

Physiological Basis for Tipburn Development in Head Lettuce

I. J. Misaghi and R. G. Grogan

Department of Plant Pathology, University of California, Davis, CA 95616. Present address of the first author: Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

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ABSTRACT

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Tipburn was induced consistently on the inner leaves of mature detached heads of lettuce that were held at 30 C for 5 days. Comparisons were made of respiration rates and metabolites of healthy and tipburned lettuce heads. Respiration rate increased with increase in temperature from 5 to 35 C, and substantial increases occurred in the levels of citrate, isocitrate, succinate, fumarate, and all of the soluble amino acids in plants subjected to 30 C as compared with heads kept at 5 C. Increases in the levels of organic and amino acids were detected before symptom development. Concentrations of water-soluble and total calcium in inner and middle leaves of mature heads were less than those in outer

leaves. Moreover, concentration of soluble and total calcium in heads of tolerant lettuce cultivars was greater than in susceptible cultivars. Typical tipburn symptoms were induced in mature heads held at 21 C by treatment with potassium salts of fumaric, succinic, and citric acids. In view of the chelating potential of organic acids, particularly citric, we hypothesize that tipburn development is a manifestation of a localized calcium deficiency that results from chelation of calcium by organic acids and other metabolites that are increased in plants during exposure to elevated temperature.

Additional key words: Etiology, physiological disorder.

Despite numerous reports regarding the influence of different factors on tipburn development (28), no definite conclusions can be drawn regarding the relative importance of the different factors on disease development. Ries et al (33) postulated that environmental factors cause an alteration in nitrogen metabolism resulting in production of toxic quantities of amino acids such as asparagine. Ashkar and Ries (1) suggested that high respiration rates during tipburn development limited protein synthesis and that protein hydrolysis continued at a rapid rate resulting in the accumulation of free amino acids, which might be toxic. Neither author, however, provided experimental data regarding the toxicity of amino acids. Tibbitts et al (40,41), Struckmeyer and Tibbitts (38), and Olson et al (29) suggested that tipburn development in lettuce resulted from rupturing of laticifers and release of latex. Release of latex was suggested to be due to excessive pressure within the differentiating laticifers developed by increased accumulation of osmotically active solutes (29,40). A relationship between tipburn and calcium deficiency also has been suggested (1,22,39). Thibodeau and Minotti (39), for example, suggested that tipburn is associated with a temporary localized shortage of soluble calcium during a period of critical demand.

We have found that tipburn can be induced consistently in mature, detached lettuce heads by holding them at 30 C for 5 days (28). The objective of this study was to determine alterations in metabolism that occur before

and during tipburn induction and to relate these findings with cultivar differences in susceptibility, calcium nutrition, and other factors that may influence tipburn incidence and severity.

MATERIALS AND METHODS

Induction of tipburn.—Mature heads of lettuce (*Lactuca sativa* var. *capitata* L. 'Calmar' and 'Calicel') selected randomly at time of commercial harvest from fields in the Salinas Valley of California were used throughout this study. Calmar is one of the most tipburn-tolerant cultivars currently grown in California, whereas growers rejected Calicel because it is more susceptible. For routine comparative studies, tipburn was induced by subjecting freshly harvested mature heads to a constant 30 C and 47 ± 4% relative humidity (RH) with 12 hr of light daily in growth chambers (28). The intensity of cool white fluorescent light in growth chambers used throughout this study was 18,000 lx.

Effect of temperature on respiration rate.—Rates of respiration were determined at different intervals from 5 to 35 C. For each temperature, four freshly harvested Calmar lettuce heads were placed in a metal respiratory container (29 × 37 × 37 cm) that was closed except for inlet and outlet tubes. Each container was flushed initially with 50 liters of N₂ for 30 min and then connected to a capillary tube that provided 10 liters/hr of unmodified air from a pressurized cylinder. Gas pressure was maintained at a constant rate by allowing excess gases to bubble through a 130-cm column of water. The percentages of O₂ and CO₂ in gas samples taken from inlet and outlet tubes

were determined at 24-hr intervals with a Carle-8000 gas chromatograph (Carle, Inc., Fullerton, CA 92631) equipped with a thermal conductivity detector, and molecular sieve and silica gel columns. Concentrations of CO₂ also were determined by the Claypool-Keefer (8) method. To determine the influence of light on the respiration rate, the experiment also was conducted in 5-liter glass jars kept at 5 to 30 C under 12 hr of cool white fluorescent light (6,000 lx). Eight heads were subjected to each temperature in each of three repetitions.

Concentration of CO₂, O₂, and ethylene inside heads.—Mature detached Calmar and Calicel lettuce heads were subjected to different constant temperatures ranging from 5 to 30 C for 5 days in environmental chambers. Gas samples (1 ml), collected from inside the heads at 1.5-, 3.0-, and 6.5-cm depths from the top of heads with hypodermic syringes at 24-hr intervals, were analyzed chromatographically for percent of CO₂ and O₂ as described earlier. Ethylene concentrations in gas samples were determined with a Carle-211 gas chromatograph equipped with a flame-ionization detector and an alumina column.

Effects of O₂, CO₂, and ethylene on tipburn incidence and severity.—The effects of reduced levels of O₂ and increased levels of CO₂ and ethylene on tipburn development were studied. Detached heads of Calmar and Calicel were placed in respiratory jars at 20 or 30 C in the dark in modified atmospheres containing various levels of O₂, CO₂, and ethylene. Each respiratory jar initially flushed with 50 liters of N₂ gas for 30 min, was then connected to a system of manometers and capillary tubes that provided a constant flow of 10 liters/hr of N₂ gas containing the desired concentrations of O₂, CO₂, and ethylene. The desired gas mixtures were prepared by mixing appropriate amounts of compressed air, compressed CO₂, liquid N₂, and ethylene using capillary glass tubes of known resistance to gas flow. Gas samples were collected from inlet tubes of the respiratory jars at intervals to confirm that desired concentrations of CO₂, O₂, N₂, and ethylene were being delivered.

The following concentrations of O₂, CO₂, and ethylene were used: (i) 0.03–0.04% CO₂ with 0.5, 1.72, 3.9, 8.1, 12.6, 16.4, and 21.7% O₂; (ii) 15.1–16.1% O₂ with 0.04, 0.05, 3.7, 6.6, 10.5, 15.8, and 17.2% CO₂; (iii) 21.1–21.4% O₂ and 0.03–0.04% CO₂ with 0, 0.31, 0.77, 1.3, 2.2, and 2.7 ppm of ethylene; and (iv) the following combination percentages of O₂ and CO₂, respectively: 21.6 and 0.05, 18.0 and 2.2, 16.0 and 3.9, 11.7 and 8.1, 7.1 and 13.3, 4.2 and 16.2, and 3.1 and 18.7. To minimize drying of the plant tissues, the gas mixtures were humidified prior to introduction into the respiratory jars. Heads were rated for incidence and severity of tipburn after treatment for 5 days. Four heads were subjected to each gas mixture in each of the three repetitions.

Determination of acetaldehyde and ethanol in healthy and tipburned tissues.—Both colorimetric and gas chromatography methods were used for acetaldehyde determination. Six freshly harvested, mature Calmar and Calicel heads were placed in respiratory jars kept at 21 or 30 C in the dark with a constant flow of 10 liters of humidified, atmospheric air per hour.

Within 5 hr after the start of the experiment, effluent gases were bubbled through 5 ml of freshly prepared 2% sodium bisulfite for 12 hr to trap acetaldehyde. The

bisulfite trapping was repeated five times during a 5-day period, and samples were kept frozen until analyzed colorimetrically for acetaldehyde as Stotz (37) described.

Volatiles also were captured by passing the effluent gas from six lettuce heads through a 40 × 0.62-cm glass column packed with Porapak Q (80–100 mesh). To elute the volatiles, the column was heated (150 C) while nitrogen gas (40 ml/min) was passing through it; the eluted volatiles were captured in a dry-ice trap. Volatiles also were collected at 24-hr intervals for 4 days from the tissue homogenates of heads that had been held at 5, 21, and 30 C during a 4-day period.

Leaves from throughout six heads, excluding the outermost leaves, were cut into small sections (3 × 3 cm), and a 100-g uniform sample was homogenized in 100 ml of 0.02 M phosphate buffer, pH 6.0, at 4 C. The homogenate was heated to 40 C in a flash evaporator, and distillate (about 5 ml) was collected in 5 ml of 4% sodium bisulfite in a cold trap under partial vacuum.

Volatiles collected from the Porapak Q column from tissue homogenates, and from gas samples taken from inside lettuce heads kept at different temperatures (described earlier), were analyzed with a Packard Model 427 dual-flame ionization gas chromatograph (Packard Instrument Co., Downers Grove, IL 60515) for the presence of acetaldehyde and ethanol. The gas chromatograph was equipped with a 180 × 0.62-cm stainless steel column packed with Porapak Q (80–100 mesh). The oven, injector, and detector temperatures were maintained at 150, 200, and 200 C, respectively. The respective flow rates of carrier N₂, H₂, and air were 20, 25, and 80 ml/min.

For all of these tests, 12 heads were subjected to each temperature in each of the three repetitions.

Comparison of organic acid and sugar contents of healthy and tipburned tissues.—Analyses were made for organic acids and sugars in central and middle leaves (situated between innermost [central] and outer portions of heads) of mature detached Calmar and Calicel lettuce heads subjected to 5 C (not conducive to tipburn development) and to 30 C (conductive to tipburn development). Samples were taken at 24-hr intervals during a 5-day period.

Extraction of organic acids and sugars from tissues and their separation and purification by cation-exchange and anion-exchange chromatography were done according to Brecht (5). To prepare trimethylsilyl derivatives, portions of purified samples representing amounts of organic acid and sugars in 2.5 g of fresh tissue were evaporated to dryness and mixed with 1.0 ml of Tri-Sil Peerce Chemical Co., Rockford, IL 61105) in a vial with a Teflon-lined cap. Vials were shaken vigorously for 3 min and kept at 65 C for 30 min prior to gas-liquid chromatography (GLC). Sugars and organic acids were analyzed with GLC according to the procedure Johnson and Carroll (21) described. The analyses were done with a Packard Model 427 dual-flame ionization gas chromatograph (Packard Instrument Co., Downers Grove, IL 60515) with a 180 × 0.62 = cm stainless steel column of 3% SE-52 coated on high performance 80–100 mesh chromosorb W. The best separation and resolution of organic acids was obtained with the following operating conditions: oven lower limit, 120 C; oven upper limit, 250 C; injector temperature, 250 C; detector temperature, 300 C;

preprogramming interval, 4 min; postprogramming interval, 1 min; and temperature programming rate 6 C/min. The best operating conditions for sugars were oven lower limit, 180 C; oven upper limit, 260 C; injector temperature, 250 C; detector temperature, 300 C; postprogramming interval, 1 min; and temperature programming rate 8 C/min, with no preprogramming interval.

Comparison of soluble amino acids of healthy and tipburned plants.—Concentrations of soluble amino acids were determined at 24-hr intervals in inner and middle leaves of mature detached Calmar and Calicel heads subjected to 5 C (not conducive to tipburn development) and to 30 C (tipburn-inducing temperature) during a 5-day period. Ten grams of leaf tissue representing a random sample from at least ten heads were kept at -70 C prior to extraction. Frozen tissue samples were placed in a prewarmed 150-ml metallic homogenizer vessel and were homogenized in 50 ml of boiling water for 2.5 min. The homogenate was transferred immediately into a 125-ml flask and placed in a boiling water bath for 10 min. The homogenate was filtered through four layers of cheesecloth and the filter residue was washed with 50 ml of boiling water. The clear filtrates were pooled, evaporated to dryness under partial vacuum at 45 C, and resuspended in 10 ml of buffered sulfosalicylic acid (8 ml of 0.15 N lithium citrate containing 1% thiodiglycol and 0.1% phenol, pH 2.2, plus 2.0 ml of 10% sulfosalicylic acid). The suspension was centrifuged at $15,000 \times g$ for 20 min and analyzed with an automatic amino acid analyzer.

Induction of tipburn with organic acids.—To determine the role of organic and amino acids, which are increased in tipburned tissues, solutions of organic acids, amino acids, and their salts at concentrations ranging from 10^{-2} to 10^{-4} M and at different pH values (2.3–8.6) were incorporated into the lettuce tissues (cultivar Monterey) by injection into heads with hypodermic syringes or by sprinkling of halves of healthy mature heads. The remaining halves were treated with water. Half-sections of heads were kept in partially closed plastic bags to decrease the loss of moisture during the test. Treated and control heads were kept at a constant 21, 26, or 30 C and $47 \pm 4\%$ RH, with 12 hr of light daily or in the dark, and were rated for tipburn severity after 3 and 5 days. This test was repeated five times with at least five heads in each repetition for each test solution.

Effects of calcium, potassium, and sodium salts on tipburn development.—Butts of detached heads were given a fresh cut and placed in 250 ml of solutions of calcium, potassium, or sodium chloride at concentrations with reduced water potentials of -7 , -14 , and -28 bars in an environmental chamber at constant 30 C and $47 \pm 4\%$ RH, with 12 hr of light daily. To differentiate the effect of salt toxicity from that of reduced water potential, butts of some heads also were placed in sucrose solutions with water potentials equal to those of the salt solutions. Control heads were placed in glass-distilled water. Heads were rated for tipburn severity after 5 days. Seven to nine heads were placed in each test solution in each of the four repetitions.

Analysis of lettuce plants for calcium content.—Glassware used for extraction of the tissues was immersed in sulfuric acid-dichromate cleaning

solution overnight, rinsed with distilled water, finally rinsed with glass-distilled water, and dried. Tissues from outer, middle, and inner leaves to be analyzed for calcium content were dried overnight in a forced-air oven at 80 C, and were pulverized to a fine powder with a mortar and pestle under liquid nitrogen.

To determine total calcium contents of the tissues, 0.1 g of an oven-dried pulverized sample was added to 3 ml of concentrated nitric acid in a 50-ml glass tube covered with a clean marble and digested over a hot plate for several hours until the solution was clear. The extract was diluted to 50 ml with 5 ml of 5% lanthanum oxide and glass-distilled water. Extracts were centrifuged at $10,000 \times g$ for 10 min and used for determination of calcium with a Perkin-Elmer Model 360 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, CT 06856).

For extraction of water-soluble calcium, 0.1 g of the oven-dried tissue was added in a test tube containing 10 ml of boiling glass-distilled water and placed immediately on a boiling water bath for 10 min. The extracts were adjusted to 50 ml with 5 ml of 5% lanthanum oxide and glass-distilled water, centrifuged at $12,000 \times g$ for 10 min, and used for calcium analysis as described above. Representative samples taken from leaves of at least two heads were used for analysis in each of the six to eight repetitions.

RESULTS

Induction of tipburn.—Symptoms of tipburn were developed consistently within 2 to 5 days on inner leaves of detached heads subjected to 30 C. No tipburn developed in comparable heads kept at 5 C (28).

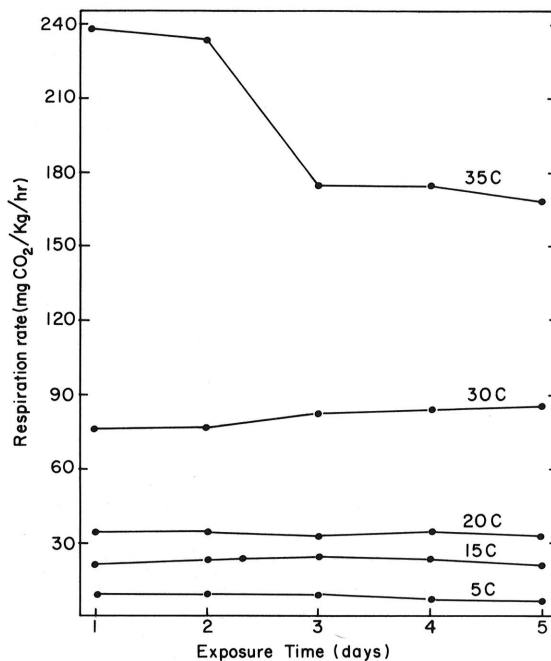


Fig. 1. Relation between temperature and respiration rate in mature detached heads of Calmar lettuce. Eight heads were subjected to each temperature in each of three replications.

Effect of temperature on respiration rate.—Respiration rate increased in direct proportion with increase in temperature from 5 to 35 C, with a large increase from 30 to 35 C (Fig. 1).

Within 24 hr after exposure to the temperature, the values of Q_{10} were between 2.0 and 2.14 over a temperature range of 5–30 C and increased to 6.4 at 35 C. Respiratory quotients (ratio of CO_2/O_2) also increased slightly with increase in temperature; at 5, 15, 20, 30, and 35 C, they were 0.9, 0.9, 1.0, 0.99, and 1.16, respectively.

Concentrations of O_2 , CO_2 , and ethylene inside heads.—Increased respiration resulted in reduction in O_2

and an accumulation of CO_2 inside heads of Calmar and Calicel. Increase of CO_2 and decrease of O_2 were more pronounced in gas samples taken from portions of heads at greater depths than those from shallower depths (Fig. 2). The changes were detectable within 1 day after exposure to 30 C, and became more pronounced up to 5 days.

The concentration of ethylene in gas samples taken from inside heads also increased with increase in temperature and with depth in the head (Fig. 3). Concentrations of ethylene in gas samples taken at 1.5- and 6.5-cm depths increased directly with increase in exposure time to 30 C.

Effects of CO_2 , O_2 , and ethylene on tipburn incidence and severity.—In mature detached heads of Calmar subjected to 30 C, increased concentrations of CO_2 and ethylene and decreased levels of O_2 resulted in a decrease in tipburn severity (Table 1). No tipburn developed in

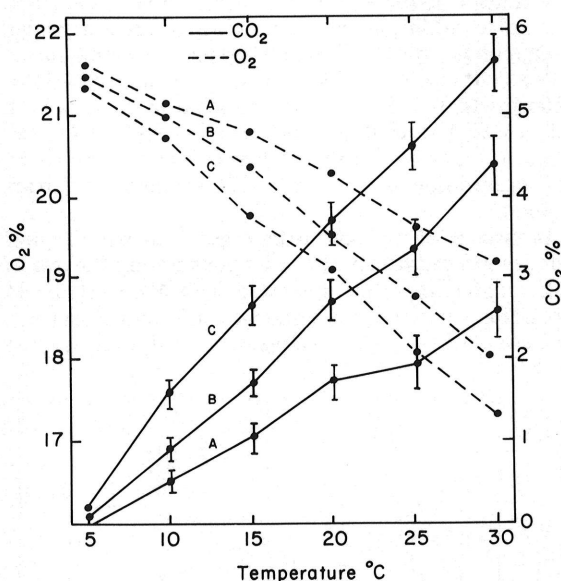


Fig. 2. Relationships between temperature and percent of O_2 and CO_2 in gas samples taken from inside mature detached heads of Calmar lettuce at 1.5- (A), 3.0- (B), and 6.5-cm (C) depths from the top, after 5 days of exposure to different temperatures. Each point represents average of 24 measurements in three replications. Vertical bars represent two units of standard deviation for CO_2 measurements. Standard deviation values for O_2 measurements were close to those for CO_2 .

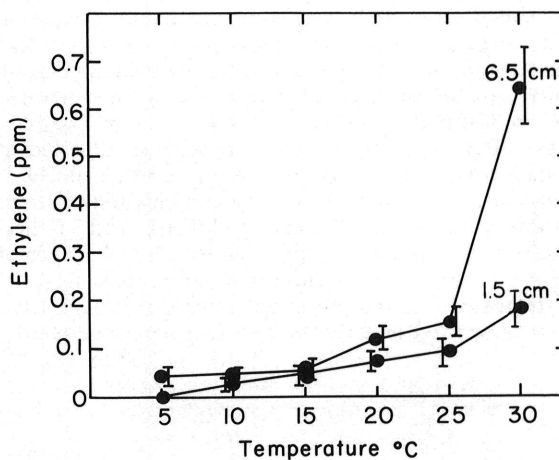


Fig. 3. Relationship between temperature and concentration of ethylene in gas samples taken from inside mature detached heads of Calmar lettuce at 1.5- and 6.5-cm depths after 5 days at different temperatures. Each point represents average of 24 measurements in three replications. Vertical bars represent two units of standard deviation.

TABLE 1. Effect of CO_2 , O_2 , and ethylene on tipburn severity in mature detached Calmar heads after 5 days at 30 C

CO_2^a (%)	Tipburn severity index ^c	O_2^b (%)	Tipburn severity index ^c	O_2 - CO_2 (%)	Tipburn severity index ^c	Ethylene ^d (μ liter/ liter)	Tipburn severity index ^c
0.04 (air)	2.6	21.7 (air)	2.4	21.6-0.05	2.3	0	2.0
0.05	2.2	16.4	2.0	18.0-2.2	2.0	0.3	1.8
3.7	1.3	12.6	1.7	16.0-3.9	1.8	0.8	1.6
6.6	1.3	8.1	1.6	11.7-8.1	1.5	1.3	1.6
10.5	1.1	3.9	1.7	7.1-13.3	1.5	2.2	1.1
15.8	0.9	1.7	1.4	4.2-16.2	1.3	2.7	1.1
17.2	0.9	0.5	1.2	3.1-18.7	1.2		

^a O_2 level varied from 15.1 to 16.1%.

^b CO_2 level varied from 0.03 to 0.04%.

^cSeverity index is mean rating of heads with tipburn symptoms on scale from 0.5 (very slight) to 5.0 (severe).

^d O_2 and CO_2 levels varied from 21.1 to 21.4% and from 0.03 to 0.04%, respectively. Four heads were subjected to each gas mixture in each of three repetitions. Within each modified atmosphere treatment, differences between tipburn severity rating of control plants kept at normal air and those kept at modified atmospheres were significant ($P = 0.05$).

plants subjected to the modified atmospheres at 20 C. The responses of cultivar Calicel were similar to those of Calmar.

Determination of acetaldehyde and ethanol in healthy and tipburned plants.—Despite a slight increase in respiratory quotients from exposure to tipburn-inducing temperatures, neither acetaldehyde nor ethanol were detected by colorimetric and gas chromatographic methods in the effluent from respiratory jars or from gas samples taken from inside heads of Calmar and Calicel kept at different temperatures. Acetaldehyde and ethanol also were not detected in tissue extracts from plants subjected to various temperatures. The lowest detection limit for ethanol and acetaldehyde in the standard solutions was 10^{-5} M.

Comparison of organic acid and sugar content of healthy and tipburned heads.—Substantial increases occurred in the levels of some organic acids in heads subjected to 30 C for 5 days as compared with those kept at 5 C. Among the organic acids, citrate, isocitrate, succinate, fumarate, and malate increased substantially in central head leaves, whereas pyruvate, which serves as fuel for the tricarboxylic cycle, was decreased (Fig. 4). Increases in the levels of organic acids were detected before symptom development, which occurred within 4 days after exposure to 30 C. In samples collected at harvest or within 5 days after exposure to 5 C or 30 C, concentrations and profiles of organic acids in central and middle leaves of Calmar (tolerant) and Calicel (susceptible) were essentially identical, but incidence of severity of tipburn in Calicel was significantly higher.

Increases of 42 and 54% and a decrease of 32% occurred in the respective levels of fructose, D-glucose, and sucrose

in inner leaves of Calmar heads subjected to 30 C for 5 days as compared with those kept at 5 C. Exposure of heads of the Calicel cultivar, which is more susceptible to tipburn, to 30 C for 5 days resulted in 45 and 60% increase in the respective levels of fructose and D-glucose and in a 48% decrease in sucrose in inner leaves as compared with inner leaves from control plants kept at 5 C for 5 days.

Comparison of soluble amino acid contents of healthy and tipburned heads.—Concentrations of some of the soluble amino acids, which increased more than 300% of control in tipburned Calmar lettuce, are shown in Fig. 5. Other amino acids and amino acid derivatives, which increased between 200 and 300% of control, were aspartate, threonine, serine, asparagine, glutamate, glutamine, proline, alanine, ornithine, histidine, arginine, γ -aminobutyrate, lysine, and glycine. The amount of increase in total soluble amino acids as a result of exposure to 30 C for 5 days was similar in heads of both tolerant and susceptible cultivars (Calmar and Calicel, respectively). Significant differences occurred, however, in concentrations of some amino acids in the two cultivars (Table 2).

Induction of tipburn with organic acids.—Typical tipburn symptoms developed on young central leaves of half sections of heads sprinkled with 6 ml of 10^{-2} M solutions of potassium salts of isocitric, fumaric, succinic, and citric acids. The respective average tipburn severity

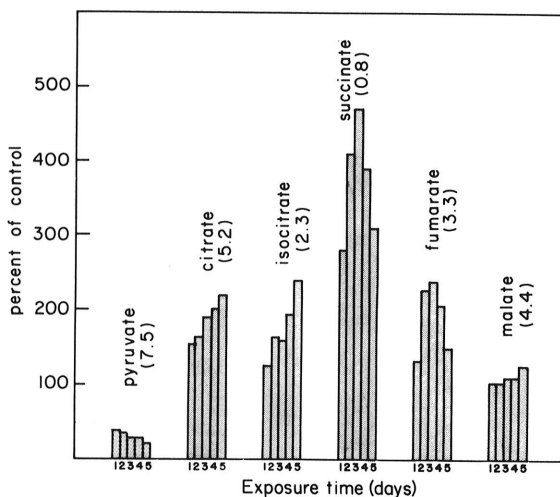


Fig. 4. Relative concentrations of some organic acids in central leaves of detached Calmar lettuce heads subjected to 30 C for 5 days expressed as percent of organic acids in central leaves of control plants kept at 5 C for 5 days. Each histogram has five levels indicating relative concentrations of acids after 1, 2, 3, 4, and 5 days of exposure to 30 C. Figures in parentheses indicate concentrations of acids as milligrams of acid per gram of dry weight in leaves of control heads kept at 5 C for 5 days. Percent figures reflect average of three separate measurements that varied between 6 and 13%.

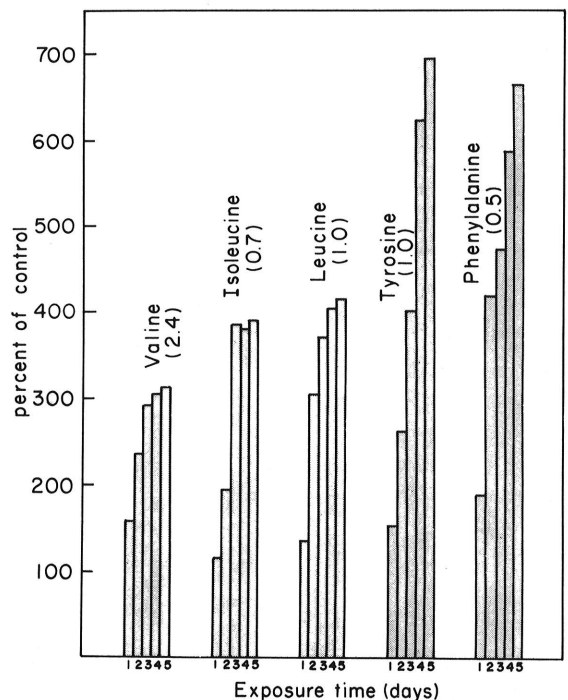


Fig. 5. Relative concentrations of some soluble amino acids in central leaves of Calmar lettuce subjected to 30 C for 5 days expressed as percent of control heads in leaves kept at 5 C for 5 days. Each histogram has five levels indicating relative concentrations of amino acids after 1, 2, 3, 4, and 5 days of exposure to 30 C. Figures in parentheses indicate concentrations of amino acids as milligrams per gram of dry weight in central leaves of heads kept at 5 C for 5 days. Percent figures are an average of three separate measurements that varied between 4 and 9%.

indexes in sections treated with water, succinic, fumaric, isocitric, and citric acids within 3 days were zero, 0.30, 0.30, 0.45, and 0.91 at 21 C and 0.98, 1.30, 1.47, 1.63, and 2.33 at 30 C. Tipburn symptoms also developed on central and middle leaves of heads that were injected with 5 ml of the above acid solutions. Within 3 days at 21 C, no tipburn developed in plants treated with water or the following: calcium salts of the above acids and free forms as well as calcium or potassium salts of tartarate, malate,

TABLE 2. Relative concentrations of soluble amino acids and amino acid derivatives in central leaves of mature detached Calmar (tolerant) and Calicel (susceptible) heads subjected to 30 C for 5 days

Amino acids and amino acid derivatives	Relative concentrations of soluble amino acids ^a	
	Calmar	Calicel
Aspartate	296	267
Threonine	182	192
Serine	131	135
Asparagine	263	467
Glutamate	102	115
Glutamine	154	196
Proline	212	326
Alanine	143	150
Valine	314	346
Isoleucine	390	180
Leucine	414	263
Tyrosine	624	360
Phenylalanine	667	321
Ornithine	143	381
Histidine	250	257
Arginine	166	60
γ -Aminobutyrate	125	407
Lysine	274	431
Glycine	120	129
Average	262	273

^aExpressed as percent of amino acids in central leaves of control plants kept at 5 C for 5 days. Each figure represents an average of three separate measurements; standard deviation ranged from 11 to 57.

TABLE 3. Effects of concentrations of salts and sucrose on tipburn development and severity in Calmar lettuce heads subjected to 30 C for 5 days

Solutions	Tipburn severity ^a at a water potential of:			
	0 bar	-7 bar	-14 bar	-28 bar
Calcium chloride	...	0	0	0
Potassium chloride	...	1.6	0.4	0
Sodium chloride	...	1.9	0.2	0
Sucrose	...	3.4	3.5	0.12
Glass-distilled water	2.3

^aFigures represent tipburn severity index, which is mean rating of tipburned heads on scale from 0.5 (very slight) to 5.0 (severe). Freshly cut butts of 10 heads were placed in each solution in each of four repetitions. Difference between 1.6 and 1.9 was significant at 5% level, whereas other differences were significant at 1% level.

oxylate, glyoxalate, and all of the free amino acids listed in Table 2.

Effect of calcium, potassium, and sodium salts on tipburn development.—In experiments in which butts of detached heads were placed in different salts, only CaCl₂ suppressed symptom development in plants subjected to concentrations equivalent to -7 bars (Table 3). The suppression of tipburn development of CaCl₂ at this concentration apparently is due to the specific effect of salt uptake rather than to reduced water potential, because more tipburn developed in plants exposed to sucrose solutions with similar water potential (-7 to -14 bars) than in those exposed to deionized water. The observed reduction or suppression of tipburn development at concentrations above -7 bars, however, could have been due to reduction of growth rate by reduced water potential (in sucrose and salt treatments) or to mineral toxicity (in salt treatments) or both.

Analyses of lettuce plants for calcium.—Concentrations of water-soluble and total calcium in inner and middle leaves of Calmar lettuce taken at harvest were less than those in outer more tipburn-resistant leaves (Fig. 6). Moreover, concentrations of soluble and total calcium in heads of tolerant cultivars (Calmar, Montemar, and Salinas) were greater than in Calicel and Monterey, which are more susceptible (Fig. 7).

DISCUSSION

Calcium nutrition generally is recognized to influence lettuce tipburn development (1,22,39). Ashkar and Ries (1) showed that in head lettuce, calcium content of the 1-

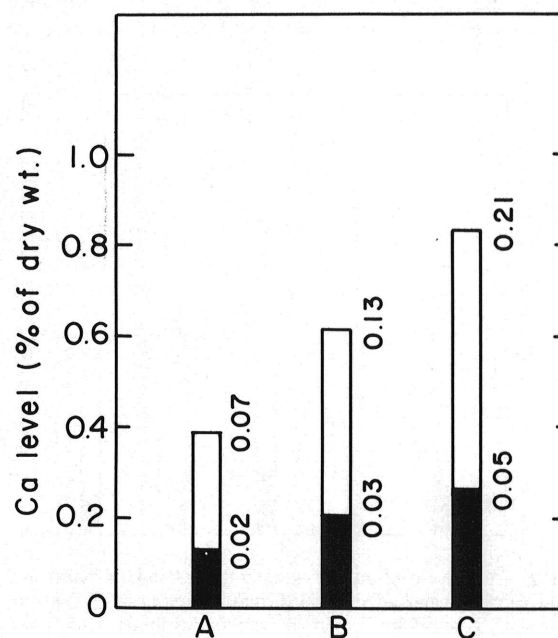


Fig. 6. Concentrations of water-soluble (shaded bars) and total calcium (unshaded bars) in inner (A), middle (B), and outer (C) leaves of field-grown mature Calmar lettuce heads. Differences were significant at 1% level. Percent figures reflect average of seven to eight measurements, with standard deviations indicated beside bars.

cm leaf margin where tipburn symptoms developed was low as compared with other parts of the leaf blade. Our results also indicated that the innermost susceptible leaves contained less calcium than the outer, more resistant leaves. Moreover, we found that concentration of soluble and total calcium in mature heads of tolerant cultivars was consistently greater than in susceptible cultivars. The involvement of calcium in tipburn development was further demonstrated by our ability to suppress tipburn in mature detached heads by placement of their butts in a 1% solution of calcium chloride during exposure to tipburn-inducing temperature. Kruger (22) and Thibodeau and Minotti (39) controlled tipburn of lettuce grown under controlled conditions by application of foliar sprays of calcium nitrate or calcium chloride solutions. Sonneveld and Van den Ende (36) also showed that soil application of calcium chloride to lettuce grown in containers reduced tipburn incidence. Twice weekly sprays of calcium to outer leaves of field-grown head lettuce, however, did not affect tipburn development (20).

Calcium nutrition also has been implicated in other physiologic disorders, such as blossom-end rot of tomato (18), tipburn of cabbage (46) and potatoes (24), blackheart of celery (17) and chicory (47), internal breakdown of apple (4), hypocotyl collar rot of beans (35,49), bitter pit of apple (2), watercore of apple (4), and brown heart of escarole (26). Moreover, timely application of calcium reduced the incidence of many of these disorders (2,4,18,26,47).

Although lettuce tipburn generally is accepted to result from calcium deficiency, little work has been done to explore possible mechanisms that bring about the deficiency. In view of the chelating potential of organic acids, particularly citrate, we believe that tipburn development is a manifestation of a localized calcium

deficiency resulting from chelation of calcium by organic acids and other metabolites that are increased in plants during exposure to elevated temperature. Our observation that the tipburn-inducing effectiveness of organic acids was directly correlated with their chelating strengths provided the strongest evidence for formation of a chelating complex between calcium and organic acids in tipburn tissue (12) (Table 4). For example, we found that more tipburn developed in heads treated with the potassium salt of citric acid, which is a strong chelator of calcium, than in those treated with potassium salts of fumaric and succinic acids, which are weaker chelators (12). Our observation that tipburn was induced in mature detached heads by application of potassium salts but not by calcium salts of citric, fumaric, and succinic acids further supported our calcium chelating hypothesis. Citric acid, which is increased in lettuce plants prior to tipburn development, is a strong chelator of calcium (7,12).

Bangerth (3) suggested that bitter pit of apple, a calcium-related physiologic disorder, is probably caused by a replacement or chelation of calcium in the plasma membrane by potassium, magnesium, hydrogen, and certain organic acids. Evans and Troxler (14) induced blossom-end rot symptoms in tomato fruits by injection of 1 ml of a solution containing 2% citric acid and suggested that citric acid as well as oxalic acid might interfere with calcium assimilation. Thibodeau and Minotti (39) showed that foliar sprays of acetate, citrate, and particularly, oxalate accelerated the development of tipburn in lettuce and suggested that "rapid growth and ensuing high respiratory rate promotes tipburn by temporarily immobilizing soluble calcium as salts of organic acids particularly oxylate." We believe, however, that localized calcium deficiency results from chelation rather than immobilization of calcium by organic acids, because the salts of organic acids, which were increased in lettuce plants undergoing tipburn development, are relatively soluble; moreover, oxalic acid, which combines with calcium to form relatively insoluble calcium oxylate, was not detected in healthy or tipburned lettuce heads.

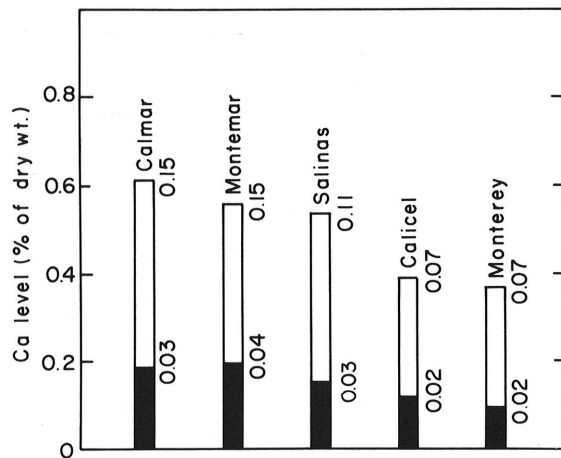


Fig. 7. Concentrations of water-soluble (shaded bars) and total calcium (unshaded bars) in mature heads of five lettuce cultivars. Representative composite samples taken from inner, middle, and outer head leaves, excluding wrapper leaves, from at least 10 heads were used for each analysis. The differences between either Calicel or Monterey and each of other three cultivars were significant at 1% level, whereas differences between other combinations were not significant at 5% level. Percent figures reflect average of six to eight measurements with standard deviations indicated beside bars.

TABLE 4. Correlation between chelating strength and tipburn-inducing ability of three organic acids increased in lettuce plants during tipburn development resulting from exposure to 30 C for 5 days^a

Acid	pK _a	Log stability constant ^b	Tipburn severity index ^c
Succinate	4.07	1.2	0.65
Fumarate	3.02	2.0	0.81
Citrate	6.4	3.1	1.60

^aHalves of healthy mature heads of Calmar were sprinkled with 5 ml of potassium salt of the acids (10⁻² M) and placed in partially closed plastic bags at 26 C in the dark for 3 days.

^bCalculated value representing chelating strength of acids for calcium at different pK_a values (12).

^cSeverity index is mean rating of tipburned heads on scale from 0.5 (very slight) to 5.0 (severe). Tipburn severity index obtained with citrate treatment (1.60) was significantly different at 1% level from those obtained with fumarate or succinate, whereas difference between succinate and fumarate treatments was not significant.

In addition to the organic acids, other metabolites such as amino acids, which are increased in lettuce plants during exposure to tipburn-inducing temperature, also can chelate calcium. In fact, chelation has been encountered with such common cell constituents as peptides and organic phosphate (6). Ashkar and Ries (1) analyzed healthy and tipburn lettuce for levels of total nitrogen, nitrate, total free amino acids, and some individual amino acids. Based on the results from these analyses, they suggested that protein hydrolysis and the resultant accumulation of toxic free amino acids may cause tipburn. Tipburn development in lettuce plants, however, seems unlikely to be due to the toxicity of amino acids, because in our study, free amino acids, including those that increased substantially in tipburned plants, did not cause any damage when they were incorporated into lettuce head tissue.

Tibbitts et al (40,41) and Olson et al (29) have proposed a different mechanism for tipburn development in lettuce. They suggested that release of latex from laticifers into the surrounding parenchyma cells resulted in collapse and necrosis of leaves, and suggested that this phenomenon is directly involved in causing tipburn. Rupturing of laticifers, however, which is consistently associated with the early symptoms of aster yellows of head lettuce (19), does not result in collapse and necrosis of leaf tissue as occurs during tipburn development. This ruptured-laticifer theory is weakened further by occurrence of tipburn in cabbage (46), potatoes (24), and sugar beets (15), which do not have laticifers.

Unavailability of chelated calcium for synthetic processes or movement of chelated calcium to other areas could effect localized calcium deficiency in leaf tissues undergoing tipburn development. Millikan and Hanger (27) showed increased mobility of calcium, which is generally immobile in tissues, in the presence of citric acid. They found that ^{45}Ca incorporated into leaves of broad bean moved only slightly from treated tissues. This immobility was overcome, however, when ^{45}Ca was mixed with EDTA or citric acid prior to its incorporation into the leaves.

The following observations provide indirect evidence for calcium redistribution in lettuce plants during tipburn development: Ashkar and Ries (1) showed that the calcium content of tipburned leaves of greenhouse-grown plants was lower as compared with healthy plants. We also found that the calcium content of marginal portions of central leaves of mature detached heads decreased between 15 and 27% ($P=0.05$) within 5 days of exposure to tipburn-inducing temperature (30 C), whereas calcium concentration in other portions of the same leaves increased proportionally and the total calcium content of the leaves remained unchanged.

Palzkill et al (30) showed that in heading cabbage plants, root pressure was required to move an adequate amount of calcium to various tissues and that calcium moved primarily to the transpiring leaves. If calcium uptake in head lettuce is influenced similarly, calcium deficiency in tipburn-susceptible central leaves of lettuce might be caused by both chelation and a reduced flow of calcium to these low-transpiring leaves under elevated temperatures, while outer high-transpiring leaves receive a sufficient supply of calcium.

Tipburn development also has been associated with

fast growth rates (9,10,31,32,39,48). For example, Cox et al (10), who measured growth rates of six lettuce cultivars, reported a positive correlation between tipburn development and increased growth rate. Corgan and Cotter (9) studied the effect of 13 different chemicals on tipburn development and found that the chemicals that reduced tipburn tended also to reduce head size. Crisp et al (11) showed that in 66-day-old boron-deficient lettuce plants, an increase in the level of the growth promoter indole-3-acetic acid (IAA) preceded tipburn development. A direct correlation between growth rate and tipburn development also was established in our study. In mature detached plants subjected to tipburn-inducing temperatures, symptoms occurred on central leaves that exhibited appreciable growth during the temperature treatment, while middle and outer leaves, which grew only slightly, were usually symptomless. Moreover, no tipburn developed on detached young or old leaves kept under different temperatures and relative humidity, or in detached mature heads in which stem tissue (butts) were excised prior to exposure of the remainder of the heads to tipburn-inducing temperatures (Misaghi and Grogan, *unpublished*). Total suppression of tipburn in heads in which butts had been excised apparently was due to reduction of growth rate because in half sections of heads subjected to temperatures ranging from 15 to 30 C, removal of stem tissue resulted in substantial decrease in the growth of inner leaves and suppression of tipburn development. Reduction in tipburn severity in detached mature heads in which stems were placed in different salts at concentrations equivalent to -28 bars also could be due to reduced growth induced by low water potential.

As Cox et al (10) pointed out, the direct correlation between tipburn development and growth rate might explain the controversy regarding factors associated with tipburn development, since the effect of many proposed tipburn-inducing factors may have mediated changes in growth rates.

The correlation between growth rate and tipburn development also involves calcium nutrition. The increased demand for calcium during growth and cell expansion coupled with a possible alteration in calcium metabolism could result in acute calcium deficiency and tissue necrosis that is characteristic of tipburn.

The reason why leaf tissues with low calcium content became necrotic and collapsed is not known. Biologic activities that are influenced by calcium nutrition and might contribute to tipburn development include membrane permeability (43,44), structural abnormalities (25), selective ion transport (13), increase in surface potential of membranes (34), protection against heavy metal toxicity (42), influencing the activity of several enzymes (50), maintenance of the plasma and vacuolar membranes (45), membrane integrity (16,23), and formation of mitochondria (23).

The soluble and total calcium content of cultivar Calmar with a high degree of field tolerance was significantly greater than of cultivar Calicel, which is very susceptible. Organic acid contents of both cultivars were similar, however, indicating that the tipburn tolerance of Calmar might be due to greater levels of calcium rather than to differences in organic acid content. Some soluble amino acids, however, particularly γ -aminobutyrate and

ornithine, which are present in significantly different concentrations in cultivars Calmar and Calicel (Table 2), may contribute to tipburn development through calcium chelation. If correlation between levels of calcium and organic and amino acids, and degrees of tolerance to tipburn is consistently established in further tests involving additional cultivars, quantitative measurement of these substances might be useful for evaluation of lettuce cultivars for tipburn tolerance in a more precise and quantitative manner.

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