LETTUCE TIPBURN AS RELATED TO NUTRIENT IMBALANCE AND NITROGEN COMPOSITION

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Abstract. Preliminary analyses of lettuce revealed that leaves with tipburn necrosis contained less calcium, and more organic nitrogen, particularly free amino acids, than normal leaves. A susceptible head lettuce, 'Great Lakes 659,' was grown under varying NO_3, Ca, Mg, and light intensities in the greenhouse and growth chambers. Tipburn was easily induces by a high NO_3 and low Ca nutritional regime. Five mM or more Ca in the nutrient solution prevented tipburn. Necrosis was aggravated by high Mg and light intensities. This disorder was accompanied by an accumulation of free amino acids, particularly aspartic and glutamic acids, and their amides. It is postulated that with Ca under stress, high N levels and conditions favoring transpiration, high temperature and light intensity, resulted in rapid nitrogen uptake. Under these environmental conditions protein synthesis probably is limited but protein hydrolysis continues at a rapid rate resulting in the accumulation of free amino acids, which may be the toxic moiety causing tipburn necrosis.

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AND

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NOTE TO THE GUIDANCE COMMITTEE

The body of this thesis is a condensed version, intended for publication in the Journal of the American Society for Horticultural Science.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Tipburn of lettuce (_Lactuca_ _sativa_ L.) is a serious problem confronting most growers. A number of investigators have approached this problem in both the greenhouse and field in different ways, including anatomical studies, morphological development of laticifers, and environmental factors related to tipburn, such as light intensity and duration, temperature, moisture, humidity and nutrient imbalance.

Tibbitts _et_ _al_. (29) studying laticifers as related to lettuce tipburn reported that the release of latex into the surrounding parenchyma cells resulted in cell collapse and necrosis of leaves. Rapid rates of growth increased tipburn severity. Olson _et_ _al_. (21) studying the morphology of laticifers, also related the rupture of laticifers with lettuce tipburn. Light intensity and duration studies by Tibbitts and Rama Rao (30) showed that both factors appreciably influenced tipburn. Tipburn was more severe at 1800 ft-c for 24 and 20 hr daily light periods than 800 ft-c for periods of 16, 12 and 8 hr. Light increased photosynthetic activity with a resultant increase in growth which lead to rupture of laticifers and injury.

Plants, other than lettuce, with laticifers in their structures, also exhibit tipburn symptoms, i.e. chicory

(*Cichorium intybus* (33) and escarole (*Cichorium endivia*) (17). However, tipburn phenomena are not restricted to plants with laticifers. Cabbage (*Brassica oleracea*) (32), celery (*Apium graveolens*) (7) and potatoes (*Solanum tuberosum*) (15) are among the horticultural crops susceptible to tipburn or tipburn-like disorder, despite the absence of laticifers in their structure.

Calcium is an essential element in the structure of pectinacious substances. A deficiency of this element causes a weakness in the cementing between cells, particularly between cells in the rapidly growing tissues. Studies of the distribution and redistribution of ^{45}Ca in bean (*Phaseolus vulgaris* L.) showed that Ca movement involved continued distribution and redistribution to newly formed tissue (9). Ca is a membrane stabilizer, and has a significant effect on membrane permeability (31). Availability of the relatively immobile Ca is reported to be of utmost importance to newly developed tissues (1, 16, 18). An antagonistic interrelationship between Ca and other cations has been reported to decrease the content of this element in the tissue of soybean (*Glycine soja*). Calcium in these plants was primarily associated with pectin of the middle lamella and/or the protoplasm (10).

Enzymes may proteolyze to amino acids when not used in the synthesis of other enzymes or proteins. There is an appreciable accumulation of free amino acids during and after plant senescence. Changes in amino acid levels in plants are found to be due to factors such as; water stress, pathological diseases and mineral deficiencies (3, 25, 27). Water stress may alter amino acid composition, increase proteolysis, interrupt protein synthesis and consistently increase proline and sometimes increases the amide compounds in plants. Pathological diseases have been associated with the accumulation of certain amino acids. Verticillium alboatrum in chrysanthemum caused an increase in proline, and wheat rust caused an accumulation of aromatic amino acids. Mineral deficiencies in tobacco resulted in an accumulation of leucine.

The control mechanism for the synthesis of several amino acids in E. coli, is well established. When specific amino acid levels increase, the synthesis of others is inhibited (8, 19). Unfortunately these studies have not been done with intact plants. The interruption of protein synthesis or proteolysis results in an accumulation of high levels of amino acids, many of which have been found to be toxic (2, 26, 27).

Retardation of leaf senescence by N6-benzyladenine in intact bean plants has resulted in an increase in chlorophyll and protein with concurrent increase in DNA and RNase activity at all stages of development (6).

Although studies of lettuce tipburn by others have shown correlations between environmental factors and the level of injury, the precise cause has not been elucidated.

This study was undertaken in an effort to determine the causes and the nature of tipburn. This information could be beneficial to the plant breeder in selecting efficiently for tipburn resistant varieties.

MATERIALS AND METHODS

Culture and Nutrition. Preliminary analysis of N, and mineral elements of lettuce leaves, 'Grand Rapids,' exhibiting tipburn as described by Tibbitts (29) were compared with those free of the necrosis. These plants were obtained from the same commercial greenhouse. Uniform head-lettuce seedlings, 'Great Lakes 659,' at the 2-leaf stage were transplanted from sand to 6-inch plastic containers filled with 60 g of vermiculite. Plants were grown in a greenhouse with a night temperature of 22°C. The temperature of growth chambers was maintained at 25°C during the day and 20°C at night. The plants were watered with 1/2 Hoagland's solution (11) with N supplied as KNO_3, Ca as $CaCl_2$ and Mg as $MgSO_4$ throughout the remaining period of growth.

The first controlled experiment consisted of application of asparagine and three levels of N (2, 4 and 16 mM). K concentration was 6, 8 and 20 mM respectively with the three N levels. Plants in the second experiment received a combination of two levels of N (6 and 24 mM) and three levels of Ca (0, 5 and 20 mM). K concentration was 10 mM with the first N level and 28 mM with the second N level. Supplemental light was furnished with an intensity of 17, 12 and 5 microwatts/cm^2 per nanometer for blue, red and far-red respectively (approx. 2000-2200 ft-c). Plants in the third test received 18 mM of N with 22 mM of K and all combinations of 0, and 20 mM Ca, with 0, 5, and 20 mM Mg and were maintained at the same light intensity. The fourth experiment was split between two growth chambers, one of which had the same light intensity as in the previous experiment, and the other received 6, 5.5 and 3.7 microwatts/cm^2 per nanometer for the three light bands (approx. 800-1000 ft-c). Treatments within a chamber consisted of all combinations of 6 and 18 mMN, 10 mM of K with the first N level and 22 mM with the second N level, 0 and 5 mM Ca, and 0 and 10 mM Mg. The fifth test was maintained under the same light regime as experiment two. Plants received nutrient solution with 18 mMN with 22 mM of K, 10 mM Mg and 0 Ca. After four weeks they received a spray of either N6-benzyladenine or 6-furfury-laminopurine at the rate of 0, 5, 10, 20 and 40 ppm. A second spray at the same rate was applied after 10 days.

Analytical Procedures. Elemental content of the margin, midrib and the remainder of the leaf blade was analyzed spectrographically (12). Total N was determined by the micro-Kjeldahl and an automated Kjeldahl procedure. Samples for the automatic analyzer were predigested in sulfuric acid. Perchloric acid and selenium were added to the mixture prior to digestion and distillation. Ammonia was determined by a color reaction with alkaline phenol and sodium hypochlorite. Total nitrogen was estimated by optical density standardized by micro-Kjeldahl analysis. Total free amino acids were assayed spectrophotometrically as described by Rosen (24). Individual amino acids were quantitatively determined in the preliminary analyses and the first experiment by the thin-layer chromatography techniques of Pataki (22). To obtain more accuracy, automatic amino acid analysis (23) procedures were utilized with citrate buffers for the next two tests. This system did not adequately separate the amides, asparagine, and glutamine. A lithium buffer system was used in later tests to accomplish this separation. The Lowry method (14) was used to measure water extractable protein. Nitrate was determined by the Lowe-Hamilton technique (13) utilizing soybean nodule bacteroids for reduction of nitrate to nitrite.

All experiments were terminated when plants were 50 to 60 days old (3 to 4 days after exhibiting visual signs of tipburn). Ratings were made of tipburn injury of inner leaves (1 = no injury, 9 = internal leaves all necrotic), after which, plants were weighed and freeze dried for later analysis. All values were expressed on a dry wt basis.

Statistical Procedures. A statistical analysis was not conducted on the preliminary analyses of lettuce from commercial greenhouses, because there were no replicates. All experiments were arranged in randomized block designs with three or more replications. The data were submitted to analysis of variance and the means compared with Duncan's Multiple Range Test. F tests were applied where relevant.

RESULTS AND DISCUSSION

Leaf-lettuce plants, 'Grand Rapids,' from a commercial greenhouse exhibiting tipburn symptoms were low in Ca, Mg, Mn and B for all leaf parts compared to normal plants (Table 1). In particular, the Ca content of the 1 cm leaf margin was low compared to the midrib and the remainder of the blade. The difference in Ca levels between tipburned and normal plants was relatively greater than that for the other elements evaluated.

The total N and, particularly the free amino acid content was considerably higher in tipburned plants (Table 2). Consequently the ratio of free amino acids to total N was higher in tipburned plants. This difference in free amino acids could not be accounted for by more dry matter per g fresh wt since the percent dry wt did not vary greatly. Of the individual amino acids that were analyzed by TLC; arginine, asparagine, aspartic acid and glutamine were appreciably higher in tipburned plants. A large portion, approximately 30% of the total N difference between tipburned and normal plants may be accounted for by the increase in free amino acids. In contrast, the nitrate content was highest in the margin of normal plants.

Steinburg *et al.* (27) associated frenching of tobacco seedlings with L-isoleucine. Tyrosine (25) and L-leucine (20)

Table 1. The elemental composition of different portions of normal and tipburned 'Grand Rapids' lettuce leaves.[a]

Leaf area	Sample	P (%)	Ca (%)	Mg (%)	Mn (ppm)	Fe (ppm)	Cu (ppm)	B (ppm)	Zn (ppm)
Margin	tipburned	0.74	0.72	0.32	30	204	10	32	34
	normal	0.59	1.10	0.37	54	194	8	39	34
Midrib	tipburned	0.62	1.32	0.14	14	59	10	22	20
	normal	0.55	1.88	0.21	20	72	3	24	26
Remainder	tipburned	0.67	0.36	0.20	22	102	3	22	25
	normal	0.60	1.75	0.24	24	109	11	25	30

[a]Each observation is the mean of three plants consisting of a composite of six leaves from each plant.

Table 2. A comparison of N fractions in normal and tipburned 'Grand Rapids' lettuce leaves.[a]

Observations (Amount/g dry wt)	Normal		Tipburned	
	Margin	Remainder	Margin	Remainder
Per cent dry wt	8.5	7.8	10.9	7.9
Total N (mg)	16.4	13.3	19.3	16.6
NO_3 (μmoles)	327	33	19	59
Total free amino acids (μmoles)	209	342	542	893
Individual amino acids (μmoles)				
Aspartic acid	37	44	40	78
Asparagine	15	20	23	34
Glutamic acid	36	30	40	32
Glutamine	22	20	37	21
Plenylalanine	18	21	11	13
Arginine	15	15	26	25

[a]Each observation is the mean of three plants consisting of a composite of six leaves from each plant.

are reported to be toxic to wheat. Audus et al. (2) showed that L-tryptophane, glycine, L-aspartic acid and D-arginine were toxic to cress seedlings. These reports along with the preliminary results in this report indicated that certain amino acids might cause tipburn in lettuce. To test this hypothesis, an experiment was conducted to determine the effect of asparagine on tipburn, since asparagine was one of the predominant amino acids in the tipburned lettuce.

Plants treated with 16 mM of N, with and without asparagine did not develop severe tipburn. However, necrosis was apparent with 16 mM of N plus three mM asparagine (Table 3). At this higher N level, the plants grew poorly and the total N, NO_3, applications of asparagine increased total N, but did not increase either NO_3, total free amino acids, or the individual amino acids analyzed including asparagine. This test failed to relate any relationship of analytical data with the occurrence of tipburn other than total N.

Nutrient imbalance has been reported by several workers to cause physiological disorders in many economic plants (5, 7, 32, 33). Hashimoto (10) reported that lack of Ca in soybean plants caused collapse of plant tissue, meanwhile K deficiency caused brittleness and buckling. Struckmeyer and Tibbitts (27), reported that Ca deficient lettuce had collapsed cells and necrosis of certain leaf areas. Boron deficiency on the other hand, caused a swelling of laticifers and extrusion of latex. However, they reported that neither

Table 3, The effect of N and asparagine applications on growth and nitrogen components of 'Great Lakes 659' lettuce leaves.

Observations[z] (dry wt basis)	Treatment (mM)					
NO_3	2	2	4	4	16	16
Asparagine	0	3	0	3	0	3
Dry wt (g/plant)	4.4a	6.0ab	6.9b	7.3b	5.9ab	6.8b
Total N (mg/g)	11.0a	11.0a	13.9ab	16.9b	27.3c	33.1d
NO_3 (µmoles/g)	Trace	Trace	Trace	Trace	919	912
Free amino acids (µmoles/g)	54a	67a	126b	201c	439d	407d
Individual amino acids (µmoles/g)						
Aspartic acid	8	11	26	30	98	101
Asparagine	1	1	3	3	3	1
Glutamic acid	5	5	8	5	16	21
Arginine	3	3	5	5	28	22

[z]Means followed by unlike letters for each observation are significantly different at .05 level.

Ca nor B deficiency symptoms truly resembled the lettuce tipburn described by Tibbitts et al. (29).

The previous observations lead to further experiments in an attempt to induce tipburn. Ca was eliminated from the growing media, leaving all other environmental factors favoring rapid growth and development unchanged. Typical lettuce tipburn developed in plants that received 24 mM N and 0 Ca. The dry wt of plants was not altered by treatment. The NO_3 content and the free amino acids were considerably higher in tipburned plants (Table 4). In general, both NO_3 and free amino acids decreased with increasing levels of Ca at both N levles. In this test, neither total N nor water extractable protein changed appreciably with different levels of Ca.

Mineral analysis indicated, as expected, Ca increased in lettuce leaves, with increasing Ca levels in the nutrient solution. The occurrence of Ca at the zero level may be explained by endogenous Ca from the seed and the presence of 0.5% Ca in vermiculite. Results of free amino acid by automatic analysis showed that serine and/or asparagine, and glutamic and/or glutamine were predominant (Table 5). Unfortunately the citrate buffer used for separation of these amino acids did not separate serine from asparagine, and glutamic acid from glutamine. Asparagine and/or serine accounted for more than 1/3 of the free amino acids where Ca was not in the nutrient solution.

Table 4. The relationship between N and Ca levels in nutrient solution with N constituents of 'Great Lakes 659' leaves.

Observation[z] (dry wt basis)	Nutrient level (mM)					
NO_3	6	6	6	24	24	24
Ca	0	5	20	0	5	20
Dry wt (g/plant)	3.5	3.7	4.2	3.2	3.6	4.5
Total N (mg/g)	24.1 a	21.0 a	21.0 a	36.7 b	30.5 c	33.3 bc
NO_3 (μmoles/g)	65 b	57 b	19 a	839 c	746 cd	586 d
Free amino acids (μmoles/g)	276 ab	194 ab	179 a	526 c	301 b	251 ab
Water extractable protein (mg/g)	61 a	58 a	67 a	84 b	72 ab	77 b
Elemental composition (amount/g)						
P (%)	1.2	0.7	0.5	0.7	0.6	0.2
Ca (%)	0.8	1.8	3.0	0.8	1.6	2.8
Mg (%)	1.2	1.0	0.9	1.2	0.6	0.6
Mn (ppm)	18	16	14	10	10	9

[z]Means followed by unlike letters for each observation are significantly different at the .05 level.

Table 5. Amino acid composition of 'Great Lakes 659' lettuce leaves receiving different levels of KNO_3 and $CaCl_2$.[a]

Amino Acids		Nutrient level (mM)					
	NO_3	6	6	6	24	24	24
	Ca	0	5	20	0	5	20
		(μmoles Amino Acid/g dry wt)					
Aspartic acid		7	16	11	26	11	27
Threonine		25	21	21	52	35	18
Serine and/or Asparagine		151	75	54	356	196	169
Glutamic acid and/or glutamine		15	19	21	54	66	30
Alanine		20	14	14	31	22	20
Valine		11	5	3	16	10	10
Isoleucine		12	4	3	14	8	8
Phenylalanine		4	3	2	6	4	4
Arginine		8	10	5	47	32	48
Total of all amino acids		307	216	228	848	557	524

[a]Each observation is the mean of two replications.

To determine if there was an interrelationship between Mg and Ca, these two elements were varied under a constant NO_3 regime of 18 mM. When the Mg level was increased in a Ca-deficient growth media, tipburn developed more rapidly and necrosis was more severe than in the previous experiment (Table 6). Calcium deficient plants were the first to show tipburn, particularly when the Mg level was at 20 mM. Apparently Mg aggravated the occurrence of tipburn. This type of elemental antagonism agrees with the findings of several workers (10, 31). None of the plants that received Ca exhibited tipburn symptoms. The total N, and NO_3 and the level of free amino acids were all higher in tipburned plants.

In the experiment to compare the incidence of tipburn at different light and nutrient levels, plants grown under high light intensity developed tipburn one week earlier than plants grown under low light intensity (Table 7). The first lettuce plants that manifested tipburn received high N, 10 mM Mg and no Ca, the second group of plants where tipburn appeared were on a regime of high N and no Ca and no Mg. Mineral analyses of lettuce leaves suggested that Mg in the nutrient media competed with Ca, reducing the Ca content of the plant tissue, which resulted in tipburn necrosis. The Ca concentration, was greater in the high light regime compared to low light. As the Ca content increased, Na content decreased. This negative correlation between Ca and Na content indicated that calcium limited Na and N

Table 6. The relationship of growth and nitrogen components of 'Great lakes 659' lettuce leaves with different levels of Ca and Mg when grown on 18 mM of NO_3.

Nutrient level (mM)		Observation[z] (Dry wt basis)				
Ca	Mg	Tipburn Ratings[a]	Dry wt (g/plant)	Total N (mg/g)	NO_3 (μmoles/g)	Free amino acids (μmoles/g)
0	0	7.0 b	4.9	36.8 b	814 b	715 b
0	5	8.0 bc	7.7	35.6 b	778 b	584 b
0	20	9.0 cd	5.4	37.4 b	716 b	643 b
20	0	1.5 a	4.7	29.7 a	467 a	377 a
20	5	1.0 a	6.0	26.8 a	359 a	370 a
20	20	1.0 a	4.9	30.0 a	376 a	429 a

[a]Rating scale; 1 = no injury, 9 = internal leaves are necrotic.

[z]Means followed by unlike letters are significant at the .05 level.

Table 7. Visual tipburn injury as related to relevant elemental components of 'Great Lakes 659' lettuce leaves.

Light Intensity	Nutrient level (mM) NO_3	Ca	Mg	Tipburn ratings[a] (Ratings of all treatments)	(Mean of high and low)	Observations[c] P (%)	Ca (%)	Mg (%)	Na (ppm)
High	6	0	0	4.5 [b]	3.8 b [z]	.45	.68	.25	721
	6	0	10	5.3	4.6 c	.54	.64	.53	658
	6	5	0	1.0	1.0 a	.30	1.56	.31	551
	6	5	10	1.0	1.0 a	.31	1.40	.37	381
	18	0	0	6.7	5.5 d	.38	.73	.27	685
	18	0	10	8.0	7.1 e	.54	.70	.60	676
	18	5	0	1.2	1.1 a	.33	1.32	.36	537
	18	5	10	1.0	1.0 a	.33	1.14	.35	488
Low	6	0	0	3.0		.58	.44	.25	740
	6	0	10	3.8		.54	.37	.48	751
	6	5	0	1.0		.34	.74	.30	604
	6	5	10	1.0		.32	.70	.42	495
	18	0	0	4.3		.46	.64	.28	968
	18	0	10	6.2		.48	.46	.46	855
	18	5	0	1.0		.29	.77	.36	558
	18	5	10	1.0		.59	.71	.51	866

[a]Rating scale; 1 = no injury, 9 = internal leaves all necrotic.

[b]The F value for interaction of nutrient level and light intensity is significant at .01 level.

[c]Correlation between Ca and Na significant at .01 level, r = -0.68.

[z]Means followed by unlike letters are significant at .01 level.

uptake. Again, the total N and free amino acid content of the injured plants was highest (Table 8). The nitrate level as in other tests was highest in the plants having tipburn. This may be the result of the lack of synthesis of nitrate reductase, to reduce the NO_3 to NO_2.

High Ca compared to low Ca levels in the nutrient solution, resulted in less accumulation of total N and free amino acids at the higher N level as compared to the lower level (Figure 1). This suggested that Ca directly or indirectly might have decreased N uptake. The decrease in N uptake may also have been due to Cl since the Ca was added as $CaCl_2$. This may account for the reduced tipburn with the addition of Ca and the interrelationships with other ions available in the nutrient media.[1]

The predominant amino acids were asparagine, glutamine, aspartic acid, glutamic acid and arginine (Table 9). The growth of tipburned leaves was completely retarded, during the last two weeks. This suggested that glucose may have become limiting which accounted for the accumulation of asparagine and glutamine, particularly under the growing conditions with high levels of NO_3.

El-Mansy _et al_. (4) reported that the shelf life of lettuce (cv. Great Lakes) treated with 6-furfurylaminopurine

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[1]Concurrently with this research, it was demonstrated that foliar applications of Ca prevented tipburn. Thibodeau, P. O. and P. L. Minotti. 1969. The influence of calcium on the development of lettuce tipburn. Proc. Amer. Cos. Hort. Sci. 94: 372-375.

Table 8. The relationship between nitrogen, calcium and magnesium levels under different light levels with the nitrogen constituents of 'Great Lakes 659' lettuce leaves.

Light Intensity	Nutrient level (mM) NO_3	Ca	Mg	Dry wt (g/plant)	Total N[a] (mg)	(Amount/g dry wt) Free amino acids[a] (μmoles)	NO_3[b] (μmoles)
<u>High</u>	6	0	0	3.3	27.2	747	92 a
	6	0	10	3.0	32.0	882	141 a
	6	5	0	3.2	28.2	693	116 a
	6	5	10	3.2	32.8	704	213 ac
	18	0	0	4.4	40.2	1431	526 bc
	18	0	10	3.3	48.8	1540	564 b
	18	5	0	3.1	38.9	1098	463 b
	18	5	10	3.1	33.6	720	490 b
<u>Low</u>	6	0	0	3.3	32.2	412	49 a
	6	0	10	3.5	33.1	558	119 a
	6	5	0	2.9	33.3	540	90 a
	6	5	10	3.1	34.6	531	202 ac
	18	0	0	3.8	43.7	684	542 bc
	18	0	10	4.4	44.3	702	535 bc
	18	5	0	2.8	37.3	621	510 bc
	18	5	10	2.9	38.2	480	609 b

[a]The F value for Ca x NO_3 interaction is significant at the .01 level.

[b]The F value for difference between NO_3 levels is significant at the .01 level.

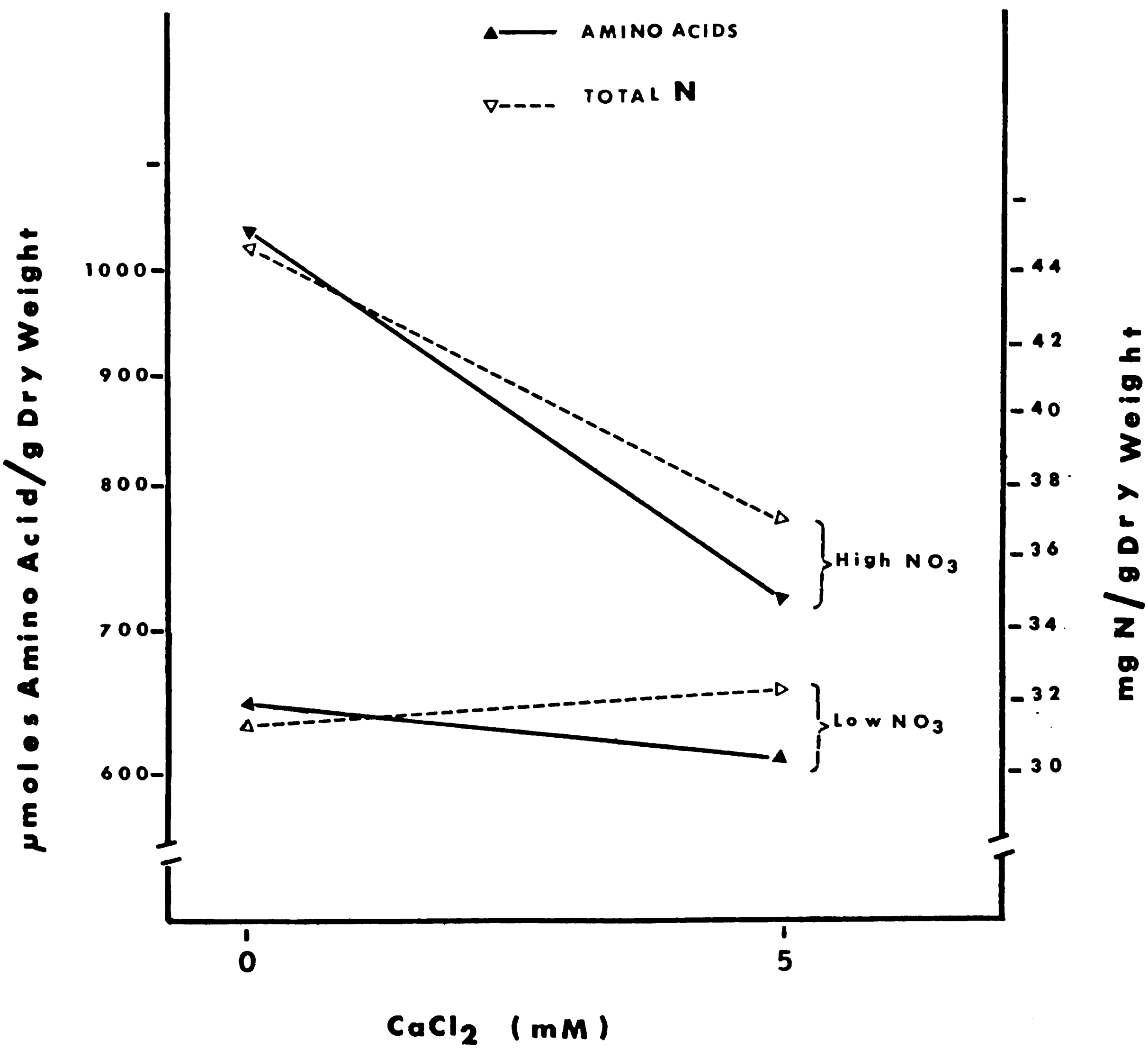

Figure 1. The amino acid and total N content of 'Great Lakes 659' lettuce leaves grown at two KNO_3 and $CaCl_2$ levels.

Table 9. A comparison of the predominant free amino acids in 'Great Lakes 659' lettuce leaves grown with varying levels of NO_3, Ca, and Mg.[a]

Amino acids (µmoles/g dry wt)	Nutrient level (mM)								
	NO_3	6	6	6	6	18	18	18	18
	Ca	0	0	5	5	0	0	5	5
	Mg	0	5	0	5	0	5	0	5
Aspartic acid		22	38	22	40	68	35	32	16
Threonine		22	28	27	24	51	98	45	28
Serine		27	30	47	40	46	53	62	42
Asparagine		40	50	18	11	157	254	80	44
Glutamic acid		94	104	124	170	178	225	141	116
Glutamine		59	68	46	22	139	420	90	39
Alanine		54	47	50	32	87	65	54	22
Valine		19	21	17	19	27	19	17	14
Isoleucine		9	10	9	10	14	16	14	8
Phenylalanine		7	7	7	7	21	15	10	6
Arginine		13	11	16	16	50	34	33	15
Total of all amino acids		713	832	731	742	1248	1615	912	707

[a]Mean of two light intensities.

and N6-benzyladenine was extended. They suggested that these chemicals acted by delaying senescence. Although the high nitrate level may be responsible for more amino acids synthesis, these levels could also result from either proteolysis or interruption of protein synthesis. These studies and the nutrient imbalance and light effects suggested an experiment to test the effect of kinetin on tipburn. Under these tipburn inducing conditions, spraying twice with N6-benzyladenine or 6-furfurylaminopurine (0, 5, 10, 20 and 40 ppm) did not have any effect on tipburn occurrence. All plants regardless of chemical or concentration showed tipburn symptoms and the dry wt, NO_3, water extractable protein and total N were not significantly altered. This did not agree with Fletcher's (6) findings reporting protein increases in intact bean leaf treated with N6-benzyladenine.

Results of controlled experiments with 'Great Lakes 659' lettuce agree with the results of the preliminary test with 'Grand Rapids' lettuce except for the NO_3 content. These results suggest that a nutrient imbalance may cause tipburn, particularly under the high light intensity values in this experiment and optimum growing conditions. All these variables had an interacting effect upon the uptake and metabolism of N. The accumulation of free amino acids which are a result rather than a cause of tipburn may contribute to the actual necrosis.

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