Europ.J.Hort.Sci., **69** (1). S. 29–36, 2004, ISSN 1611-4426. © Verlag Eugen Ulmer GmbH & Co. Stuttgart

Effects of Relative Air Humidity, Light, and Calcium Fertilization on Tipburn and Calcium Content of the Leaves of *Eustoma grandiflorum* (Raf.) Shinn.

N. Islam, G. Grindal Patil, S. Torre and H. R. Gislerød
(Dept. of Plant and Environmental Sciences, Agricultural University of Norway, Ås, Norway)

Summary

Tipburn is a physiological leaf disorder that reduces the quality of a cut flower production of *Eustoma* in the greenhouse. The disorder may be affected by environmental factors, and in several experiments we have studied if relative air humidity (RH), daily lighting period, photosynthetic photon flux density (PPFD), and calcium (Ca) fertilization affect the incidence of tipburn and Ca content of the leaves. Constant high RH or a few hours of high RH at night caused a higher incidence of tipburn than a low RH. High PPFD or a long lighting period caused tipburn only at high RH. Incidence and severity of tipburn differed in the cultivars tested. The onset of tipburn symptom appeared in the young developing leaves from one to two weeks before until after visible flower bud development. Plants that took longer time to flower and gained high shoot biomass also had a higher incidence of tipburn than those flowering early and with a low shoot biomass. There was always less Ca in leaf tips with tipburn than in those without, and the threshold level for visible symptoms seemed to be 3.5 mg g^{-1} leaf dry weight. High Ca concentrations in the nutrient solution increased the Ca content in the whole leaf, but had little or no effect on incidence of tipburn. In conclusion, tipburn in *Eustoma* was an effect of constant high RH, high RH at night, or high dry matter accumulation per plant. These factors influenced the translocation of Ca to the leaf tips.

Zusammenfassung

Wirkung von relativer Luftfeuchte, Licht und Calciumdüngung auf Spitzenbräune und Calciumgehalt der Blätter von *Eustoma grandiflorum* (Raf.) Shinners. Spitzenbräune ist eine physiologische Blattkrankheit, die die Qualität der im Gewächshaus produzierten Schnittblumen von *Eustoma* vermindert. Die Störung scheint durch Umweltfaktoren verursacht zu sein. In zahlreichen Experimenten wurde hier untersucht, ob die relative Luftfeuchte (RH), die tägliche Belichtungsdauer, die Photonenflussdichte (PPFD) und die Calciumdüngung (Ca) das Auftreten von Spitzendürre und den Ca-Gehalt der Blätter beeinflusst. Konstant hohe RH oder hohe RH während einiger Nachtstunden führte häufiger zu Spitzendürre. Eine hohe PPFD oder eine lange Belichtungsdauer führte nur bei hoher RH zu Spitzendürre. Auftreten und Schwere des Befalls variierten bei den untersuchten Sorten. Die ersten Symptome der Spitzendürre traten in der Phase 1–2 Wochen vor bis nach Sichtbarwerden der Knospen auf. Bei Pflanzen, die später zur Blüte kamen und mehr Sprossbiomasse entwickelten, trat häufiger Spitzendürre auf als bei frühblühenden mit geringerer Sprossmasse. Pflanzen mit Spitzendürre wiesen immer weniger Ca in den Blattspitzen auf als Pflanzen ohne Symptome. Der Schwellenwert für sichtbare Symptome scheint bei 3.5 mg g^{-1} Blatttrockengewicht zu liegen. Hohe Ca-Konzentrationen in der Nährlösung erhöhte den Ca-Gehalt im ganzen Blatt, hatte aber wenig oder keinen Einfluss auf das Auftreten der Spitzendürre. Zusammenfassend kann gesagt werden,, dass Spitzendürre an *Eustoma* eine Folge von konstant hoher RH, hoher RH während der Nacht oder hohem Trockenmassenaufbau pro Pflanze war. Diese Faktoren beeinflussten den Transport des Ca in die Blattspitzen.

Key words. daily lighting period – leaf tips – PPFD – Ca – RH

Introduction

Popularity of *Eustoma grandiflorum* (Raf.) Shinn. as a cut flower is increasing. Tipburn, a physiological leaf disorder, limits a high quality of *Eustoma*. Effects of environmental factors have been studied in many crops in relation to tipburn or chlorosis, such as: air humidity (RH) in sweet pepper (BAKKER 1989), strawberry (BRADFIELD and GUTTRIGE 1979; TAKEDA et al. 1991; CHOI et al. 1997), cabbage (PALZKILL and TIBBITTS 1977), cucumber (Bakker et al. 1987; ADAMS and HAND 1993), and tomatoes (HOLDER and COCKSHULL 1990; ADAMS 1991; CHOI et al. 1997); temperature in Chinese cabbage (ALONI et al. 1986; IMAI 1987; LANDER et al. 1989; KAO and WU 1992) and lettuce (ROSEN 1990; WISSEMEIER 1996) and photoperiod in tomato (BRAD-



Fig. 1. Defined stages of tipburn development (TB 1 = tipburn 1; TB 2 = tipburn 2; TB 3 = tipburn 3) in *E. grandiflorum*.

LEY and JANES 1985; LOGENDRA et al. 1990), eggplant (HORI et al. 1987; MURAGE et al. 1996), chrysanthemum and radish (WARRINGTON and NORTON 1991). WOLTZ and HARBAUGH (1986) have also mentioned that many environmental factors induced bract necrosis in poinsettia and that this was linked to a reduced Ca content. There is no published report on the effects of environmental factors (e.g. photosynthetic photon flux density (PPFD), RH, and daily lighting period) on incidence of tipburn in *Eustoma*.

In *Eustoma*, tipburn symptoms typically appear first at the tip of young leaves and gradually leaves are deformed, become necrotic and, in extreme cases, the growing point dies (Fig. 1). Tipburn generally develops at the leaf margin or shoot apex and might be associated with low concentration of calcium (Ca) in the affected tissue (THIBODEAU and MINOTTI 1969; BANGERTH 1979). According to SAURE (1998), tipburn is a necrosis at the margins of young developing leaves mainly in head forming vegetables such as lettuce, white cabbage, Chinese cabbage, and Brussels sprouts. Ca is relatively immobile in the plant and deficiency symptoms typically appear first in the young tissue. Transpiration is the main driving force for Ca transport in plants, since Ca moves along with water in the xylem (CLARKSON 1984). In a study by GISLERØD et al. (1987), a reduced amount of Ca was found in tomato plants grown at high (90–95 %) compared to low RH (70–75 %). Increasing air humidity reduces transpiration and transport of Ca (EHRET and HO 1986), which may lead to Ca deficiency symptoms in the upper, rapidly expanding leaves (WINSOR and ADAMS 1987).

Ca is recognized as an essential nutrient for plant growth since the mid-1800 s. Low Ca accumulation causes various physiological disorders in the plant organs, and 35 Ca deficiency disorders have been observed in a variety of crops (SHEAR 1975). Ca is an integral part of the cell wall providing cell wall rigidity (PALTA 1996) and is also essential for cell division and expansion (WHITE 2001). Ca deficiency has disruptive effects on the formation and stability of the mitotic spindle and microtubules.

Since environmental factors and Ca affect plant quality in many crops, several experiments were conducted to investigate the effect of different environmental factors such as PPFD, RH, daily lighting period, and Ca fertilization on growth and Ca content of leaves of *Eustoma*, with special emphasis on tipburn.

Materials and Methods

Plant material and growing conditions

Four experiments (Expt.) were conducted. In all of them, seedlings of *Eustoma grandiflorum* (Raf.) Shinn. were bought from Holland (Hamer Flower Seeds Ltd.) and kept under short day conditions (10 h light), 21±1 °C, with 85 μmol m^{-2} s^{-1} supplementary PPFD for 2–3 weeks before starting the experiments. The plants were grown in plastic pots with a standard fertilized peat medium that contained 2 kg N-P-K (11-5-17) fertilizer, 8 kg dolomitic lime and 0.2 kg fritted trace elements (F. T. E. No. 36) per m^3 of sphagnum peat, giving 2400 g Ca per m^3 peat. Plants were watered daily with a complete nutrient solution. The exception was Expt. 4 where plants were grown in water culture.

Except when indicated otherwise, additional light was provided by high-pressure sodium lamps (Philips SON/T) for 20 h per day, temperature was set at 21 °C and ventilation at 23 °C, RH was set at 75 %, and 800±50 μmol mol^{-1} CO_2 were supplied when ventilators were closed. The artificial light went off when the out door light level exceeded 300 μmol m^{-2} s^{-1}. The light was measured by using a Li-Cor quantum radiometer, model LI-185, at pot height at the beginning of the experiments. The number of plants per treatment was 60 in experiments (Expts.) 1, 2 and 3, and twelve in Expt. 4, that also had two replications.

Expt. 1. Effect of RH and PPFD on tipburn and Ca content of the leaves.. Eleven week old seedlings of 'Echo Blue' and 'Kyoto Yellow' were grown under high (90±5 %) and low RH (65±5 %), with (PPFD of 120 μmol m^{-2} s^{-1}) or without additional light. The experiment was carried out during May to August under natural day length (average 18 h). The daily additional lighting period was weekly adjusted to the natural day length. Shadow curtains were used to reduce (30 %)

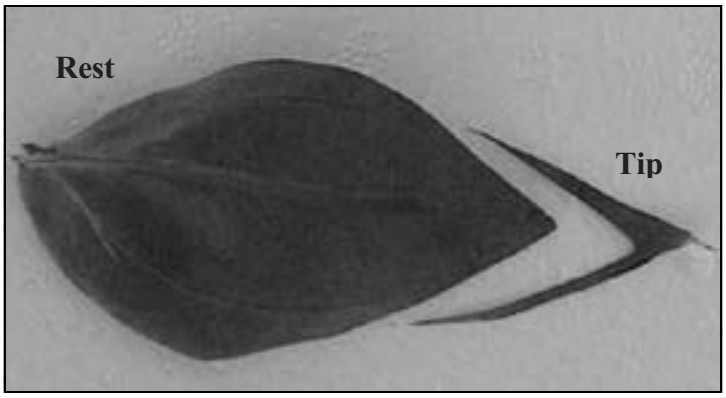


Fig. 2. Section of leaf showing the tip and rest part of leaf used for analysis in Expt. 1 (Table 1).

the natural light levels when the out door level exceeded 400 μmol m^{-2} s^{-1}.

Expt. 2. Effect of increasing PPFD on tipburn. Twelve week old seedlings of 'Echo Blue' and 'Fuji Deep Blue' were grown from January to May under four PPFD levels (60, 120, 180, and 240 μmol m^{-2} s^{-1}±10 %). The PPFD levels were established on four tables. A Priva recorder unit was placed between the two middle light levels. Due to the limitations of the greenhouse system it was not possible to keep the same RH and temperature in all the treatments. Measurements in the canopy showed that the highest PPFD (240 μmol m^{-2} s^{-1}) raised the air temperature to 23 °C and reduced RH to 70 %, while at the lowest PPFD (60 μmol m^{-2} s^{-1}) a lower temperature (20 °C) and a higher RH (90 %) were recorded. Leaf temperatures were similar to the canopy temperature under the highest and the lowest PPFD level (ISLAM 2002).

Expt. 3. Effect of light period and PPFD on tipburn. Eleven to twelve week old seedlings of 'Polestar Blue Picotee', and 'Kyoto Purple' were grown in three greenhouse compartments from January to May under three PPFD levels (60, 120 and 240 μmol m^{-2} s^{-1}±10 %). Plants were grown in the standard fertilized peat medium as in other experiments but with 20 %addition of perlite (V/V). Each compartment was divided with white reflecting curtains (from 18:00 to 8:00) and two daily lighting periods of 16 h (from 4:00 to 20:00) and 24 h were established. A Priva recorder unit was placed under the 24 h lighting periods. The temperature in the canopy under the highest PPFD increased to 23 °C during daytime (light on) and decreased to 19 °C at night (light off), while the RH increased to 90 %only at 16 h (ISLAM 2002). Similar leaf temperatures as in the canopy were recorded under the highest PPFD. Deviations in RH and observed temperature during the day and night were smaller at lower PPFD levels.

Expt. 4. Effect of RH and Ca fertilization on Ca content of the leaves. The experiment was conducted during July to September in growth chambers placed in a greenhouse as described by TORRE and FJELD (2001). Seedlings of 'Fuji Deep Blue' and 'Kyoto Yellow' were grown at low (70±5 %) and high RH (90±5 %) with supplementary light (PPFD of 75 μmol m^{-2} s^{-1}). Seedlings were grown in plastic boxes (0.4 m x 0.6 m x 0.15 m = 0.036 m^3) containing complete nutrient solutions with different Ca concentrations (30, 150 and 300 mg l^{-1}) established by adding $CaCl_2$. The nutrient solution was changed weekly. The electric conductivity of the solutions was 2.5–2.7 mS cm^{-1} and the pH 5.5–6.0.

Registrations

Onset of tipburn was identified by the appearance of one or more necrotic lesions at the leaf tips and the severity was followed daily until the development of tipburn stopped at the time the visible bud (VB) appeared and the shoot started to elongate. The leaves were visually rated for severity of tipburn (TB) on a scale from 1 to 3; TB 1, when only small necrotic lesion developed in one or one pair of leaf tips; TB 2, when two to three pairs of leaves were affected or the severity increased in the first pair of leaves; TB 3, when more than three pairs and/or the growing points were affected (Fig. 1). Appearance of the flower bud was defined when the first flower bud became visible (≥2 mm in diameter) after carefully unfolding the surrounding leaves. Shoot dry weight was measured on 14 plants per treatment at anthesis of the third flower in Expts. 1, 2 and 3.

Calcium and potassium determinations

Leaf samples were collected at the time of tipburn incidence from seven to eight plants per treatment in Expts. 1 and 4. The leaf tips from Expt. 1 were cut off (app. 10 % of fresh weight of leaf) and analyzed separately (Fig. 2). Analysis of oven dried leaf samples were carried out by using inductive coupled plasma-atomic emission spectroscopy (ICP-AES). In Expt. 1, the uppermost two to three pairs of developed leaves were collected. In Expt. 4, leaf samples without tipburn symptom from the 4^{th} to the 6^{th} leaf pair from the box surface (lower leaf) and the uppermost developed leaves (upper leaf) were collected from plants grown at 150 and 300 mg l^{-1} Ca. The Ca content of the whole leaf was analyzed. Plants grown with 30 mg l^{-1} Ca in the nutrient solution died a few weeks after starting the experiment and no leaf sample was available.

Table 1. Effect of relative humidity (RH) and additional photosynthetic photon flux density (PPFD, 18 h) on severity (TB 1 to 3, see Fig.1) of tipburn (n=60), time to visible bud (VB) and onset of tipburn (n=44), and shoot dry weight (n=14) in *E. grandiflorum* 'Kyoto Yellow'. Standard errors of the mean (SE) are given for shoot dry weight, while for days from seeding SE was always ≤1.0. For the days to onset of tipburn 'n' was dependent on the number of affected plants (Expt. 1).

RH (%)	PPFD (μmol m^{-2} s^{-1})	Percent plants affected			Number of days from seeding		Shoot dry weight (g)
		TB 1	TB 2	TB 3	VB	Onset of TB	
65	0	28	3	0	135	132	12.1±0.8
65	120	58	24	0	127	128	15.0±0.3
90	0	96	73	35	134	121	11.4±0.6
90	120	100	93	70	131	117	16.2±0.6

Table 2. Content of calcium (Ca) and potassium (K) (mg g^{-1} dry weight) in the leaves with (n=5) or without (n=7) tipburn of *E. grandiflorum, taken from* 'Echo Blue' *and* 'Kyoto Yellow' grown under different air humidity and photosynthetic photon flux density. Standard errors of the mean (SE) are given (Expt. 1).

	Leaf tips			Rest part of the leaves		
	Ca	K	K/Ca	Ca	K	K/Ca
With tipburn	2.8±0.2	17.3±1.4	6.2±0.7	2.9±0.2	23.1±2.2	7.9±0.6
Without tipburn	4.8±0.4	19.3±1.9	4.2±0.6	2.9±0.2	26.7±1.9	9.3±0.7

Table 3. Effect of additional photosynthetic photon flux density (PPFD, 20 h) on severity (TB 1 to 3, see Fig.1) of tipburn (n=60), time to visible bud (VB) and onset of tipburn (n=44), and shoot dry weight (n=14) in *E. grandiflorum* 'Fuji Deep Blue'. Standard errors of the mean (SE) are given for shoot dry weight, while for days from seeding SE was always ≤.9. For the days to onset of tipburn 'n' was dependent on the number of affected plants (Expt. 2).

PPFD (μmol m^{-2} s^{-1})	Percent plants affected			Number of days from seeding		Shoot dry weight (g)
	TB 1	TB 2	TB 3	VB	Onset of TB	
60	60	30	12	139	141	17.7±0.7
120	25	5	0	127	137	14.5±0.8
180	23	5	0	119	129	15.5±0.9
240	10	0	0	116	128	14.7±1.0

Results

Expt. 1. Effect of RH and PPFD on tipburn and Ca content of the leaves

A higher number of plants were affected by tipburn (TB) at high (95 %) than at low (65 %) RH in 'Kyoto Yellow', and more of the plants reached the most severe stage of tipburn (TB 3) (Table 1). The incidence of tipburn was also higher in the plants grown with additional light than without. In 'Echo Blue' only TB 1 symptoms were recorded in a few plants under high RH (data not shown).

The onset of tipburn appeared approximately two weeks before flower buds were visible (VB) under a high RH and almost at the time of VB under low RH. The appearance and severity of tipburn stopped after the shoot started to elongate. The severely damaged growing points (TB 3) in 'Kyto Yellow' under high RH and additional PPFD made it difficult to record the original time to VB development, and apparently the number of days to VB increased in 'Kyoto Yellow' (Table 1). The TB symptoms appeared 11 days earlier under high than low RH, and four days earlier with additional PPFD than without. After onset of tipburn, it took on average 4–5 days to develop more sever symptoms (TB 1 to TB 2 or TB 2 to TB 3) independent of RH and light treatments (data not shown). The plants grown with additional PPFD had higher shoot dry weight compared to those grown without.

Analysis of leaf tips showed that both the Ca and potassium (K) content were lower in leaf tips with tipburn than in those without, but the effect on Ca content was most pronounced (Table 2). In general, there were minor differences in Ca content in the remainder of the leaves with or without tipburn, while in leaves without tipburn the rest part was always lower in Ca than the tips. A higher K/Ca ratio in the tips was found in leaves with tipburn than in those without (Table 2). The analysis presented in Table 2 is an average of two cultivars, where ' Kyoto Yellow', the most susceptable cultivar to tipburn, had a lower Ca content in the tips than 'Echo Blue'.

Table 4. Effect of additional photosynthetic photon flux density (PPFD) and daily lighting period on severity (TB 1 to 3, see Fig.1) of tipburn (n=60), time to visible bud (VB) and onset of tipburn (n=44) and shoot dry weight (n=14) in *E. grandiflorum* 'Kyoto Purple'. Standard errors of the mean (SE) are given for shoot dry weight, while for days from seeding SE was always ≤0.7. For the days to onset of tipburn 'n' was dependent on the number of affected plants (Expt. 3).

Daily lighting period (h)	PPFD (μmol m^{-2} s^{-1})	Percent plants affected			Number of days from seeding		Shoot dry weight (g)
		TB 1	TB 2	TB 3	VB	Onset of TB	
16	60	22	11	0	151	147	30.6±1.1
	120	87	65	45	148	142	38.0±2.4
	240	97	85	55	146	135	51.9±2.4
24	60	2	0	0	138	145	23.8±1.0
	120	10	3	0	134	130	36.0±1.8
	240	50	16	10	125	126	32.4±1.8

Expt 2. Effect of increasing PPFD on tipburn

The effect of PPFD on the incidence and severity of tipburn differed with the cultivars. Incidence of tipburn was recorded mainly in 'Fuji Deep Blue', while 'Echo Blue' was almost unaffected. Therefore, only the data from 'Fuji Deep Blue' are presented. At the lowest PPFD, where the RH in the canopy was high (90 %), the incidence of tipburn was the highest (60 %affected plants), while under the highest PPFD, where RH in the canopy was low (70 %), incidence of tipburn was lowest (10 %affected plants) (Table 3). Moreover, the severity of tipburn was also highest at the lowest PPFD. Plants with TB 3 occurred only at the lowest PPFD, and TB 2 was recorded up to 180 μmol m^{-2} s^{-1}. When the bud development was delayed by a low PPFD the onset of tipburn was also delayed. Thus, onset of tipburn at the lowest PPFD was almost at the time of VB, and about 12 days after the time of VB under the highest PPFD (Table 3). The plants grown under the lowest PPFD had the highest shoot dry weight among all PPFD levels.

Expt. 3. Effect of light period and PPFD on tipburn

Sensitivity for the incidence and severity of tipburn was different among the cultivars. The 'Kyoto Purple' was severely affected, while 'Polestar Blue Picotee' was almost unaffected (data not presented). The incidence and severity was higher at 16 h than at 24 h daily lighting period, and higher at the highest (240 μmol m^{-2} s^{-1}) than at the lowest (60 μmol m^{-2} s^{-1}) PPFD (Table 4). Under a 16 h lighting period, incidence of tipburn was the highest (97 %of the plants affected) under the highest PPFD, where RH at night also raised to 90 %, while under the lowest PPFD (75 %RH) the incidence was reduced (22 %affected). Plants grown under the lowest PPFD with 24 h lighting period had almost no tipburn symptom.

The onset of tipburn appeared a few days before or after VB; almost at the time of VB in 24 h ligthing period and 11 days earlier at 16 h ligthing period under the highest PPFD (Table 4). The plants under the highest PPFD at 16 h lighting period had severely damaged growing points (TB 3), that made it difficult to record the time to VB. This may explain the increased number of days to observed VB in 'Kyoto Purple'. Increasing the PPFD level increased the shoot dry weight and the effect was greater under a 16 h lighting period compared to 24 h ligthing.

Expt. 4. Effect of RH and Ca fertilization on Ca content of the leaves

RH had no effect on Ca content of the whole leaves (Fig. 3), but the incidence of tipburn was higher at high than at low RH (data not shown). Increasing the Ca concentration in the nutrient solution from 150 to 300 mg l^{-1} increased the Ca content in the leaves. Lower Ca contents were found in the upper than the lower leaves. In the lower leaves, Ca contents were almost doubled when the plants were grown in 300 compared to 150 mg l^{-1}Ca, but the effect was less noticeable in the upper leaves (Fig. 3). The Ca content was higher in 'Kyoto Yellow' than in 'Fuji Deep Blue'.

Discussion

The incidence and severity of tipburn in *Eustoma* increased with increasing RH, although the cultivars differed in sensitivity. Sensitive cultivars were easily affected by tipburn at high RH, even by a few hours high RH at night. A high RH and tipburn is positively correlated in many crops (SAURE 1998). Opposite observations to the results in *Eustoma* were found in cabbage and lettuce (COLLIER and TIBBITTS 1984; VAN BERKEL 1988), where high RH during the night limited tipburn.

The incidence and severity of tipburn may increase with the light intensity and under long photoperiods (PRESSMAN et al. 1993; GAUDREAU et al. 1994). Shading reduced the intensity of tipburn (WISSEMEIER 1996) and THIBODEAU and MINOTTI (1969) found no tipburn under low light intensity. However, our results indicate that a high PPFD or a long lighting period (24 h) do not always cause tipburn in *Eustoma*, and low PPFD does not prevent tipburn incidence except at low RH. The effect of additional light on tipburn may not necessarily be due to improved light condition, but also to a change in temperature and thus RH in the canopy.

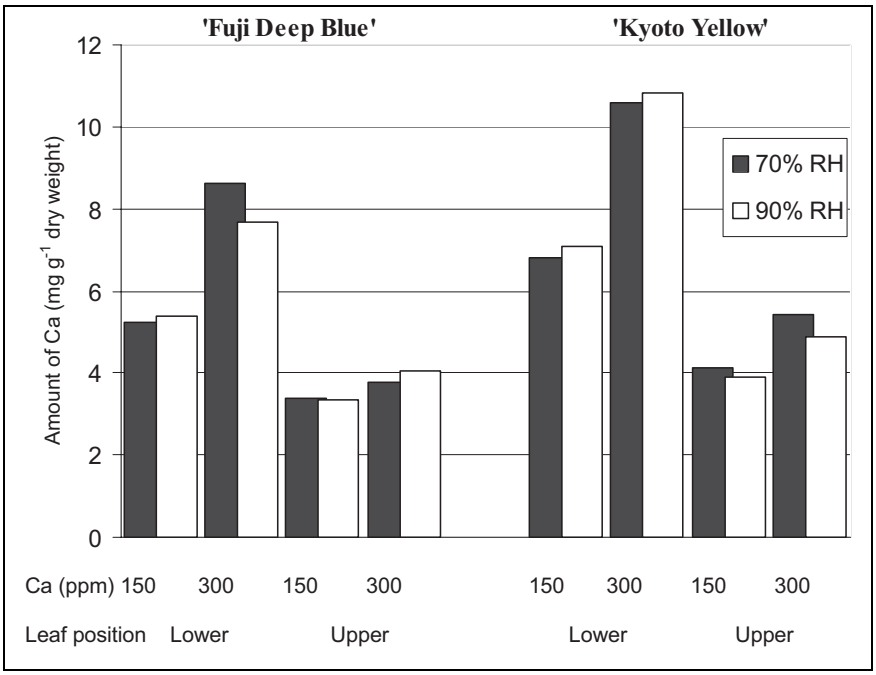


Fig. 3. Effect of air humidity (RH) and Ca concentration of the nutrient solution on Ca content in leaves without tipburn from two positions (upper: uppermost developed leaf, and lower: 4th –6th leaf pair from the box surface) of *E. grandiflorum* 'Fuji Deep Blue' *and* 'Kyoto Yellow' grown in water culture (Expt. 4).

Under the highest PPFD, when the light was turned off after 16 h, the leaf and air temperatures decreased a few degrees and the RH increased (Expt. 3), and this was probably enough to increase the incidence of tipburn as compared to 24 h ligthing.

Uptake and accumulation of Ca is dependent upon transpiration. High RH is known to reduce transpiration, and thereby the Ca transport to young developing leaves and low-transpiration organs (HO 1989; BAKKER 1990; ADAMS and HOLDER 1992; ADAMS and HAND 1993; TORRE et al. 2001), However, in *Eustoma* plants, RH seems to have minor effect on the total Ca content. A lower Ca content was found in the upper leaves, but there was no prominent difference between plants grown at high (90 %) and low RH (70 %). Further, no significant difference in Ca content was found between whole leaves developed at high or low RH. On the other hand, a lower Ca content was found in leaf tips of leaves with tipburn. This suggest that tipburn in *Eustoma* is related to a lower accumulation of Ca to the leaf tips, probably due to inadequate transport of Ca to the tips. Similar results are found for bract-necrosis in Poinsettia (JACQUES et al. 1990; STRØMME et al. 1994), suggesting that it is the distribution of Ca in the leaf rather than the total Ca content that matters.

The threshold value for the Ca content in the leaf tips for being affected by tipburn seems to be 3.5 mg g^{-1} dry weight (DW). The Ca content in the remainder of the leaf was about 3.0 mg g^{-1} DW, and was not correlated with the severity of tipburn.

Plants affected by tipburn are often heavier than others (WIEBE 1975; PALZKILL et al. 1980) as also found in our experiments. It is therefore not meaningful to discuss tipburn and Ca content irrespective of the growth of the plants. The heavy shoot growth and restriction of root development in plants grown in pots may be one of the reasons for tipburn development in *Eustoma.* High RH enhanced the relative growth and individual leaf area of cucumber plants (VAN DE SANDEN and VEEN 1992). Similarly, the plants grown with high PPFD at 16 h lighting period had a higher dry weight and total leaf areas compared to those grown at 24 h (unpublished data), and were more susceptible to tipburn than others. A deficiency of Ca causes membrane leakage, dissolution of the middle lamella and associated changes in the cell wall (FERGUSON and DRØBAK 1988; POOVAIAH 1988) and a high growth rate of low-transpiring organs may increase the risk of Ca content in the tissue falling below the critical levels for stability of cell wall and membrane integrity (MARSCHNER 1995). Restriction of root growth in small containers appears to increase the hydraulic resistance of the root system and to reduce transpiration (HAMEED et al. 1987), thus interfering with the translocation of Ca from root to shoot (AL-SAHAF 1984). Root restriction reduced the Ca content of the young leaves causing tipburn in Chinese cabbage (FRETT et al. 1988).

Increasing Ca levels in the nutrient solution increased Ca content in the whole leaves, which is in agreement with findings by others (ADAMS and HOLDER 1992; TORRE et al. 2001). Adding lime to the medium gave a higher Ca content in the leaves to some extent, but did not prevent the tipburn (unpublished data). Ca deficiency in the low transpiring organ is a result of undersupply of Ca, but not necessarily because of lack of Ca at the root surface (GISLERØD 1999). A reduction in the RH seems therefore to be more important to reduce the incidence of tipburn than adding more Ca to the nutrient solution. Similar is found for bract-necrosis in *Rosa* (TORRE et al. 2001). Even a foliar application of a solution containing 375 or 750 mg l^{-1} Ca to a tipburn susceptible cultivar, grown under high RH, gave no reduction in tipburn compared with pure water (unpublished data). Our result differs from the findings in poinsettia (WOLTZ and HARBAUGH 1986; STRØMME et al. 1994), where foliar application of Ca es-

sentially prevented bract necrosis. However, a waxy layer on the leaf surface of *Eustoma* may restrict the Ca absorption through the cuticle.

Our results suggest that constant high RH or a few hours during the night inhibit translocation of Ca to the leaf tip and promote development of tipburn in *Eustoma*. In particular, tissue sensitivity to tipburn seems to be greater during the time period of two to three weeks before and after VB development (eg. 15–20 weeks from seeding), but the sensitivity varies among cultivars.

To avoid tipburn, maintaining a low RH (<80 %) is important, especially two to three weeks before and after VB and during the dark period of the day. If possible, the cultivation of sensitive cultivars should be avoided and controlling the temperature and RH carefully during the time of the year with high temperature fluctuations is important.

Acknowledgements

The authors are grateful to Dr. Peter Adams for his useful comments and critical review of this paper. We thank Vigdis Revhaug for the photos and technical assistance. The analyses of leaf samples were all carried out by the Agricultural Service Laboratory at Soil Research Institute, Aas.

References

ADAMS, P. 1991: Effect of diurnal fluctuations in humidity on the accumulation of nutrients in the leaves of tomato (*Lycopersicon esculentum*). J. Hortic. Sci. **66**, 545–550.

ADAMS, P. and R. HOLDER 1992: Effect of humidity, Ca and salinity on the accumulation of dry matter and Ca by the leaves and fruit of tomato (*Lycopersicon esculentum*). J. Hortic. Sci. **67**, 137–142.

ADAMS, P. and D.J. HAND 1993: Effect of humidity and Ca level on dry-matter and Ca accumulation by leaves of cucumber (*Cucumis sativus* L.). J. Hortic. Sci. **68**, 767–774.

ALONI, B., T. PASHKAR and R. LIBEL 1986: The possible involvement of gibberellins and calcium in tipburn of Chinese cabbage: study of intact plants and detached leaves. Plant Growth Reg. **4**, 3–11.

AL-SAHAF, F.H. 1984: The effect of root confinement and calcium stress on the physiology, morphology and cation nutrition in tomatoes. Ph. D. Thesis, Lincoln University, New Zealand.

BAKKER, J.C., G.W.H. WELLES and J.A.M. Van UFFELEN 1987: The effect of day and night humidity on yield and quality of glasshouse cucumber. J. Hortic. Sci. **62**, 361–368.

BAKKER, J.C. 1989: The effects of air humidity on growth and fruit production of sweet pepper (*Capsicum annum* L.). J. Hortic. Sci. **64**, 41–46.

BAKKER, J.C. 1990: Effect of day and night humidity on yield and fruit quality of glasshouse tomatoes (*Lycopersicon exculentum* Mill.). J. Hortic. Sci. **65**, 323–331.

BANGERTH, F. 1979: Calcium-related physiological disorders of plants. Annu. Rev. Phytopathol.**17**, 97–122.

BRADFIELD, E.G. and C.G. GUTTRIDGE 1979: The dependence of calcium transport and leaf tipburn in strawberry on relative humidity and nutrient solution concentration. Ann. Bot. **43**, 363–372.

BRADLEY, F.M. and H.W. JANES 1985: Carbon partitioning in tomato leaves exposed to continuous light. Acta Hortic. **174**, 293–301.

CHOI, J.A., G.C. CHUNG and S.R. SUH 1997: Effect of night humidity on the vegetative growth and the mineral composition of tomato and strawberry plants. Sci. Hortic. **70**, 293–299.

CLARKSON, D.T. 1984: Calcium transport between tissues and its distribution in the plant. Plant Cell Environ. **7**, 449–456.

COLLIER, G.F. and T.W. TIBBITTS 1984: Effect of relative humidity and root temperature on calcium concentration and tipburn development in lettuce. J. Am. Soc. Hortic. Sci. **109**, 128–131.

EHRET, D.L. and L.C. HO 1986: Translocation of calcium in relation to tomato fruit growth. Ann. Bot. **58**, 679–688.

FERGUSON, I.B. and B.K. DRØBAK 1988. Calcium and the regulation of plant growth and senescence. HortScience **23**, 262–266.

FRETT, J.J., J.W. KELLY, B.K. HARBAUGH and M. ROH 1988: Optimising nitrogen and calcium of *Lisianthus*. Commun. in Soil Sci. Plant Anal. **19**, 13–24.

GAUDREAU, L., J. CHARBONNEAU, L-P. VEZINA and A. GOSSELIN 1994: Photoperiod and photosynthetic photon flux influence growth and quality of greenhouse-grown lettuce. HortScience **29**, 1285–1289.

GISLERØD, H.R., A.R. SELMER-OLSEN and L.M. MORTENSEN 1987: The effect of air humidity on nutrient uptake by some greenhouse plants. Plant and Soil **102**, 193–196.

GISLERØD, H.R. 1999: The role of calcium on several aspects of plant and flower quality from a floricultural perspective. Acta Hortic. **481**, 345–351.

HAMEED, M.A., J.B. REID and R.N. ROWE 1987: Root confinement and its effects on the water relations, growth and assimilate partitioning of tomato (*Lycopersicon esculentum* Mill.). Ann. Bot. **59**, 685–692.

HO, L.C. 1989: Environmental effect on diurnal accumulation of ^{45}Ca by young fruit and leaves of tomato plants. Ann. Bot. **63**, 281–288.

HOLDER, R. and K.E. COCKSHULL 1990: Effects of humidity on the growth and yield of tomatoes. J. Hortic. Sci. **65**, 31–39.

HORI, Y., M. TATSUMI and K. SHIRASHI 1987: Studies on the growth of vegetables in relation to light conditions. II. The effect of prolonged illumination on the growth of vegetables. Natl. Hortic. Res. Inst. Bull. **68**, 173–185 (in Japanese with English summary).

IMAI, H. 1987: NH_4-N toxicity and calcium deficiency in tipburn and internal rot in Chinese cabbage. In: Proceedings of a seminar on improved vegetable production in Asia. FFTC/ASPAC book Series No **36**, Taipei, 21–48.

ISLAM, N. 2002: Effects of climatic and nutritional factors on flowering, growth, and keeping quality of *Eustoma grandiflorum* (Raf.) Shinn. Ph. D. Thesis, Agricultural University of Norway. ISBN 82-575-0532-3.

JACQUES, D.J., R.E.J. BOERNER and J.C. PETERSON 1990: Effect of Ca supply and stress on uptake and translocation of Ca in two poinsettia cultivars. Environ. Exp. Bot. **30**, 525–531.

KAO, T.C. and C.H. WU 1992: Vegetable growth performance in semitropical air-inflated greenhouse. Paper No. 924006, Am. Soc. Agric. Engineers, p. 21.

LANDER, C., B. SCULLY and J.B. STOREY 1989: The evaluation of production practices in early season lettuce. J. Rio Grande Valley Hortic. Soc. **42**, 99 (abstr.).

LOGENDRA, S., J.D. PUTMAN and J.W. JANES 1990: The influence of light period on carbon partitioning, translocation and growth in tomato. Sci. Hortic. **42**, 75–83.

MARSCHNER, H. 1995: Mineral Nutrition of Higher Plants. Academic Press, London, UK.

MURAGE, E.N., Y. SATO and M. MASUDA 1996: Relationship between dark period and leaf chlorosis, potassium, magnesium and calcium content of young eggplants. Sci. Hortic. **66**, 9–16.

PALTA, J.P. 1996: Role of Ca in plant responses to stresses: linking basic research to the solution of practical problems. HortScience **31**, 51–57.

PALZKILL, D.A. and T.W. TIBBITTS 1977: Evidence that root pressure flow is required for calcium transport to head leaves of cabbage. Plant Physiol. **60**, 854–856.

PALZKILL, D.A., T.W. TIBBITTS and B.E. STRUCKMEYER 1980: High relative humidity promotes tipburn on young cabbage plants. HortScience **15**, 659–660.

POOVAIAH, B.W. 1988: Calcium and senescence. *In* Senescence and Ageing in Plants. In: NOODEN, L.D. and A.C. LEOPOLD (eds.): Academic Press, New York, USA, 369–389.

PRESSMAN, E., R. SHAKED and L. ARCAN 1993: The effect of flower inducing factors on leaf tipburn formation in Chinese cabbage. J. Plant Physiol. **141**, 210–214.

ROSEN, C.J. 1990: Leaf tipburn in cauliflower as affected by cultivar, calcium sprays, and nitrogen nutrition. HortScience **25**, 660–663.

SAURE, M.C. 1998: Causes of tipburn disorder in leaves of vegetables. Sci. Hortic. **76**, 131–147.

SHEAR, C.B. 1975: Calcium-related disorders of fruits and vegetables. HortScience **10**, 361–365.

STRØMME, E., A.R. SELMER-OLSEN, H.R. GISLERØD and R. MOE 1994: Cultivar differences in nutrient absorption and susceptibility to bract necrosis in poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch). Gartenbauwissenschaft **59**, 6–12.

TAKEDA, F., M.E. WISNIEWSK and D.M. GLENN 1991: Occlusion of water pores prevents guttation in older strawberry leaves. J. Am. Soc. Hortic. Sci. **116**, 1122–1125.

THIBODEAU, P.O. and P.L. MINOTTI 1969: The influence of calcium on the development of lettuce tipburn. J. Am. Soc. Hortic. Sci. **93**, 454–461.

TORRE, S. and T. FJELD 2001: Water loss and postharvest characteristics of cut roses grown at high or moderate relative humidity. Sci. Hortic. **89**, 217–226.

TORRE, S., T. FJELD and H.R. GISLERØD 2001: Effects of air humidity and K/Ca ratio of the nutrient supply on growth and postharvest characteristics of cut roses. Sci. Hortic. **90**, 291–304.

VAN BERKEL, N. 1988: Preventing tipburn in Chinese cabbage by high relative humidity during the night. Netherlands J. Agric. Sci. **36**, 301–308.

VAN DE SANDEN PIETER, A.C. M. and B.W. VEEN 1992: Effects of air humidity and nutrient solution concentration on growth water potential and stomatal conductance of cucumber seedlings. Sci. Hortic. **50**, 173–186.

WARRINGTON, I.J. and R.A. NORTON 1991: An evaluation of growth and development under various daily quantum integrals. J. Am. Soc. Hortic. Sci. **116**, 544–551.

WHITE, P.J. 2001: The pathways of calcium movement to the xylem. J. Exp. Bot. **358**, 891–899.

WIEBE, H.J. 1975: Relations between the water regime of the plants and the occurrence of the interior sheet necroses with white Kohl. Gartenbauwissenschaft **40**, 134–138.

WINSOR G.W. and P. ADAMS 1987: Diagnosis of Mineral Disorders in Plants. Glass-house Crops, Vol. 3, HMSO, London.

WISSEMEIER, A.H. 1996: Calcium-Mangel bei Salat (*Lactuca sativa* L.) und Poinsettie (*Euphorbia pulcherrima* Willd. Ex Klotzsch): Einfluβ von Genotyp und Umwelt. Verlag Ulrich E. Grauer, Stuttgart, ISBN 3-86186-150-X, pp. 299.

WOLTZ, S.S. and B.K. HARBAUGH 1986: Calcium deficiency as the basic cause of marginal bract necrosis of 'Gutbier V-14' poinsettia. HortScience **21**, 1403–1404.

Received August 29, 2003 / Accepted February 2, 2004

Addresses of authors: Nazrul Islam, Grete Grindal Patil, Sissel Torre and Hans Ragnar Gislerød (corresponding author), Dept. of Plant and Environmental Sciences, Agricultural University of Norway, Ås, Norway, e-mail: hans.gislerod@ipm.nlh.no.