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Flower Longevity in Ten Cultivars of Cut *Ranunculus asiaticus* L. as Affected by Ethylene and Ethylene Inhibitors

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Summary

The role of ethylene on ranunculus cut flower senescence was investigated. Ten *Ranunculus asiaticus* cultivars ('Pluto', 'Juny', 'Shangai', 'Auriga', 'Bianco2', 'Ken', 'Dido', 'Lulù', 'Saigon' and 'Green') were treated with 2 mM silver thiosulfate (STS), 4 mM amino-oxyacetic acid (AOA), and 377 nL L^{-1} 1-methylcyclopropene (1-MCP, Ethylbloc™) for 14 h and with 8 μL L^{-1} ethylene for both 14 and 72 h. Evaluation of postharvest performance was based on: visual check for symptoms of senescence alteration (VS), stem fresh weight (FW), petal colour, ethylene production, and leaf chlorophyll content. Results showed genotype differences for all parameters. Senescence was usually reached about 13 d after the beginning of the experiment. In untreated flowers, 'Green' was the longest-lived (16.2 d) and 'Lulù' the shortest-lived (11.1 d).

Statistical analysis showed an interaction between cultivar and treatment. In general, AOA and STS treated flowers lasted more than the control. These molecules are inhibitors of ethylene biosynthesis or ethylene action respectively. However, they can also act as antibacterial agents. More in detail, AOA extended the longevity in four out of ten cultivars ('Lulù', 'Pluto', 'Bianco2' and 'Green') and STS in two ('Pluto' and 'Bianco2'). Exogenous ethylene application did not negatively affect any of the investigated cultivars. Overall it can be concluded that the new ranunculus cultivars tested are ethylene insensitive. Therefore special precautions against exposure to ethylene are not needed.

Key words. anti-ethylene molecules – buttercup – postharvest – vase life

Introduction

Ranunculus asiaticus L. is an important cut flower in the Mediterranean area. From as far back as the early 18th century, ranunculus has been widely bred (Costa et al. 2007). Double flower types with long and strong stems were developed, making the flowers suitable for cut flower commercial production, and possibly for pot production of the dwarf types (Kenza et al. 2000).

Breeding programs for this plant are quite complex since the species, under Mediterranean conditions, is heterothallic and the homogeneity of the populations is not always guaranteed (Beruto and Debergh 2004). Therefore, at present the cut flower production relies to a large extent on the vegetative propagation of selected genotypes (Beruto et al. 1996). Tissue culture is an attractive option to induce accelerated propagation of performing and healthy genotypes of ranunculus for which a better production schedule can be envisaged (Beruto 2002).

The longevity of cut stems is affected by production practices as well as proper postharvest treatments. The vase life may be extended by floral preservatives. Many products provide a carbohydrate source as well as a biocide such as silver nitrate or 8-hydroxyquinoline sulphate (HQS) that inhibit bacterial or fungal proliferation and maintain the hydraulic conductance of the stem (Kumar et al. 2008). Some floral preservatives include an inhibitor of ethylene biosynthesis or ethylene action. In many species, ethylene may have a detrimental effect on the vase life of cut stems, resulting in the senescence or abscission of leaves and flowers (van Doorn and Woltering 2008). Early senescence may be related to ethylene exposure, mainly occurring in the postharvest shipping and marketing environment, and to endogenous ethylene production. As reported by Kumar et al. (2008), ethylene sensitive flowers can be classified into three types:

1. Flowers where senescence is regulated by the increased amount of ethylene production during ageing or following pollination (e.g. carnation and petunia);
2. Flowers in which ethylene sensitiveness and production increase when they are pollinated (e.g. cyclamen);
3. Flowers sensitive to ethylene upon bud opening but do not produce elevated amount of the hormone with ageing (e.g. rose).

Generally speaking, flowers belonging to *Ranunculaceae* family are regarded as highly ethylene-sensitive (Woltering and van Doorn 1988).

The action of amino-oxyacetic acid (AOA) (Baker et al. 1982) and cobalt ions (Lau and Yang 1976) is well known for blocking ethylene biosynthesis. However, ethylene biosynthesis inhibitors do not protect flowers from the effect of exposure to exogenous ethylene (Newman et al. 1998).

The blocked action of ethylene is mainly based on the competition between the hormone and other molecules at the binding site. The usefulness of the silver thiosulfate (STS) to overcome problems associated with exposure to ethylene gas has already been well established (VEEN and VAN DE GEIJN 1978; NICHOLS et al. 1982). From about 15 years on, the research of alternative molecules free of heavy metals led to the discovery and use of 1-methylcyclopropene (1-MCP), a gas acting at low concentrations (ppb range) and that could be administered even for few hours (SISLER and SEREK 1997; SEREK et al. 2006). 1-MCP appeared to give ethylene protection equally as STS (SEREK et al. 1994; BLANKENSHIP and DOLE 2003) in several cut flowers, such as *Dianthus caryophyllus* (SINGH et al. 2007), *Epidendrum ibaguense* (FINGER et al. 2008), *Matthiola incana* (MUNETO et al. 2007), *Freesia* and *Chamelaucium* (DOLE et al. 2005) and *Dendrobium* (UTHAICHAY et al. 2006).

In *Ranunculus*, flowers last up to 20 d on plant, but only about nine days in vase (HALEVY and MAYAK 1979; KENZA et al. 2000). Cultivation practices can affect postharvest quality (BERNSTEIN et al. 2005). Despite its commercial importance, few studies have been carried out to date on cut ranunculus flowers with the aim of increasing postharvest longevity and investigating the possible involvement of ethylene in senescence processes. According to KENZA et al. (2000), flower quality of *R. asiaticus* 'Gold' was not improved by treatments with AOA or STS, suggesting that ranunculus flowers are not ethylene-sensitive. EVANS et al. (2002) reached similar conclusions in *R. lyallii* Hook. In contrast, PISKORNIK et al. (1995) reported that STS and cobalt ions increased flower longevity of cut Persian ranunculus, while AOA exerted some phytotoxic effects. Lastly, according to MENSUALI SODI et al. (2002), treatments with AOA and ACC reduced ethylene biosynthesis in *R. asiaticus* and the endogenous ethylene production was in turn correlated to flower colour. More in detail, white flowers produced the least ethylene and had the longest vase life, AOA enhanced the longevity especially of yellow and orange flowers.

As the involvement of ethylene in the regulation of cut ranunculus flower senescence appeared debated, we further investigated the possible action of both endogenous and exogenous ethylene on flower senescence in several *R. asiaticus* cultivars. At first, differences in postharvest longevity of ten commercially promising buttercup selections were evaluated. Then, the ethylene role in flower senescence and the efficacy of treatments with claimed ethylene inhibitory effects were investigated.

Materials and Methods

Plant materials

Cut flowers at closed and pigmented bud stage of ten buttercup cultivars were provided by Biancheri Creations Company (Camporosso Mare, Imperia, Italy). The cultivars were produced through *in vitro* propagation and had different flower colours: 'Pluto' (red), 'Juny' (yellow), 'Shangai' (pink), 'Auriga' (yellow-orange),'Bianco2' (white), 'Ken' (white with purple stripes), 'Dido' (orange), 'Lulù' (pink), 'Saigon' (red) and 'Green' (green). Cut flowers were gathered from plants originating from tuberous roots obtained from *ex vitro* plantlets and supplied between 15th January and 15th April 2006. Cultivation was carried out in an unheated greenhouse, on benches equipped with a drop-irrigation system and filled with a substrate of pumice supplemented with a low percentage of peat. Planting density was 12–14 plants m^{-2}.

Experimental trials

After delivery to the postharvest laboratory, stems were immediately placed in tap water and recut, labelled, weighed and treated. Three floral preservatives and two ethylene treatments were evaluated and compared to one control in

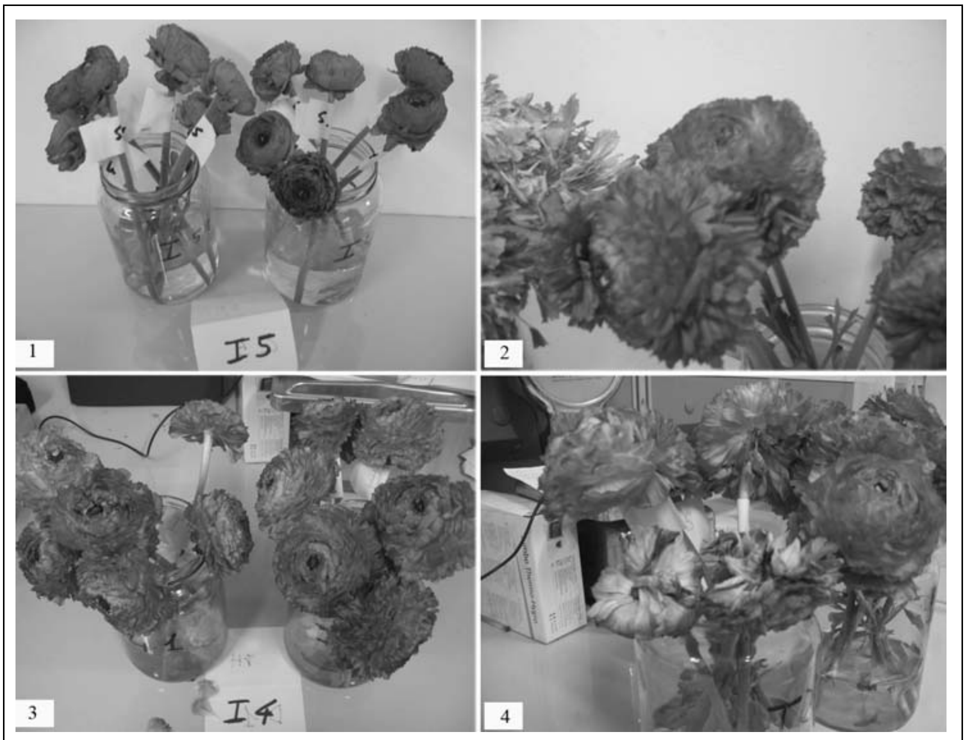

Fig. 1. Time course of senescence in untreated stems of *R. asiaticus* 'Saigon'. The four stages of visual senescence (VS) are: 1 = no alterations; 2 = slight alterations (early petal colour variation); 3 = evident alterations (petal colour variation, petal abscission, and early wilting); and 4 = total alterations (wilting and bent neck).

tap water. For each cultivar, six cut flowers (each stem 30 cm long) were placed for 14 h in tap water, 2 mM STS solution or 4 mM Amino-oxyacetic acid (AOA) solution; and six cut flowers were placed in tap water, enclosed in an air-tight cabinet (112 L) and treated with Ethylbloc™ powder (0.14 % w/w active ingredient; Agrofresh Inc. Auckland, New Zealand) to produce 377 nL L^{-1} 1-methylcyclopropene (1-MCP) for 14 h or exposed to 8 μL L^{-1} ethylene both for 14 and 72 h. After treatments, stems were placed in vases containing tap water (pH 7.4, 427 μS cm^{-1} E.C., 23 F). The experiment was performed twice, in a standard vase life room at 20 ± 2 °C, 60 % RH, and 46 μmol m^{-2} s^{-1} cool white light as measured at flower height with light meter (model HT307; HT, Faenza, Italy) for 12 h per day from 06.00 am to 06.00 pm. The experimental conditions were meant to closely simulate interior home conditions.

Data collection and statistical analysis

Evaluation of postharvest performance was based on: visual check for symptoms of senescence alteration (VS), stem fresh weight (FW), petal colour, ethylene production, and leaf chlorophyll content.

Visual check and fresh weight determinations were carried out daily, according to Scariot et al. (2008). VS was recorded as the number of days after delivery (day 0) that flowers reached the end of their longevity due to bent neck or advanced signs of fading on all petals. The visual symptoms of senescence (wilting, colour variation and bent neck) were evaluated daily, following four stages: 1 = no alterations; 2 = slight alterations (early petal colour variation); 3 = evident alterations (petal colour variation, petal abscission, and early wilting); and 4 = total alterations (wilting and bent neck) (Fig. 1). In many flowers, FW at first usually increases during development and then at later stages decreases resulting similar to that earlier development. In this study, also in absence of visual symptoms, cut flowers were considered senescent when FW went back to the starting value (ΔFW = 0). The first evaluation method is useful to establish the aesthetic quality trend during the vase life, the second to describe disturbances in water balance. However, daily measuring of the FW variation could cause embolisms in the stems.

Table 1. Effect of 2 mM silver thiosulfate (STS), 377 nL L^{-1} 1-methylcyclopropene (1-MCP), 4 mM amino-oxyacetic acid (AOA), 8 μL L^{-1} ethylene applied for 14 h and tap water on the longevity of ten cultivars of *Ranunculus asiaticus*. In parentheses, evaluation of differences among untreated cultivars is reported. Values refer to the longevity (days) estimated both as visual senescence (VS) and fresh weight variation (FW).

Cultivar	Method	Longevity (d)				
		STS	1-MCP	AOA	Ethylene	Control
‘Lulù’	VS	11.1 b*	11.8 b	13.3 a	12.3 b	11.1 b (c)
	FW	7.3 ab	7.0 ab	8.4 a	6.8 b	7.3 ab (c)
‘Shangai’	VS	13.3 a	11.9 a	12.7 a	11.9 a	12.5 a (bc)
	FW	8.2 a	7.8 a	8.8 a	7.0 a	7.7 a (bc)
‘Dido’	VS	12.7 ab	13.1 a	12.2 ab	11.8 b	12.7 ab (bc)
	FW	4.9 a	4.3 a	3.8 a	3.7 a	3.8 a (de)
‘Auriga’	VS	12.8 a	12.5 a	12.5 a	13.3 a	12.3 a (bc)
	FW	11.3 a	10.4 a	9.6 a	9.9 a	10.8 a (a)
‘Juny’	VS	14.1 a	12.7 a	12.5 a	13.3 a	12.3 a (bc)
	FW	12.6 a	9.0 b	10.6 ab	11.2 ab	10.3 b (a)
‘Saigon’	VS	13.5 b	14.1 ab	13.7 b	15.3 a	13.8 b (b)
	FW	3.0 a	4.3 a	3.2 a	3.4 a	2.8 a (e)
‘Pluto’	VS	16.5 a	13.5 bc	14.7 b	11.9 c	12.3 c (bc)
	FW	10.6 a	11.5 a	10.5 a	9.2 a	9.3 a (ab)
‘Ken’	VS	13.7 a	14.8 a	14.5 a	13.9 a	14.1 a (b)
	FW	7.5 a	7.6 a	5.2 b	6.4 ab	5.4 b (d)
‘Bianco2’	VS	16.0 a	14.7 ab	16.5 a	14.8 ab	13.5 b (b)
	FW	11.3 a	11.3 a	12.3 a	10.5 a	10.8 a (a)
‘Green’	VS	15.7 b	16.2 b	18.3 a	17.1 ab	16.2 b (a)
	FW	9.0 ab	5.8 b	10.5 a	8.6 ab	7.3ab (c)

*Mean values of each cultivar showing the same letter are not statistically different at P≤0.05 (according to Tukey’s test).

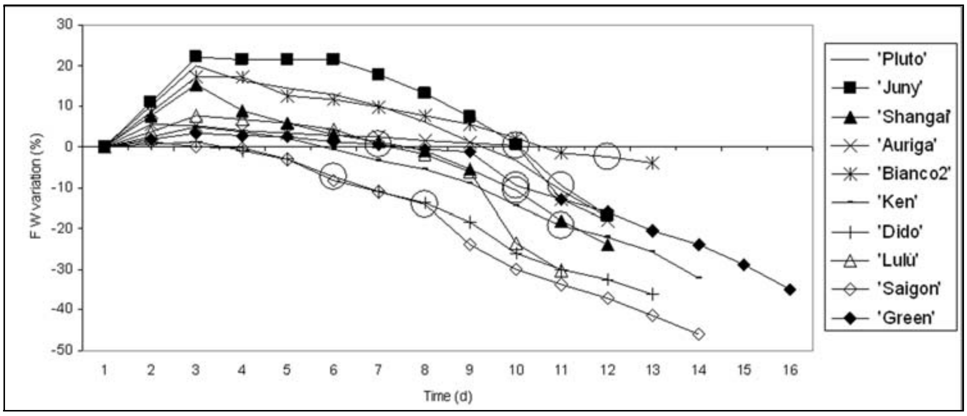

Fig. 2. Average daily stem fresh weight (FW) variation in untreated flowers of ten cultivars of *R. asiaticus*. Circles point out when the flower longevity based on visual check for symptoms of senescence alteration was at stage 2 (vase life end).

Petal colour was measured on 12 petals (4 external, 4 median and 4 inner) from 4 flowers per cultivar every two days using the Spectrophotometer CM-2600 Konica Minolta Sensing Inc. (Osaka, Japan), L* a* b* colour space (L* = brightness; a*+ = red light and a*- = green light; b*+ = yellow light and b*- = blue light).

After seven days from the beginning of each treatment, endogenous ethylene, produced by two stems of each cultivar kept for 14 h in sealed tubes, was measured by means of the DANI 8510 Gas Chromatograph (DANI Instruments S.p.A., Cologno Monzese, Italy) and calculated using the DDS 1032 v.1.4.10 software.

Leaf chlorophyll content was evaluated using the Chlorophyll Meter SPAD-502 Konica Minolta Sensing Inc. (Osaka, Japan). Measurements were performed on four leaves per stem each two days.

Data were subjected to univariate analysis of variance and Tukey's test ($P<0.05$), using the software SPSS Inc. (Chicago, Illinois).

Results and Discussion

The present study aimed to investigate vase life differences among new ranunculus cultivars and ethylene role on cut flower senescence. Postharvest performances were therefore evaluated at first on untreated flowers and then comparing the different treatments.

Ranunculus senescence was estimated by means of visual check (VS) and fresh weight (FW) variation (Table 1). In untreated flowers, the longevity based on VS was different among cultivars with 'Green' the longest-lived (16.2 d) and 'Lulù' the shortest-lived (11.1 d). On average, senescence was generally reached about 13 d after the beginning of the experiment. In previous studies, flowers lasted 11.5 d in *R. asiaticus* (Bernstein et al. 2005) and 10 d in *R. lyallii* (Evans et al. 2002). The longevity based on FW was about 7 d, with 'Bianco2' (10.8 d), 'Auriga' (10.8 d), 'Juny' (10.3) and 'Pluto' (9.3 d) the longest-lived and 'Dido' (3.8 d) and 'Saigon'(2.8 d) the shortest-lived. In the graph showing the average daily stem FW variation (%) for each cultivar (Fig. 2), untreated flowers appeared to increase their weight within the first three days. Then flower weight decreased depending on the genotype. $\Delta FW = 0$ was reached before cultivars lost their aesthetic quality (stage 2), except for 'Lulù' and 'Juny' in which these two moments are coincident. Therefore, FW variation measurements appeared to be inappropriate to evaluate the flower longevity. Commercially, the vase life ends at stage 2

Table 2. Mean values of the chlorophyll content (express as SPAD values) in ten cultivars of *R. asiaticus* treated with 1-methylcyclopropene (1-MCP), amino-oxyacetic acid (AOA), silver thiosulfate (STS), ethylene applied for 14 h and tap water (control) after 3 d of vase treatment. In parentheses, evaluation of differences among untreated cultivars is reported.

Cultivar	SPAD (a. u.)				
	STS	1-MCP	AOA	Ethylene	Control
'Lulù'	55.30 a*	49.01 ab	49.20 ab	46.02 ab	40.22 b (d)
'Shangai'	53.87 a	54.32 a	56.49 a	58.89 a	52.38 a (bc)
'Dido'	57.90 ab	54.79 b	57.08 ab	55.63 b	59.79 a (ab)
'Auriga'	54.97 a	60.86 a	56.85 a	57.51 a	59.13 a (ab)
'Juny'	56.71 a	58.04 a	50.69 a	55.28 a	51.48 a (bc)
'Saigon'	69.17 a	50.34 ab	57.40 ab	58.63 ab	42.27 b (d)
'Pluto'	66.21 a	60.93 a	61.33 a	68.76 a	57.72 a (abc)
'Ken'	60.80 a	57.73 a	54.52 a	52.68 a	61.08 a (a)
'Bianco2'	54.57 a	53.95 a	53.84 a	50.19 a	53.68 a (abc)
'Green'	65.53 a	57.52 a	62.24 a	57.98 a	58.14 a (abc)

*Mean values of each cultivar showing the same letter are not statistically different at $P\leq0.05$ (according to Tukey's test).

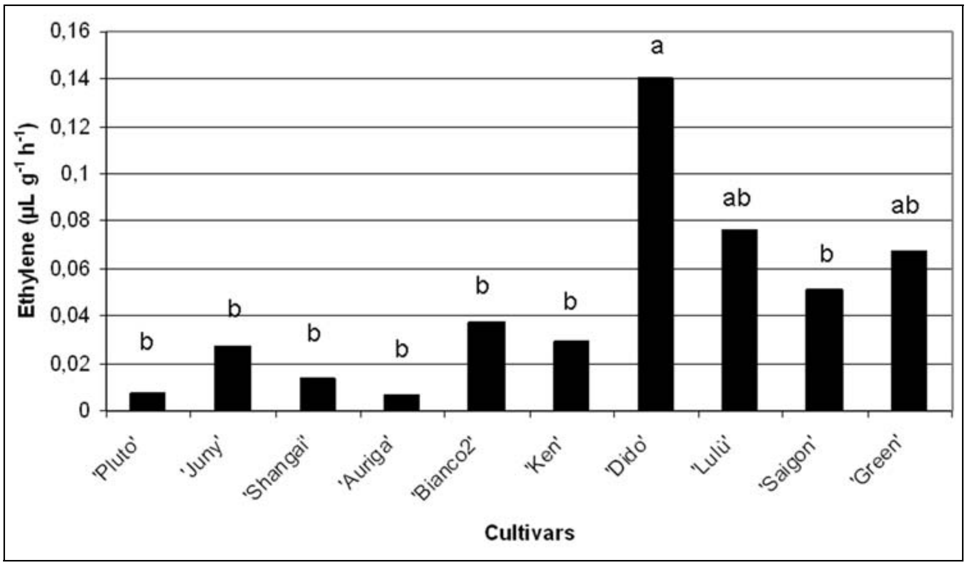

Fig. 3. Mean values of ethylene production of ten untreated cultivars of *R. asiaticus* at the 7th day from the beginning of the experiment. Mean values of each cultivar showing the same letter are not statistically different at $P \leq 0.05$ (according to Tukey's test).

(Fig. 2). This stage occurred about after 6 d in 'Dido' (63 % longevity length), 7 d in 'Lulù' (63 %), 8 d in 'Saigon' (44 %), 10 d in 'Auriga' (90 %), 'Green' (63 %), 'Shangai', 'Juny', and 'Pluto' (80 %), 11 d in 'Ken' (80 %) and 12 d in 'Bianco2' (90 %).

Statistical analysis showed an interaction between cultivar and treatments. Overall, AOA and STS treated flowers lasted more than the control (data not shown). In Table 1, treatment efficacy was analyzed by cultivar. In agreement with Mensuali Sodi et al. (2002), AOA treatment postponed the appearance of senescence alterations in 'Lulù', 'Green', 'Pluto' and 'Bianco2'. By contrast, no correlations with petal colour were observed. Even if flowers were treated with quite AOA high concentration, phytotoxic effects were not found, unlike Piskornik et al. (1995). In 'Pluto' and 'Bianco2' the senescence was delayed by STS treatment, too. This result differs from what observed in *R. lyallii* where STS failed to increase the vase life (Evans et al. 2002). 1-MCP and ethylene (14 h) treated flowers performed as well as the control, except in 'Saigon' for which, unexpectedly, ethylene increased the vase life. Similar results were obtained in *R. lyallii* (Evans et al. 2002). In 'Dido', 1-MCP treated flowers performed better than flowers exposed to ethylene (14 h). Generally in the pollutant environments, ethylene concentration does not overcome 3 $\mu L\ L^{-1}$. So flowers can be classified as ethylene sensitive or not over this concentration (Ferrante and Reid 2006). The responsiveness of flowers to ethylene varies depending on the physiological age and state of the tissue at the time of exposure. Usually, the ethylene sensitivity of flowers increases with age from anthesis to senescence in many ethylene-sensitive species. In *R. lyallii,* cut flowers were exposed at 1, 2, 5 and 50 $\mu L\ L^{-1}$ for 1, 4, 8, 24 and 48 h and all the treatments failed to accelerate the senescence (Evans et al. 2002). Similarly, in our experiment ethylene, applied with an amount higher than usual both for 14 and 72 h (data not shown), did not negatively affect any of the investigated cultivars. Overall it can be concluded that the new ranunculus cultivars tested did not show any ethylene responses, in agreement with Kenza et al. (2000).

AOA extended the longevity in four out of ten cultivars ('Lulù', 'Pluto', 'Bianco2' and 'Green'), STS in two ('Pluto' and 'Bianco2'). AOA acts in blocking ethylene biosynthesis while STS, as well as 1-MCP, by protecting flowers from the effect of endogenous and exogenous ethylene. However, AOA and STS can act as antibacterial agents, which inhibit bacterial growth. The silver ion is well known to have a bactericidal property (Veen 1983). The ability of AOA as an antimicrobial agent is attributed to the maintenance of low pH which results in a non-conducive environment for bacterial growth (Chandran et al. 2006).

Leaf chlorophyll content (SPAD) was genotype-dependent. In Table 2, SPAD values after 3 d of vase life are reported. The highest chlorophyll content was found in 'Ken', the lowest in 'Lulù' and 'Saigon'. These cultivars are also the only two in which STS treatment preserved SPAD values better than in the control. In 'Dido', both 1-MCP and ethylene treated flowers showed a decrease in chlorophyll. The other preservative treatments did not affect the leaf chlorophyll content. At the second survey (5 d) differences were no longer detectable (data not shown).

Low variations of the chromatic parameters a*, b* and L* were considered as an indicator of colour maintenance. Results showed that genotype differences were more influent than treatments (data not shown). Cultivars with white ('Bianco2' and 'Ken') and pink ('Shangai' and 'Lulù'') flowers preserved more their early petal colour. Cultivars with red ('Pluto' and 'Saigon') flowers changed more, especially in the a* component. In green flowers ('Green'), the lowest variation of the red component (a* = 10.5 %) and the highest variation of both the yellow component (b*= 50.8 %) and brightness (L*= 22.1 %) were observed.

In untreated flowers, differences in ethylene production were detected among cultivars (Fig. 3). 'Dido' produced double the amount of ethylene (0.140 $\mu L\ g^{-1}\ h^{-1}$) compared to 'Lulù'' (0.077 $\mu L\ g^{-1}\ h^{-1}$), twenty times higher compared to 'Auriga' (0.007 $\mu L\ g^{-1}\ h^{-1}$) and five times higher compared to 'Juny' and 'Ken' (0.028 and 0.029 $\mu L\ g^{-1}\ h^{-1}$, respectively). By contrast, in treated flowers (data not shown), ethylene production was not statistically different from the control.

In conclusion, the involvement of ethylene in the regulation of cut ranunculus flower senescence was debated.

In this study, exogenous ethylene application did not anticipate senescence in the newer varieties of ranunculus tested. Therefore in agreement with EVANS et al. (2002) and KENZA et al. (2000) special precautions against exposure to the ethylene are not needed. The provided data could be of benefit to growers and those involved in shipping.

In order to increase cut ranunculus longevity, future studies should be addressed to investigate other flower treatments, such as cycloheximide (SHEIKH and SIKANDAR 1997), N-lauroylethanolamine (ZHANG et al. 2007), and cetyl pyrine bromide (ZHANG et al. 2006).

Differences in genotype were observed related to several parameters. This information could be useful in order to select new valuable accessions for the flower market and new genetic resources for breeding programs.

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