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Testing irrigation, day/night foliar spraying, foliar calcium and growth inhibitor as possible cultural practices to reduce tipburn in lettuce

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Corriveau, J., Gaudreau, L., Caron, J., Jenni, S. and Gosselin, A. 2012. **Testing irrigation, day/night foliar spraying, foliar calcium and growth inhibitor as possible cultural practices to reduce tipburn in lettuce**. Can. J. Plant Sci. **92**: 889–899. Most of the lettuce produced in Quebec, Canada, is grown in organic soils in the area south of Montreal. Regularly, producers experience tipburn damage to their crop, a physiological disorder associated with Ca deficiency along the margins of young actively growing leaves. Therefore, active research is ongoing to reduce damage associated with this disorder. Two greenhouse trials on Romaine lettuce (*Lactuca sativa* L. 'Sunbelt') were conducted to measure the effect of day and night foliar water spraying, irrigation, foliar application of prohexadione calcium (a growth inhibitor) and foliar application of Ca on lettuce growth and incidence of tipburn. None of the treatments had a significant effect on biomass, dry weight, leaf number or leaf area in lettuce. However, the results show that frequent foliar applications of Ca as low as 90 mg L^{-1} Ca^{2+} resulted in a significant decreases in the number of leaves and percent leaf area with tipburn, and significant increases in Ca content in young leaves. Foliar water spraying, irrigation and foliar application of prohexadione calcium resulted in no significant differences in tipburn in greenhouse experiments. As greenhouse and field conditions may differ importantly, Ca application should be tested further at the field scale.

Key words: Tipburn, irrigation, foliar water spraying, prohexadione calcium, foliar application of calcium, lettuce

Corriveau, J., Gaudreau, L., Caron, J., Jenni, S. et Gosselin, A. 2012. **Étude de l'irrigation, des pulvérisations diurnes/nocturnes d'eau sur les feuilles, de l'application de calcium aux feuilles et de l'usage d'un inhibiteur de croissance en tant qu'éventuelles pratiques culturales visant à atténuer la brûlure apicale chez la laitue**. Can. J. Plant Sci. **92**: 889–899. Les laitues du Québec (Canada) sont pour la plupart cultivées sur des sols organiques dans la région située au sud de Montréal. Les producteurs enregistrent couramment des pertes en raison de la brûlure apicale, problème de nature physiologique lié à une carence en Ca qui se manifeste à la bordure des feuilles en croissance. On poursuit activement des recherches dans l'espoir d'atténuer les dommages attribuables à ce problème. Les auteurs ont procédé à deux essais en serre sur de la laitue Romaine (*Lactuca sativa* L. 'Sunbelt') pour jauger les effets des pulvérisations diurnes et nocturnes d'eau sur les feuilles, de l'irrigation, de l'application de prohexadione-calcium (un inhibiteur de croissance) aux feuilles et d'une application foliaire de Ca sur la croissance de la laitue et sur l'incidence de la brûlure apicale. Aucun traitement n'a modifié de façon significative la biomasse, le poids sec et le nombre de feuilles ni la superficie de ces dernières. Néanmoins, les résultats indiquent que l'application fréquente d'une aussi faible quantité de calcium que 90 mg de Ca^{2+} par litre aux feuilles entraîne une diminution sensible du nombre de feuilles atteintes par la brûlure apicale ainsi que de la partie de la surface foliaire affectée, tout en accroissant significativement la teneur en Ca des jeunes feuilles. La pulvérisation d'eau sur les feuilles, l'irrigation et l'application de prohexadione-calcium aux feuilles n'ont pas donné lieu à une diminution importante de la brûlure apicale lors des essais en serre. Puisque les conditions dans les serres et sur le terrain peuvent varier considérablement, il conviendrait d'entreprendre d'autres essais pour vérifier l'effet des applications de Ca au champ.

Mots clés: Brûlure apicale, irrigation, pulvérisation d'eau sur les feuilles, prohexadione-calcium, application foliaire de calcium, laitue

In Canada, 70 418 tonnes of lettuce are produced annually. Quebec is the largest producer of lettuce in Canada, with close to 2800 ha planted to lettuce, and a total production of 59 312 tonnes [Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) 2009]. Romaine lettuce is planted on approximately 30 to 40% of the lettuce crop by area (Larochelle 2012, personal communication, Prisme Consortium Company, Québec, QC). Lettuce is grown primarily on organic soils, and close to 75% of the Quebec production area is located in the southern Montreal region at Napierville. Each year, producers experience tipburn damage, a physiological disorder frequently observed in lettuce, which can cause heavy yield and financial losses. Tipburn appears as a brown or a black necrosis along the margins of young actively growing leaves.

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The major uptake pathway for Ca by plants is through the absorption of water by their roots during the transpiration process (Kirkby and Pilbeam 1984). The middle and outer leaves receive more water and Ca because they have a higher transpiration rate than the young inner leaves particularly when the inner leaves are enclosed by the outer leaves. The water flux produced by transpiration is not the only mechanism of Ca transport. Translocation of Ca to young expanding leaves can also be improved by promoting root pressure, a phenomenon that occurs primarily at night, when the transpiration rate is low and the soil is moist (Palzkill and Tibbitts 1977). Low leaf transpiration causes an increase in the concentration of mineral elements in the roots, resulting in plant demand for water from the soil. The entry of water in the roots creates root pressure, which drives Ca transport to the young leaves [Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ) 2005]. A number of studies have been conducted on the various factors influencing Ca transport mechanisms by changes in water flux. According to the studies, tipburn development is influenced by several environmental, biotic and agronomic factors that act directly on Ca transport through the plant (Saure 1998).

Despite the large number of studies, few practical and effective methods have been developed to minimize crop losses associated with tipburn. Proper irrigation management, foliar application of Ca and the use of resistant cultivars are the methods currently used and studied. The use of growth regulators has also been studied, but there are no growth regulators registered in Canada/North America for use on lettuce.

Plamondon-Duchesneau (2011) investigated the effect of different irrigation thresholds on tipburn incidence. She observed no significant reduction in the number of leaves showing tipburn between the different soil matric potentials, i.e., -10, -20, -30 and -50 kPa. In contrast, Bert and Honma (1975) observed a higher incidence of tipburn in lettuce at soil matric potentials of 0 and -20 kPa than at -40 or -200 kPa.

Cox and Dearman (1981) reported a decrease in tipburn resulting from the application of trickle irrigation combined with night misting; 72% of the plants in the control treatment (irrigated by rain) were affected by tipburn, compared with 16% of the plants treated by trickle irrigation combined with night misting. Jenni and Stewart (2008) reported an 80% decrease in tipburn incidence compared with the control treatment when misting was applied to endive plants when the air temperature at plant height reached 28°C.

There is considerable disagreement among the results of experiments on the effectiveness of foliar application of Ca in reducing tipburn incidence. Its effectiveness appears to vary with the timing and frequency of applications and with the concentration of Ca used. Several authors reported a reduction in tipburn incidence with foliar application of Ca (Thibodeau and Minotti 1969; Sonneveld and Van Den Ende 1975; Maroto et al. 1986; Pressman et al. 1993; Saleh 2009), whereas others observed no significant reduction (Walker et al. 1961; Corgan and Cotter 1971; Misaghi et al. 1981; Jenni and Stewart 2008).

Because the appearance of tipburn is often associated with a high growth rate, researchers have tested the effectiveness of the use of growth regulators. Obispo (1997) observed that lettuce plants treated with paclobutrazol (Cultar) were less affected by tipburn than those treated with daminozide (B-Nine). In addition, in some cases, the application of daminozide reduced tipburn in cabbage (Aloni 1986). Also, Handley and Moran (2009) observed that prohexadione calcium applied as a root dip and foliar spray tends to cause a reduction in strawberry plant dry weights.

As can be seen, there is considerable disagreement among the results of the studies and a limited range of recommendations available to growers to minimize the incidence of tipburn. Given the varying degree of effectiveness of the methods studied to reduce the incidence of tipburn and the fact that the effects vary as a function of climate, we conducted two greenhouse experiments aimed at quantifying the effects of day and night foliar water spraying, irrigation, foliar application of Ca and the application of prohexadione calcium (prohexadione, BASF company, North Carolina) on Romaine lettuce growth and incidence of tipburn under conditions representative of the climate of eastern North America.

MATERIALS AND METHODS

Two experiments were conducted in Quebec, Canada, in the Envirotron greenhouse at Université Laval, Quebec City, Canada (lat. 46°49′N, long. 71°15′W), with the first experiment, an irrigation/spray study, that was repeated and a second experiment, a foliar calcium study, which was undertaken only once. The time period of these separate experiments is shown in Table 1. Romaine lettuce (*Lactuca sativa* L. 'Sunbelt', Central Valley Seeds Inc., Sanger, CA) seeds were planted in peat blocks. After 14 d of growth, the plants were transplanted to containers filled with relatively undecomposed organic soil Typic Mesisol (Soil Classification Working Group 1998) obtained from a commercial field in the Sherrington area (lat. 45.1328°N, long. 73.5252°W). In exp. 1 (Irrigation/Spray Experiment), three lettuce plants were transplanted into thirty 84-L containers (56.5 cm × 36.5 cm × 45 cm deep), with the three plants spaced 10 cm apart within each container. In exp. 2 (Foliar Ca Experiment), two lettuce plants were transplanted into each of sixteen 64-L containers (50 cm × 39.5 cm × 46 cm deep), with the two plants spaced 30 cm apart within each container. Organic soil blocks were collected at the farm of Delfland Inc. in Napierville, QC, using metal casts, mechanically driven into the soil to limit disturbance and to preserve the soil structure. Before transplanting lettuce, soil analysis (Mehlich III) were performed (Ca: 9310 kg ha^{-1},

Table 1. Schedule of the Irrigation/Spray Experiment and Foliar Ca Experiment. The experiments were conducted during the year 2010

		Seedling	Transplant	Beginning of main plot treatments	Beginning of subplot treatments	Harvest
Irrigation at soil matric potential/Spray Experiment	Run 1	Jun. 17	Jul. 02	Jul. 15	Jul. 30	Aug. 19
	Run 2	Sep. 04	Sep. 27	Oct. 12	Oct. 18	Nov. 04
Foliar Ca Experiment	–	Oct. 04	Oct. 22	Nov. 08	–	Dec. 08

P: 194 kg ha^{-1}, K: 645 kg ha^{-1} andMg: 868 kg ha^{-1}), and nitrogen (calcium nitrate) was applied to a rate of 55 kg ha^{-1}. According to CRAAQ fertilization reference guide (Centre de référence en agriculture et agroalimentaire du Québec 2003) for an organic soil, Ca, P, K and Mg were at appropriate levels. To promote seedling survival and growth following transplantation, small plants were irrigated when necessary until the start of the treatments.

Temperatures were set to be maintained at 22°C during the day and 17°C during the night. Relative humidity was set to be maintained at 50% during the day and 75% during the night. However conditions varied from these set points (Table 2), particularly during the day, as irradiance levels and outside temperature reached high values. A second run (Run 2) was performed for exp. 1 and plants from the Irrigation/Spray Experiment Run 2 and the Foliar Ca Experiment were grown under a 14-h photoperiod with supplemental HPS lighting, which provided a photosynthetic photon flux density of 180 μmol m^{-2} s^{-1}. The plants from Irrigation/Spray Experiment Run 1 did not receive supplemental lighting because of the adequate natural radiation. The plants were irrigated using a drip irrigation system and irrigation scheduling was performed using wireless electronic tensiometers (Irrolis T80, Hortau Inc., Lévis, QC).

Table 2. Temperature and relative humidity measured in greenhouse during the Irrigation/Spray Experiment and Foliar Ca Experiment

	Minimum	Maximum	Mean
Irrigation at soil matric potential/Spray Experiment Run 1			
T °C day	17.2	30.2	23.4
T °C night	16.9	22.1	19.4
RH day	39	91	64
RH night	64	92	79
Day vapor pressure deficit (kPa)	1.2	0.4	1.0
Night vapor pressure deficit (kPa)	0.7	0.3	0.4
Irrigation at soil matric potential/Spray Experiment Run 2			
T °C day	15	25	21
T °C night	15	18	16.5
RH day	19.5	84	49
RH night	38	82	68
Day vapor pressure deficit (kPa)	1.4	0.5	1.2
Night vapor pressure deficit (kPa)	1.0	0.4	0.6
Foliar Ca Experiment			
T °C day	15.5	25	20.5
T °C night	15.5	18	17
RH day	22	76	41
RH night	46	76	60
Day vapor pressure deficit (kPa)	1.5	0.8	1.5
Night vapor pressure deficit (kPa)	1.0	0.5	0.8

The Irrigation/Spray Experiment was repeated once and the results of Runs 1 and 2 were pooled. This experiment was arranged in a split-plot design with five randomized complete blocks. Six treatments were compared in the main plots and three other treatments were compared in the subplots. The subplots consisted of the three single plants within each container. In the main plots, six treatments were studied: control, day foliar water spraying, night foliar water spraying, irrigation at −30/−50 kPa, irrigation at −50/−30 kPa and foliar application of prohexadione (prohexadione calcium, BASF Company, Research Triangle Park, NC). The plants from the control, day foliar water spraying, night foliar water spraying and foliar prohexadione calcium application treatments were irrigated using a drip irrigation system controlled by the tensiometers when the soil matric potential reached −30 kPa. The day and night foliar water spraying were performed using an automatically controlled system of nozzles (Cross Cool-Net 5 L/h, Netafim, Tel Aviv, Israel). Day foliar water spraying was applied once a day in the middle of the day for 1 min; night foliar water spraying was applied once a night 1 h before sunrise for 1 min. After a 1-min spray, the leaves were all well wet and droplet formation was observed. After approximately 1 h, the leaf surfaces were dry again. Hence, the leaf wetness treatment lasts at least 1 h. Plants from the −30/−50 kPa irrigation treatment were irrigated when the matric potential reached −30 kPa during the first 2 wk of the treatment. They were then irrigated when the matric potential reached −50 kPa until harvesting. For the −50/−30 kPa treatment, irrigation was applied when the matric potential reached −50 kPa during the first 2 wk of the treatment. Irrigation was then applied when the matric potential reached −30 kPa until harvesting. The minimal soil matric potentials recorded were −30 or −50 kPa, depending of the irrigation set point. When irrigation stopped, the soil matric potential reached around 0 to −10 kPa. Last, the foliar treatment with prohexadione consisted of a foliar application of a 100 mg L^{-1} solution of prohexadione calcium 21 d before harvesting for Run 1 and 28 d before harvesting for Run 2. Each container of three lettuces had a tensiometer, and the irrigation was applied when the average soil matric potential for the same treatment reached the threshold −30 or −50 kPa value, depending on the treatment. During the first 2 wk of growing, the three lettuces of the same container received 1.5 L when the soil matric potential reached the threshold and later, up to harvest, lettuces received 2.1 L by irrigation. Water consumption was recorded from flowmeters installed on irrigation lines (one per treatment) with totalizers. Since leaching did not occur with this amount of water applied, water uptake was estimated by dividing the total amount of water used for the whole treatment by the plant fresh weight and the total length in days of the experiment, neglecting surface evaporation, as good surface coverage was observed for most of the experiment.

In the subplots, three treatments were studied: a control treatment (without foliar application), foliar application of water and foliar application of Ca. For the foliar Ca applications, young lettuce leaves were sprayed to droplet formation with a 360 mg L^{-1} calcium chloride solution (Dow Chemical, Calgary, AB) twice a week. Spraying was done with a hand sprayer, and a plastic bag was used to cover the other two plants in the same container to avoid spray drift during the spraying operation. The spray covered the internal leaf surfaces quite efficiently with this type of lettuce, because the leaf architecture funneled water towards the plant's heart, as opposed to the leaf architecture of butterhead and crispy lettuces, which is more likely to direct water towards the outside of the plants (umbrella effect). Cross-contamination was hence not expected because of this very localized foliar application and because adjacent plants were protected by plastic bags. Also, given the low volume application of solution (up to droplet formation) and the fact that old leaves were covering part of the soil surface, significant contamination of the soil was not expected. Indeed, no change in soil matric potential was observed following spraying, indicating no significant infiltration of solution or water into the soil. In addition, the amount of calcium brought into the soil through surface contamination by spraying would have been extremely limited, given the fact that the soil calcium levels were already very high. Therefore, cross-contamination was neither expected nor supported by the data. The lettuce plants of Runs 1 and 2 received six and five applications (over 21 and 18 d), respectively. The foliar application of water was done in the same manner as the foliar application of Ca.

For the Foliar Ca Experiment, a randomized complete block design comprising four replicates and four treatments was used. The two lettuce plants per container received the same treatment and were therefore the sampling units. Four treatments were studied: a control treatment (without foliar application), and foliar applications of Ca at 90, 180 and 360 mg L^{-1}. For the foliar Ca applications, young lettuce leaves were sprayed to droplet formation with a calcium chloride solution twice a week. Spraying was done with a hand sprayer. The plants received a total of eight applications. They were irrigated using a drip irrigation system controlled by electronic tensiometers when the soil matric potential reached −20 kPa. During the first 2 wk of growing, the two lettuces in a container received 0.8 L when the soil matric potential reached the threshold and, until harvest, lettuces received 1.4 L by irrigation. This experiment was not repeated, as it was a first step to more extensive fieldwork.

The dates of seeding, transplanting, beginning of treatments and harvest, for both experiments are presented in Table 1. In both experiments, biomass, dry weight, leaf area, leaf number and Ca content of young leaves were determined at the time of harvest. The leaf area was measured using a leaf area meter (3100, Li-Cor Inc., Lincoln, NE). This measurement was not taken in Run 2 of the Irrigation/Spray Experiment. Leaf Ca content was analyzed by atomic absorption spectrometry (atomic absorption spectrometer 3300, Perkin-Elmer, Überlingen, Germany) using the method described in Perkin-Elmer Corporation (1982). The plants were first dried at 60°C, ground (Retsch, Brinkmann ZM–1, Haan, Germany) with a 0.5-mm screen and heat treated (Thermolyne, F62735, Dubuque, IA). To assess the incidence and severity of tipburn, each plant was examined every 1 or 2 d to note the date of appearance of the first symptoms during the course of the experiment (data not reported as no treatment effect was observed). In general, the first symptoms of tipburn appeared 4 wk after transplanting. At the time of harvest, the number of young leaves showing tipburn and the leaf area affected in square centimeters were evaluated for each plant. The evaluation of leaf area affected by tipburn was effectuated by using an artisanal leaf area framework (0 to 16 cm^2). Each young leaf was compared with different leaf area on the framework. The first 15 outer leaves were considered mature and all other leaves over 2 cm in length were considered young. Continuous measurements of the soil matric potential were taken by the irrigation control system and the quantities of water applied were obtained for the Irrigation/Spray Experiment.

For the Irrigation/Spray Experiment, data analysis was performed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) in accordance with a split-plot design combining data from both runs, to increase the degrees of freedom of the analysis. The pooling of runs did not affect the conclusions on treatment effects, as the statistical analysis of pooled runs showed that the experiment (or Run) effect was not significant, as well as the interaction between experiments (or Run) and treatments. To meet the assumptions of normality and homogeneity of variance, a square-root transformation (SQRT) was used on the "dry weight of the young leaves per plant" variable, a log transformation (LOG) was used on the "number of leaves per plant" variable, a square-root transformation (SQRT) was applied to the "number of young leaves with tipburn per plant" and "total leaf area with tipburn per plant'' variables and a log transformation (LOG) was applied to the ''Ca concentration of young leaves per plant'' variable. For these variables, data presented in tables were back transformed for ease of presentation.

For the Foliar Ca Experiment, data analysis was performed using the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC) in accordance with a randomized complete block design. To meet the assumptions of normality and homogeneity of variance, a log transformation (LOG) was applied to the ''% of plant area with tipburn'' variable. For these variables, data presented in tables were back transformed for presentation. The analysis of variance was preferred to a regression approach, as the goal was not to determine the response curve but rather to determine the minimum calcium concentration that would significantly reduce tipburn at an acceptable level relative to the control.

For both experiments, normality was accepted when $P > 0.01$ using the Shapiro-Wilk test or as confirmed by visual analysis of normal distribution plot. Homogeneity was analyzed by visual inspection of residual plot. For each variable, when the statistical model was significant at $P < 0.05$, the least square means (LSmeans) were compared.

RESULTS AND DISCUSSION

Irrigation, Foliar Water Spraying and Prohexadione Treatments

Growth and Development

Plants from Run 1 received an average of 6 L more water than plants from Run 2 (Table 3). Average water uptake was 0.8 mL g^{-1} d^{-1} for Run 1 and 0.5 mL g^{-1} d^{-1} for Run 2. Because plants from Run 1 were cultivated during the summer, the temperature and relative air humidity conditions favored higher water use by the plants and more rapid drying of the soil. Plant above-ground biomass was between 318 and 342 g and the dry weight of young leaves ranged from 6.1 to 8.0 g. The total number of leaves per plant was between 37 and 41. The irrigation, foliar water spraying and foliar prohexadione treatments had no significant effects on the growth parameters analyzed (Table 4).

The purpose of the −30/−50 and −50/−30 kPa treatments was to reduce growth rate by modifying soil water availability during plant growth. However, the biomass, dry weight of the young leaves and total leaf number in these two treatments were not significantly reduced by the different irrigation thresholds applied during growth. For the −30/−50 kPa treatment of Run 2, these results can be explained by the average soil matric potential. The average matric potential tension was maintained at −12.5 kPa when the irrigation threshold was −50 kPa (Table 3). The soil matric potential of this treatment was much higher than the expected average tension of −25 kPa. The matric potential of −25 kPa was not reached because the −50 kPa irrigation threshold was applied only 9 d prior to harvest and the soil did not have time to reach the average matric potential of −25 kPa. The same scenario was observed for the −50/−30 kPa treatment. The anticipated average tension of −15 kPa was not reached when the irrigation threshold was −30 kPa and the

Table 3. Average matric potential, quantity of water applied and daily water uptake of the lettuce plants for each treatment in Irrigation/Spray Experiment Run 1 and 2

Treatments	Average matric potential (kPa)	Quantity of water applied (L)	Water uptake (ml g^{-1} d^{-1})
Run 1			
Control (−30kPa)[z]	−16.7	33.3	0.9
Day foliar water spraying[z]	−17.2	27.4	0.8
Night foliar water spraying[z]	−17.7	26.5	0.8
−30 kPa	−20.1		
−30/−50 kPa		29.6	0.9
−50 kPa	−26.9		
−50 kPa	−29.7		
−50/−30 kPa		28.1	0.8
−30 kPa	−21.8		
Prohexadione (foliar spray of 100 ppm)[z]	−18.5	32.1	0.8
Run 2			
Control (−30kPa)	−19.5	23.9	0.5
Day foliar water spraying	−22.3	24.1	0.5
Night foliar water spraying	−22.7	21.9	0.5
−30 kPa	−17.0		
−30/−50 kPa		20.6	0.4
−50 kPa	−12.5		
−50 kPa	−24.7		
−50/−30 kPa		21.8	0.5
−30 kPa	−25.3		
Prohexadione (foliar spray of 100 ppm)	−14.9	25.8	0.6

[z]The plants from the control, day foliar water spraying, night foliar water spraying and prohexadione treatments were irrigated when the soil matric potential reached −30 kPa.

Table 4. Effect of irrigation, foliar water spraying, foliar application of prohexadione and foliar application of Ca on aboveground fresh biomass, dry weight of young leaves and the total number of leaves in lettuce plants in Irrigation/Spray Experiment

Main plot treatments	Aboveground biomass (g)	Dry weight of young leaves per plant (g)[z]	Number of leaves per plant
Control (−30 kPa)	336	7.6	40
Day foliar water spraying	323	6.1	39
Night foliar water spraying	318	6.5	38
Irrigation −30/−50 kPa	342	8.0	38
Irrigation −50/−30 kPa	327	6.9	37
Prohexadione (foliar spray of 100 ppm)	326	7.0	42
Subplot treatments			
Control (no foliar application)	320	6.7	39
Control (water)	335	7.2	39
Ca	331	7.1	39
P value			
Main plot treatments	0.8652NS	0.0865NS	0.0828NS
Subplot treatments	0.3576NS	0.2095NS	0.6287NS
Main plot × Subplot	0.5837NS	0.5402NS	0.7412NS

The averages of Runs 1 and 2 were pooled.
[z]On a lettuce, the first 15 outer leaves were considered mature and all other leaves over 2 cm in length were considered young.

average tension of this treatment therefore remained constant throughout the entire crop cycle (−25 kPa). Our results agree with those of Bergeron-Piette (2011). The different average matric potentials measured during her experiment (ranging from −12.28 to −22.85 kPa) had no significant effect on the total fresh weight of lettuce plants. For Run 1, the average matric tensions desired for the −30/−50 and −50/−30 kPa treatments were reached. However, these two treatments did not reduce biomass. These results do not agree with those of Plamondon et al. (2011), who studied the effect of different irrigation thresholds (−10, −20, −30 and −50 kPa) on Romaine lettuce biomass. Their results show that biomass in the −50 kPa irrigation threshold treatment was significantly lower than that of the other irrigation treatments (−10, 20 and −30 kPa). In contrast, in our experiment, the −50 kPa irrigation threshold was not reached until approximately 2 wk prior to harvest, while in the experiment of Plamondon et al. (2011), the thresholds were reached 29 d prior to harvest. The shorter duration of our drier treatments relative to Plamondon et al. (2011) could account for the results obtained on the growth parameters analyzed.

The application of night foliar water spraying had no significant effect on plant growth compared with the control treatment. These results agree with those of Cox and Dearman (1981), who observed no significant differences in biomass following night foliar water spraying of lettuce plants.

Day foliar water spraying also had no significant effect on lettuce biomass. These results do not agree with those of a previous trial (Corriveau et al. 2011). In that trial, day foliar water spraying significantly increased lettuce biomass, by 31% compared with the control. In contrast, Jenni and Stewart (2008) observed no significant difference in biomass or leaf number after applying day misting when the temperature around the plants was above 28°C.

Foliar application of Prohexadione calcium had no significant impact on plant biomass. These results do not agree with those of Handley and Moran (2009), who observed that prohexadione calcium applied as a root dip and foliar spray tended to reduce dry weight and leaf number in strawberry plants. Prohexadione calcium did not have the same effect on lettuce as on strawberries. The effect of prohexadione calcium appears to vary not only as a function of the time, frequency and rate of application, but also as a function of the plant material used.

Tipburn and Ca Concentration of Young Leaves

The number of young leaves showing tipburn ranged from 3.7 to 6.3 per plant, and the total leaf area affected ranged from 1.2 to 3.2 cm^2. None of the irrigation, foliar water spraying or foliar prohexadione application treatments resulted in a significant reduction in tipburn incidence compared with the control treatment (Table 5). Usually, on a practical level a surface corresponding to 4.5 cm^2 or more would cause the lettuce plant to be discarded. In the Irrigation/Spray Experiment, the level observed for all treatments was low enough to commercialize them, despite the plants being relatively small in size. The Ca concentration of young leaves ranged from 3.75 to 4.95 mg g^{-1}. Young leaves from the night foliar water spraying treatment had a significantly lower Ca concentration than plants from the control treatment, but no differences in tipburn with day foliar application (Table 5).

Rapid growth rate is a factor that increase tipburn incidence. Plants affected by tipburn often have larger, wider leaves than unaffected plants (Tibbitts et al. 1965; Palzkill et al. 1980). KiYoung and YongBeom (2003) also observed that rapid growth rate was highly

Table 5. Effect of irrigation, foliar water spraying, foliar application of prohexadione and foliar application of Ca on the number of young leaves with tipburn, the leaf area with tipburn and the Ca concentration of young lettuce leaves in Irrigation/Spray Experiment

Main plot treatments	Number of young leaves with tipburn per plant[z]	Total leaf area with tipburn per plant (cm^2)	Ca concentration of young leaves per plant ($mg\ g^{-1}$)[z]
Control (−30 kPa)	5	1.4	4.48*bc*
Day foliar water spraying	4	1.7	4.07*ab*
Night foliar water spraying	6	3.2	3.75*a*
Irrigation −30/−50 kPa	4	1.8	4.60*bc*
Irrigation −50/−30 kPa	5	1.2	4.68*bc*
Prohexadione (foliar spray of 100 ppm)	6	2.2	4.95*c*
Subplot treatments			
Control (no foliar application)	7*a*	3.3*a*	4.20*a*
Control (water)	6*a*	1.9*a*	4.27*a*
Ca	3*b*	0.5*b*	4.81*b*
P value			
Main plot treatments	0.4115NS	0.1827NS	0.0031
Subplot treatment	<0.0001	<0.0001	<0.0001
Main plot × Subplot	0.1449NS	0.3167NS	0.4479NS

The averages of Run 1 and 2 were pooled.
[z]On a lettuce, the first 15 outer leaves were considered mature and all other leaves over 2 cm in length were considered young.
a–c Values within a column and at each main/subplot level followed by the same letter were not significantly different at $P<0.05$ according to the LSMeans test.

positively correlated with tipburn. Moreover, a previous study demonstrated positive correlation between lettuce plant biomass and the percentage of tipburn per plant (Corriveau et al. 2011). However, in the present experiment, none of the irrigation, foliar water spraying, foliar calcium or foliar prohexadione treatments had a significant effect on the growth parameters analyzed. Hence, it may explain why no effect of the different treatments on tipburn was observed.

The −30/−50 and −50/−30 kPa treatments did not result in significant reductions of either biomass or tipburn incidence or severity. Moreover, the treatments neither hindered nor promoted absorption of Ca by the roots, with the leaf Ca concentration of plants from these treatments not being significantly different from that of the control plants. These results agree with those of Plamondon (2011), who measured the effect of different irrigation thresholds (−10, −20, −30 and −50 kPa) on the incidence of tipburn in lettuce. None of these irrigation treatments significantly reduced the number of young leaves showing tipburn.

Day foliar water spraying did not reduce tipburn. The purpose of this treatment was to reduce leaf temperature to create conditions favorable to non-expression of tipburn. Temperature is a factor that can influence the appearance of tipburn (Misaghi and Grogan 1978). In addition to acting on plant growth rate, temperature affects the plant's transpiration rate and therefore the level of water and Ca absorption. A study by Misaghi and Grogan (1978) on the relationship between temperature and tipburn incidence in lettuce shows that the higher the air temperature, the more severely affected the plants. Jenni and Stewart (2008) reported that sprinkler irrigation applied when the air temperature at plant height was over 28°C resulted in a significant decrease in tipburn incidence in endive. In our experiment, our day temperature set point was 22°C, which was much lower than that reached in the field by Jenni and Stewart (2008), although a maximum of 30°C was recorded in our greenhouse for several hours (Table 2). In addition, measurements with a porometer (Delta-T Devices, AP4, Cambridge, United Kingdom) did not show any changes in the stomatal conductance of the plants following day foliar water spraying (data not presented). Day foliar water spraying likely did not affect plant transpiration and therefore did not increase Ca absorption, perhaps due to the non-excessive temperature conditions in the greenhouse or to the short duration of the leaf surface treatment.

The objective of night foliar water spraying was to reduce the incidence of tipburn by promoting maximum hydration of tissues by root pressure (Cox and Dearman 1981). Root pressure is a process that occurs primarily at night, when the transpiration rate is low and the soil is moist. A number of authors have reported decreases in tipburn incidence by reducing the transpiration rate of plants at night by increasing the relative humidity of the greenhouse or covering the plants with bags (Palzkill et al. 1977; Collier and Tibbitts 1984; Holtschulze 2005). In our experiment, night foliar water spraying did not reduce tipburn incidence or severity. This treatment may not have reduced the transpiration rate of the lettuce plants at night enough to promote full expression of root pressure. Our results may be explained by the fact that night foliar water spraying was initiated shortly before sunrise. In the experiments of Palzkill et al. (1977), Collier and Tibbitts (1984) and Holtschulze (2005), the treatment lasted the entire night. In our experiment, night foliar water spraying likely reduced plant transpiration for only 1 or 2 h at the end of the night and the start of the day, which may not have been sufficient to promote Ca transport by root pressure. Young leaves from this treatment had the lowest Ca concentration but the concentration was not different from the day spray treatment. This may be due to the range of calcium content, which was not low enough to create symptoms, or to the fact that tipburn symptoms may be associated with local calcium deficiency, which may not be revealed by analysis of the whole leaf Ca content. Obviously, it could be argued that irrigating frequently or misting leaves at regular intervals should show more effect. However, such scenarios were not tested, as they would not be applicable at the field scale, from the growers' feedback obtained.

The objective of the application of prohexadione calcium was to reduce the incidence of tipburn by reducing the aerial growth of the lettuce plants. However, prohexadione did not reduce the lettuce biomass or the tipburn incidence or severity. In addition, the Ca concentration of plants from this treatment was not higher than that of plants from the control treatment.

Foliar Applications of Calcium

Growth and Development

In the Irrigation/Spray Experiment, fresh biomass values were 320, 335 and 331 g and the dry weights of young leaves were 6.7, 7.2, and 7.1 g for the control, foliar application of water and foliar application of Ca, respectively (Table 4). The total number of leaves per plant was 39 for the three treatments. In the Foliar Ca Experiment, the lettuce biomass was between 340 and 398 g and the leaf area ranged from 4762 to 5215 cm^2 (Table 6). The total number of leaves per plant was between 40 and 44. None of the foliar application of Ca treatments had an effect on the growth parameters analyzed. The Ca treatments did not have an effect on the biomass of the lettuce plants. This result agrees with those of other studies (Holtschulze 2005; Jenni and Stewart 2008).

Tipburn

In the Irrigation/Spray Experiment, foliar application of Ca resulted in a reduction in the number of young leaves with tipburn, i.e., 2.5 compared with 6.7 with the control and 5.7 with the foliar application of water (Table 5). The application of Ca also significantly reduced the total leaf area affected per plant, with only 0.5 cm^2 compared with 3.3 and 1.9 cm^2 for the control and the application of water, respectively. In the Foliar Ca Experiment, the number of young leaves affected per plant ranged from 6.0 to 15.8 (Table 7). The total leaf area affected per plant ranged from 0.8 to 6.5 cm^2. Last, the percentage of each plant showing tipburn ranged from 0.03 to 0.36%. For these three variables, the results obtained were different between treatments.

In the Irrigation/Spray Experiment, the foliar application of Ca reduced the number of young leaves showing tipburn and the total leaf area affected compared with the control and water treatments. In the Foliar Ca Experiment, the 90, 180 and 360 mg L^{-1} Ca treatments reduced the total leaf area affected by tipburn as compared with the control, with no differences between the concentrations. In addition, the 90 and 360 mg L^{-1} treatments reduced the number of tipburned young leaves and the percent of plant area affected with tipburn. On a practical basis, a surface corresponding to 4.5 cm^2 or more would discard the lettuce plant. In the Irrigation/Spray Experiment, the level observed for all treatments was low enough to commercialize them. In the Foliar Ca Experiment, only the level observed corresponding to the control treatment was severe enough to prevent their commercial harvest. The results of the different studies suggest that tipburn incidence and severity in lettuce could be reduced by frequent foliar application of Ca. Tipburn appears at the margins of young actively growing leaves when the demand for Ca exceeds the plant's capacity to provide the necessary Ca. Foliar application of Ca provided the quantity necessary for proper growth of the young leaves. In a second study, a 100 mg L^{-1} chelated calcium solution was applied only twice during the growth cycle. The foliar Ca applications did not significantly reduce tipburn (Corriveau et al. 2011). The possible reasons for the differences between studies can be attributed to frequency of application, to the chelated form of Ca, or both. In fact, the use of the chelated nutrient form usually does not increase absorption in relation to organic salts. It seems that an effect of chelates on leaf ability to take up nutrients is related to some properties of the chelate itself (Wojcik 2004). Maroto et al. (1986) observed a reduction of tipburn in Chinese cabbage by means of foliar application of a calcium-rich fertilizer once a week. Tzortzakis (2009) controlled tipburn in Romaine lettuce through foliar application of a 15-mM calcium nitrate solution twice a week. Jenni and Stewart (2008) studied the effect of misting and foliar Ca application on tipburn incidence and severity in chicory. Foliar Ca was applied at a rate of 2.5 L of Calcimax per 1000 L of water one to three times a week. The foliar Ca had no effect on the incidence of tipburn. However, it did result in a reduction (58%) in tipburn severity when applied in

Table 6. Effects of foliar application of different rates of Ca on aboveground fresh biomass, leaf area and number of leaves in lettuce plants in Foliar Ca Experiment

Calcium foliar application (mg L^{-1} Ca^{2+})	Aboveground biomass (g)	Leaf area (cm^2)	Number of leaves per plant
Control (no foliar application)	358	4762	43
90	340	4898	43
180	398	5215	44
360	359	5100	40
P value	0.2744NS	0.5248NS	0.5346NS

Table 7. Effect of foliar application of different rates of Ca on the number of young leaves with tipburn, the total leaf area with tipburn, the percentage of tipburn and the Ca concentration of young lettuce leaves in Foliar Ca Experiment

Calcium foliar application ($mg\ L^{-1}\ Ca^{2+}$)	Number of young leaves with tipburn per plant[z]	Total leaf area with tipburn per plant (cm^2)	% plant area with tipburn	Ca concentration of young leaves per plant ($mg\ g^{-1}$)[z]
Control (no foliar application)	16*a*	6.5*a*	0.36*a*	4.33*a*
90	6*b*	1.2*b*	0.05*b*	4.866*ab*
180	13*ab*	2.9*b*	0.12*ab*	4.98*b*
360	7*b*	0.8*b*	0.03*b*	5.21*b*
P value	0.0497	0.0016	0.0118	0.0475

[z]On a lettuce, the first 15 outer leaves were considered mature and all other leaves over 2 cm in length were considered young.
a, *b* Values within a column followed by the same letter were not significantly different at $P < 0.05$ according to the LSMeans test.

non-misted plants. Thibodeau and Minotti (1969) controlled tipburn and increased the Ca concentration of Butterhead lettuce leaves by frequent (every day or other day) applications of foliar $Ca(NO_3)_2$ or $CaCl_2$. Saleh (2009) obtained similar results by applying Ca (0.7% of Calfruit, 15% CaO) to lettuce leaves at a frequency of three times every 2 wk. Therefore, foliar Ca appears to be effective against tipburn if applied frequently (weekly or every other week or so). In work conducted by Misaghi et al. (1981), the foliar application of 4.7 g L^{-1} calcium chloride or 7.1 g L^{-1} calcium nitrate four times during the 10-wk crop cycle did not reduce tipburn or increase the Ca concentration of the lettuce leaves. In the light of the results of the present study and those of Thibodeau and Minotti (1969), Maroto et al. (1986), Jenni and Stewart (2008), Saleh (2009) and Tzortzakis (2009), the frequency of Ca applications (only four times in 10 wk) could explain the lack of response obtained by Misaghi et al. (1981).

Leaf Calcium Concentration

In the Irrigation/Spray Experiment, the leaf Ca concentration of plants that received foliar Ca applications was higher, with 4.81 mg g^{-1} compared with 4.20 and 4.27 mg g^{-1} for the control treatment and control water treatment, respectively (Table 5).

In the Foliar Ca Experiment, the Ca concentration of young leaves ranged from 4.33 to 5.21 mg g^{-1} (Table 7). The Ca treatments increased the Ca concentration of young leaves, but only foliar Ca applications at 180 and 360 mg L^{-1} increased the Ca content of young leaves compared with the control.

Tipburn is caused by a Ca deficiency in the margin of young actively growing leaves. In our experiment, foliar Ca applications increased the leaf Ca concentration. Ca can penetrate the leaf cuticle through the stomata or ectodesmata (Wojcik 2004). By increasing the leaf Ca concentration, the foliar application of Ca reduced tipburn incidence and severity. Barta and Tibbitts (1986) showed that Ca concentrations in tipburned young leaves were lower than in unaffected leaves. Thibodeau and Minotti (1969) were also successful in reducing tipburn and increasing the Ca concentration of lettuce leaves through frequent Ca applications. Ca concentrations measured in this study are comparable with those obtained by other authors. In their study, Barta and Tibbitts (1991) measured the Ca concentrations in lettuce leaves. According to that study, tipburned (injured) areas on the 14th leaf had Ca concentrations of 0.2 to 0.3 mg g^{-1} dry weight and not tipburned (uninjured) areas of tipburned leaves contained from 0.4 to 0.5 mg g^{-1} dry weight. In addition, Ca concentrations in uninjured 14th and 5th leaves were 1.1 and 1.6 mg g^{-1}, respectively. These results show that Ca concentrations vary from leaf to leaf. Saleh (2009) measured a Ca concentration of 5.23 mg g^{-1} in young leaves. In the Irrigation/Spray Experiment and the Foliar Ca Experiment, Ca concentrations, which ranged from 3.75 to 5.21 mg g^{-1}, correspond to the results obtained by Barta and Tibbitts (1991) and Saleh (2009). In the analysis of Ca concentration, all affected and unaffected young leaves were taken together. Therefore, given the variability observed by Barta and Tibbitts (1991), the measurements of Ca concentrations in the young leaves of this study are consistent with those of the authors mentioned.

The many factors and processes involved in the expression of tipburn are still poorly understood. The results of this study either contradict or support the results of many other studies on tipburn. The many differences in the results of the studies demonstrate the complexity of the phenomenon. Further field experiments will have to be conducted to confirm these results and if so, to more accurately determine the timing, frequency and rate of Ca applications. In addition, the effectiveness of foliar application of Ca on head (iceberg) or leaf lettuces should be investigated as this study is specific to Romaine lettuce. Other strategies for the application of prohexadione calcium and other growth regulators could also be studied.

CONCLUSION

Foliar applications of Ca reduced the number of young leaves with tipburn and the total leaf area affected and increased the Ca concentration in young leaves compared with controls. The results of the different studies suggest that tipburn incidence and severity in Romaine lettuce could be reduced by use frequent foliar application of Ca. Tipburn appears at the margins of young actively growing leaves when the demand for Ca exceeds the plant's capacity to provide the necessary Ca. Foliar application of Ca provided the quantity necessary for proper growth of the young leaves. The irrigation, foliar water spraying and foliar prohexadione application treatments had no significant effect on plant growth or incidence of tipburn. Therefore, based on greenhouse experiments, this indicates that on a practical basis, only foliar Ca application may be efficient to reduce tipburn at the field scale and consequently, this avenue should be tested further.

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