Effect of mild vacuum packaging on cut *Matthiola incana* L. flowers vase life

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Abstract

Mild vacuum (MV) packaging was compared with conventional cold wet storage on cut stock (*Matthiola incana* L.). The cut stock flowers were selected and cut to 50 cm and stored in water (wet storage) or placed in plastic bags under MV at 4 °C for six days. The effect of storage was compared with non-stored cut flowers. The postharvest quality of cut flowers was evaluated by monitoring vase life, water uptake, alcohol dehydrogenase activity (ADH), ethylene production, total chlorophyll and SPAD measurements. The MV packaging allowed storing the stock flowers for three days, without any visible injury and with a vase life of about 7 – 8 days. MV storage caused an accumulation of ethylene and CO_2 in bags, 919.9 pl ml^{-1} and 21382 µl l^{-1}, respectively. Only flowers stored for 6 days in mild vacuum showed quality reduction. The leaves appeared dehydrated; with local transparencies of cuticles and a green-gray colour. The ADH was higher in open flowers (2.9 nmol mg^{-1} protein min^{-1}) compared with other organs in MV storage method, and the highest concentration was recorded after 6 days storage. At the end of vase life stock flowers wet stored showed a greater production of ethylene (0.75 nl h^{-1} g^{-1} FW) then MV (0.3 nl h^{-1} g^{-1} FW). The MV system can be used in *M. incana* if storage or transportation is limited to three days.

Keywords: ADH, ethylene, postharvest, storage, stock.
Abbreviations: ADH-alcohol dehydrogenase; MV- Mild vacuum; C – Control wet.

Introduction

The commercial value of cut flowers is limited be affected by flower (individual flowers or inflorescence) and/or leaf senescence, especially yellowing. The senescence can be dramatically accelerated by ethylene in sensitive flowers or by other factors like water stress, hormone imbalance, nutrient starvation and environmental conditions such as temperature, relative humidity, etc. (Teixeira Da Silva, 2003). In particularly, leaf yellowing is a form of senescence that is both highly programmed and genetically regulated. Leaf yellowing can compromise the commercial values of flowers. It depends from their sensitivity to yellowing that varies from the species and cultivars. The postharvest factors that enhance the leaf yellowing can be sub-optimal storage conditions, presence of ethylene, darkness, leaf age or mechanical injury (Ferrante et al., 2009). Many ornamentals, both potted plants and cut flowers, exposed to exogenous ethylene show leaf yellowing (Woltering (1987; Raffeiner et al., 2009; Ferrante et al., 2012). Optimal environmental conditions during storage and transportation are extremely important for preserving quality during postharvest handling of ornamental perishables (Cid, 1992; Dodge et al., 1998; Çelikel and Reid, 2002). Floriculture items often have to be transported to long distances before they reach the selling markets with the risk of quality losses. The transport of cut flowers and foliage is very expensive; therefore, packaging techniques may play an important role in reducing the volume. This aim can be achieved by using vacuum packaging that removes air inside packages (Pacifici et al., 2008a, 2008b). Vacuum packaging or modified atmosphere packaging (MAP) can extend the shelf-life of many intact and fresh-cut horticultural products (Gorny, 1997; Murcia et al., 2003). MAP can be created inside a package either passively

through product respiration or actively by replacing atmosphere in the package with a desired gas mixture. Passively generated MAP can evolve within a sealed package through consumption of O_2 and production of CO_2 by respiration (Kader and Watkins, 2000). The progressive reduction of oxygen can cause environmental hypoxia phenomena. The effect of hypoxia on the senescence of horticultural crops includes a progressive decrease in the respiration rate, a delay in the onset of the climacteric rise in ethylene evolution and a decrease in the rate of ripening (Burg and Burg, 1967; Kanellis et al., 1989a; 1989b; 1991; Solomos, 1994). Induction of alcohol dehydrogenase (ADH) during anoxic conditions has been observed in many plant species (Ellis et al., 1999; Tadege et al., 1998; Xie and Wu, 1989). Low oxygen stress dramatically alters the pattern of gene expression (Sachs and Ho, 1986; Sachs et al., 1996), and several plants have evolved a series of adaptive physiological and biochemical changes which enhance their ability to survive in these adverse conditions (Richard et al., 1994; Plaxton, 1996; Vartapetian and Jakson, 1997). The survivability of several species exposed to mild hypoxia during a subsequent period of severe hypoxia or anoxia was greatly improved (Johnson et al., 1989; Andrews et al., 1994; Ellis et al., 1999). The ADH has been extensively studied over the last 20 years in relation to anaerobic and hypoxic responses and tolerance (Sachs et al., 1980; Freeling and Bennett, 1985). The ADH gene was the first (Hagermann and Flesher, 1960), molecular marker of hypoxic and anoxic responses (Dolferus et al., 1985; Chang and Meyerowitz, 1986). The aim of this work was to evaluate the effect of mild vacuum packaging on the postharverst quality of cut stock flowers of *Matthiola incana* after different storage period as

Table 1. Determination of ethylene and CO_2 changes in mild vacuum (MV) plastic bags with and without cut flowers. Data are means ± standard errors.

Treatments	Ethylene (pl ml^{-1})		CO_2 (µl l^{-1})	
	3 storage days	6 storage days	3 storage days	6 storage days
MV without cut stock	34.8±1.50	35.7±12.99	522 ±105.2	166±42.3
MV with cut stock	791.5±48.00	919.9±70.71	17059±502.6	21382±1901.4

Table 2. Total chl (a+b) content and SPAD values of cut stock flowers (*M. incana.*) during vase life in control (T0), cold wet (C) or mild vacuum dry (MV) stored for 3 or 6. Value are means ± standard errors (chl n = 6 and SPAD n=18). Data were subjected to Anova one way; treatment whit different letters are significantly different according to the Tukey test (*** = P≤0.001).

	Chl a+b (µg mg^{-1})			SPAD (a.u.)		
	Days vase life			Days vase life		
	1	3	6	1	3	6
T0	1.49±0.0128a	1.35±0.0351b	0.84±0.028a	53.87±0.644b	54.97±0.920a	36.20±1.817ab
T3C	1.46±0.0125a	1.37±0.0353b	0.81±0.0394a	58.95±1.025a	53.36±0.579a	41.14±1.369a
T3MV	1.47±0.0178a	1.36±0.0619b	0.66±0.0486a	53.37±1.669b	50.40±1.251ab	32.95±1.631b
T6C	1.35±0.0080b	0.84±0.0460c	0.81±0.0636a	59.52±0.605a	38.16±1.218c	30.95±2.369b
T6MV	1.52±0.0273a	2.15±0.1141a		56.38±0.694ab	46.87±2.365bc	
ANOVA one way						
P value	***	***	ns	***	***	***

an alternative to wet cold storage. Cut stock flowers were selected for their commercial value in the flower industry and because both the flower and leaf senescence limit the postharvest life. Generally cut stock flowers are wet cold stored, using different preservative compounds directly added in the vase solution (Ferrante et al., 2006; Regan and Dole, 2010).

Results and Discussion

In our experiments, a new mild vacuum packaging technique (100-500 mbar) was applied to cut stock flowers (Fig. 1) and was compared with conventional wet storage at low temperature. The mild vacuum packaging allowed the storage of stock flowers for three days, without visible injury, even if a slight reduction of the vase life was observed. Mild vacuum packaging induced environmental changes in sealed bags, as gas composition mainly due to ethylene and CO_2, therefore the atmosphere inside is a modified atmosphere (Kader and Watkins, 2000).

Vase life, leaf yellowing and flower senescence

Generally the vase life of stock flowers was defined by both leaf yellowing and floret wilting, which were affected by time and storage methods (Çelikel and Reid, 2002; Ferrante et al., 2004, 2009). In this work the storage treatments affected the vase life, shortened meanly by leaves deterioration rather than flowers. In mild vacuum treatment, the leaves of cut flowers were damaged after 6 days of storage and senescence symptoms appeared as soon as after one day during the vase life. The flower life on the inflorescence was not affected by storage method and duration (Fig. 2.). After 6 days of mild vacuum storage, leaves showed severe injury and turned in a silver colour. This phenomenon was probably caused by epidermal detachment from the mesophyll (Fig. 3) and determined the end of vase life in these cut flowers (Fig. 2b and 2c). Statistical analysis showed a significant interaction between treatments and storage time for leaves senescence (Fig. 2a) and whole stems (Fig. 2c). Similar responses to mild vacuum packaging are also observed in *Eucalyptus parvifolia* Cambage, where the MV packaging did not cause damage during first 6 days of storage (Pacifici et al., 2008b).

Water uptake and weight variation

In MV method, flowers were stored dry and water uptake

Fig 1. Mild vacuum sealed bags with three cut stock flowers.
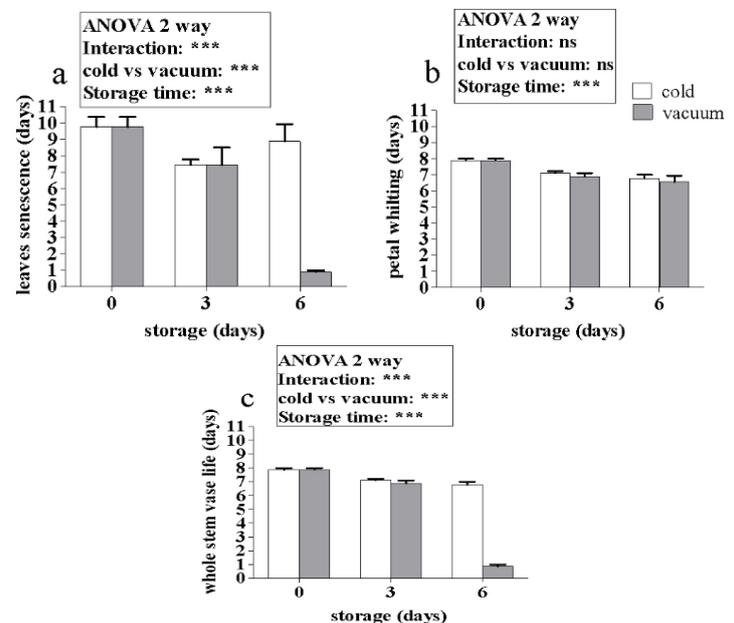
Fig 2. Vase life of cut stock flowers (*M. incana*) cold wet (C) or mild vacuum dry (MV) stored for 3 or 6 days. The vase life is reported on the base of leaves yellowing (a), petals wilting (b), and whole stem quality (c). The vase life of the whole stems is the minor value reported in figures a and b, and summarized in figure c. Values are means ± standard errors. Data were subjected to two-way ANOVA (n=6). (*P<0.05; **P<0.001; ***P<0.001).

during the vase life was measured for understanding if the hydraulic conductance can be impaired during the storage period. Results showed that water uptake of cut flowers

stored with the two methods had the same trend. Indeed, the cut flowers stored for three days, in C and MV, showed different water uptake during the first day of vase life after storage. The cut flowers stored in MV had higher water uptake than cold stored after 1 and 2 days of permanence in the postharvest evaluation room (Fig. 4a, b). After six days of storage the water uptake showed an erratic trend for both storage methods without significant differences between them during the post-storage period. In particular the water uptake drastically declined after two days of vase life with the lowest values (2-5 H_2O g d^{-1} flower $stem^{-1}$) while after three days the water uptake increased and showed the highest values (35-40 H_2O g d^{-1} flower $stem^{-1}$). After seven days of permanence in the postharvest room the water uptake of cut flowers was not different between flowers stored for three or six days in both storage methods but was lower than control. Statistical analyses showed that storage method, C or MV, significantly influenced the water uptake after one and seven days of vase life, while storage duration always affected the water uptake. The weight of not stored cut flowers (Fig. 5) increased at the third day of vase life as reported for the many cut flower species (Van Doorn, 1997).

ADH enzyme activity

Since cut flowers stored in MV might be exposed to limited oxygen conditions because the air is almost completely removed from the bag the ADH activity was monitored. Preliminarily, to know the response of flower tissues to limited oxygen conditions, cut flowers were exposed to anoxic conditions (100% N_2) for 42 h at 20±1°C. The ADH activity was 163.3±31.86, 574.9±149.40 and 717.4±72.17 nmol mg^{-1} protein min^{-1} in leaves, buds and open flowers, respectively. Cut flowers maintained in air, at 20±1°C were used as control and the ADH activity values were 22.26 nmol mg^{-1} protein min^{-1} in leaves tissue while, in open flowers tissue, the enzyme activity was 45.87 nmol mg^{-1} protein min^{-1} and in flower buds was 63.95 nmol mg^{-1} protein min^{-1}. The ADH enzyme activity values measured in cut flowers stored in C or MV conditions for 3 ad 6 days are reported in Fig. 6, whit similar levels of ADH enzyme activity in leaves of control and in samples stored for three days in C or MV. Similar levels of ADH enzyme activity were observed in leaves of control and in samples stored for three days in C or MV (Fig. 6.a), while it increased in cut flowers stored for six days in MV bags (51.56±6.721 nmol mg^{-1} protein min^{-1} Fig. 6a). The ADH activity in open flowers, detached from the inflorescence, was different after three and six days of storage in MV compared to C (Fig. 6b). No significant differences were observed in the enzyme activity of flower buds in both methods (Fig. 6c). In many works the induction of ADH and activation of fermentation was considered a strategy for plants to survive under low oxygen condition (Kennedy et al., 1992; Ricard et al., 1994; Drew, 1997). During vacuum storage, gas concentration continuously changes, due to plant tissue respiration (Knee and Aggarwal, 2000), in fact the stock flowers were in shortage of O_2. In our case during storage low temperature (4±1°C) and low oxygen concentration into sealed bags were present. *M. incana* showed higher ADH activity in open flowers and this can be easily explained since flowers are the most active metabolic organ in plants. These results were observed also in carnation, where petals showed ADH activities in response to hypoxia similar to roots, but different from shoots or leaves (Chen and Solomos, 1996). The highest ADH activity was observed for leaves stored for six days in MV (Fig. 6). The ADH activity in MV stored flowers was anyway lower than

Fig 3. Damage on 6 days mild vacuum cut stock flowers stored: particular of leaf damage with dehydration after two days of vase life.

the enzyme activity observed in flowers under anoxic chamber. Therefore, the anoxic stress in MV was not severe. The ADH activity observed in *M. incana* MV stored were comparable to that reported by other species after hypoxia stress as lettuce (Hisashi, 2000; 2001), carnation (Chen and Solomos, 1996), gipsophila (Nimitkeatkai et al., 2005). Then *M. incana* showed good resistance to hypoxia during the first MV storage days, but successively after six days the limited oxygen concentration may explain the leaf damage. The ADH activity was also shown to be involved in conversion of volatiles to the corresponding alcohols, and it has been implicated in aromatic production compounds in Petunia flowers (Garabagi et al., 2005). Therefore it is important for off-odor or off-flovour production that may compromise the flower commercialization. Moreover the role of ADH during senescence is yet unclear (Yamada et al., 2007). In some species such as carnation flowers can activate mechanisms to sense and adapt to hypoxic stress, this aspect may be very important for the flowers that have to be stored in controlled atmosphere (Chen and Solomos, 1996).

Ethylene production from cut stock tissues

The ethylene analysis was performed because this hormone can be considered a marker of stress status and aging of plants or tissue parts.
Preliminary work was performed to evaluate ethylene accumulation in the bags during the storage. Ethylene and CO_2 concentrations inside the MV packages with or without flowers were determined at the end of three and six days of storage (Tab. 1). Abiotic ethylene concentrations in the empty bags did not change between the two tested periods of storage while a low decline was observed in CO_2 concentration. Biotic ethylene and CO_2 produced by cut stock stems sealed in bags ranged from 791.5±48.0 to 919.9±70.71 pl ethylene ml^{-1} and 17059±502.6 to 21382±1901.4 µl CO_2 L^{-1} after three and six days of storage respectively (Tab. 1). The abiotic ethylene released from the plastic bags was very low and under the physiological action level (Reid, 1995). Moreover, some authors (Abeles et al., 1992; Dodge et al., 1998) argue that the presence of exogenous ethylene during storage can affect the longevity and quality of cut flowers, at very low concentrations (0.1-1 µL L^{-1}). Therefore the amount of exogenous ethylene monitored in our mild vacuum system cannot be responsible for senescence processes. Ethylene production rate from leaves, open flowers and buds of cut stocks was monitored during vase life (Fig. 7). Ethylene
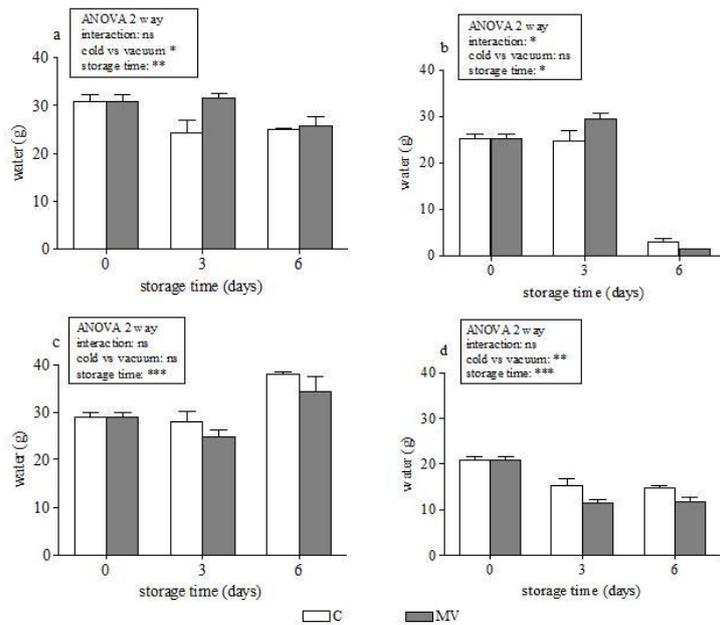
Fig 4. Daily water uptake (H_2O g d^{-1} flower stem^{-1}) of cut stock flowers (*M. incana*) cold wet (C) or mild vacuum cold dry (MV) stored for 0 or 3 or 6 days on the first day (a), second (b), third (c) and seventh (d) days of vase life. The values are means ± standard errors. Data were subjected to ANOVA 2-way (n=6). (*P<0.05; **P<0.001; ***P<0.001)
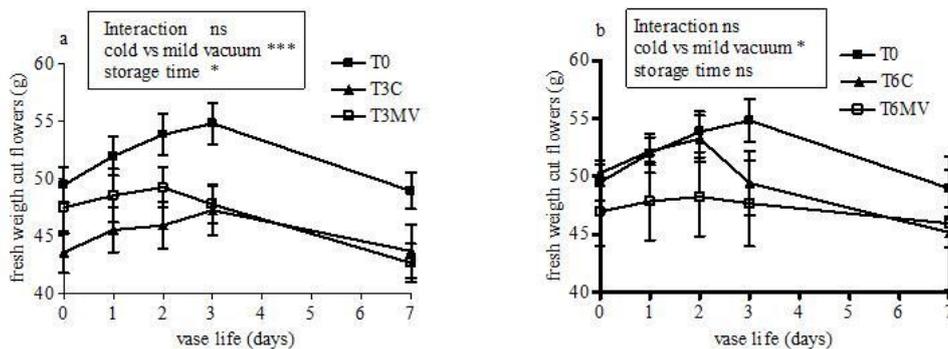
Fig 5. Daily weight (g flower stem^{-1}) during vase life of cut stock flowers (*M. incana*) not stored (T0), cold wet (T3C and T6C) or mild vacuum cold dry stored (T3MV and T6MV) after 3 (a) or 6 (b) storage days. Values are means ± standard error.
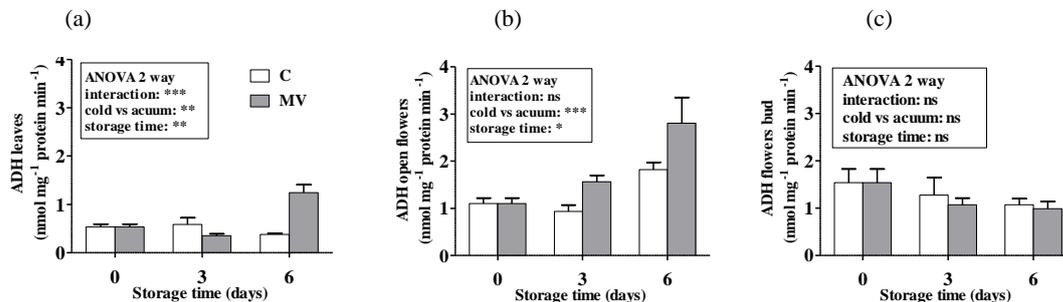
Fig 6. ADH activity (nmol mg^{-1} protein min^{-1}) in leaves (a), open flowers (b) and flowers bud (b) at the end of postharvest storage in cut stock stems (*M. incana*) control (T0), cold wet (c) or mild vacuum stored dry (MV) for 3 or 6 days. Value are means ± standard error. Data were subjected to ANOVA 2-way (n=6). (*P<0.05; **P<0.001; ***P<0.001).

production was lower in open flowers compared to bud and leaves which showed the highest values. Ethylene evolution in open flowers stored for three days was lower in MV compared to C (Fig. 7.a), while no differences were observed in open flowers obtained from cut stems stored for six days in both storage methods (Fig. 7.b). For flower buds the ethylene production at the beginning of the vase life was lower in stored flowers than in control. After seven days of vase life, ethylene production from buds increased and the flowers stored with C method showed the higher values (Fig 7.c and d). In cut flowers stored for three days ethylene evolution from leaves increased at the end of the vase life evaluation period (7 days) (Fig. 7.e). While in cut flowers stored for six days in MV method, a ethylene peak was observed in leaves at day 3 and then decreased again after seven (Fig. 7.f). Results obtained showed that leaf yellowing was not significantly correlated with ethylene production. Analogous results were found in other species of *Brassicaceae* family as *Eruca sativa* (Koukounaras et al., 2007) and *Brassica oleracea* var. *italica* (King and Morris, 1994; Tian et al., 1995). On the contrary other works showed that ethylene and leaf yellowing were strongly related (Mattoo and Aharoni, 1988; Able et al., 2003; 2005). Ethylene production was higher in leaves rather in flowers. These results were in agreement with previous works (Ferrante et al., 2009). Çelikel and Reid (2002) reported that *M. incana* cut flowers were very susceptible to ethylene exposure, in fact 1 μL L^{-1} ethylene concentration for 2 days at 20±1°C resulted in 100 % petal abscission. Flowers stored in mild vacuum treatments were exposed to higher ethylene concentration than control, and no effects were observed on flower longevity and quality until 3 days of storage at 4°C. The lower ethylene production found in the T3MV was presumably due to less stressful conditions than for short period of time (3 days). Contrary, T6MV showed a major ethylene production respect to T6C because leaves were much more damaged. It is well known that ethylene production is associated with stress conditions. Generally, T3C and T6C showed an increase between the third and seventh day of vase life, suggesting an increased state of stress of cut stocks.

SPAD and chlorophyll determination

Chlorophyll content was determined with two different methods. Table 2 includes SPAD values and chlorophyll spectrophotometrically determined. The chlorophyll content diminished during vase life in both control and treatments. The mean values ranged from 1.53±0.027 μg mg^{-1} (or 14 μg cm^{-2}) at beginning of vase life to 0.696±0.0486 μg mg^{-1} (or 4 μg cm^{-2}) at the end of vase life. After three days of storage the total chlorophyll content was reduced by 10 % in control and in treatments at the third day of vase life, and 40 % in those flowers stored for six days of storage. Cold wet storage showed leaf yellowing after 3 days of vase life, while flowers in cold mild vacuum storage showed severe leaf damage after 2 days of vase life. Similar results were observed using SPAD measurements. SPAD values ranged from 59.52±0.605 (T6C at the first day of vase life) to 30.95±2.369 (T6V at the last day of vase life). At the end of the vase life, cut flowers showed a dramatic degradation of chlorophyll in each treatment, while cut stock stems stored for six days in mild vacuum method showed higher total chlorophyll. The correlation between the two methods was significant with *r*=0.9495 (Fig 8). Mean values of chlorophyll reported here are ~~was~~ similar to those found by Ferrante et al., (2004; 2009) which reported values between 3 and 35 μg cm^{-2}. The use of

easy and non destructive instrument for chlorophyll characterization, SPAD, is very important for quickly qualitative analysis in the market. Our results showed a good correlation between SPAD and spectrophotemer values. Unfortunately in different species and conditions this correlation may not be significant (Yadava, 1986; Pacifici et al., 2008b). The spectrophotometric measurements determine the amount of chlorophyll pigment present in the tissue, while the measurement performed by SPAD depends on the transmission of light through the leaf tissue (Monje and Bugdee, 1992). This, in turn, does not depend only on pigment concentration in the tissue, but also by the dispersion of light by refraction of the leaf surfaces and distribution of pigments in the leaf (Vogelmann, 1989). The light scattering depends on the distribution of cells in the leaf tissues and the most component of dispersion seems to be the gaps between the cells and air spaces (Vogelmann, 1989). It is reported in the literature that the use of SPAD led to an overestimation of the effective chlorophyll content in leaves when real concentration exceed 600 mg m^{-2} or less was 100 mg m^{-2}, within in this range correlation was found (*r*=0.93). Nevertheless in our case, although the chlorophyll concentration in the samples of *M. incana* was less than 100 mg m^{-2} the correlation was also significant (*r*=0.9496). Therefore, non-destructive chlorophyll analysis can be used in cut stock flowers for quality evaluation.

Materials and methods

Plant material

Cut stock (*Matthiola incana* L.) flowers were obtained from a commercial company (Floratoscana, Pescia - PT - Italy) and transported to the postharvest laboratory of the Department of Biologia delle Piante Agrarie of University of Pisa. Cut flowers were selected and trimmed to a length of 50 cm to provide homogenous samples and basal leaves were removed. Experiments were carried out in the postharvest evaluation room under the following environmental conditions: 20±1°C, 15 μmol m^{-2} s^{-1} of photosynthetic photons flux density (PPFD), relative humidity 60-70% and 12 h photoperiod with cool-white fluorescent lamps.

Storage conditions

The experiments were composed by a basic control (non stored flowers), cold wet (C, flowers stored in water) and cold mild vacuum dry (MV, flowers stored in plastic bag with air removed). The basic control was represented by flowers directly placed in the postharvest room in glass bottles containing 400 ml deionised water for evaluating the potential vase life. The C treatment was performed placing cut stock flowers in distilled water at 4±1°C in darkness for 3 or 6 days (T3C and T6C). The MV was carried out packaging three cut stock stems (in average 150 g FW) for each bag (vacuum bag 30 cm x 60 cm, Polyamide 20 μm – Polyethylene 80 μm extrusion laminated, oxygen permeability 50 cm^3/m^2 d bar, thickness 100 μm, ORVED, Musile di Piave, VE, Italy) sealed with vacuum packing machines (vacuum ranged from 100 to 500 mbar) at 4±1°C for 3 or 6 days (T3MV and T6MV). The MV package was performed removing the air until the two layers of the bag touch each other without removing all the air. The effect of storage on vase life of cut *M. incana* was determined by taking out samples after 3 and 6 days, and transferred in the postharvest evaluation room.
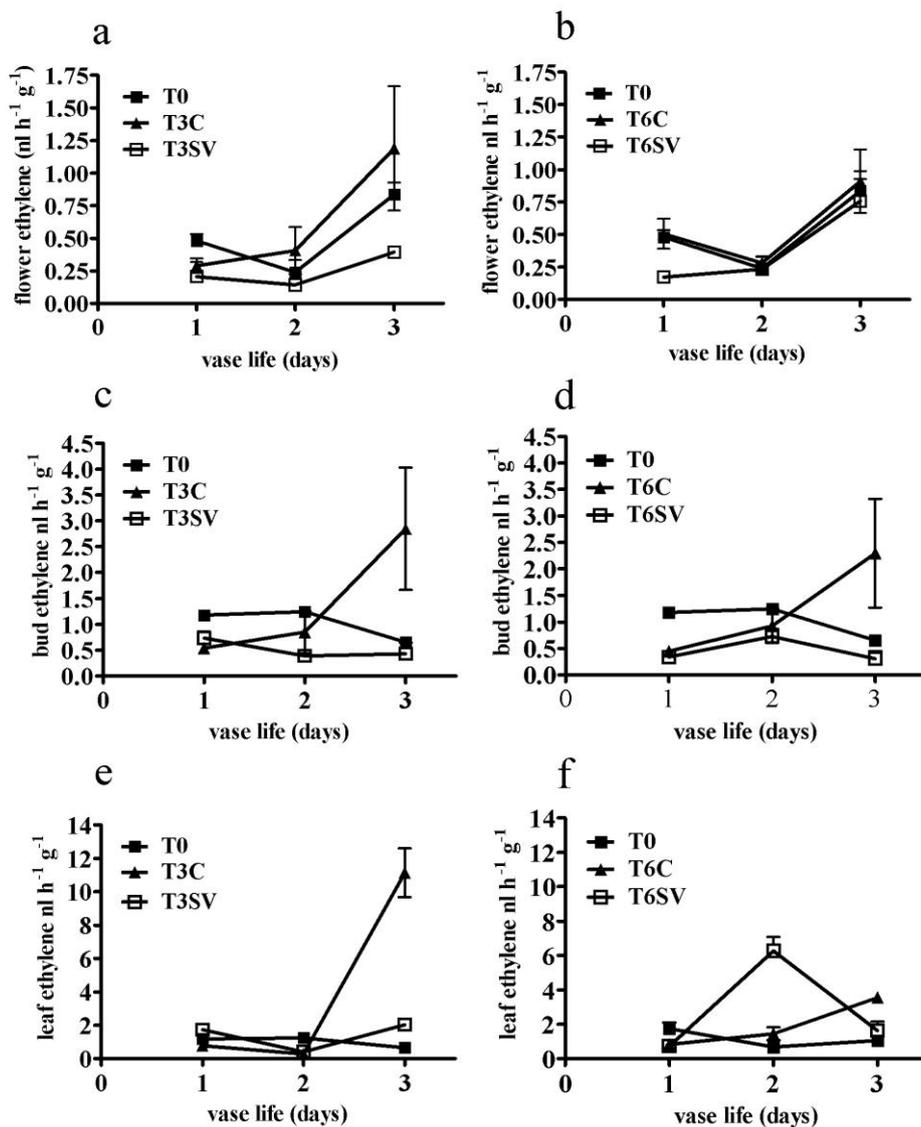
Fig 7. ethylene production (nl h^{-1} g^{-1} fw) from open flower (a and b), buds (c and d), leaves (e and f) during vase life in cut stock flowers (*m. incana*) not stored (t0), cold wet (t3c and t6c) or mild vacuum cold dry (t3mv and t6mv) stored after 3 or 6 storage days. values are means ± standard error.

$$y = 3.008x - 40.544$$
$$r = 0.9496$$
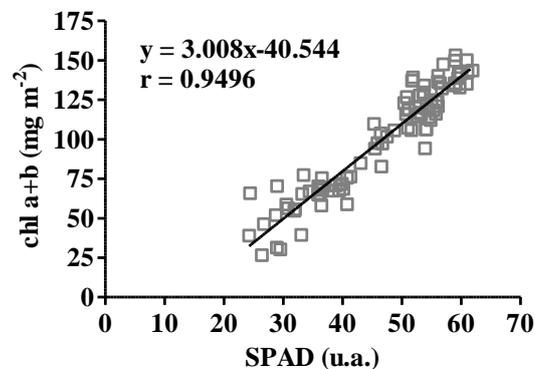
Fig 8. Correlation between the concentration values of total chlorophyll (chl a+b: mg m^{-2}) by spectrophometric analysis and SPAD index values (a.u. arbitrary units).

Vase life evaluation

The vase life was visually evaluated by monitoring leaf yellowing and floret wilting. The first symptoms of senescence were determined by daily observations of medial or apical leaf yellowing or withering and petal abscission. Vase life within each storage treatment was considered completed when 75% of flowers were wilted. Leaves were considered senesced when at least 50% of the area was chlorotic. The useful vase life was considered as the minor value between the leaf yellowing or withering and flower senescence.

Water uptake and weight variation determination

The daily water uptake was measured by gravimetric method, by weighing the glass bottles containing deionised water with or without flower, and expressed as a daily rate for a cut stock (H_2O g d^{-1} flower stem^{-1}). Also the daily weight stock variation was determined with the same method (g d^{-1} whole stem^{-1}).

Alcohol dehydrogenase assay

The ADH enzyme activity was monitored in the different flower organs in samples stored in both storage conditions. The endogenous ADH activity was measured with modified method described by Chung and Ferl (1999). For each treatment four samples were collected from leaves, open flowers and buds tissue (1 g for each sample). Tissues were powdered in liquid nitrogen using a mortar and pestle. One gram of tissue powder was extracted with 3 ml of 50 mM Tris-HCl (pH 8.0) and 15 mM L-(−)-Dithiothreitol (Sigma, Milan Italy). The homogenate was centrifuged at 12.000 g for 15 min at 4±1°C. The ADH activity was spectrophotometrically measured by monitoring the oxidation of NADH at 340 nm of reaction solution composed by 752 μl of reaction buffer (50 mM Tris HCL at pH 9.0 and 0.867 mM NAD), 40 μl supernatant, mixed three time and maintained at room temperature for 5 minutes. Just before starting spectrophometric analysis was added 208 μl of ethanol 96% were added. Protein was determined by the method of Bradford (1976) using bovine γ-globulin as a standard. For the calculation of ADH activity the molar extinction coefficient of NADH ($6.22*10^3$ mol cm^{-1}) was utilized. The results were expressed as mM min^{-1} mg^{-1} of protein.

Ethylene and CO_2 determination

Ethylene and CO_2 analyses were carried out in the plastic bags. Six plastic bags were place in the postharvest room containing 5 glass tubes (15.0 x 1.9 cm) to reproduce a similar volume occupied by the three stock flowers. Ethylene accumulation was initially determined into the bags with and without cut stock, in order to evaluate the ethylene released from the plastic materials of the bags. Ethylene production was also measured by enclosing six disks (∅ 1.0cm) of leaves in airtight containers or three open or three bud flowers in airtight containers. Two ml gas samples were taken from the headspace of the containers after 1 h incubation at room temperature. The ethylene an CO_2 concentrations were measured by a gas chromatograph (HP5890, Hewlett-Packard, Menlo Park, CA) using a flame ionization detector (FID) and thermal conductivity detector (TCD), respectively for ethylene and CO_2; a stainless steel column (150 x 0,4 cm ∅ packed with Hysep T), column and detector temperatures of 70°C and 350°C, respectively, and nitrogen carrier gas at a flow rate of 30 ml min^{-1} and 45 ml min^{-1} for ethylene and CO_2 respectively. Quantification was performed against an external standard and results were expressed on a fresh weight basis (nl h^{-1} g^{-1} F.W.). In preliminary experiment the fresh and the dry weight of leaf disk samples of cut stock were determined at the opening of sealed bags and during vase life to evaluated the water content in the tissue.

SPAD and chlorophyll determination

Three measurements for each leaf were taken with SPAD (Soil Plant Analysis Development) a non-destructive chlorophyll meter (SPAD-502, Konica Minolta Sensing, INC., Japan), three basal and medium leaves for each storage treatment. The same leaves were used for chlorophyll content determination. Chlorophyll content was determined by extraction with methanol (99%) and samples (6 for each treatment) were kept in darkness at 4±1°C for 24 h. The absorbance readings were performed at 665.2 and 652.4 nm. Total chlorophyll content was calculated as described by Lichtenthaler (1987).

Statistical Analysis

The data are reported in figures as mean with standard errors. Nine stems were used for each storage treatment: six replicates were used for vase life and for chlorophyll determination; three replicates for ethylene determination in C and MV treatments for flowers and leaves respectively and eighteen replicates for SPAD determinations. Data of the vase life, water uptake, ADH activity were subjected to ANOVA two way analysis. Chlorophyll and SPAD values were analysed by ANOVA one way and differences among means were determined by Tukey's post-test.

Conclusion

In conclusion, the results of this work suggest that mild vacuum packaging can be used for storing cut stock flowers if transportation or storage is limited to three days. In this period of time, no differences of vase life and quality were found in cut flowers stored in C or MV. Therefore, MV can be used for lowering the transportation costs by increasing the loading capacity per unit of volume of cut flowers. Moreover, the absence of water facilitates all handling operations, contributing to a reducing in the high costs that characterize this sector. However further studies must be carried out for extending this packaging technique to other ornamentals.

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