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Effects of Relative Humidity and Root Temperature on Calcium Concentration and Tipburn Development in Lettuce

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Abstract. **Growth chamber studies were undertaken with a tipburn-sensitive cultivar of romaine lettuce (*Lactuca sativa* L. cv. Lobjoits Green Cos) grown under a photosynthetic photon flux density of 320 μmol $s^{-1}m^{-2}$ for 16 hours; light and dark temperatures were 26.0° and 12.5°C, respectively. As the relative humidity (RH) during the light period was decreased from 74% to 51%, growth was retarded, Ca concentration increased, and the onset of tipburn delayed. Decreasing RH during the dark period from 95% to 90% reduced growth and resulted in lower CA concentrations and earlier tipburn development. Further decreases from 90% to 65% caused no additional change in growth or tipburn response. Root temperatures of 23.5°, compared with 15.0°, slightly increased Ca concentration but induced earlier tipburn development. Ca concentrations were increased and tipburn delayed by humidity conditions which provided large diurnal fluctuations in water potential in the plant and which encouraged root pressure flow during the dark period. Elevated root temperatures did not provide expected increases in Ca accumulation in young leaves.**

Tipburn, a physiological disorder of lettuce, is a serious limitation to the production of high-quality crops both in the field and the greenhouse (5). The necrosis which occurs at the leaf margin or apex is associated with low concentrations of Ca in the affected tissue (1, 14). Injury occurs even when there is an adequate supply of Ca to the roots. This is because Ca moves by mass flow in the transpiration stream and therefore accumulates in those parts of the plant which transpire freely (1, 17). Tissue which develops injury is enclosed partially or totally by outer leaves and so has a lower net transpiration rate and consequently less Ca than outer leaves.

Tipburn development is under the control of both genotype (2, 4, 6, 9, 11) and environment (9, 15). Progress in understanding the developmental physiology and control of tipburn has been impeded by the increased sensitivity of lettuce plants to the disorder in controlled environments. Symptoms develop rapidly even in cultivars which are known to have a high level of field resistance.

The Ca concentration of low-transpiring leaves of cabbage (13, 16), cauliflower (10), and strawberries (3) was increased and necrosis prevented when plants were subjected to high RH in the dark, low RH in the light, and soil conditions that encouraged rapid water uptake by the roots. These environmental conditions maximize diurnal fluctuations in plant water potential (10, 16) and encourage root pressure flow during the dark period (3, 12), which enhance Ca movement to these tissues.

By analogy, Ca-related injuries in lettuce might be avoided if plants are grown under conditions which maximize root pressure flow and the diurnal fluctuation in plant water potential. Experiments were carried out at 2 different root temperatures with various combinations of light- and dark-period RH. We report the effects on tissue Ca concentration, tipburn development, and plant growth.

Materials and Methods

Plant propagation. Seeds of 'Lobjoits Green Cos' romaine lettuce were sown into moistened peatlite in 12.7-cm-diameter, white, plastic pots. There were 10 seeds per pot and the seedlings were grown for 14 days from sowing in a reach-in growth chamber in the Univ. of Wisconsin Biotron. Seedlings were thinned to 3 per pot 7 days after sowing (3 days after emergence).

The photoperiod was 16 hr from cool-white fluorescent and incandescent lamps with input wattage of 70% and 30%, respectively. The mean photosynthetically active radiation (PAR) at plant height, measured with a LI-COR quantum sensor, was 325 ± 15 μmol $s^{-1}m^{-2}$. Light-and dark-period temperatures measured with copper-constantan thermocouples were 23.5° ± 0.5°C and 10.5° ± 0.5°, respectively. RH was monitored with a lithium chloride sensing element which was calibrated daily with a thermocouple psychrometer. RH was 75% ± 5% in the light and 80% ± 6% in the dark. Four times each day each pot received about 100 ml of nutrient solution (8) in which the Ca concentration was 10^{-4}m.

Postpropagative plant culture and experimental treatments. Eight small chambers, 30 × 30 × 20 cm in height, were constructed from ultraviolet-transmitting Plexiglas GII-UVT (Rohm and Haas, Inc.) and an 11.7-cm-diameter a hole was cut in the base of each to accommodate the aerial parts of the plants. The base of the chamber was 16 cm above bench height with Plexiglas supports at each corner, 2 of the supports being water reservoirs, each 1200 ml in volume. The chambers were placed in a walk-in, controlled-environment room.

Plants were grown with a 16-hr photoperiod under 34% and 66% input wattage from incandescent and cool-white fluorescent lamps, respectively. The mean PAR at plant height inside the chambers was 320 ± 10 μmol $s^{-2}m^{-2}$. Mean air temperatures in the chambers, measured daily with a copper constantan thermocouple, were 26° and 12.5°C in the light and dark, respectively, with a variation of ± 1.8° between the chambers.

Seven experiments were carried out; within any one experiment there was one RH treatment in combination with 2 root

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temperatures and each treatment was replicated with 4 plants in separate containers. The RH treatments were 51%, 62% and 74% during the light period and 65%, 72%, 90%, and >95% during the dark period. The light- and dark-period treatments were conducted with corresponding dark- and light-period humidities of 95% and 62%, respectively. With the exception of >95% RH, the humidity of the room air was controlled to the described level and the air drawn into the chambers at 20 liters min^{-1} with squirrel-type fans. Room humidity was monitored as described earlier. The fans drawing air through the chambers were switched off for humidities >95% and, oil-free diaphragm pumps were used to bubble room air at 5 liters min^{-1} through a water reservoir in one support leg of the chamber.

Root temperature was controlled by surrounding the pots with a coil of flexible, polyvinylchloride tubing through which water was pumped from a temperature-controlled bath. The warm root temperatures, measured daily with a copper-constantan thermocouple in the center of the pots, were 25.5 ± 2.0°C and 21.8 ± 2.0° in the light and dark, respectively; and the cool root temperatures were 15.9 ± 1.0° and 13.9 ± 1.0° in the light and dark, respectively.

Plant selection and data collection. Uniform plants were selected 14 days after sowing and pots paired on the basis of length of the 4th true leaf and apparent size. One of the 3 seedlings in each pot was harvested for dry weight determinations and one discarded. The pot with remaining seedling was placed in the Plexiglas chambers in the treatment room. One pot of each pair was maintained with a cool root zone and the other with a warm root zone. The average length of the 4th true leaf varied from 2.7 to 4.5 cm and plant dry weight varied from 0.026 to 0.039 g in the different experiments.

Plants were maintained under treatment conditions for 14 days. The date and the number of the leaf on which tipburn first appeared on each plant were recorded. Tipburn was identified by the appearance of one or more necrotic lesions, usually at the leaf apex but occasionally on the leaf margin. At the end of the treatment period, the aerial portion of each plant was harvested by cutting at the cotyledonary node. Records were taken of the length and width of leaves 7, 12, 17, and 22 (numbered from the cotyledons). The number of each leaf with tipburn and the dry weight of the top of the plant were determined. The small, inner leaves between 1 and 3 cm in length were analyzed for Ca content and the results compared with those from an analyses of the remaining leaves and stem of the plant. Each sample was dried at 95°C, subsampled, and ashed at 550° for 4 hr for Ca analysis. The ashed sample was dissolved in 1.0 M HCl (5.0 ml) to which $LaCl_3$ was added and the Ca concentration was measured using a Perkin Elmer (Model 2380) atomic absorption spectrophotometer.

Table 1. The effects of relative humidity (RH) during the light period and root temperature on the number of plants with tipburn, and the time taken from sowing to tipburn development.

Light period RH[z] (%)	No. plants with tipburn[y]		Time taken from sowing to tipburn (days)[x]	
	Root temp		Root temp	
	15°C	23.5°	15°	23.5°
51	0	3	---	28.0
62	2	3	28.0	27.3
74	4	4	25.5	23.8

[z]Dark-period RH >95%.
[y]Four plants in each treatment.
[x]Mean days derived from only those plants that developed tipburn.

Table 2. The effects of relative humidity (RH) during the dark period and root temperature on the number of plants with tipburn, and the time taken from sowing to tipburn development.

Dark period RH[z] (%)	No. plants with tipburn[y]		Time taken from sowing to tipburn (days)[x]	
	Root temp		Root temp	
	15°C	23.5°	15°	23.5°
65	4	4	26.3	23.5
72	4	4	26.3	24.3
90	4	4	24.8	23.0
95	2	3	28.0	27.3

[z]Light period RH > 62%.
[y]Four plants in each treatment.
[x]Mean derived from only those plants that developed tipburn.

Results

Tipburn development. There was no evidence that any particular combination of conditions effectively prevented tipburn, but its onset was delayed by certain treatments. Tipburn was delayed by reducing the RH during the light period (Table 1) and by increasing it above 95% during the dark period (Table 2). Tipburn developed later at the lower root temperature in most cases (Tables 1 and 2). Plants maintained at 51% RH during the light period, and with cool root temperatures, did not develop tipburn by harvest at 28 days; but on the basis of other studies (T.W Tibbitts, unpublished) we assumed that the plants would have developed tipburn if grown for a longer period.

Growth. Plant dry weight and the length and width of leaf 12 increased linearly with increases in light-period humidity (Table 3). In the dark period, plant dry weight and the length of leaf 12 were increased significantly by only the highest humidity treatment (Table 4). The treatment effects on leaf width were insignificant. Growth was very much faster at the higher root temperature in both light- and dark-period humidity treatments (Tables 5 and 6).

Leaf calcium concentration. The Ca concentration of the inner, low-transpiring leaves was less than 1.4 mg Ca g^{-1} dry weight (DW) in all treatments and only about 1/10th of the concentration in the remainder of the plant. The Ca concentration of the inner leaves increased with decreasing RH during the light period (Table 3). In contrast, the calcium concentration of inner leaves of plants did not increase with decreases in RH during the dark period. Highest calcium concentrations, instead, were dark-period RH level (>95%). Dark-period RH treatments below 95% had lower calcium concentrations and all were at similar levels (Table 4). Plants grown at root temperatures of 23.5°C had marginally higher Ca concentrations in inner leaves than plants grown at root temperatures of 15° (Tables 5 and 6). The Ca concentration of the remainder of the plant was between 9.5 and 10.6 mg Ca g^{-1} DW in the different humidity and root temperature treatments.

Discussion

The delay in tipburn development with low humidity levels in the light period was associated with slower growth and increased Ca concentrations in the leaf tissue. The time taken to

Table 3. The effects of relative humidity (RH) during the light period on plant dry weight, length and width of leaf 12, and the calcium concentrations in the inner leaves and the remaining leaves and stems.

Light period RH[z] (%)	Dry wt[y] (g)	Length and width of leaf 12[y] (cm)		Calcium concn (mg Ca g^{-1} dry wt)[y]	
				Inner leaves	Remaining leaves and stem
51	2.84	12.64	9.88	1.30	10.41
62	3.66	14.32	10.06	1.13	9.99
74	4.61	15.38	10.31	0.94	8.88

[z]Dark period RH >95%.
[y]Mean of 3 plants per treatment.

Table 4. The effects of relative humidity (RH) during the dark period on plant dry weight, length and width of leaf 12, and the calcium concentrations in the inner leaves and the remaining leaves and stems.

Dark period RH[z] (%)	Dry wt[y] (g)	Length and width of leaf 12[y] (cm)		Calcium concn (mg Ca g^{-1} dry wt)[y]	
				Inner leaves	Remaining leaves and stem
65	3.00	12.21	9.11	0.86	10.10
72	2.84	12.25	9.23	0.82	10.68
90	2.85	12.40	9.16	0.87	10.28
>95	3.66	14.32	10.06	1.12	9.99
SE	0.12	0.56	0.30	0.05	0.18

[z]Light period RH > 62%.
[y]Mean of 8 plants per treatment.

the onset of tipburn was correlated negatively with dry weight but was unrelated to ontogenetic age. It is reported that slow-growing plants are resistant to tipburn (7, 15), apparently because the demand for Ca is reduced. It also has been suggested that the increased Ca level which occurs with slower growth at reduced humidities results from increased transpiration and thus increased water transport to the developing leaves (13, 16). Although saturated humidities at night increased the rate of growth, tipburn was reduced probably as a result of root pressures that developed during the dark period which promoted Ca transport to the young, expanding leaves. It has been shown in studies with cabbage (12) that substantial quantities of Ca are moved to young tissues when environmental conditions encourage the development of root pressure and subsequent guttation from the leaves. The results therefore indicate that humidity has distinctly different regulatory effects on tipburn during the light and the dark period. Nonetheless, the combination of low humidity during the day and saturated moisture conditions at night would act together to provide a large fluctuation in plant water potential which could encourage Ca movement to the young leaves and delay tipburn as shown for cabbage (16).

It was anticipated that the higher root temperature, in combination with saturated humidity during the dark period, would increase root pressure flow and thereby increase the Ca concentrations in young leaves above those found in similar tissues of plants grown at the lower root temperature. The small increase in Ca concentration observed with the higher root temperature was not great enough to have a significant effect in reducing tipburn injury. In other unreported studies, tipburn developed readily when the root temperature was 20°C, thus it appears that root temperature regulation between 15 and 25° will not be effective in preventing tipburn.

The Ca concentration of 1 mg g^{-1} found in the inner leaves was considerably less than the level of 3 mg g^{-1} which has been reported in other tipburn-injured tissue (10). Thus, even though there were increases of 0.3 to 0.5 mg g^{-1} of Ca under particular growing conditions, significantly greater increases appear to be required to avoid Ca-deficiency injuries.

Table 5. The effect of root temperature for light-period humidity treatments on plant dry weight, length and width of leaf 12, and the calcium concentrations in the inner remaining leaves and stems.

Root temp (°C)	Dry wt[z] (g)	Length and width of leaf 12[z] (cm)		Calcium concn (mg Ca g^{-1} dry wt)[z]	
				Inner leaves	Remaining leaves and stem
15	3.23	13.18	9.33	1.09	9.48
23.5	4.17**	15.05**	10.83**	1.15	10.03**

[z]Mean of 12 plants per treatment.
**Significantly different from 15°C treatment at 1% level.

Table 6. The effect of root temperature for dark-period humidity treatments on plant dry weight, length and width of leaf 12, and the calcium concentrations in the inner leaves and the remaining leaves and stem.

Root temp (°C)	Dry wt[z] (g)	Length and width of leaf 12[z] (cm)		Calcium concn (mg Ca g^{-1} dry wt)[z]	
				Inner leaves	Remaining leaves and stem
15	2.67	11.84	8.96	0.87	9.97
23.5	3.50**	13.75**	9.82**	0.97*	10.56**

[z]Mean of 16 plants per treatment.
*,**Significantly different from 15°C treatment at 5% (*) and 1% (**) level.

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