ELECTRON MICROSCOPICAL,
GREENHOUSE AND FIELD STUDIES OF
TIPBURN OF HEAD LETTUCE

by

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ABSTRACT

The ultrastructural changes in head lettuce leaf tissue undergoing tipburn development were examined. In the early stages of disease development the initial change observed was separation of the plasmalemma from the cell wall in parenchyma cells. In more advanced stages of tipburn development membranes of organelles in parenchyma cells progressively degenerated. In severely tipburned tissue only remnants of disintegrated organelles remained in parenchyma cells. Rupturing of laticifers did not seem to play a primary role in initial or intermediate stages of tipburn development. Although, latex within laticifers did become progressively granular.

Several chemicals were evaluated for controlling tipburn in the field. Soil amendments which lower the soil pH resulted in a slight reduction in tipburn incidence, while foliar application of chelated calcium did not result in any consistent decrease in tipburn incidence.

A study was conducted to determine if tipburn tolerance of lettuce cultivars could be improved by selecting plants with high levels of calcium. No correlation was found between the calcium content of selected parent plants and that of their first progeny. It was concluded that selection of plants with high calcium content cannot be used as a means of improving tipburn tolerance of lettuce cultivars.

CHAPTER 1

INTRODUCTION

Tipburn, a non-parasitic disease of lettuce, occurs throughout the world. This disease is particularly important in spring planted head lettuce grown in Arizona and California, where the warm temperatures during head maturation favor tipburn development. Therefore, the time and place of commercial planting is usually chosen to avoid warm weather. When favorable conditions for disease development occur nothing can be done to prevent severe losses.

The disease is characterized by necrotic breakdown of marginal tissue of leaves within the heads and a darkening of veins. The loose outer leaves of the head are not usually affected, therefore symptoms are evident only after removal of the outer leaves. This hidden symptomology prevents selection of non-infected heads, thus fields with even a small percentage of diseased plants are often abandoned. Other than the use of few partially tolerant cultivars, no satisfactory control method has been developed.

The effect of temperature on disease development was determined quantitatively by Misaghi and Grogan (35). These workers were able to induce tipburn in healthy, detached field grown lettuce heads by exposing them to 24-33 C for four to seven days in growth chambers. They showed that the internal temperature of mature heads in the field during sunny days was usually about 6 C higher than ambient, which might explain occurrence of tipburn in the field when ambient temperatures do not exceed 24 C which is the minimum temperature for tipburn induction under laboratory conditions. Misaghi and Grogan also reported that the rate of respiration in heads held at 35 C was six times that of heads held at 20 C (36).

The most consistent difference between tolerant and susceptible cultivars is the level of total calcium. Misaghi and Grogan (36) showed that mature heads of tolerant cultivars contained higher levels of soluble and total calcium than susceptible cultivars. They suggested that tipburn development is a manifestation of localized calcium deficiency resulting from chelation of calcium by organic acids and other metabolites which increase in plants as a result of increased rate of respiration during exposure to elevated, tipburn-inducing temperatures. Symptoms are most severe in the leaf margins because chelation and the slow rate of translocation of calcium deprive the tissue of this vital element.

CHAPTER 2

ELECTRON MICROSCOPICAL STUDY OF HEAD LETTUCE LEAF TISSUE UNDERGOING TIPBURN DEVELOPMENT

Introduction and Objectives

Calcium is known to play a key role in many physiological activities of the cell and in membrane stability against various stress factors (10, 17). Changes in membrane permeability and integrity brought about by calcium deficiency could exert profound effect on cellular metabolism leading to cell death. Results of a number of studies with plants grown under calcium deficient conditions have shown that necrosis and tissue collapse were associated with breakdown of membranes. Membrane irregularities were found in cells from meristems (29) and roots (32) of barley grown under calcium deficient conditions. Disintegration of the plasmalemma and tonoplast were common features of tissue from potato sprouts showing symptoms of calcium deficiency (21). Extensive disintegration of membranes also was found in apple tissues showing symptoms of bitter pit, a calcium-related disorder (30), and internal breakdown (16).

The objectives of this study were to determine ultrastructural changes in tissue of head lettuce during tipburn development and to establish whether or not tissue breakdown is preceded by disruption of cellular membranes.

Materials and Methods

Several mature healthy and tipburned heads of Calmar Lettuce (Lactuca sativa var. Capitata) were selected from a field in Yuma, Arizona. Uniform leaves showing different levels of tipburn symptoms at the margins were selected. Small sections (1 X 5 mm) were cut at the margin of the leaves from tissues showing mild, intermediate and severe tipburn symptoms. Tissue samples were also collected from mature heads in which tipburn was induced by exposing the heads to 30 C in growth chambers according to a method described earlier (35). Controls consisted of tissue samples collected from the margin of the leaves of healthy plants which were held at 5 C and from unaffected margins of leaves of diseased plants.

Tissue sections were fixed in 1% glutaraldehyde in .066 M Sorensen's phosphate buffer, pH 7.0. The fixative solution was changed three times at two-hour intervals and the tissues were rinsed in Sorensen's buffer for six hours with three changes at two-hour intervals. Post fixation was accomplished with 2% osmium tetroxide in Sorensen's buffer for four hours with one solution change. Fixation was done at 22 C with constant rotation in corked vials. Additional tissue samples were fixed in 3% glutarahldehyde in 0.05 M collidine buffer, pH 7.2, for twelve hours at 4 C. Fixed tissue was then rinsed in buffer with three changes during a one-hour period. Samples were postfixed in 2.5% aqueous potassium permanganate for one hour at 4 C. Fixed tissue was washed with deionized water.

Samples were dehydrated in an alcohol series of 12.5, 25, 50, and 75% (10 min per solution) with two final rinses in absolute ethanol and two rinses in propylene oxide. Tissues were placed in a mixture of propylene oxide and Spurr's low viscosity resin (1:1, v/v) and the propylene oxide was allowed to evaporate overnight. Tissues were then embedded in Spurr's resin and the resin was polymerized at 70 C for 8-10 hr (46).

Thin sections were cut from selected specimens with a Porter Blum MT2B ultramicrotome and expanded with trichloroethelene vapor. Sections showing silver-gold refraction colors were placed on 150 mesh carbon coated grids and 300 mesh naked grids. Grids were submerged in a 2% aqueous uranyl acetate for thirty min at 60 C (57). After allowing the solution to attain room temperature the sections were passed through three rinses of distilled and deionized water and then into Reynolds lead citrate (41) on dental wax in a covered petri dish for fifteen min at room temperature. Sections were then washed in a .02 N NaOH followed by deionized water. The sections were then viewed with a Hitachi 500 electron microscope at an accelerating voltage of 75 kV.

Results

The most prominent ultrastructural changes observed in lettuce tissue during the initial stages of disease development was the disruption of the plasmalemma in parenchyma cells and their separation from the cell wall (Figs. 1B and 3B). In intermediate stages of disease development, mitrochondria (Fig. 2), lysosomes, peroxisomes and tonoplasts showed progressive degeneration. Laticifers with intact

Fig. 1. Parenchyma cells from healthy lettuce leaf tissue (A) and from leaf tissue exhibiting progressive necrosis (B,C,D) during tipburn development.

Glutaraldehyde-$KMnO_4$ fixation, X 3500. B, disruption of the plasmalemma (arrow) is apparent in the initial stages of disease development. C, a nucleus (n) and vacuolated chloroplasts (vc) are the only organelles left in a cell at intermediate stages of disease development. D, only a few degenerated organelles are seen in a cell from severely damaged tissue.

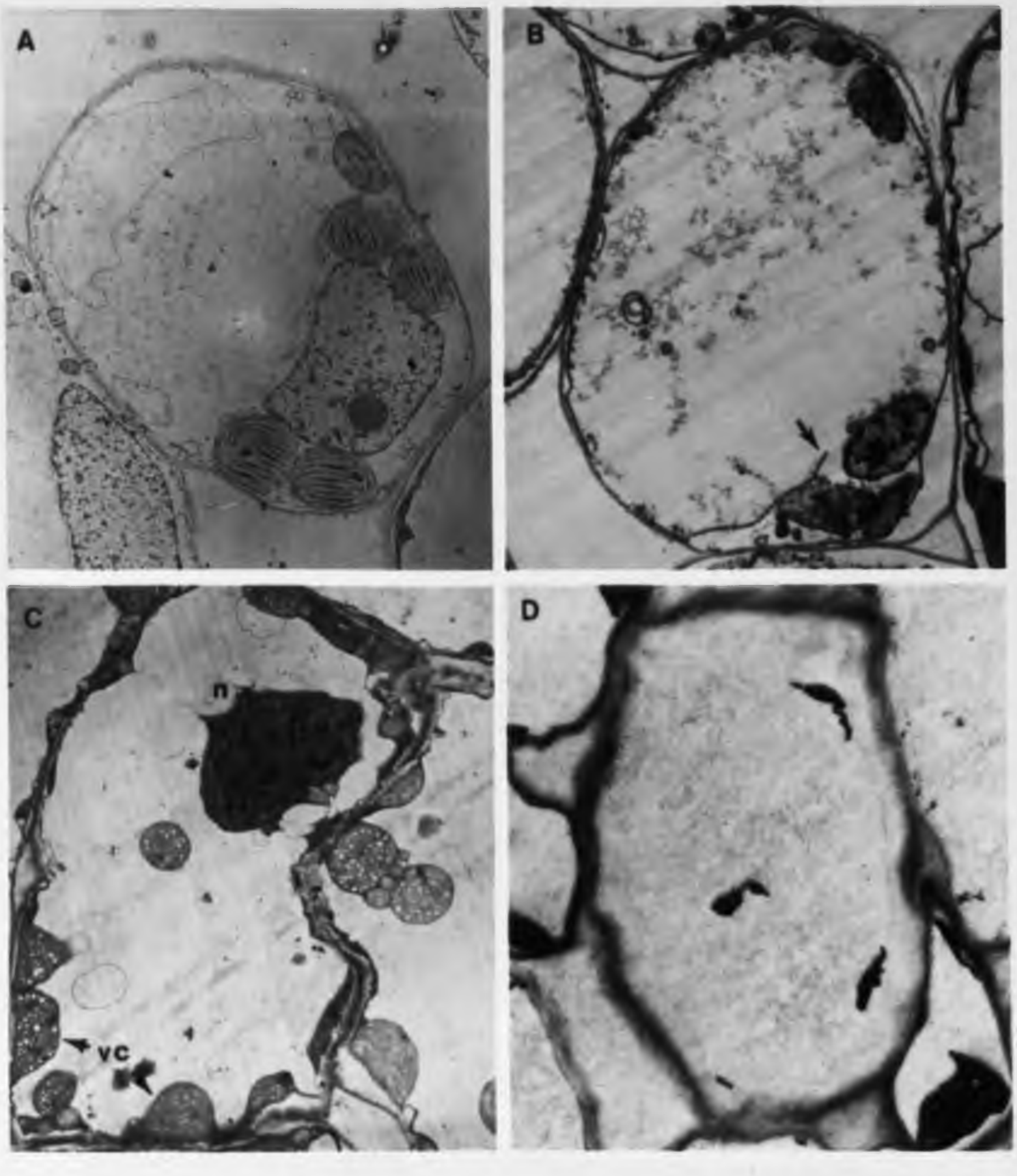

Fig. 1. Parenchyma cells from healthy lettuce leaf tissue (A) and from leaf tissue exhibiting progressive necrosis (B,C,D) during tipburn development.

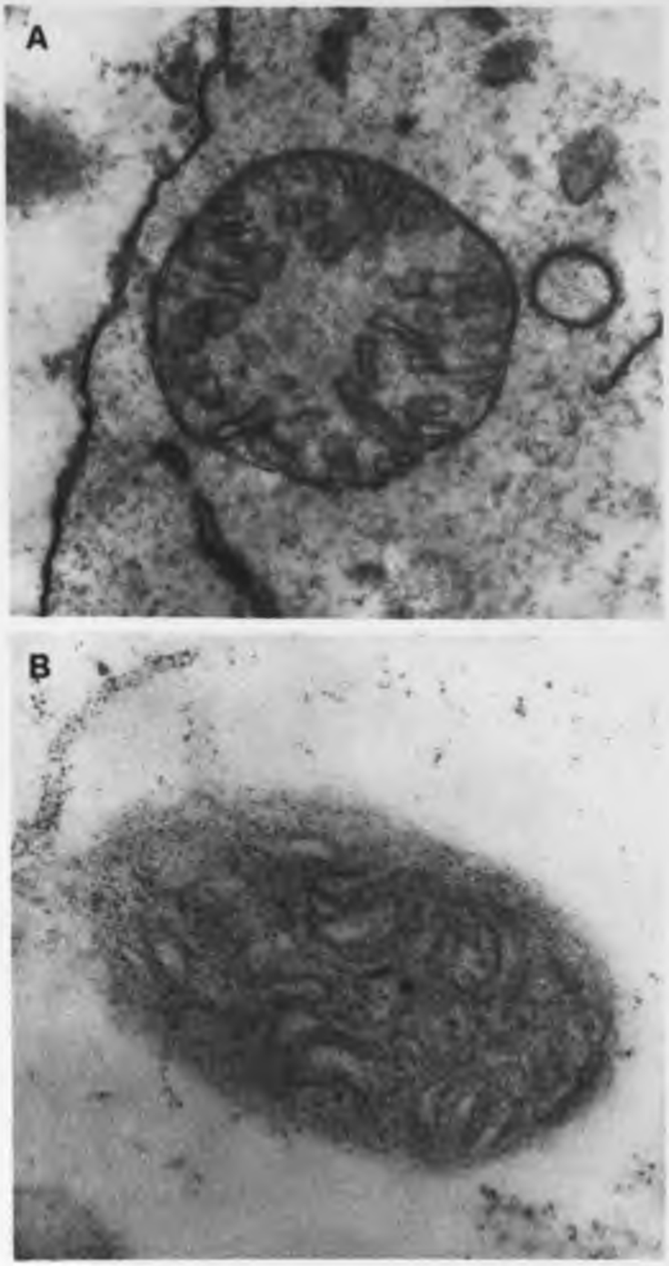

Fig. 2. Mitochondria from healthy lettuce leaf tissue (A) and tissue at intermediate stages of tipburn development (B).

Glutaraldehyde-$KMnO_4$ fixation, X 50,000. The membrane in the mitochondrion from the diseased tissue are degenerated.

Fig. 3. Laticifers and parenchyma cells from healthy lettuce leaf tissue (A) and from tissues exhibiting progressive necrosis during tipburn development (B,C).

Glutaraldehyde O_sO_4 fixation, X 3,000.
B, laticifers (1) in the initial stages of disease development remain intact while in adjacent parenchyma cells the plasmalemma (p) is separated from the cell wall. C, the organelles are disintegrated in parenchyma cells bordering a laticifer in a tissue at advanced stages of necrosis. Although the latex within the laticifer has become granular, the laticifer cell wall is intact.

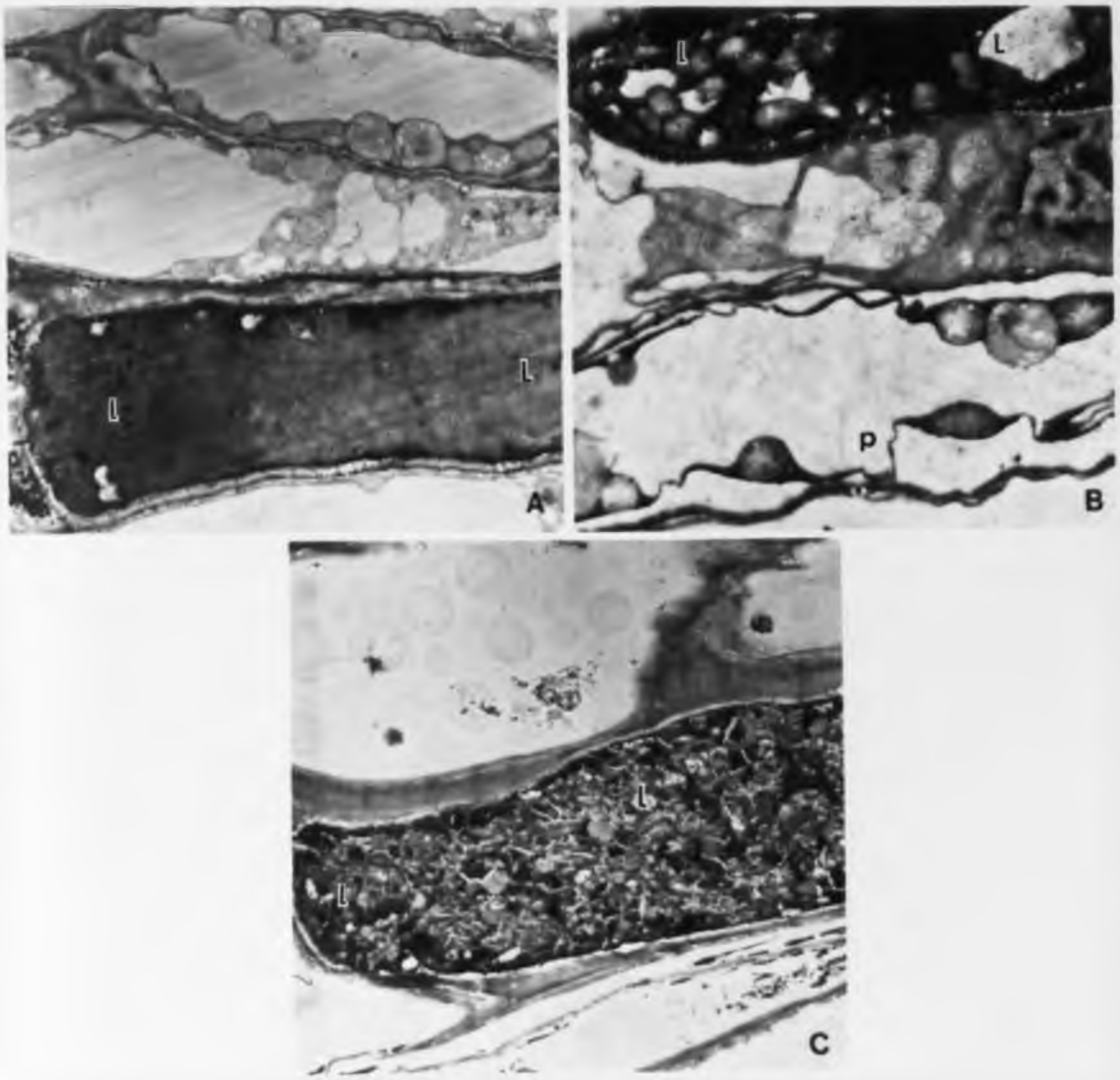

Fig. 3. Laticifers and parenchyma cells from healthy lettuce leaf tissue (A) and from tissues exhibiting progressive necrosis during tipburn development (B,C).

membranes and cell walls were surrounded by moderately to severely damaged parenchyma cells (Fig. 3B and C) and the latex within intact laticifers became progressively granular (Fig. 3). In more advanced stages of the disease only nuclei and vacuolated chloroplasts remained relatively intact indicating that they were more resistant to degeneration than other organelles (Fig. 1C). In advanced stages of necrosis, when all the cells surrounding laticifers exhibiting gross degeneration, laticifers occasionally had burst, exuding latex into intercellular spaces. Eventually, empty parenchyma cells became devoid of any organelles and contained only residual deposits along the cell walls (Fig. 1D).

Discussion

Disruption of plasmalemmae, tonoplasts and other membranes were the most prominent structural changes observed in cells of lettuce tissue undergoing tipburn development. The progressive separation of the plasmalemma from the cell wall and the disruption of the plasmalemma (Fig. 1B) and organelle membranes (Fig. 2) appear to be responsible for the initiation of necrosis observed in tipburned tissues. The ultrastructural changes observed in tipburned tissue were similar to those in tissues of other plants subjected to calcium-deficient conditions (16, 21, 29, 30, 32). Similarity of ultrastructural changes in tipburned and calcium deficient tissue lend support to a suggestion made by us (36) and others (2, 25, 49) that tipburn is a manifestation of a calcium deficient condition. The involvement of calcium in tipburn is also supported by observations that the calcium content of the margin of lettuce leaves, where tipburn symptoms generally developed were lower than other parts of the leaf blade (2). We have also found that the innermost, susceptible leaves of head lettuce contained less calcium than the outermost resistant leaves (36).

The reason why leaf tissue with low calcium becomes necrotic and collapses is not known. Calcium is known to be involved in many vital processes including membrane permeability (53,55), selective ion transport (13), increasing membrane potential (44), membrane integrity (15,27), maintenance of plasma and vacuolar membranes (54), protection against heavy metal toxicity (52), influencing the activity of several enzymes (23) and formation of mitchondria (27).

Tibbits et al. (50,51), Struchmeyer and Tibbits (47) and Olson et al. (38) suggested that tipburn development in bibb 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 as a result of increased accumulation of osmoticly active solutes (38,50). They suggested that latex contains toxic polyphenols which cause cell collapse as they diffuse through the intercellular spaces.

Ruptured laticifers were found only in tissues during advanced stages of disease development but not at early or intermediate stages, while disorganized parenchyma cells were consistently found at early, intermediate and later stages of the disease. Moreover, intact laticifers were frequently found among heavily damaged parenchyma cells in tissue exhibiting advanced necrosis (Fig. 3B). If rupturing of laticifers is the trigger mechanism for tissue necrosis associated with tipburn, ruptured laticifers should have been seen in tissues at both early and late stages of disease development. Moreover, as pointed out earlier, rupturing of laticifers which is consistently associated with the early symptoms of aster yellows of head lettuce (20), does not result in collapse and necrosis of leaf tissue as occur during tipburn development. This ruptured laticifer theory is weakened further by occurrence of tipburn in cabbage (56), potatoes (28), and sugar beets (14), which do not have laticifers.

Although rupturing of laticifers have been reported to cause tipburn in bibb lettuce grown in the laboratory (38, 47, 51), it has been suggested that tipburn symptoms vary depending on the cultivar and growing conditions (48). It is possible therefore that rupturing of laticifers, which plays a major role in tipburn of bibb lettuce, does not contribute or contributes only indirectly to tipburn in field grown head lettuce.

Results of this study show that the physical alteration of membranes are the earliest and probably the initial events in tipburn development, and rupturing of laticifers is not the primary cause of tipburn in head lettuce. However, the possibility of involvement of laticifer rupture as a factor contributing to the disease cannot be ruled out.

CHAPTER 3

EVALUATION OF SEVERAL SOIL AND FOLIARLY APPLIED CHEMICALS FOR CONTROLLING TIPBURN OF HEAD LETTUCE

Introduction and Objectives

Calcium related disorders in some plants have been reported to be controlled by soil and foliar application of calcium salts (3, 4, 11, 19, 31, 58). However, attempts to reduce the incidence of tipburn using these techniques have met with limited success. Kruger (25) and Thibodeau (49) controlled tipburn of head and bibb lettuce grown under controlled conditions by foliar sprays of calcium nitrate or calcium chloride solutions. Sonneveld et al. (45) demonstrated that daily foliar and soil application of calcium chloride to head lettuce grown in containers reduced tipburn incidence. Misaghi and Grogan (36) were also able to suppress tipburn in mature detached heads of lettuce by placement of their cut stems in a 1% solution of calcium chloride during exposure to tipburn-inducing temperatures in the laboratory. In spite of successful control of tipburn in the laboratory, no effective control of tipburn has been developed for field grown lettuce (9, 22, 37).

The reason why foliar application of calcium salts has been ineffective in increasing the calcium content of inner susceptible leaves of head lettuce is probably due to slow mobility of calcium in plant tissue (5). Once calcium is deposited on outer leaves it apparently either does not penetrate the leaf tissue or moves very slowly to areas of new growth or localized deficiency. Millikan and Hanger (33) demonstrated increased mobility of calcium when it was mixed with EDTA or citric acid prior to its incorporation into leaves of broad bean. Thus, redistribution of calcium in lettuce may be improved by the use of chelators of calcium.

An alternative to foliar application of calcium salts for controlling tipburn is soil application of pH-modifying chemicals, such as sulfuric acid and sulfur, which have been shown to increase mineral availability in alkaline soils (6, 40, 42, 43, 59). Large amounts of calcium, in the form of calcium carbonate and other salts are present in soils of Arizona and California, but most of it is unavailable for plant use. Calcium carbonate and calcium phosphate are highly insoluble in soils with a pH of 7.0-8.5. We therefore rationalized that by lowering the soil pH with acid-forming compounds calcium might become more soluble in the soil allowing an increase in calcium uptake by the plant and a decrease in tipburn incidence.

Materials and Methods

Two field plots were established during the 1979 growing season. The first was in the Mesa Experiment Station, Mesa, Arizona, and the second was in a commercial field in Willcox, Arizona. Both were completely randomized plot designs with four replications. Each replication included two adjacent rows twenty-five feet long consisting of 150-200 plants.

Mesa Field Plots

Treatments included in this plot were concentrated sulfuric acid at 1000 and 2000 pounds per acre, soil sulfur at 1000 pounds per acre and Iron-Sul at 1000 pounds per acre. (Iron-Sul is a commercial mine product of equal parts sulfuric acid, sulfur and iron sulfate, Duval Corp., Tucson, Arizona.) These materials were placed in four-inch-deep furrows at the top of the beds and were covered two days prior to direct seeding. 'Empire' head lettuce was grown in the plot according to standard commercial methods.

Rating of Plants for Tipburn

At maturity twelve, uniform, mature heads were selected from each replication. Heads were rated for tipburn incidence and severity using a laboratory method described by Misaghi and Gorgan (35). For induction of tipburn the freshly harvested heads were subjected to a constant 30 C ± 3% in growth chambers in the dark for five days. Tipburn severity was estimated on a scale from 0.5 to 5.0 to indicate slight to severe symptoms.

Analysis of Lettuce Tissue for Total Calcium

Six mature, uniform heads of lettuce were randomly selected from each replication at the time of commercial harvest. After removal of wrapper leaves, heads were cut into four sections and one-fourth of each of the six heads was combined to constitute one representative sample. The leaf tissues were dried for 48 hr in a forced air oven at 60 C and ground in a Wiley mill using a forty-mesh screen.

Glassware used for analysis of tissue was immersed in sulfuric acid, "Nochromix" (Godax Laboratories, Inc., New York, NY) cleaning solution overnight. It was then rinsed twice with distilled water, and deionized water and dried.

Dried, ground samples (0.1g each) were placed in porcelain crucibles and ashed 16 hr at 500 C. The ash was added to 50 ml of .05 M hydrochloric acid containing 0.5% lanthanum oxide. Calcium concentrations of tissue extracts were determined with a Unicam SP 90 atomic absorption spectrophotometer. Two analyses were performed per sample.

Willcox Field Plot

Soil treatments included in this plot were ammonium thiosulfate at thirty gallons per acre, ammonium thiosulfate at sixty gallons per acre, soil sulfur at 1000 pounds per acre, and Iorn-Sul at 1000 pounds per acre. These materials were placed in four-inch-deep furrows cut on both sides of the beds halfway between the top and the bottom, five weeks after direct seeding.

Foliar treatments included in this plot were Link Ca-Zn (5% calcium and 2.5% zinc organic complex, Wilbur-Ellis Co., Fresno, CA) at three pints per 100 gallons per acre mixed with "Charge" (a surfactant, 30% aklarylnonephenol, Wilbur-Ellis Co., Fresno, CA) at three pints per 100 gallons per acre, Link Ca-Zn at six pints per 100 gallons per acre mixed with "Charge" at three pints per 100 gallons per acre, Link Ca-Zn at 6 pints per 100 gallons per acre without "Charge", 1.5% calcium citrate at 100 gallons per acre, 1.5% calcium citrate at 100 gallons per acre mixed with "Charge" at 3 pints per 100 gallons per acre, "Charge" only at 3 pints per 100 gallons per acre and water at 100 gallons per acre. These materials were applied with a hand-held sprayer, three times, at five, eight and ten weeks after planting. The first two treatments were applied prior to head formation and the final one was applied after heads were formed.

Rating of plants for tipburn and analysis of lettuce tissue for total calcium were performed as described above.

Results

The effect of different soil treatments on tipburn severity and on the level of tissue calcium are shown in Table 1. Although some treatments resulted in appreciable decreases in tipburn severity and increases in tissue calcium compared to controls, none of the differences were statistically significant at the 5% level, using an F test.

Foliar application of test materials did not result in statistically significant changes in the severity of tipburn or in the levels of tissue calcium (Table 2).

Discussion

Soil applications of sulfuric acid as well as other sulfur containing compounds have been used as a means to increase the availability of iron and phosphorus (6, 40, 42, 59) by slightly reducing the soil pH. Results of this study showed that such treatments might also render calcium more available to lettuce plants.

Table 1. Effect of different soil treatments on the level of tissue calcium and severity of tipburn.

Treatment	Rate	Cultivar	Location	increase (+) or decrease (-) in tipburn severity[1]	% increase (+) or decrease (-) in tissue calcium[1]
Ammonium Thiosulfate[3]	30 gal/A	Calmar	Willcox, AZ	-25	+13
Ammonium Thiosulfate[3]	60 gal/A	Calmar	Willcox, AZ	-30	+2
Sulfur[3]	1000 lb/A	Calmar	Willcox, AZ	-15	-9
Sulfur[2]	1000 lb/A	Empire	Mesa, AZ	-33	+7
Iron-Sul[2,4]	1000 lb/A	Empire	Mesa, AZ	-34	-9
Iron-Sul[3]	1000 lb/A	Calmar	Willcox, AZ	-26	-3
Sulfuric Acid[3]	1000 lb/A	Calmar	Willcox, AZ	-10	-25
Sulfuric Acid[2]	1000 lb/A	Empire	Mesa, AZ	-30	+10
Sulfuric Acid[2]	2000 lb/A	Empire	Mesa, AZ	-20	-14

1. % change relative to controls. Figures represent average of 4 replications.
2. Materials were placed in four-inch-deep furrows at the top of beds and were covered two days prior to direct seeding.
3. Materials were placed in four-inch-deep furrows on both sides of the beds halfway between the top and the bottom, five weeks after direct seeding.
4. A granular product consisting of ferrous sulfate, sulfuric acid, and soil sulphur, Duval Corporation, Tucson, Arizona.

The differences were not significant at the 5% level using an F test.

Table 2. Effect of different foliar treatments on the level of tissue calcium and tipburn severity in Calmar lettuce.

Treatment[1]	Rate	% increase (+) or decrease (-) in tissue severity[2]	% increase (+) or decrease (-) in tissue calcium
Calcium Citrate 1.5%	100 gal/A	+6	+16
Calcium Citrate 1.5% + surfactant	100 gal/A 3 pt/100 gal/A	+10	+9
Link[3]	3 pt/100 gal/A	-14	-28
Link Surfactant[4]	3 pt/100 gal/A 3 pt/100 gal/A	-12	-1
Link Surfactant	6 pt/100 gal/A 3 pt/100 gal/A	+14	-8
Surfactant	3 pt/100 gal/A	+36	-23
Water	100 gal/A	-4	-30

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1. Materials were applied at five, eight and ten weeks after direct seeding.

2. % change relative to control. Figures represent averages of four replications.

3. Organic Calcium, Zinc Complex, Wilbur-Ellis Co., Fresno, CA.

4. 30% Akylarylnoneylphenol, Wilbur-Ellis Co., Fresno, CA.

Sulfuric acid reacts with the soil immediately whereas other sulfur containing compounds are oxidized gradually by the following reaction: $S + 3/20 + H_2O \rightarrow H_2SO_4$. Various species of the genus Thiobacillus are the most prominent microorganisms involved in this process. Soil which is moist, warm and well areated promotes rapid conversion of sulfur. Ammonium thiosulfate and iron sulfate are also converted into acids in the soil. Ammonium thiosulfate breaks down into ammonium sulfate and elemental sulfur when applied to the soil. The elemental sulfur reacts as described above while the ammonium component is oxidized to nitrate which also is an acid forming reaction.

$$2NH_4 + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

$$2NO_2^- + O_2 \rightarrow 2NO_3$$

Sulfuric acid is formed through hydrolysis of iron sulfate by the following reaction:

$$FeSO_4 + 2H_2O \rightarrow Fe(OH)_2 + H_2SO_4$$

Although lowering the pH of field soil is economically prohibitive because of the large amount of ammendments required, smaller quantities can be effective when applied in a band next to the plant.

All of the soil treatments in this experiment slightly decreased the severity of tipburn, however, the differences were not significant at the 5% level. Changes in tissue calcium did not always correlate with changes in tipburn severity. These variable results reflect some of the problems involved in attempting to increase calcium absorption from the soil. Variation in soil character as well as nutrient availability have been shown to influence calcium uptake by plant roots. Studies have indicated that NH_4^+, Al^+, Mn^+, K^+, and M_g^+ inhibit calcium absorption by roots (24). Therefore, even if calcium is present in sufficient quantities, the presence of competing cations may interfere with calcium absorption.

Foliar and soil application of calcium salts have been reported to reduce the incidence of a number of calcium related disorders other than tipburn (3, 4, 19, 31, 58). The reason why calcium treatment is not effective in controlling tipburn of head lettuce is not known. Unlike head lettuce, susceptible parts of other plants suffering from calcium related disorders can be sprayed with calcium salts throughout the growing season. Susceptible inner and middle leaves of head lettuce, however, are not exposed and cannot be sprayed directly after head formation. In order for calcium application to be effective after head formation, the calcium must be redistributed to the inner leaves. Apparently, this type of movement does not take place. The reason for the lack of mobility lies in the fact that calcium does not move in the transpirational stream by bulk flow but moves by a process of exchanges with negatively charged molecules within the xylem. Thus, movement of calcium lags behind that of water and monovalent cations (5). Chelating agents form uncharged or negatively charged complexes with calcium preventing its absorption to exchange sites (33).

The overall results of this experiment show that soil ammendments which reduce soil pH or foliar application of chelated calcium do not cause statistically significant reductions in the incidence of tipburn or increases in the calcium content of inner leaves of head lettuce.

CHAPTER 4

IMPROVING TIPBURN TOLERANCE OF LETTUCE CULTIVARS BY SELECTING PLANTS WITH HIGH CALCIUM CONTENT

Introduction and Objectives

Differences in calcium content between various plant species has been shown to be under genetic control (26). In addition, differences in calcium uptake and distribution as well as susceptibility to calcium related disorders among plant cultivars have been reported for a number of crops including lettuce (7, 8, 12, 18, 36, 39). Although lettuce cultivars are often inbred as many as ten to twenty generations, large variations are observed among individual plants of one cultivar with respect to several characteristics such as germination, maturation rate, head size, as well as tipburn incidence and severity. Misaghi et al. were able to demonstrate a significant variation in calcium content among individual plants of a single cultivar (34).

Although a direct correlation between the level of tissue calcium and tolerance to tipburn in lettuce has been well established (36), calcium uptake ability has never been selected by lettuce breeders.

The objectives of this study were as follows:

1. To determine if the tissue calcium content of lettuce plants is under monogenic or multigenic control.
2. To determine the possibility of improving tipburn tolerance of lettuce cultivars by selecting plants with high levels of tissue calcium.

Materials and Methods

Twenty mature, uniform Vanguard lettuce plants (L. sativa var capitata) were selected from a commercial field in Marana, Arizona. Middle and inner leaves were collected and analyzed for calcium as previously described (see Field Study, Materials and Methods). The terminal buds of these plants were left intact on the plant. These plants were then transplanted into three-gallon pots filled with a uniform, pasteurized soil mixture in greenhouses where they eventually formed seed. Seeds were collected from plants with high, intermediate low tissue calcium. Seeds were exposed to fluorescent light for 48 hr and then planted. Five plants from the progeny (S_1) were selected from each of the ten parent (P_1) plants. Tissue from all S_1 plants were analyzed for total calcium after 14 wks of growth.

The experiment was repeated once using the greenhouse for growing both P_1 and S_1 plants. Nitrogen, phosphorus and potassium but no calcium were supplied to plants in quantities sufficient to promote rapid growth.

Results

Results of the calcium analysis of P_1 and S_1 generations (Table 3) failed to show a direct correlation between the calcium content of the parent selection and their first progenies. The random distribution (Table 4) shows that selection of progenies with highest calcium content cannot be used as a means for improving tipburn tolerance of lettuce cultivars.

Table 3. Calcium content in parts per million of lettuce leaf tissue from parent selection (P_1) and their first progenies (S_1).

	Plant No.	P_1	S_1[a]
CALMAR	1	6842	3619
	2	6831	2833
	3	6493	2687
	4	5879	2687
	5	5374	2623
	6	5119	2784
	7	4440	2867
	8	4212	2867
	9	4071	2885
	10	2718	3962
VANGUARD	1	5599	4212
	2	5456	4040
	3	5521	4146
	4	4938	3170
	5	4906	4418
	6	4721	4566
	7	4598	4294
	8	4473	3625
	9	4298	3709
	10	4243	5052

[a] Average values of five different S_1 plants from the same P_1 parent.

Correlation coefficients for Calmar and Vanguard were 0.3998 and 0.1183 respectively. Neither were significant at the 5% level.

Table 4. Correlation between calcium content of parent selections and their S_1 progeny.

Ranking of the parent plants based on calcium content		Percent of plants in S_1 generation with low, intermediate and high calcium content
VANGUARD	Low	66% low 0% intermediate 33% high
	Intermediate	25% low 50% intermediate 25% high
	High	0% low 100% intermediate 0% high
CALMAR	Low	33% low 33% intermediate 33% high
	Intermediate	66% low 33% high
	High	50% high 50% intermediate

Discussion

Historically the use of resistant cultivars has been one of the most successful and economical methods for controlling plant diseases. Lettuce breeders have selected lines for tipburn resistance and have been able to develop some moderately tolerant cultivars. "Calmar" and "Salinas" are the most tolerant cultivars used today by lettuce growers in Arizona and California. Progress in developing more tipburn resistant lines has been very slow because of the absence of a genetic marker for tipburn tolerance.

Identification of a genetic marker for tipburn tolerance such as tissue calcium, would facilitate selection of resistant lines in breeding programs. Collier et al. (7) demonstrated that high concentrations of chlorogenic acid in lettuce plants are associated with a high incidence of tipburn at maturity. They felt that analysis for chlorogenic acid concentrations in young plants could be helpful in screening cultivars against tipburn. Misaghi and Grogan (36) demonstrated that lettuce cultivars resistant to tipburn have a higher total calcium content than susceptible ones. Moreover, their preliminary data (34) demonstrated a significant variation in calcium content among individual plants of a single cultivar. They suggested selection of individual plants within cultivars with high calcium content might lead to development of more resistant lines. However, results of the experiment reported here failed to show that calcium can be used as a marker to select lettuce plants for tipburn tolerance.

The failure of this experiment may be due to the use of heavily inbred lines. Both Calmar and Vanguard have been inbred 10-12 generations theoretically creating a highly homogenous population. Parent-offspring correlations within pure lines are known to be very low (1). Variation in calcium content among individual plants of a single cultivar in the field might be due to differences in environmental conditions, such as soil type, interfering cations or nutrient availability. However, the differences observed in greenhouse plants cannot be explained on this basis.

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