Calcium Localization and Tipburn Development in Lettuce Leaves during Early Enlargement

Daniel J. Barta¹ and Theodore W. Tibbitts
Department of Horticulture, University of Wisconsin, Madison, WI 53706

Abstract. Tissue concentrations of Ca, Mg, and K were determined across immature leaves of lettuce (Lactuca sativa L. ‘Buttercrunch’) at different stages of enlargement using electron microprobe x-ray analysis. The analysis was with a wavelength dispersive spectrometer to permit detection of low concentrations of Ca. Patterns of mineral accumulation in immature leaves that were exposed were compared to patterns of accumulation in leaves that were enclosed within a developing head. The leaves developing without enclosure were free to transpire and developed normally whereas leaves developing with enclosure were restricted in transpiration and developed an injury that was characteristic of Ca deficiency. In the exposed leaves, Ca concentrations increased from an average of 1.0 to 2.1 mg·g–1 dry weight (DW) as the leaves enlarged from 5 to 30 mm in length. In the enclosed leaves, Ca concentrations decreased from 1.0 to 0.7 mg·g–1 DW as the leaves enlarged from 5 to 30 mm in length. At the tips of these enclosed leaves a larger decrease was found, from 0.9 to 0.3 mg·g–1 DW during enlargement. Necrotic injury first became apparent in this tip area when the concentration was ≈0.4 mg·g–1 DW. Magnesium concentrations across the exposed leaves were similar to concentrations across the enclosed leaves, and did not change with enlargement. Magnesium concentrations averaged 3.5 mg·g–1 DW in both enclosed and exposed leaves during enlargement from 5 to 30 mm. In both exposed and enclosed leaves, K concentrations increased during enlargement from 40 to 60 mg·g–1 DW. Potassium concentrations were highest toward the leaf apex and upper margin where injury symptoms occurred, and this may have enhanced injury development. This research documents the critical low levels of Ca (0.2 to 0.4 mg·g–1 DW) that can occur in enclosed leaves of plants and which apparently leads to the marginal apex necrosis of developing leaves seen frequently on lettuce and other crops.

Materials and Methods

Plants of Lactuca sativa ‘Buttercrunch’, a butterhead lettuce, were grown in a walk-in growth chamber (model CEL 512-37; Sherer-Gillett, Marshall, Mich.). Six seeds were sown in each of 60 1-L white plastic pots, containing a medium of 1 peat : 1 vermiculite (v/v). The pots were watered four times daily to excess with a modified, half-strength Hoagland nutrient solution (Hammer et al., 1978) at a pH of 6.4. Positions of the pots within the growth room were randomly changed several times during the course of the experiment. A 16-h photoperiod was provided by cool-white fluorescent lamps at a photosynthetic photon flux of 350 ± 35 µmol·m–2·s–1, averaged across the pots over the length of the experiment. Air temperature and relative humidity averaged 20 ± 1 °C and 65% ± 5%, respectively, during both the light and dark periods.

After 12 d, the pots were thinned to the modal two plants based on the size of the first true leaf. The pots were thinned to one plant per pot by harvests when leaf 5, the outer exposed leaf, reached a length of 5 mm to 30 mm (Fig. 1). Twelve plants were harvested at about daily intervals to obtain plants with leaf 5 at lengths of (average ± range) of 5 ± 0.5, 10 ± 0.5, 20 ± 1.0, and 30 ± 0.5 mm. The remaining plant in each pot was grown for an additional period of 5 d and plants harvested again at about daily intervals when leaf 14, an enclosed leaf, reached lengths (average ± range) of 5 ± 1.1, 10 ± 1.0, 20 ± 2.0, and 30 ± 2.5 mm. At each harvest, the individual leaves on each plant were observed for the presence of injury symptoms. Only one leaf from each of two plants from each harvest was selected that had a length closest to the target

Enclosure of leaves at the growing point of plants leads to reduced Ca concentrations and frequently a tipburn injury of these leaves. (Barta and Tibbitts, 1986; Bradfield and Guttridge, 1979; Palzkill et al., 1980; Tibbitts et al., 1983; Van Berkel, 1988). The enclosure reduces transpiration and thus reduces Ca transport (Collier and Tibbitts, 1982; Marschner, 1995), as most Ca transport in plants occurs mainly in the xylem by mass flow (Clarkson, 1984). This mass flow results from several factors including transpiration, root pressure, and diurnal changes in water stress (Marschner, 1983). Although Ca is generally considered phloem immobile, the phloem does contain low concentrations of Ca (Bangerth, 1979; Clarkson, 1984) and thus provides some Ca to tissues.

Enlarging leaves have a high requirement of Ca for formation and expansion of cell walls (Clarkson, 1984; Demarty et al., 1984; Marschner, 1995). When young leaves become photosynthetically competent, the rate of nitrate reduction is initially high, leading to the formation of organic acids which have a high affinity for Ca (Kirkby and Knight, 1977; Van Egmond and Breteler, 1972). Thus developing tissues require a continuous, adequate supply of Ca during enlargement.

It was the purpose of this study to determine Ca concentrations in areas of developing lettuce leaves during the early stages of leaf enlargement, and compare these to concentrations of K and Mg in these same areas of the leaves. Patterns of nutrient accumulation in exposed leaves that can transpire freely were compared to patterns in enclosed leaves that were restricted in transpiration and subject to Ca deficiency injuries. An analysis procedure was used that permitted an accurate determination of the anticipated low concentrations of Ca in small discrete areas of these leaves.
The depth of beam penetration was determined to be between 50 and 55 µm and the beam irradiated $1 \times 10^{-4}$ mm$^2$ of tissue. Thus, each analysis involved measurement of the average concentration of element in 10 to 30 cells. The beam did not penetrate the leaf completely and only 40% to 70% of the leaf cross-section was analyzed. However, initial studies had shown that concentrations within interveinal areas of these immature leaves were similar throughout the leaf cross-section (Barta and Tibbitts, 1991b). These similar concentrations within the leaf were also found in beech (Fagus sylvatica L.) leaves with electron microprobe analysis (Thie et al., 1995). Analyses were performed through the lower epidermis, with the leaf surface parallel to the mount and perpendicular to the electron beam. This orientation was found to be most useful for estimates of the average concentration of Ca, Mg, and K across the leaf profile.

The measured concentrations of each element are presented on illustrations of idealized leaves drawn from leaf clearings showing locations of major veins. The results from the two replicate leaves were combined and are reported as average concentrations at each location across the leaf.

### Results

Tipburn injury began to develop in plants at 22 d after seeding. Marginal injury first occurred on each plant on the ninth, tenth or eleventh leaf and continued on all successive leaves that developed. Injury symptoms were first apparent on leaves when they were 20 to 30 mm in length. When the enclosed 14th leaves were 20 mm long, 33% had tipburn, and when 30 mm long, 83% showed injury. Injury was restricted to the leaf apex and distal margin and was characterized by water-soaking, laminal and veinal necrosis, and laticifer rupture. None of the exposed leaves exhibited any injury symptoms.

Calcium concentrations across the sampled exposed and enclosed leaves are presented in Fig. 2. When 5 mm long, Ca concentrations were relatively uniform across both leaves, and averaged 1.0 mg·g$^{-1}$ DW. At 10 mm in length, a slight reduction in Ca concentrations was observed in the distal portion of both leaves, and this decrease was more pronounced in the enclosed leaves. By 20 mm long, Ca concentrations in the exposed leaf had increased dramatically, particularly toward the apex, reversing the earlier pattern of greater accumulation toward the base. Calcium concentrations were highest along the midrib and upper margin. The highest concentration of Ca in the 20 mm leaf, 3.1 mg·g$^{-1}$ DW, was found at the terminus of the midvein, at the leaf apex. At 30 mm in length, a further increase in Ca across the exposed leaf was noted. Calcium was highest at the upper margin and along the midrib, and generally decreased both laterally and basipetally. In contrast, Ca concentrations across the enclosed leaves continued to decrease during enlargement, particularly in the distal marginal areas. At 30 mm in length, Ca concentrations as low as 0.2 mg·g$^{-1}$ DW were found along the margin associated with areas of injury development as noted by the shading in these figures. Calcium concentrations in basal areas remained relatively unchanged during this development period. Average Ca concentrations for the leaves were 1.0, 0.9, 1.6, and 2.1 mg·g$^{-1}$ DW for the exposed leaves and 0.9, 0.7, 0.7, and 0.7 mg·g$^{-1}$ DW across the enclosed leaves, when 5, 10, 20, and 30 mm long, respectively. Thus, exposed leaves exhibited a large increase in Ca over this period of leaf enlargement whereas enclosed leaves exhibited no increase.

Magnesium concentrations across the sampled exposed and enclosed leaves when 5, 10, 20, and 30 mm long are presented in Fig. 3. Magnesium concentrations were relatively uniform across both leaves throughout enlargement, most values were between 2.5 and 4.5 mg·g$^{-1}$ DW. There were no apparent differences in...
concentration between the exposed and enclosed leaves, with the exception that some severely injured areas on the 30-mm-long enclosed leaves had higher Mg concentrations. Average Mg concentrations for the leaves were 3.8, 3.6, 3.2, and 3.5 mg·g⁻¹ DW for the exposed leaf and 3.4, 3.6, 3.3, and 3.4 mg·g⁻¹ DW for the enclosed leaf, when 5, 10, 20, and 30 mm long, respectively.

Potassium concentrations across the sampled exposed and enclosed leaves when 5, 10, 20, and 30 mm long are presented in Fig. 4. In contrast to Mg, large gradients in K concentration developed across both leaves during enlargement. Potassium concentrations tended to be greater in an acropetal direction but less laterally from the midrib. The gradients were small when leaves were 5 mm long, and became greater during enlargement. At 30 mm in length, K levels in the apical regions were almost 2-fold higher than in the base. The highest K concentrations were found at the terminus of the midvein at the leaf apex. The gradient of decreasing K from midrib to margin was less consistent in the enclosed leaves than the exposed leaves.

Average K concentrations for the leaves were 46, 50, 56, 64 mg·g⁻¹ DW for the exposed leaves and 38, 42, 46, and 50 mg·g⁻¹ DW for the enclosed leaves, when 5, 10, 20, and 30 mm long, respectively.

**Discussion**

The tissue concentration of Ca found in this study to encourage injury to lettuce was ≤0.4 mg·g⁻¹ DW and is considerably less than the critical levels reported previously for calcium-related injuries in leaf tissues by most researchers (Cresswell, 1991; Huett, 1994; Thibodeau and Minotti, 1969). However these previous analyses of leaf tissue were confounded by 1) increases in Ca concentrations occurring after initiation of injury and before sampling for analyses, 2) unavailability of procedures to analyze small discrete areas of tissue, and 3) inclusion of veinal tissue which had higher concentrations of Ca (Barta and Tibbitts, 1991a). This Ca concentration of 0.4 mg·g⁻¹ is considerably below the concentration (1 to 2 mg·g⁻¹ DW) that Loneragan and Snowball (1969) proposed as a functional minimum for normal growth of dicotyledonous plants. Nonetheless these low concentrations of Ca are similar to the concentrations reported for Ca-related physiological disorders of fruit and certain storage organs (Al-Ani, 1978; Cerda et al., 1979; Fallahi et al., 1988). Fruit and storage tissues, as potatoes (*Solanum tuberosum* L.), provide large amounts of uniform tissue for effective analysis of low concentrations of Ca by atomic absorption spectrometry.

The declining gradient in Ca concentration from base to apex in Ca deficient leaves described in this paper is consistent with observations of patterns of ⁴⁰Ca-deficient leaves reported by others (Chiu and Bould, 1976; Millikan and Hanger, 1964; Pressman et al., 1993)), with Ca accumulation generally confined to vascular tissues in the basal portion of the leaf. Tissues at the apex and distal margin are first to mature, because cell elongation and maturation occur basipetally during leaf ontogeny, once veination has been established (Olson et al., 1969). The supply of Ca is apparently not sufficient to sustain normal development at the apex and distal areas once the leaf initiates rapid elongation and maturation. Injury development also follows this basipetal progression of deficiency.

Reasons for the decrease in Ca concentration in marginal areas
The locally high levels of Mg associated with some for lettuce grown under controlled environment conditions (Berry et al., 1981). The concentrations of Mg and K found in the leaves are typical of several species (45:2:1), but not the apoplastic fluid within the xylem (2:0.2:1) (Hocking, 1980, Jeschke and Pate, 1991; Pate and Hocking, 1978). The similarity in ratios of K:Mg:Ca in the young growing leaves and in the phloem suggests that the phloem may be supplying a significant portion of the Ca early in leaf ontogeny. A leaf is heterotrophic early in its development, depending upon phloem transport for carbon, water, and many essential mineral nutrients for growth. Phloem elements differentiate ahead of the tracheary elements in lettuce (Olson et al., 1969), and are the first functional conducting tissue available to cells in a developing leaf. Because the Ca requirement of the interveinal tissues of the 5 to 10 mm-long leaves is very low, ≈1 mg·g−1 DW, the low levels of Ca in the phloem sap could be adequate to sustain early leaf growth before xylem differentiation is completed.

Further research is needed to understand how physiological and developmental changes in young growing leaves affect the magnitude and mode of Ca transport. It is of particular importance to understand how these changes interact under the environmental conditions that encourage Ca-related injuries.

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