During the initial 7 days of vase life, there were no differences in solution uptake between ethylene-treated and non-ethylene-treated flowers that had been pre-treated with STS (Fig. 3C, D). In 1-MCP pre-treated flowers, after day 3, there were no differences in solution uptake by those ethylene-treated and those not ethylene treated (Fig. 3C, D).

Discussion

'First Red' rose is a climacteric cultivar as characterised by a pronounced peak in ethylene production that is evident during postharvest evaluation in the vase (Fig. 2). Consequently, ethylene treatment reduced the vase life of cut 'First Red' rose (Table 1). Shortened vase life was associated with abnormal opening (star shape) and inhibition of opening. Reid *et al.* (1989) reported that a very low concentration of ethylene (0.5 $\mu l\ l^{-1}$) markedly inhibited the opening of cut rose flowers, and Serek *et al.* (1994) found treatment of *Rosa hybrida* 'Victory Parade' with 1 $\mu l\ l^{-1}$ ethylene decreased its vase life. Faragher and Mayak (1984) suggested that reduced vase life of roses after long term low temperature storage is associated with increased ethylene production.

In an ethylene-free environment, 1-MCP pre-treatment did not increase the vase life of 'First Red' rose. However, following ethylene treatment, the 1-MCP pre-treatment maintained vase life at the level as in the absence of ethylene but markedly increased vase life when the comparison is made with those flowers not having the pre-treatment (Table 2). Cameron and Reid (2001) reported that pre-treatment with 1 $\mu l\ l^{-1}$ 1-MCP for 2 h completely inhibited ethylene-induced petal abscission in *Pelargonium peltatum*. Kebenei *et al.* (2003) reported that pretreatment of sweet pea flowers with 200 $nl\ l^{-1}$ 1-MCP for 6 h at 20 °C protected against ethylene (1 $\mu l\ l^{-1}$) and prolonged display life to almost 7 days as compared with 3 days for the control. Pretreatment with 150 $nl\ l^{-1}$ 1-MCP for 6 h in the absence of exogenous ethylene neither extended vase life nor improved quality of the Asiatic lilies or *Lilium longiflorum* flowers (Elgar *et al.* 1999). However, 1-MCP pre-treatment of the hybrid Asiatic lily cultivar 'Cordelia' that was subsequently exposed to 10 $\mu l\ l^{-1}$ ethylene for 24 h gave a vase life extension of 1.4-fold; from 4.7 days (control) to 6.7 days. Our results on 'First Red' rose showing no effect of 1-MCP on vase life in the absence of ethylene, is therefore consistent with published reports.

STS pre-treatment had greater efficacy against ethylene treatment than did 1-MCP pre-treatment (Table 2). STS pre-treatment complete protection of cut 'First Red' rose flowers, returning a vase life of 19.4 days, much the same as that of non-ethylene treated flowers (20.9 days). Reid *et al.* (1989) reported that the effect of exogenous ethylene (0.5 $\mu l\ l^{-1}$) on rose flowers could be overcome by pre-treatment with STS at a rate of 0.5 µmol $stem^{-1}$.

Elgar *et al.* (2003) reported that exposure of *Leucocoryne coquimbensis* inflorescences to 8 $\mu l\ l^{-1}$ ethylene for 24 h reduced its vase life from 10 to 5 days and pre-treatment with 1 mM STS for 2 h protected flowers, giving a vase life of 9.1 days. Similarly, Macnish *et al.* (2000) reported that STS treatment was more effective in providing waxflower with long term protection against ethylene than was 1-MCP. Pre-treatment with 10 $nl\ l^{-1}$ 1-MCP for 12 h at 20 °C protected waxflower sprigs against ethylene for about 4 days, but pre-treatment with 0.5 m $mol\ l^{-1}$ STS under the same conditions conferred protection for 10 days. Newman *et al.* (1998) reported that STS treatment was more effective than 1-MCP treatment in affording long-term protection of developing buds on cut *Gypsophila paniculata* inflorescences against ethylene. They proposed that the STS complex remained available in the inflorescence and capable of binding to receptors formed as the buds opened into flowers.

STS and 1-MCP pre-treatments both delayed loss of flower fresh weight and increased solution uptake in cut 'First Red' rose flowers (Fig. 3). Delay in fresh weight loss by 1-MCP pre-treatment in ethylene-treated flowers has also been reported for *Boronia hetrophylla* (Macnish *et al.,* 1999) and *Petunia hybrida* 'Pink Cascade' (Serek *et al.,* 1995). Similarly, STS pre-treatment was effective in this context for *Verticordia nitens* (Joyce and Poole, 1993).

Overall, ethylene production and sensitivity are key issues in the postharvest longevity of cut 'First Red' rose, which is a cultivar of major commercial importance. STS and 1-

MCP pre-treatments can inhibit ethylene effects and thereby extend the display life of this cultivar. In this capacity, STS pre-treatment has greater efficacy than 1-MCP pre-treatment. This difference may be attributable to a residual 'pool' of STS in pre-treated flowers that yields silver ions to block newly forming ethylene binding sites.

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Table 1. Effect of various ethylene concentrations (1, 10, 100 µl l^{-1}) on vase life of cut 'First Red' rose flowers. The L.S.D. ($p \leq 0.05$) for comparison among treatment means is 1.546. Means (n = 10) followed by the same letter do not differ significantly.

Ethylene concentration (µl l^{-1})			
0 (control)	1	10	100
11.7 a	9.6 b	8.9 b,c	7.9 c,d

Table 2. Effect of 1-MCP (1µl l^{-1}) and STS (0.5mM) pre-treatments on vase life of ethylene and non-ethylene treated cut 'First Red' rose flowers. The L.S.D. ($p \leq 0.05$) for comparison among treatment means is 1.8. Means (n = 9) followed by the same letter do not differ significantly.

Treatments	Vase life (days)
Ethylene treated	
STS (0.5mM)	19.4 a,b
Control for STS	10.8 g
1-MCP (1µl l^{-1})	13.2 c,d,e
Control for 1-MCP	10.6 g,h
Non-ethylene treated	
STS (0.5mM)	20.9 a
Control for STS	13.3 c,d
1-MCP (1µl l^{-1})	13.9 c
Control for 1-MCP	12.7 c,d,e,f

LIST OF FIGURES

Fig 1.

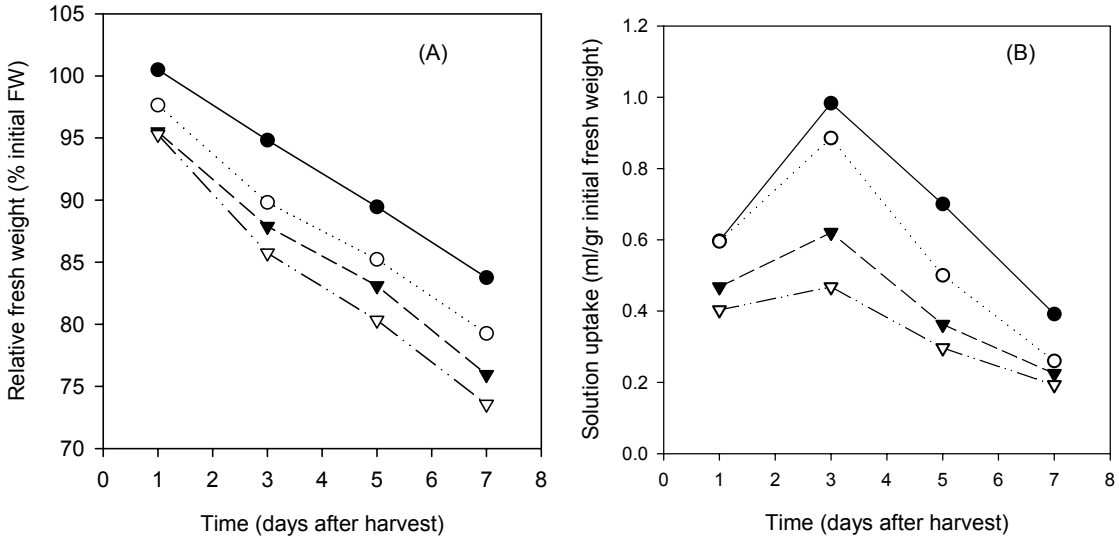

(A)
Relative fresh weight (% initial FW)
105
100
95
90
85
80
75
70
0
1
2
3
4
5
6
7
8
Time (days after harvest)
(B)
Solution uptake (ml/gr initial fresh weight)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
6
7
8
Time (days after harvest)

Fig 2.

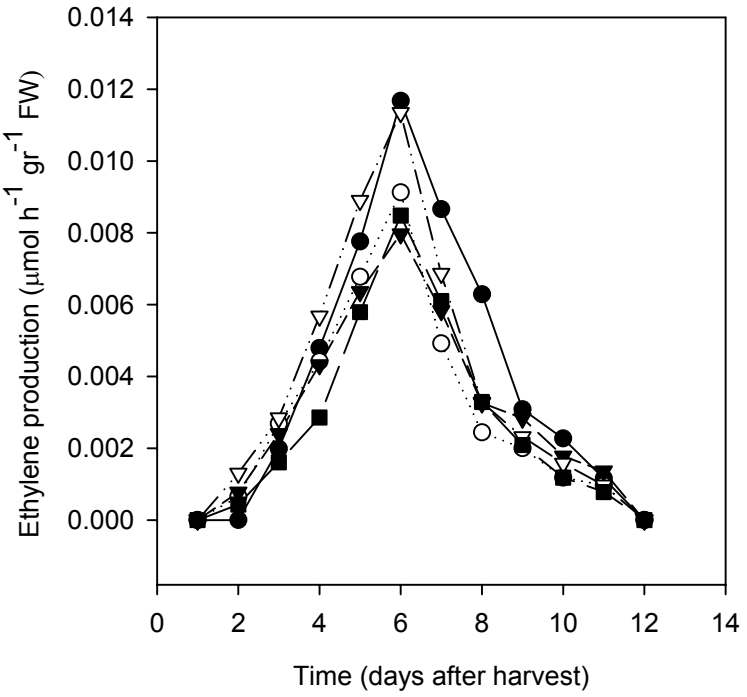
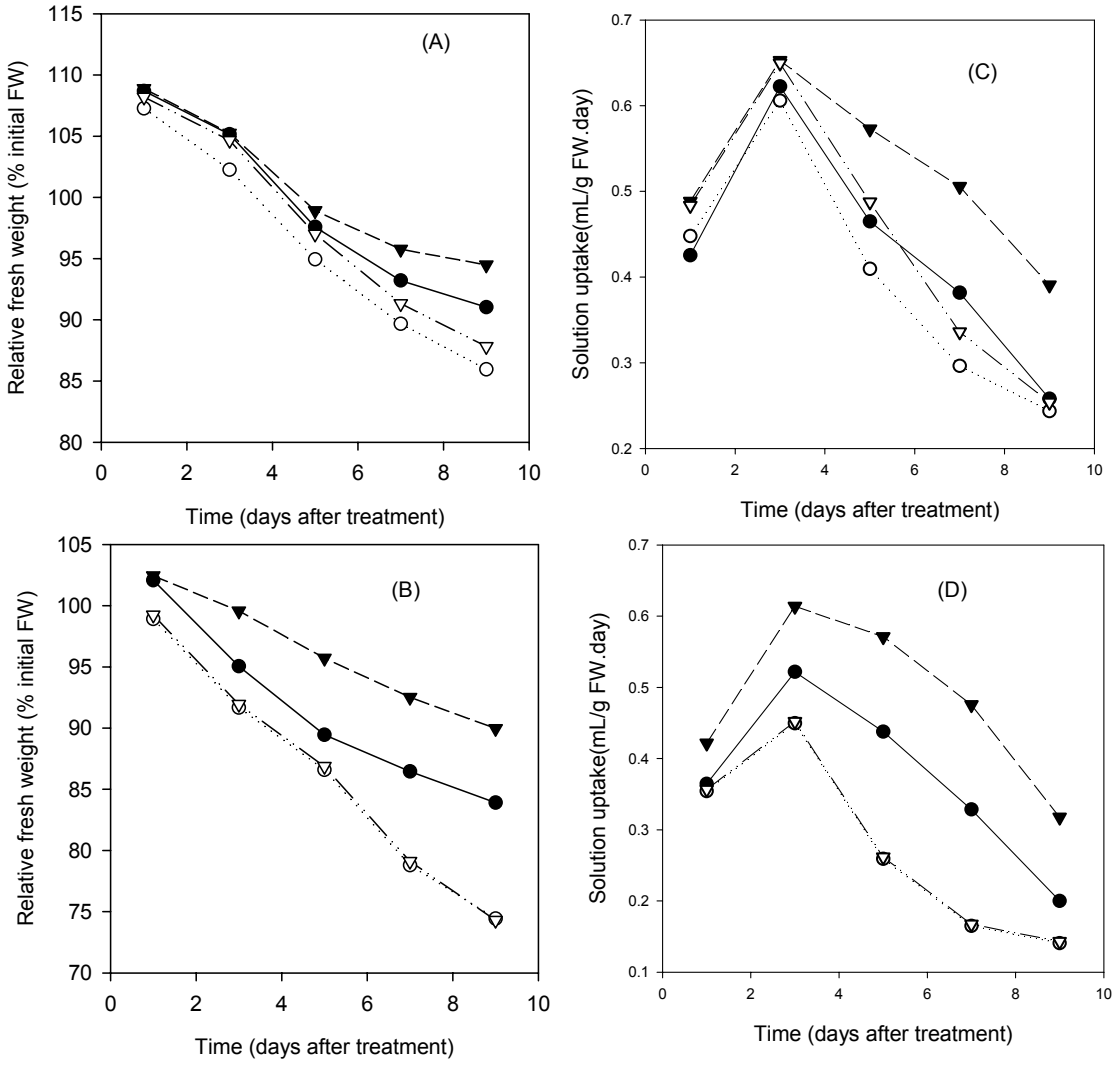

0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
Ethylene production (µmol h-1 gr-1 FW)
0
2
4
6
8
10
12
14
Time (days after harvest)

Fig 3.


(A)
Relative fresh weight (% initial FW)
Time (days after treatment)
(C)
Solution uptake(mL/g FW.day)
Time (days after treatment)
(B)
Relative fresh weight (% initial FW)
Time (days after treatment)
(D)
Solution uptake(mL/g FW.day)
Time (days after treatment)