Adv. Hort. Sci., 2015 29(2-3): 103-108

Evaluation of postharvest storage and treatments in cut ruscus foliage

R. Bulgari(*), M. Negri, A. Ferrante
Dipartimento di Scienze Agrarie e Ambientali, Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy.

Key words: *Danae*, glycerol, leaf senescence, Thidiazuron.

Abstract: *Danae racemosa* (L.) Moench is an important cut foliage in the ornamental market. During postharvest, leaf senescence symptoms are leaf yellowing, weight loss and/or abscission of leaves. The aim of this work was to evaluate the effect of pre-treatments for 24 h with glycerol and Thidiazuron (TDZ) on *Danae* vase life before and after storage. Treatments were applied in vase water containing glycerol 0.1, 1 or 10 mM, 10 µM TDZ and a combined treatment of 10 µM TDZ plus 10 mM glycerol. The effect of treatments was evaluated through the determination of vase life, chlorophyll content, chlorophyll *a* fluorescence parameters, sucrose and total sugars content. The cut foliage were stored in sealed plastic bags and placed in a dark room at 4°C for two months. The vase life, before and after storage, was determined in a controlled chamber set to 20°C and RH 50-60% with a light intensity of 20 µmol m^{-2} s^{-1}. Results demonstrated that 10 µM TDZ plus 10 mM glycerol was the most effective treatment in maintaining quality during vase life of stored and not stored cut foliage.

1. Introduction

Cut foliage represents an important part of the floricultural industry. They are used as fillers in floral bouquets especially for cut flowers without leaves such as gerberas. The vase life of cut foliage is usually longer than cut flowers, but it may become shorter when they are stored for long periods. Storage methods, wet or dry, affect the vase life of cut foliage (Ferrante *et al*., 2002 a) and play a crucial role for preserving quality: it is important, in particular, to delay the symptoms of leaf senescence considering that the intensity of leaf color is the most important quality parameter (Pacifici *et al*., 2007) and closely associated with the marketability of ornamental cut foliage. During postharvest, quality losses of cut foliage are essentially represented by leaf senescence. The most common symptoms are leaf yellowing, leaf desiccation and/or leaf abscission, and weight losses (Pacifici *et al*., 2007). Leaf yellowing is common in many cut flowers such as *Alstroemeria*, *Chrysantemum*, lilies, tulips, but also in some cut foliage such as cut *Danae racemosa* foliage (van Doorn *et al*., 1992; Ferrante *et al*., 2002 b, 2003, 2005). Postharvest treatments with cytokinins are able to delay leaf yellowing in many cut flowers, but a substituted phenylurea, the Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-yl urea, TDZ), with cytokinin-like activity, has been found to be very effective in many cut flowers sensitive to leaf yellowing (Ferrante *et al*., 2002 b); application of low concentrations of TDZ delayed leaf yellowing (Ferrante *et al.,* 2002 b, 2003; Mutui *et al.*, 2005; Jiang *et al.*, 2008).

During the postharvest chain, another important problem for cut foliage is weight loss. Since these ornamental items are sold by weight, any weight loss is directly translated into economic loss. Cut foliage is essentially composed of branches with leaves, therefore, weight losses are due to water losses by transpiration. During postharvest, the hydraulic conductance of the branches or cut stems progressively declines due to vessel blockages from bacteria growth, embolism, etc. (van Doorn, 1997).

Cut flowers and branches treated with glycerol are able to enhance osmotic potential and absorb great amounts of water, avoiding reduction of the water potential. Glycerol has been used to preserve ornamental plant materials (Dubois and Joyce, 1992) as it causes a greater water removal due to an increment of osmotic potential. In this way, glycerol treatment reduces leaf weight loss, as well as water loss, which reflects on extending vase life (Shanan and Shalaby, 2011).

In this study, the possibility to extend as much as possible the storage of cut foliage in a cold room at 4°C for 60 days was evaluated with the aim of maximizing cut *Danae* availability on the market. This outlook has practical value for some cooperatives and commercialization companies as extention of the storage period could cover periods when production is lacking (Pacifici *et al*., 2013).

(*) Corresponding author: roberta.bulgari@unimi.it

Received for publication 26 September 2014
Accepted for publication 26 March 2015

2. Materials and Methods

Plant materials

Cut branches of *Danae racemosa* (L.) Moench. [=*Ruscus racemosus* L.] were provided by a commercial company (Floratoscana, Pescia, PT, Italy) and transported to the postharvest laboratory of the Department of Agricultural and Environmental Sciences of the University of Milan. Cut branches were trimmed to a length of 70 cm to provide homogenous samples for the experiments.

Chemical treatments and storage

Cut foliage was pre-treated for 24 h with a solution containing 0.1, 1 or 10 mM glycerol, 10 µM TDZ and a combined pre-treatment of 10 µM TDZ plus 10 mM glycerol, while control samples were held in distilled water. After pretreatments, the plant material was stored in vases containing distilled water at 20°C and 50-60% relative humidity. Each treatment was composed of eight replicates. A part of the cut branches treated with 10 mM glycerol, 10 µM TDZ and combined treatment 10 µM TDZ plus 10 mM glycerol were stored for 60 days in polyethylene bags at 4°C.

The gas composition in terms of oxygen and carbon dioxide percentages was monitored by means of a "Binder Combigas GA-m³" (from Binder, D) portable gas analyzer equipped with an electrochemical cell for oxygen and CO_2 measurement and an infrared dispersion cell for NH_3 determination.

Vase life evaluation

After storage, cut branches were transferred to a growth chamber with controlled temperature (20°C), relative humidity (60-70%), and light intensity (10-15 µmol m^{-2} s^{-1} PPFD for 12 h per day). Non-stored cut branches (control) were used for vase life assessment immediately after harvest. Ten branches for each storage period were placed in individual bottles for vase life analysis. Vase life was determined as the number of days from when branches were placed in the water to the onset of leaf senescence, yellowing or abscission.

Chlorophyll determination and chlorophyll a fluorescence

Chlorophyll content was measured using a chlorophyll meter (CL-01, Hansatech, UK) that provides an indicator of green color of leaves. This device determines relative chlorophyll content *in vivo* using dual wavelength optical absorbance (620 and 940 nm wavelength).

Chlorophyll *a* fluorescence was measured with a portable Handy Plant Efficiency Analyzer (PEA, Hansatech, UK). Leaves were dark-adapted for 30 min. Using a leaf clip (4 mm diameter), a rapid pulse of high intensity light of 3000 µmol m^{-2} s^{-1} (600 W m^{-2}) was absorbed by the leaf inducing fluorescence, which was measured by the sensor. The fluorescence parameters were calculated automatically. JIP analysis was performed to determine the Performance Index (PI).

Sugars determination

In order to quantify sucrose and total sugars content, about 0.5 g of leaves were ground in 10 ml of distilled water. The homogenate was centrifuged at 10000 rpm for 5 min. For sucrose determination, 0.2 ml of extract were added to 0.2 ml NaOH 2N and incubated at 100°C for 10 min; then 1.5 ml of resorcinol were added and incubated at 80°C for 10 min. A resorcinol solution was prepared by adding 35 mg of resorcinol and 90 mg of thiourea in 250 ml HCl 30%, mixed with 25 ml of acetic acid and 10 ml of distilled water. Samples were cooled at room temperature and spectrophotometer readings were performed at 500 nm. A calibration curve was built with sucrose standards at 0, 0.5, 1, 1.5, 2 mM. Total sugars were calculated by anthrone method: 0.2 g of anthrone were melted in 100 ml of H_2SO_4 and shacked for 30-40 min; 0.2 ml of diluted extract was added to 1 ml of anthrone solution, cooled in ice for 5 min and mixed thoroughly. Samples were incubated at 95°C for 5 min and then cooled on ice. Absorbance readings were measured at 620 nm and a calibration curve was built with glucose standards at 0, 1, 2, 3 and 4 mM. Leaf extracts were diluted 1:10 for total sugars assay and used pure for sucrose assay.

Statistical analysis

Experiments were performed in a completely randomized experimental design with eight replicates for each treatment. The data are reported as means with standard errors. Data presented in Table 2 were subjected to one-way ANOVA.

3. Results

Gas compositions inside plastic bags

The gas composition was determined after 30 and 60 days of storage. Results showed that oxygen was lower in the plastic bags containing cut branches treated with TDZ plus glycerol. At the same time, also higher values of NH_3 were found in the TDZ+Gly treatment (Table 1).

Vase life and chlorophyll content

The vase life was significantly improved by the combined treatment TDZ plus glycerol (33.6 days in average) in comparison with the control (average 21.2 days) and the other treatments (21.8 to 23.6 days) (Table 2). Vase life was significantly reduced in cut foliage stored for 60 days, but treatment with TDZ plus glycerol gave the longest vase life, 22.6 days on average. Vase life of the control was 13.8 days, while in the treatment with glycerol and TDZ alone it was 10.2 and 15.2 days, respectively (Table 2).

The chlorophyll content was higher in all treatments compared to control during the first 15 days of vase life, then declined in 10 mM glycerol (this treatment had the best efficiency among the glycerol treatments) while cut foliage treated with TDZ showed chlorophyll reduction after 20 days. The combined TDZ plus glycerol treatment did not show any chlorophyll reduction until 22 days (Fig. 1). After storage the best results were obtained with TDZ and TDZ plus glycerol treatment. TDZ retained the

Table 1 - Gas compositions inside the plastic bags during storage

Days	Gases	Blank	Treatments			
			Control	10 mM Gly	TDZ	TDZ+Gly
30	CO_2 (%)	0	0.30	0.60	0.10	0.3
	O_2 (%)	20	12.70	19.90	20.90	13
	NH_3 (µL L^{-1})	2	15.00	0.00	12.00	17
60	CO_2 (%)	0.00	0.50	0.40	0.40	0.3
	O_2 (%)	20.90	20.30	19.50	20.00	13
	NH_3 (µL L^{-1})	0.00	8.00	8.00	8.00	17

Table 2 - Vase life before and after storage of cut foliage treated with glycerol (0.1, 1 or 10 mM), 10 µM TDZ or a combination 10 µM TDZ plus 10 mM glycerol

Vase life of fresh harvest cut foliage						
	Control	Glycerol			TDZ	TDZ + glycerol
		0.1 mM	1 mM	10 mM	10 µM	10 µM + 10 mM
Vase life (d)	21.2±1.09 b	21.8±0.45 b	22.2±0.45 b	22±0 b	23.6±1.34 b	33.6±1.14 a

Storage and vase life post-storage				
	Control	Glycerol	TDZ	TDZ + glycerol
		10 mM	10 µM	10 µM + 10 mM
Storage (d)	60	60	60	60
Post-storage vase life (d)	13.8±1.09 b	10.2±1.12 b	15.2±0.45 b	22.6±2.61 a
Storage + vase life (d)	73.8±1.09 b	70.2±1.12 b	75.2±0.45 b	82.6±2.61 b

Values are means with standard errors (n=5).
Data were subjected to one-way ANOVA analysis and different letters indicate significant differences for P<0.05.

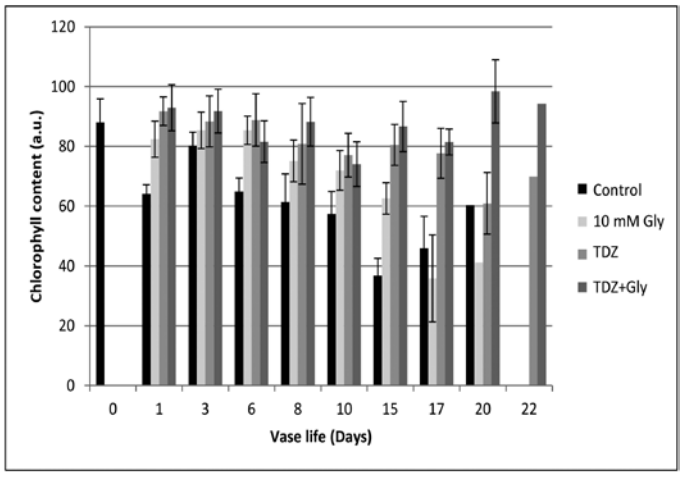

Fig. 1 - Chlorophyll content measured from cut *Danae* branches during vase life with a chlorophyll meter and expressed as relative units. Values are means with standard errors (*n=8*).

chlorophyll content in the treated cut foliage until 10 days, then chlorophyll started to decline. In the combined treatment TDZ plus glycerol, the chlorophyll values remained unchanged and similar to the fresh cut foliage (80 a.u.) until 20 days of vase life (Fig. 2).

Chlorophyll a fluorescence measurements

Chlorophyll *a* fluorescence was measured during storage by way of a non-destructive method to evaluate the leaf health status of *Danae* leaves during vase life, before and after storage. Among the different parameters and indexes calculated, the performance index (PI) is reported in figure

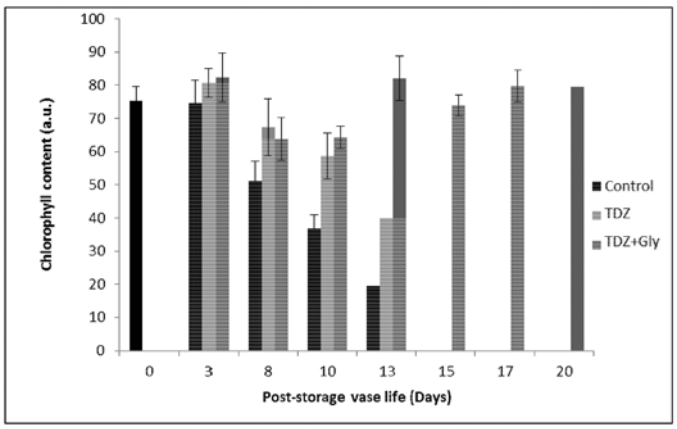

Fig. 2 - Chlorophyll content during vase life of cut *Danae* branches stored for 60 days. Chlorophyll was measured using a chlorophyll meter and expressed as relative units. Values are means with standard errors (*n=8*).

3 and 4. In particular, the PI measured during the vase life of non-stored cut foliage did not change in the combined treatment, while it declined in the control and glycerol treatments. Instead, in the TDZ treated cut branches, the PI declined after eight days of vase life (Fig. 3). After two months of storage, the PI was lower in all treatments and dropped faster in the control and TDZ, while in the TDZ plus glycerol it slightly declined after eight days and remained unchanged until 20 days of vase life (Fig. 4).

Sugars content

The sucrose content of cut foliage at harvest was 15 mg/g FW on average and declined during vase life. In glycerol treatments, and in particular in the 10 mM treatment, the reduction was faster and showed lower values. After 14 days of vase life, higher values were found in TDZ and TDZ plus glycerol. After 19 days only the cut branches treated with TDZ and glycerol were alive and the sucrose content was 4.5 mg/g FW on average (Fig. 5). In stored cut branches the sucrose content was five fold lower compared to the fresh harvested branches. The sucrose content declined in TDZ after eight days of storage and ranged from 1.5 to 2.2 mg/g FW until 15 days of vase life. In TDZ plus glycerol treatment the cut branches did not show reduction of sucrose content until 15 days of vase life and a significant decline was observed after 20 days of vase life (Fig. 6).

Total sugars showed a similar trend of sucrose content during vase life before and after storage. The higher values were found in TDZ plus glycerol treatment, especially at the end of vase life (data not shown).

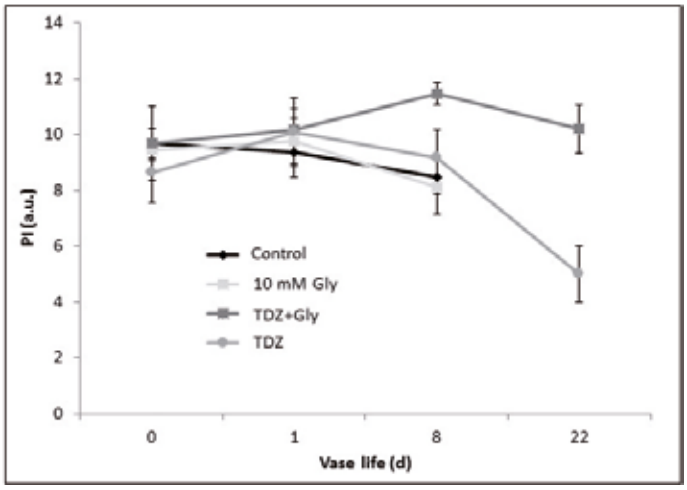

Fig. 3 - Performance index (PI) measured on cut foliage during vase life determination. Values are means with standard errors (n=5).

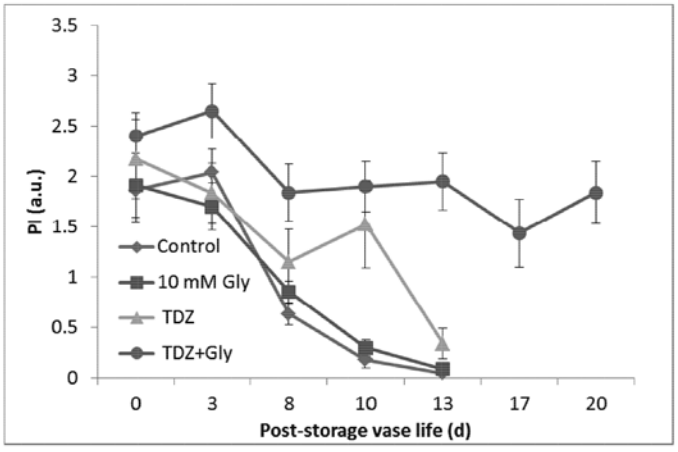

Fig. 4 - Performance index (PI) measured on cut foliage stored for 60 days and then transferred to 20 °C for vase life determination. Values are means with standard errors (n=5).

4. Discussion and Conclusions

The visual appearance of cut foliage is the most important quality parameter, and preservative treatments for

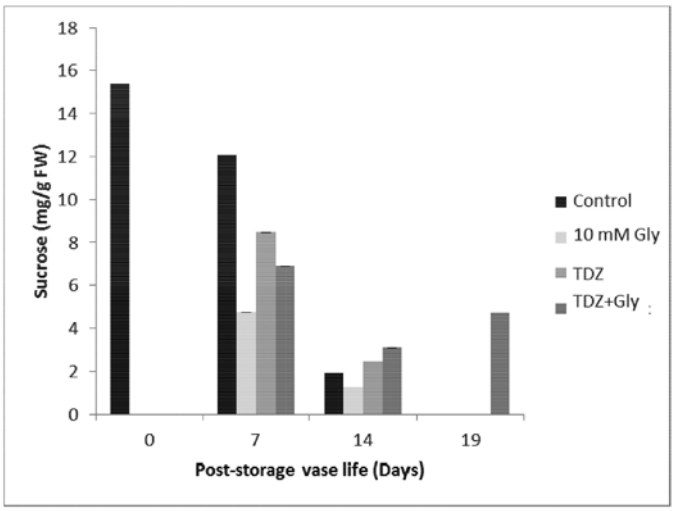

Fig. 5 - Sucrose content measured in leaves of cut *Danae* branches treated with distilled water (control), 10 mM Glycerol, 10 µM TDZ or 10 µM TDZ plus 10 mM Glycerol during vase life. Values are means with standard errors (n=3).

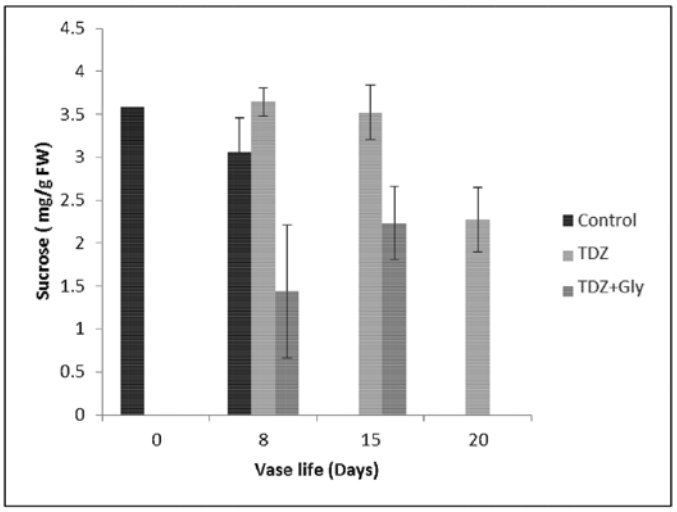

Fig. 6 - Sucrose content measured in leaves of stored cut *Danae* branches treated with distilled water (control), 10 µM TDZ or 10 µM TDZ plus 10 mM Glycerol during vase life. Values are means with standard errors (n=3).

these ornamental items are aimed at delaying leaf senescence or yellowing, which can occur during long-distance transportation or long storage periods. In addition to external quality, another important parameter to control in cut foliage postharvest is weight loss. Since cut foliage is sold on the basis of weight, any weight losses translate into economic losses. *Danae* plants are in active vegetative growth from September to May, thus there are three months without harvest and a lack of cut branches on the market. Flower markets and cooperatives are interested in storing as much cut foliage as possible. Cut *Danae* branches can be stored under mild vacuum packages or in water for two months while maintaining satisfactory vase life (Pacifici *et al*., 2014). However, preservative treatments are needed to delay leaf yellowing during post-storage vase life. The positive effect of cytokinin treatments on chlorophyll retention was observed in cut eucalyptus branches (Ferrante *et al*., 2002 b) and TDZ has been used in cut flowers to inhibit leaf yellowing in sensitive species such as *Alstroemeria*, chrysanthemum and tulips (Ferrante *et al*., 2002 a, 2003, 2005). Our results indicate that cut branches treated with TDZ plus glycerol provide the best post-storage performance. TDZ alone reduced chlorophyll decline but TDZ with glycerol showed a synergistic effect; the positive effect of the combination was observed in all parameters measured.

Chlorophyll *a* fluorescence measurements can be used during postharvest to evaluate the health status of leaves. In the ornamental field, chlorophyll *a* fluorescence and relative derived parameters have been used to evaluate quality losses in cut *Eucalyptus* and *Danae* branches (Pacifici *et al*., 2008, 2013). In potted *Bougainvillea* plants, the use of chlorophyll *a* fluorescence parameters were used to evaluate the efficiency of ethylene inhibitors during the post-production stage (Ferrante *et al.,* 2012). In our experiments, chlorophyll *a* fluorescence showed that TDZ plus glycerol maintained leaf functionality as demonstrated by the Fv/Fm ratio (data not shown) and PI.

Storage of cut *Danae* branches in polyethylene bags at 4°C for 60 days gave good results since the cut foliage was available on the market for more than 80 days from harvest. The reduced gas exchanges avoided excessive water loss by transpiration and probably also conditioned respiration, as demonstrated by the gas compositions at the end of storage, especially in the bags containing cut branches treated with TDZ plus glycerol. Cut *E. parvifolia* branches treated with glycerol showed longer life compared with control (Ferrante *et al.*, 2001) and analogous results were found in *E. cinerea* (Campbell *et al*., 2000). Glycerol uploaded in the cell increases osmotic capacity and induces an initial stress which can be observed by measuring ethylene production (Ferrante *et al*., 2001), but it also helps maintain water balance.

Sugars represent the energy source for stored products and are usually associated with vase life. Among the sugars, sucrose represents one of the most important energy reserves and its degradation provides a direct substrate for respiration (Reid, 1991; Ferrante and Reid, 2006). In the present study, the cut foliage treated with TDZ showed higher sugars content. Further studies are needed to understand sugar metabolism in TDZ treated cut foliage after storage.

These results can be further evaluated in combination with postharvest treatments and the use of passive refrigeration systems, which have been applied to different perishable foods (Costa *et al*., 2013).

In conclusion, the results obtained from this investigation suggest that pulse treatment for 24 h with TDZ and glycerol can be used to extend the vase life of stored and non-stored cut *Danae* branches. However, further studies are required at physiological and biochemical level to elucidate the biological pathways affected by TDZ and glycerol.

Acknowledgements

This work was funded by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF) under the project TRACEFLOR.

The work reported in this paper was presented at the "POSTHARVEST2014 Reducing Postharvest Losses to Feed the World Congress" held in Barletta, Italy, on 22-23 May 2014.

References

CAMPBELL S.J., OGLE H.J., JOYCE D.C., 2000 - *Glycerol uptake preserves cut juvenile foliage of* Eucalyptus cinerea. - Australian Journal of Experimental Agriculture, 40(3): 483-492.

COSTA C., ANTONUCCI F., MENESATTI P., PALLOTTINO F., BOGLIONE C., CATAUDELLA S., 2013 - *An advanced colour calibration method for fish freshness assessment: A comparison between standard and passive refrigeration modalities.* - Food and Bioprocess Technology, 6(8): 2190-2195.

DUBOIS P., JOYCE D., 1992 - *Preservation of fresh cut ornamental plant material with glycerol.* - Postharvest Biology and Technology, 2(2): 145-153.

FERRANTE A., HUNTER D. A., HACKETT W. P., REID M. S., 2002 a - *Thidiazuron - a potent inhibitor of leaf senescence in Alstroemeria.* - Postharvest Biology and Technology, 25(3): 333-338.

FERRANTE A., MENSUALI-SODI A., SERRA G., TOGNONI F., 2001 - *Water consuption, chlorophyll contents and vase life of cut* Eucalyptus parvifolia Cambage *foliage.* - Workshop on green cut foliage. - ACE International Flortecnica, Santa Flavia 4 May 2001 PA, 5: 197-203.

FERRANTE A., MENSUALI-SODI A., SERRA G., TOGNONI F., 2002 b - *Effects of ethylene and cytokinins on vase life of cut* Eucalyptus parvifolia *Cambage branches.* - Plant Growth Regulation, 38: 119-125.

FERRANTE A., MENSUALI-SODI A., TOGNONI F., SERRA G., 2005 - *Postharvest studies on leaf yellowing of chrysanthemum cut flowers*. - Adv. Hort. Sci., 19(2): 81-85.

FERRANTE A., REID M.S., 2006 - *Postharvest physiology of cut flowers*. - Italus Hortus, 13(4): 29-41.

FERRANTE A., TOGNONI F., MENSUALI-SODI A., SERRA G., 2003 - *Treatment with Thidiazuron for preventing leaf yellowing in cut tulips and chrysanthemum.* - Acta Horticulturae, 624: 357-363.

FERRANTE A., TRIVELLINI A., BORGHESI E., VERNIERI P., 2012 - *Chlorophyll a fluorescence as a tool in evaluating the effects of ABA content and ethylene inhibitors on quality of flowering potted* Bougainvillea*.* - The Scientific World Journal, ID 684747: 1-11.

JIANG C.Z., WU L., MACNISH A.J., KING A., YI M., REID M. S., 2008 - *Thidiazuron, a non-metabolized cytokinin, shows promise in extending the life of potted plants*. - Acta Horticulturae, 847: 59-66.

MUTUI T.M., MIBUS H., SEREK M. 2005 - *Effects of thidiazuron, ethylene, abscisic acid and dark storage on leaf yellowing and rooting of Pelargonium cutting*. - J. Hort. Sci. Biotech., 80: 543-550.

PACIFICI S., BURCHI G., DEL CARLO A., FERRANTE A., 2013 - *Effect of storage temperature and duration on vase life of cut* Ruscus racemosus *L. foliage*. - Acta Horticulturae, 970: 69-74.

PACIFICI S., BURCHI G., DEL CARLO A., FERRANTE A., 2014 - *Mild vacuum packaging for long storage of cut* Danae racemosa *(L.) Moench foliage*. - Acta Horticulturae, 1071:143-148.

PACIFICI S., FERRANTE A., MENSUALI-SODI A., SERRA G., 2007 - *Postharvest physiology and technology of cut eucalyptus branches: a review.* - Agr. Med., 137: 124-131.

PACIFICI S., MENSUALI-SODI A., FERRANTE A., SERRA G., 2008 - *Comparison between conventional and vacuum storage system in cut foliage*. - Acta Horticulturae, 801: 1197-1204.

REID M.S., 1991 - *Effects of low temperatures on ornamental plants*. - Acta Horticulturae, 298: 215-224.

SHANAN N.T., SHALABY E.A., 2011 - *Influence of some chemical compounds as antitranspirant agents on vase life of* Monstera deliciosa *leaves*. - Afr. J. Agric. Res., 6(1): 132-139.

VAN DOORN W.G., 1997 - *Water relations of cut flowers*. - Horticultural Rev., 18: 1-85.

VAN DOORN W.G., HIBMA J., DE-WIT J., 1992 - *Effect of exogenous hormones on leaf yellowing in cut flowering branches of* Alstroemeria pelegrina L. - Plant Growth Regul., 11: 445-448.