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Effects of Pre- and Postharvest Conditions on Vase Life of *Eustoma grandiflorum* (Raf.) Shinn.

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Summary

Postharvest quality of *Eustoma grandiflorum* (Raf.) Shinn. was investigated after growing plants at different photosynthetic photon flux density (PPFD), lighting periods, supplements of lime to the peat medium and different levels of boron fertilization. The plants were harvested at three open flowers and were placed in glass bottles with 500 ml vase solution of the desired preservatives under the following conditions: 21 °C, 45 % relative humidity (RH) and 12-h photoperiod with a PPFD of 15 μmol m^{-2} s^{-1} provided by white fluorescent tubes. Wilted flowers, leaves and buds were recorded every day and postharvest life was evaluated. In general, an increase in preharvest PPFD level increased the vase life. In our first experiment, the plants grown with a 20-h lighting period under a low PPFD (60 μmol m^{-2} s^{-1}) with high RH (90 %) in the canopy showed the shorter vase life than those grown under a high light level (240 μmol m^{-2} s^{-1}) and low RH (70 %). In the second experiment, a few hours of high RH at the canopy level during night with a 16-h lighting period and a PPFD of 240 μmol m^{-2} s^{-1} resulted in a shorter vase life than 24-h and constant low RH. When temperature and RH were better controlled in a third experiment under a PPFD of 105 μmol m^{-2} s^{-1}, the vase life was slightly longer in stems from 16-h than from 24-h lighting period. Compared to their long vase life (26 to 28 days), the differences (2–4 days), however, must be regarded as small, and we therefore suggest that there is no adverse effect of 24-h lighting on vase life. Vase life was only affected when the commercial preservative Chrysal Clear (CC) or sucrose with 8-hydroxyquinoline sulphate (HQS) was used in the vase solution, and not when either sucrose or HQS was compared with the water control (reverse osmosis water). These results suggest that high PPFD, long lighting period, low RH during the growing period and use of sugar combined with HQS in the vase solution, are all important factors for prolonging the vase life of *Eustoma*.

Zusammenfassung

Wirkung von Vor- und Nacherntebedingungen auf die Haltbarkeit von *Eustoma grandiflorum* (Raf.) Shinn.. Die Nacherntequalität von *Eustoma grandiflorum* (Raf.) Shinn. wurde an Pflanzen untersucht, die zuvor bei unterschiedlichen Photonenflussdichten (PPFD), Belichtungszeiten, Zugaben von Kalk zum Torfsubstrat und unterschiedlichen Bordüngestufen kultiviert worden waren. Die Pflanzen wurden geerntet, sobald drei Blüten geöffnet waren und in 500 ml Glasflaschen mit Wasser, dem das jeweilige Haltbarkeitsmittel zugefügt wurde, bei 21 °C, 45 % relativer Feuchte (RH) und 12-h Licht mit einer PPFD von 15 μmol m^{-2} s^{-1} aus weißen Leuchtstoffröhren, aufgestellt. Welke Blüten, Blätter und Knospen wurden jeden Tag erfasst und die Haltbarkeit ermittelt. Im Allgemeinen verlängerte eine höhere PPFD die Haltbarkeit. Im ersten Versuch, wiesen die Pflanzen, die im 20-Stunden-Tag mit geringer PPFD (60 μmol m^{-2} s^{-1}) und hoher RH (90 %) im Blattbereich kultiviert wurden, eine geringere Haltbarkeit auf als jene, deren Kultur bei Starklicht (240 μmol m^{-2} s^{-1}) und geringerer RH (70 %) durchgeführt wurde. Im zweiten Versuch führten wenige Nachtstunden mit hoher RH im Blattbereich in Verbindung mit einer 16-h-Belichtungsdauer und einer PPFD von 240 μmol m^{-2} s^{-1} zu geringerer Haltbarkeit als 24-h Licht und konstant geringer RH. Als Temperatur und RH im dritten Versuch besser kontrolliert wurden, war die Haltbarkeit bei einer PPFD von 105 μmol m^{-2} s^{-1} nach einem 16-h Tag besser als nach einem 24-h Tag. Bezogen auf die lange Haltbarkeit (26 bis 28 Tage) muss eine Verlängerung um 2–4 Tage allerdings als gering angesehen werden, so dass wir vorschlagen, nicht von einer negativen Wirkung des 24-Stunden-Tages auf die Haltbarkeit zu sprechen. Das Aufkalken des Torfsubstrats oder die Düngung mit Bor hatte keine signifikante Wirkung auf die Haltbarkeit. Die Haltbarkeit wurde nur beeinflusst, wenn das Handelsprodukt Chrysal Clear (CC) oder Saccharose in Kombination mit 8-Hydroxyquinolinsulfat (HQS) für das Vasenwasser verwendet wurden. Bei alleiniger Verwendung von Saccharose oder HQS wurden im Vergleich zur Kontrolle (Umkehrosmosewasser) keine Unterschiede sichtbar. Die gefundenen Ergebnisse zeigen, dass sowohl hohe PPFD, lange Belichtungszeiten, geringe RH in der Wachstumsphase als auch die Verwendung von Saccharose in Kombination mit HQS im Vasenwasser wichtige Faktoren zur Verlängerung der Haltbarkeit von *Eustoma* sind.

Key words. Light level – lighting period – relative humidity – sucrose – vase life

Introduction

The popularity of *Eustoma* as a cut flower is increasing due to its long vase life and beautiful colours. Year round production of *Eustoma* is achieved in Norway with supplementary lighting. Since the photosynthetic activity in the interior environment is limited due to low light conditions, optimal light conditions during the growth period might be of importance to ensure high carbohydrate levels in the plants at harvest and thereby prolong the vase life. Increasing the photosynthetic photon flux density (PPFD) during the growing period increased the vase life in roses (FJELD et al. 1994), while high RH greatly reduced the vase life by restricting their ability to control water loss (MORTENSEN and FJELD 1998).

The vase life of *Eustoma* can be extended by postharvest chemical treatment. The long vase life influences the market value of *Eustoma,* and strongly affects consumer satisfaction and repeated purchasing (ONOZAKI et al. 2001). The cut surfaces of the plants are susceptible to attack by bacteria and fungi, resulting in pathological breakdown and death (KADER 1992). The biocides in floral preservatives maintain clarity in the solution and prevent blockage of xylem elements by micro organisms (KNEE 2000).

During the vase period the small buds of *Eustoma* do not open fully and the colour of petals is only slightly expressed. Additional sugars play an important role in keeping the quality of cut flowers, and therefore adding sucrose with a germistat to improve the vase life of *Eustoma* is suggested (ICHIMURA 1998). ICHIMURA and KORENAGA (1998) showed that the vase life of cut *Eustoma* flowers might be significantly improved by 2 % sugars and 8-hydroxyquinoline sulphate (HQS). Postharvest chemical treatment with inhibitors of ethylene biosynthesis such as α-aminoisobutyric acid, or an inhibitor of ethylene action, such as silver thiosulphate, can delay the onset of flower senescence (VEEN 1979; ONOZAKI and YAMAGUCHI 1992; ONOZAKI et al. 1998).

HALEVY and MAYAK (1979) suggested using deionised water in postharvest research. However, the consumers normally use tap water, and therefore the appropriateness of deionised water as a control solution in vase life studies is questionable (VAN MEETEREN et al. 2000). Tap water may vary significantly in mineral composition and is therefore unsuitable as a standard treatment. VAN MEETEREN et al. (2000) claim that Ca and Cu are nearly always present in tap water at varying concentrations and hence could be an important source of variability of the vase life.

Due to long vase life of cut *Eustoma*, there have been few studies on postharvest physiology (HALEVY and KOFRANEK 1984; SHIMAMURA and OKABAYASHI 1997). However, the effect of sucrose (ICHIMURA 1998), role of ethylene (ICHIMURA et al. 1998), temperature, 8-hydroxyquinoline sulphate, sucrose (ICHIMURA et al. 1999), and aluminium sulphate (LIAO et al. 2001) have earlier been studied. We are not aware of any published report on the effect of preharvest growing conditions on the postharvest life of *Eustoma*. The aim of our studies was therefore to investigate the effect of preharvest factors such as PPFD, lighting period, RH and supplement of lime and boron (B) on the postharvest life of *Eustoma*. In addition, the effect of preservatives on vase life and quality was studied.

Materials and Methods

Plant material and postharvest conditions

Three experiments were carried out during January to May with twelve-week-old seedlings of 'Echo Blue' and 'Fuji Deep Blue' in Expt. 1 and eleven to twelve week old seedlings of 'Polestar Blue Picotee' and 'Kyoto Purple' in Expts. 2 and 3. Stems were harvested when they had at least three open flowers. The plants were grown at a density of 60 plants per m^2 and eight to ten plants per treatment were tested for keeping quality. Stems were cut off at a length of 60 cm and placed in the vase solution after removing the lower leaves, allowing no leaf in the vase solution. Each stem was placed in a glass bottle with ca. 500 ml of the desired solution in a room at 21± 0.5 °C, 45± 2 % RH and 12-h photoperiod with a PPFD of 15 μmol m^{-2} s^{-1} provided by white fluorescent tubes (TDL 33).

The vase solutions were made with reverse osmosis (RO) water (pH 7.5) and were as follow:

1. 200 mg l^{-1} HQS,
2. 200 mg l^{-1} HQS plus 20 g l^{-1} sucrose (HQS + Suc.),
3. 10 g l^{-1} Chrysal Clear (CC, commercial preservative from Pokon and Chrysal, Naarden, The Netherlands).

Control stems were held in RO water. In addition, stems were also held in tap water (pH 7.3) in some trials to compare the effect of tap and RO water. The pH of the solution with HQS + Suc. was 4.5, and with CC 3.3 and 3.5 before and after the testing period, respectively. Additional vase solutions were added when needed.

Registrations

Number of flower buds and flowers was counted at the start and the end of vase life. Number of days to first wilting flower, leaf or bud and end of vase life was recorded daily. The end of vase life was determined by the number of days from harvest until the leaves and/or buds were wilted or more than 50 % of the open flowers were wilted and no longer had decorative value. The fresh weight of stems and the amount of water uptake were measured daily at the same time of the day to determine the increase or decrease of fresh weight. Corrections were made for daily evaporation rate through the bottle mouth.

Preharvest growing conditions

Experiment 1: Effect of PPFD. Seedlings were grown in a greenhouse under natural day length and supplement of four different PPFD (20-h day^{-1}) levels (60, 120, 180 and 240 μmol m^{-2} s^{-1} ± 10 %) with high-pressure sodium lamps (HPS). The set temperature was 21 °C and RH 75 %. Due to the limitations of the greenhouse system it was not possible to keep the same temperature and RH in all the treatments. Therefore, the plants grown under the highest PPFD had air canopy temperatures of 23 °C and 70 % RH, while at the lowest PPFD the temperature was 20 °C and RH

90 % at the time of harvest. Similar leaf temperatures were recorded.

Experiment 2: Effect of PPFD and daily lighting period. Seedlings were grown in greenhouse compartments under natural day length with three PPFD levels of additional light (60, 120 and 240 μmol m^{-2} s^{-1} ± 10 %) with HPS in combination with daily lighting periods of 16-h (from 4:00 to 20:00) or 24-h. To establish the two lighting periods each compartment with a specific PPFD level was divided with white reflecting curtains (from 18:00 to 8:00). A Priva recorder unit was placed under the 24-h lighting treatment to control and record the climatic conditions. The temperature in the canopy under 240 μmol m^{-2} s^{-1} PPFD increased to 23 °C during daytime (light on) and decreased to 19 °C at night (light off), while the RH increased to 90 % during the night only with 16-h lighting period. Under lower PPFD levels the RH and the temperature were only slightly different from the set temperature and RH.

Experiment 3: Effect of lime, boron (B) and lighting period. Seedlings were grown with 16-h and 24-h daily lighting periods under natural day light with additional PPFD of 105± 10 μmol m^{-2} s^{-1} and 75 % RH. In addition, three lime and three B treatments were applied. Lime treatments were established by adding 0, 8 and 16 kg 45 % CaO per m^3 to a standard fertilized (2.0 kg N-P-K + 0.2 kg Fritt per m^3) and limed peat medium (8 kg Dolomite limestone, pH of 5.3 before adding extra lime). The plants in the lime treatment were watered daily with a complete nutrient solution with electric conductivity of 1.7 to 2.7 mS cm^{-1}. Three levels of B were established (0.21, 0.68 and 1.17 ppm) by adjusting the content in the nutrient solution, while all other nutrients were kept.

Statistical analysis

Data were subjected to analysis of variance with single plant as replicate. The cultivars were analysed separately.

Results

Experiment 1: Effect of PPFD

Increasing PPFD from 60 to 240 μmol m^{-2} s^{-1} significantly increased ($P<0.001$) vase life. The use of HQS + Suc. or CC also significantly ($P<0.001$) increased the vase life compared to HQS (Fig. 1). However, the effect of PPFD on vase life was only noticed when HQS + Suc. or CC was added in the vase solution indicating an interaction ($P<0.001$) between PPFD and preservatives. There was no effect of adding only HQS or sucrose on vase life compared to RO water control (data not shown). The vase life was 11–12 days with HQS, while with CC it increased from 22 to 28 days from the lowest to the highest PPFD level. The results were similar for both cultivars. Sucrose combined with HQS solution almost doubled the vase life compared to HQS or RO water, but vase life was still shorter than with CC (Fig. 1).

There was no clear effect of preharvest growing conditions on percent of flowers opening during vase life. Only 10 to 20 % of the flower buds opened if treated with only HQS, while HQS + Suc. and CC resulted in opening of 50 to 70 % of the flower buds (data not shown). Generally, buds did not wilt or they wilted almost at the end of vase life when CC or HQS + Suc. were used and no signs of wilted leaves or first flower were observed within the first 10 to 12 days (data not shown). With HQS + Suc. and CC more buds (≥2 mm diameter) developed into flowers with colour. Flowers opening in RO water and HQS solution did not show the original colour, for example, the petals of blue flowers became pale blue or white.

Experiment 2: Effect of PPFD and daily lighting period

An increase in PPFD from low (60 μmol m^{-2} s^{-1}) to moderate PPFD (120 μmol m^{-2} s^{-1}) increased the vase life significantly, but there was no further effect of an increase from 120 to 240 μmol m^{-2} s^{-1} (Table 1). The

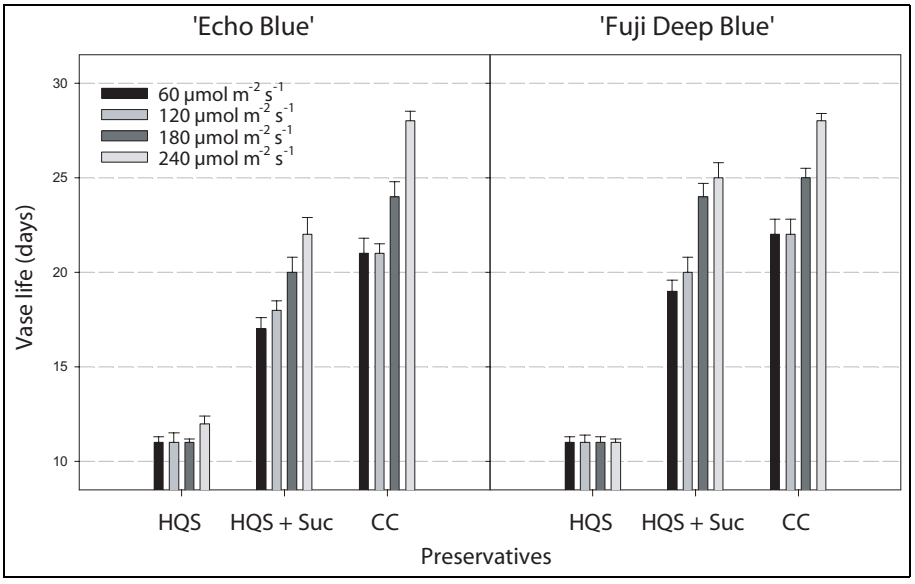


Fig. 1. Effect of photosynthetic photon flux density and different preservatives (HQS: 200 mg l^{-1}; HQS + Suc.: 200 mg l^{-1} HQS plus 20 g l^{-1} sucrose; CC: 10 g l^{-1} Chrysal Clear) on vase life of cut stems of *E. grandiflorum* 'Echo Blue' and 'Fuji Deep Blue' grown with a 20-h daily lighting period (Expt. 1). The vertical bars indicate ± SE, n=10 plants.

Table 1. Effect of preharvest photosynthetic photon flux density (PPFD) and daily lighting period (16-h and 24-h) on vase life in days of cut stems of *E. grandiflorum* 'Polestar Blue Picotee' and 'Kyoto Purple' kept in Chrysal Clear solution (Expt. 2). Standard errors of the mean are given (± SE, n=8 plants).

	'Polestar Blue Picotee'			'Kyoto Purple'		
PPFD (µmol m^{-2} s^{-1})	60	120	240	60	120	240
16-h lighting period	22 ± 0.5	26 ± 1.0	19 ± 0.6	21 ± 0.4	22 ± 0.4	18 ± 0.5
24-h lighting period	19 ± 0.6	24 ± 0.9	23 ± 0.7	19 ± 0.7	21 ± 1.0	22 ± 0.1

plants with a 16-h lighting period under the highest PPFD had shorter vase life than a 24-h lighting period, while it was the opposite under a low and moderate light level (Table 1).

Fresh weight of the cut stems treated with CC increased about 25 % during the first 12–14 days (Fig. 2). Thereafter, the fresh weight decreased, and more rapidly from the lowest than the highest PPFD treatment. Stems of 'Polestar Blue Picotee' grown with a 24-h lighting period under the lowest PPFD did not increase in fresh weight during vase life with RO water, while stems in tap water showed an increase in fresh weight from day one to end of the measurements on day seven (data not shown).

Rate of water uptake was significantly higher ($P<0.001$) in stems from the 24-h than in those from the 16-h treatment, independent of PPFD level (Fig. 3). Water uptake rate was higher in CC solution and in tap water compared to RO water (data not shown). There was no clear effect of preharvest growing conditions on percent buds opening during the vase period. In 'Polestar Blue Picotee', 55 to 70 %, and in 'Kyoto Purple' 40 to 54 % of flower buds opened when stems were kept in CC solution independent of PPFD levels (data not shown).

Experiment 3: Effect of lime, boron (B) and lighting period

The vase life of cut stems from the 16-h light treatment was significantly ($P<0.001$) longer than those from the 24-h treatment (Fig. 4). No significant effects on vase life were found after adding lime to the standard fertilized peat medium, or after increasing B-fertilization during the growing period. Adding the highest level of lime or B increased vase life by one to two days only with a 16-h lighting period (Fig. 4).

Discussion

Vase life of cut stems of *Eustoma* increased with increasing PPFD level. The effect was, however, different among the cultivars. A similar result was also found in roses (FJELD et al. 1994). The prolonged vase life of stems grown under the highest PPFD might be related to the high light level, but also the low RH in the canopy that occurred as a result of increased temperature. Shorter vase life under the lowest PPFD might be related to both low light and high RH (Expt. 1). Stems grown under the lowest PPFD combined with high RH in the canopy (Expt. 1) or under a 16-h lighting period,

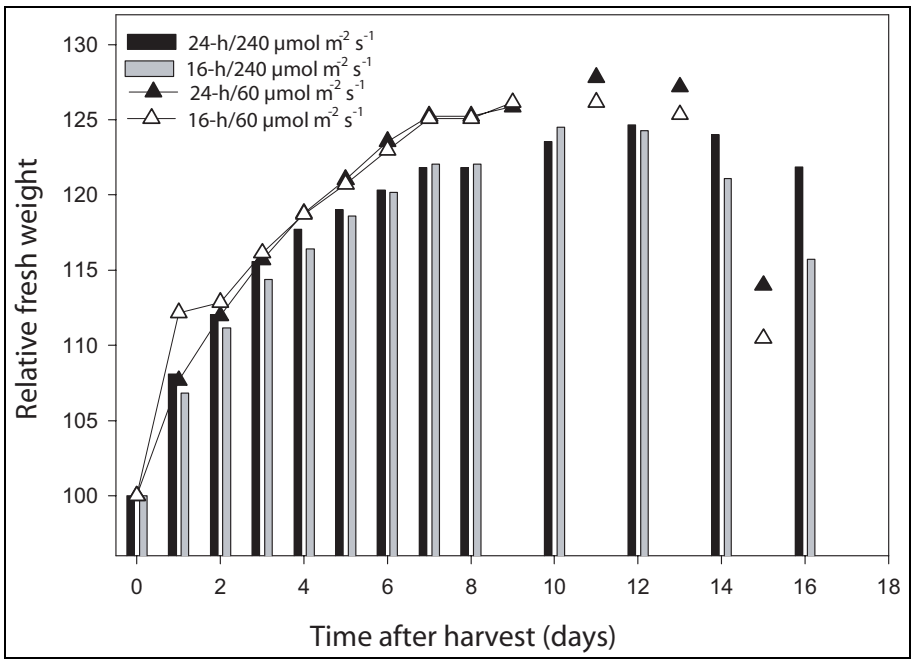


Fig. 2. Effect of photosynthetic photon flux density and daily lighting period (16-h and 24-h) on relative fresh weight of cut stems of *E. grandiflorum* 'Kyoto Purple' kept in Chrysal Clear during vase life (Expt. 2), n=8 plants.

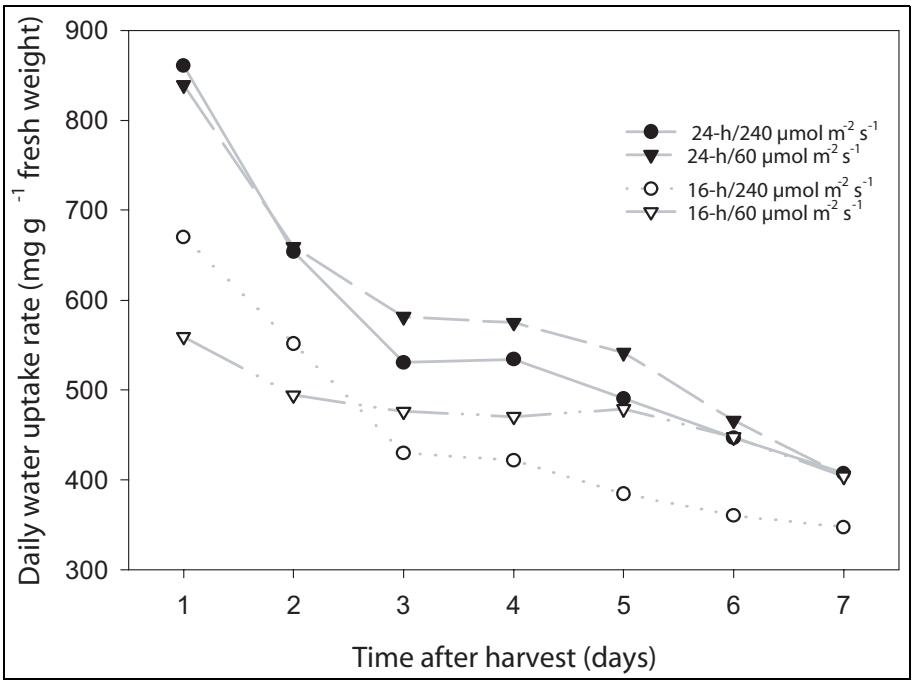


Fig. 3. Effect of photosynthetic photon flux density and daily lighting period (16-h and 24-h) on water uptake rate of cut stems of *E. grandiflorum* 'Kyoto Purple' kept in Chrysal Clear during vase life (Expt. 2), n=8 plants.

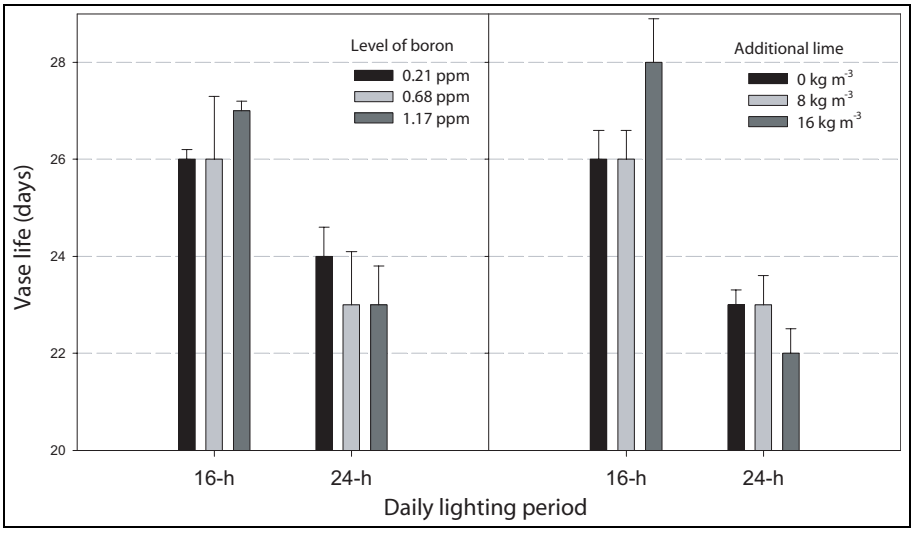


Fig. 4. Effect of daily lighting period, level of boron in the nutrient solution and additional lime in standard fertilized peat on vase life of cut stems of *E. grandiflorum* 'Polestar Blue Picotee' grown with additional PPFD of 105 µmol m^{-2} s^{-1} and kept in Chrysal Clear (Expt. 3). The vertical bars indicate ± SE, n=8 plants.

high PPFD and high RH at night (Expt. 2) had the shortest vase life and also the highest shoot biomass (ISLAM 2002). These results may indicate that high plant biomass and/or a plant grown at high RH may result in shorter vase life. A high RH also lead to shorter vase life in roses (MORTENSEN and FJELD 1998).

MORTENSEN and FJELD (1998) found that a 24-h ligthing period reduced the vase life of roses significantly. Some experiments in *Eustoma* indicate a few days reduction (Fig. 4), another experiment showed no difference (Table 1). Increasing the lighting period from 16-h to 24-h increased the rate of water uptake by the stems during vase life. Obviously, a higher rate of water loss will lead to a stronger water stress (MAYAK et al. 1974).

The reason for a shorter vase life with 24-h lighting in roses was that stomata were kept in an open position. The results in our work indicate that this can also be the case for *Eustoma*. Constant high RH or for a few hours during night seems therefore to affect vase life negatively and also the Ca translocation to the leaves (ISLAM 2002). Roses do not develop tip burn because of high RH as observed in *Eustoma*. A reason for shorter vase life in *Eustoma* as a result of high RH during the growing period could be a combination of reduced stomata functioning and less Ca to flower and flower stem. Additional Ca to the plant tissue is found to delay senescence (FERGUSON and DROBAK 1988).

Vase life was significantly increased with HQS + Suc. or CC solution compared to HQS, and the effect was greater if grown under the highest PPFD (Fig. 1). Our results are therefore different from those in roses (FJELD et al. 1994), where addition of sugar to the vase solution eliminated the effect of different light levels during the growing period. The effect of sugar on the improvement of vase life is not only due to the effect

of osmolytes, but also as a source of substrates of respiration and synthetic materials (ICHIMURA 1998). In our studies, CC was found to be more effective than HQS + Suc. in both cultivars. Sucrose may lead to the inhibition of ethylene production, but not the ethylene action (ICHIMURA 1998). CC may also have an inhibitory effect on ethylene.

VAN DOORN (1997) suggested that vascular blockage of stems in water controls caused water deficit, which shortened the vase life. HQS, an effective germistat, inhibits vascular occlusion and extends the vase life of cut rose flowers (ICHIMURA et al. 1999), but there was no positive effect of HQS alone on the vase life of *Eustoma* compared with treatment with RO water. No effect of adding HQS on vase life suggested that there was no problem of vascular occlusion by bacteria during the two weeks after starting the keeping quality test. The response to germicides is highly variable among species (FUJINO et al. 1983). The combined treatment with HQS + Suc. extended the vase life and promoted the flower opening. This was not found with sucrose alone, most likely because sucrose encourages multiplication of bacteria, which eventually will block the xylem vessels, while biocides can prevent this (KNEE 2000). We therefore suggested that the increased level of soluble carbohydrate is responsible for the increased vase life of *Eustoma,* but a germicide is needed to this effect.

The percentage of flowers that opened with normal colour was higher in HQS + Suc. and CC solution compared to HQS and RO water. Treatment with sucrose increased the anthocyanin concentration in petals as well as extended the vase life of *Eustoma* flowers, and sucrose may therefore be involved in the anthocyanin biosynthesis gene expression (ICHIMURA 1998). Carbohydrate supply seems sufficient for pigmentation, and other metabolites may not be required in pigment formation in *Eustoma* (KAWABATA et al. 1999).

Serious leaf damage was observed in a trial with 40 g l^{-1} sucrose irrespective of preharvest treatments (data not shown). This might be the effect of osmotic disruption and cell death caused by osmotic agents in the preservatives, as has been observed in other species (HALEVY and MAYAK 1981; MARKHART and HARPER 1995).

ICHIMURA et al. (1998) indicated that flower senescence in *Eustoma* is at least partially regulated by autocatalytic ethylene production. They found that flowers were not sensitive to ethylene at anthesis, but became more sensitive with increasing senescence. In our case, the vase life of *Eustoma* was found to be 12–14 days in HQS and RO water. ICHIMURA et al. 1998 described a low ethylene production rate from a whole flower until 6 days after harvest and then reached a peak 12 days after harvest. *Eustoma* flowers are naturally pollinated and ICHIMURA and GOTO (2000) found that pollination markedly accelerated petal senescence of *Eustoma* cut flowers. They also suggested that the acceleration of flower senescence by pollination is mediated by ethylene.

No adverse effect of the 24-h light treatment was found on vase life. High PPFD, long photoperiod and low RH during the growing period, as well as the use of sugar combined with HQS during the vase period, may therefore be important factors for a long vase life of *Eustoma*.

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