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CONTROLLED ENVIRONMENT LIFE SUPPORT SYSTEM: CALCIUM-RELATED LEAF INJURIES ON PLANTS

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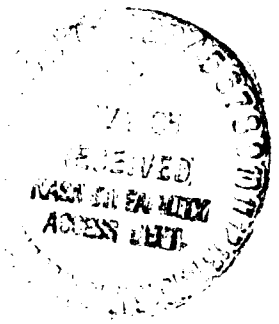

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SUMMARY

Plants grown in controlled environments are subject to certain physiological injuries and growth restrictions that are rarely encountered in outside environments. When growth rates are forced with favorable environmental conditions many injuries develop which may seriously limit growth. Thus, in life support systems where it is essential to maximize growth rates of plants, these injuries could be serious limitations to plant production.

Calcium deficiency injury is of considerable concern to certain species of plants when grown in controlled environments. The injury develops in tissues that have low rates of transpiration, thus injury is found in enlarging leaves within enclosed growing points, storage tissues in the soil, and enlarging fruits with impermeable surface layers. The injury develops because there is inadequate Ca movement to these non-transpiring tissues. The injury develops even though adequate amounts of Ca are provided in the soil or nutrient medium. Previous research has demonstrated that significant quantities of calcium can be translocated to all tissues, including non-transpiring tissues, if the atmosphere is saturated with water during the night cycle and root pressures develop to force water through the plant.

This research study was focused on a calcium-related injury in lettuce termed 'tipburn'. It affects the young developing leaves as they become enclosed during head formation. It is a good model system to study because the injury can be induced with good predictability and lettuce is one of the crops chosen by the CELSS program for concentrated study. Investigations were undertaken to study a number of different procedures, that would have the potential for encouraging movement of calcium into the young developing leaves

and to study the time course and pattern of calcium accumulation in developing leaves to provide a basis for developing effective control procedures for this injury.

Plants were maintained either in peat-vermiculite media or in liquid culture in the different studies. Seedlings were grown for 14 days from sowing in a reach-in growth chamber. At 14 days after planting, the seedlings were transferred to other growing rooms for experimental treatments. Plants were grown for an additional 14 to 20 days until tipburn developed and harvests were made. Similar light, temperature and humidity conditions were maintained in each study unless changes were required for specific treatments. The lighting was with cool-white fluorescent lamps with a 16-hr photoperiod. The mean photosynthetically active radiation (PAR) at plant height was maintained between 300 and 350 umol s^{-1} m^{-2} in the different studies. Light- and dark-period temperatures were held between 20 and 24°C. RH was maintained between 60 and 80% in both the light and dark.

Studies on Injury Prevention: a) Investigations were undertaken to increase relative humidity to saturation during the dark period and to alter root temperatures to promote the development of root pressures. Study was made of calcium accumulation in the young developing leaves and data taken on tipburn development.

Tipburn was delayed by reducing the RH during the light period and by increasing it above 95% during the dark period . Tipburn developed later at the lower root temperature of 15°C than at the higher temperature of 23.5°C.

The Ca concentration of the inner leaves increased with the decreasing RH during the light period. In contrast, the calcium concentration of inner leaves of plants did not increase with decreases of RH during the dark

period. Highest calcium concentations, instead, were present in the plants maintained at the highest dark-period RH level (>95%). Plants grown at root temperatures of 23.5°C had marginally higher Ca concentrations in inner leaves than plants grown at root temperatures of 15°C. The Ca concentration of the remainder of the plant was similar in the different humidity and root temperature treatments.

Although saturated humidities at night increased the rate of growth, tipburn was reduced probably as a result of root pressures that developed during the dark period which promoted Ca transport to the young, expanding leaves. Nonetheless, the combination of low humidity during the day and saturated moisture conditions at night would act together to provide a large fluctuation in plant water potential which would encourage Ca movement to the young leaves and delay tipburn.

The small increase in Ca concentration observed with the higher root temperature was not great enough to have a significant effect in reducing tipburn injury. Thus it appears that root temperature regulation between 15 and 25°C will not be effective in preventing tipburn.

b) A study was undertaken to maximize water stress during the day and minimize stress during the night in an attempt to induce greater calcium transport to the tipburn-sensitive developing leaves and thus decrease or eliminate tipburn. The water stress on the plants was altered diurnally through regulation of the irradiance, temperature and humidity level.

Although this study was developed to modulate the environment so that a greater fluctuation in plant water stress would be produced in plants than under constant conditions, no difference in water stress was produced between the modulating and constant environments. Consequently, there was little difference in tipburn development under the two environments. This study

indicates that more drastic environmental modulations must be undertaken to have a significant effect on plant water stress. This probably could be accomplished by utilizing growth chambers fitted with HID lamps to increase irradiance significantly and installing fans to direct a large air movement over the plants during the light period.

c) Investigations were undertaken to determine if shortened L:D cycle lengths, in combination with elevated atmosphere moisture levels during the dark portion of the cycle, would prevent leaf injury. It was hypothesized that the elevated moisture levels during the extra dark periods would provide more effective root pressure transport of calcium during these dark periods.

The use of a 8:4 light dark cycle instead of a 16:8 cycle provided no reduction in tipburn nor caused any increase in calcium concentration in the leaves. It was interesting to note that the shoot dry weight of the plants under the two cycles was similar, suggesting that there is no exacting requirement of a 24 hour photoperiod for lettuce growth.

d) The nutrient concentration was reduced to promote greater root pressure during the dark period and thus obtain greater calcium transport into the developing leaves.

The use of low concentrations of nutrient solution delayed tipburn. Tipburn was delayed from 23 to 27 days after seeding and occurred first on the 13th leaf with nutrient concentrations having a conductivity of .305 dSm^{-1} (1 decisieman m^{-1} = 1 mmhos cm^{-1}) compared to occurring first on the 10th leaf with a conductivity of 1.170 dSm^{-1}. The common solution utilized for nutrient culture studies has a conductivity of approximately 1.200 dSm^{-1} (Hammer et al., 1978) and is approximately one-half strength of the original Hoaglands solution. There was also an increase in calcium in both the inner and outer leaves, indicating that there may have been a greater calcium

transport in plants maintained with the lower nutrient concentration. The increase in the inner leaves is of questionable significance, however, because of the wide variability in levels found in the four plants. Two plants had nearly the same levels of calcium as found in the plants maintained at the higher nutrient concentrations. The reduced strength solution produced significantly smaller plants and thus the tipburn delay may be the result of slowed growth rather than the increased calcium transport to the inner leaves.

e) Another study was performed to test whether vibration caused by plant shaking would increase calcium transport to the tipburn-sensitive leaves at the growing point or provide structual strengthening to decrease or eliminate tipburn injury.

The shaking of plants did not prevent tipburn although there was an indication that tipburn was delayed. Shaking delayed tipburn one day and the first leaf tipburning was the 12th rather than the 10th leaf. Interestingly, there was also indication that the shaking treatment increased the shoot dry weight.

Studies of Calcium Accumulation in Developing Leaves: a) The growing points of one-half of the plants in the study were sheathed with an enclosure made from aluminized polyethylene film to mimic a heading condition. The sheaths were fitted over the growing point beginning with leaf 7, as numbered from the base, and included all younger leaves. The young leaves were covered for a 4 day treatment period.

At harvest, tipburn injury was present on all plants included in the enclosure treatment, but was present on only one of the untreated control plants. The calcium concentration was only .63 mg/g dry weight in the covered

inner leaves, 57% lower than the uncovered controls. The magnesium concentration of the inner leaf tissue was only slightly affected by the enclosure treatment. The magnesium concentration of the covered leaves was only about 3% lower than the uncovered controls. This research documents that enclosure of leaves at the growing point, as would occur with normal head development, is sufficient to create a limiting condition of calcium in the tissue capable of initiating tipburn injury.

b) The calcium concentration of the inner tipburn-susceptible leaves was determined at different times over the day to find if low calcium concentrations tend to occur during a particular part of the day and thus give information indicating when and how calcium moves to the developing tissues.

The monitoring of calcium at four different times indicated a reduction of calcium concentrations during the day and an increase at night. A low level of 0.047% Ca was seen in the inner tipburn sensitive leaves near the end of the light period and a high of 0.059% Ca at the start of the light period. There was a similar pattern of decrease and increase in the outer enlarging leaves but concentrations were approximately 15 times greater. This data documents that there is a measurable dilution of calcium in the inner leaves during the light period; indicating that plants would be most susceptible to calcium-related injuries toward the end of the light period.

c) The x-ray diffraction microprobe, utilizing a wavelength dispersion detection system, was found to be useful for monitoring low levels of calcium in lettuce tissue. The beam width was set at 50 micrometer so that it was possible to monitor very localized areas of leaves.

This study documented that there are very low levels of calcium present in developing leaves and significantly lower levels toward the margins of leaves than in midveins. This was shown in 2 cm long leaves, with both the

tip and side margin having only one-tenth the calcium found in the midrib near the base. In contrast, the larger exposed leaf had no significant calcium gradient toward the tip and margins. This x-ray probe data documents the very low concentrations found in the tip and marginal areas of leaves that are the locations of tipburn injury in lettuce.

INTRODUCTION

Plants grown in controlled environments are subject to physiological injuries and growth restrictions that are rarely encountered in outside environments. When growth rates are forced with favorable environmental conditions many injuries develop which may seriously limit growth. Thus, in life support systems where it is essential to maximize growth rates of plants, these injuries are serious limitations to plant production.

Calcium deficiency injury is of considerable concern to certain species of plants when grown in controlled environments. It is of major significance to lettuce production in greenhouses (Klo.s, 1952) and when grown in growth chambers. It is impossible to grow lettuce free of this problem in growth chambers under favorable radiation and temperature levels unless plant growth is retarded by water stress. Ca-related injuries have been observed in many other plants and in different organs of the plants. It has been observed in the developing leaves of escarole, cabbage, celery, Brussel sprouts, chicory, carrots and strawberries; flower parts of cauliflower and strawberries; storage tissues of tomatoes, carrots, parsnips and peanuts; and fruit of tomatoes, peppers, watermelon, apples, pears, avacado, prunes and cherries and mango (Shear, 1975).

The injury becomes more serious as the growth rate of the plants is increased with more favorable growing environments (Tibbitts and Rao Roma Rao, 1968; Shear, 1975). For example, the injury has been observed in corn only when plants are grown under very high intensities of light (observations in University of Wisconsin Biotron).

Large differences in sensitivity to injury have been noted with different cultivars of different species as lettuce (Krueger, 1966), potatoes (Collier et al., 1980), Brussel sprouts (Priestly and North, 1962) and tomatoes (Greenleaf and Adams, 1969).

The injury develops in tissues that have low rates of transpiration, thus injury is found in enlarging leaves within enclosed growing points (particularly with plant species as lettuce and cabbage that develop heads), storage tissues in the soil, and enlarging fruits with impermeable surface layers. The injury develops because there is inadequate Ca movement to these non-transpiring tissues (Shear, 1975; Thibodeau and Minotti, 1969). This type of injury is restricted to a deficiency of Ca (and possibly B) because this nutrient is moved only in the xylem stream and not translocated through phloem elements as are other nutrients such as K and P. The injury develops even though adequate amounts of Ca are provided in the soil or nutrient medium (Takano and Sisa, 1964), and is more serious as the total nutrient concentration of the media is increased (Bradfield and Guttridge, 1979; Mason and Guttridge, 1975). Injury has even been increased when the nutrient concentration is elevated with Ca salts (Walker et al., 1961; Guttridge et al. 1978). A recent report indicates that the osmotic pressure of the nutrient medium must be maintained at or near 0.25 atmosphere, to prevent injury (Bradfield and Guttridge, 1979). This concentration is less than the standard one-half strength modified Hoagland's nutrient solution (0.40 atmosphere) utilized in most liquid culture studies.

Previous research has also demonstrated that significant quantities of calcium can be translocated to all tissues, including non-transpiring tissues, if the atmosphere is saturated with water and root pressures develop to force water through the plant (Palzkill and Tibbitts, 1978). With saturation of the atmosphere over the night period, it has been shown that calcium concentrations can be increased and injuries to cabbage plants prevented in growth chambers (Palzkill, Tibbitts and Williams, 1976). This procedure also has effectively prevented a calcium deficiency injury in strawberries (Bradfield and Guttridge, 1979). However, we have not been able to prevent

injury to lettuce with this procedure, but have found that calcium levels in susceptible tissues were increased, though not above, the threshold for injury.

It has been suggested that these Ca deficiency problems are particular problems in controlled environments because the humidity levels during the dark period are usually considerably below saturation levels so that root pressure rarely develops. Also, the root media cools significantly during each dark period so that water uptake by the roots is retarded and thus the potential for root pressure development is less.

Research has shown that daily fluctuations in water potential, high water stress during the day and low water stress at night, will inhibit injury in cabbage because this promotes water, and hence calcium movement, via xylem transport (Wiebe et al., 1974).

This research has been undertaken to investigate a number of different procedures, that would have the potential for encouraging movement of calcium into the young developing leaves. Data has been obtained on the calcium increases and the inhibition of tipburn development in the developing leaves. In addition, investigations have been undertaken to study the time course and pattern of calcium accumulation in developing leaves to provide a basis for developing effective control procedures for this injury.

MATERIALS AND METHODS

General

Plants were maintained either in peat-vermiculite media or in liquid culture in the different studies. For the peat-vermiculite studies, lettuce was sown into moistened peatlite in plastic containers. Seedlings were grown for 14 days from sowing in a reach-in growth chamber. The lighting was with cool-white fluorescent lamps with a 16-hr photoperiod. The mean photosynthetically active radiation (PAR) at plant height, measured with a LI-COR quantum sensor, was maintained between 300 and 350 umol s^{-1} m^{-2} in the different studies. Light- and dark-period temperatures were held between 20 and 24°C. RH was monitored with a lithium chloride sensing element which was calibrated daily with a thermocouple psychrometer. RH was between 60 and 80% in both the light and dark. With an automatic watering system, each container received about 100 ml of nutrient solution 4 to 6 times each day (Hammer et al., 1978) in which the Ca concentration was 10^{-4}M.

At 14 days, the seedlings were transferred to other growing rooms for experimental treatments. Similar light, temperature and humidity conditions were maintained during this period unless changes were required for specific treatments.

For the liquid culture studies lettuce was grown in a reach-in cabinet equipped with a solution culture system (Tibbitts et al., 1978) with one liter containers and a circulation rate of 85 ml per minute per container. Seeds were germinated on black rayon cloth in nutrient solution and transplanted 2 per pot when the hypocotyls reached 25 mm long. The seedlings were supported by ethanol washed, autoclaved white foam plugs painted black on top to prevent light penetration to the root zone. A modified half-strength Hoagland

solution was used as described by Hammer et al. (1978). Solution pH was maintained at 5.75 ± .25 pH using an automatic pH controller by addition of 0.1 N HCl or 0.1 N KOH. Old solution was continuously bled from the system and fresh solution re-added at a rate sufficient to hold its ionic strength to within 1% of the fresh solution.

Fourteen days from seeding the plants were transferred to a walk-in growth room and transplanted into a similar recirculating solution culture system provided with a circulation rate of 200-400 ml per pot per minute. The seedlings were planted, 1 per container, and left in this system for the remainder of the experiment. The light, temperature, and humidity conditions in the liquid culture studies were similar to those maintained in the peat vermuculite studies unless indicated differently in specific studies below.

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

Studies on Injury Prevention

Relative Humidity: Investigations were undertaken to increase relative humidity to saturation during the dark period in order that root pressures would develop. Study was made of calcium accumulation in the young developing leaves and data taken on tipburn development.

Lettuce, cultivar, Lobjoits Green Cos., was sown and grown in one liter white plastic containers filled with peat-vermuculite for these studies. The fourteen day old seedlings were subjected to experimental treatments in small chambers, 30 x 30 x 20 cm in height constructed from ultraviolet-transmitting Plexiglas GII-UVT (Rohm and Haas, Inc.). An 11.7 cm diameter hole was cut in the base of each to accommodate the aerial parts of the plants. The base of the chamber was 16 cm above bench height with Plexiglas supports at each corner, 2 of the supports being water reservoirs, each 1200 ml in volume. The top of the plant containers were positioned flush with the base of the chamber. The chambers were placed in a walk-in, controlled environment room.

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

Mean air temperatures in the chambers, measured daily with a copper constantan thermocouple, were 26.0° and 12.5°C in the light and dark, respectively, with a variation of $\pm$ 1.8° between the chambers.

In these studies, as in the root temperature studies following, the lighting with cool-white fluorescent lamps was supplemented with incandescent lamps having an input wattage of 70 and 30%, respectively.

Root Temperatures: The root temperature was independently regulated from shoot temperature to determine if a significant improvement in uptake of calcium and/or increased root pressure flow could be obtained and thus provide adequate calcium to the developing leaves. For these studies the root temperature was controlled by surrounding the pots with a coil of flexible, polyvinylchloride tubing through which water was pumped from a temperature-controlled bath. The warm root temperatures, measured daily with a copper-constantan thermocouple in the center of the pots, were 25.5 $\pm$ 2.0°C and 21.8 $\pm$ 2.0° in the light and dark, respectively; and the cool root temperatures were 15.9 $\pm$ 1.0° and 13.9 $\pm$ 1.0° in the light and dark, respectively.

ENVIRONMENTAL MODULATION: Weibe, Schatzler and Kuhn (1977) have shown that calcium is transported to non-transpiring tissues as a result of hydration and dehydration under fluctuating environmental conditions. It was the purpose of this study to determine if an environment which maximizes water stress during the day and minimizes stress during the night could induce greater calcium transport to the tipburn-sensitive developing leaves and thus decrease or eliminate tipburn.

The water stress on the plants was fluctuated diurnally through regulation of the irradiance, temperature and humidity level. The pattern of environmental regulation for this study is shown in Figure 1. Fluctuations were timed so that the average temperature, relative humidity and irradiance over each period of 24 hours was equal to a comparison treatment maintained at

350 $umol^{-1}m^{-2}$ irradiance for 16 h, 20°C light and dark temperature and 61% light and dark relative humidity. The fluctuating environment was initiated 14 days after seeding.

Buttercrunch lettuce was grown in solution culture for this study. Thirty-two uniform plants were selected 14 days after seeding and transferred to one liter containers in a walk-in growing room. In an effort to eliminate some of the variability in calcium accumulation in the partially enclosed inner leaves. the growing points of one-half of the plants were sheathed with an enclosure made from aluminized polyethelene film as detailed in the leaf enclosure study. The sheaths were fitted on the plants six days after transfer to the walk-in growth room. After an additional four days of growth the plants were harvested for calcium and magnesium analysis

Light-Dark Cycle Length: Investigations were undertaken to determine if shortened L:D cycle lengths, in combination with elevated atmosphere moisture levels during the dark portion of the cycle, would prevent leaf injury. It was hypothesized that the elevated moisture levels during the extra dark periods would provide more effective root pressure transport of calcium during these dark periods. It is believed that the rapid development of tipburn in controlled environments is due to leaf growth rates in excess of calcium supply. Leaf growth during a long light cycle may deplete calcium transported to non-transpiring tissues during the previous dark cycle. If the photoperiodic cycle is shortened while retaining the same light:dark ratio, the light cycle is effectively split into smaller photosynthetic periods with less calcium demand between supply events. The plant may have enough calcium available during these shorter episodes of growth to last until the next supply event.

Plants of cultivar, Buttercrunch, were grown in solution culture for these studies under a light:dark cycle of 16:8 hr. At 14 days after seeding, plants were transferred to separate treatment chambers as described in the Humidity and Root Temperatures studies and maintained under light:dark cycles of 16:8, and 8:4 hr. Thus each treatment had the same number of light and dark hours during each 24-hour period. The plants were exposed to near saturated humidity levels during each dark period by directing moist air from an atomizing humidifier into the chambers. During each light period 20 l/min of room air at 50% RH was pulled through the chambers with squirrel-cage fans.

The light and humidity treatments were maintained for 14 days until tipburn had been initiated on the plants. Two experiments were undertaken at the 16:8 hr. and one at the 8:4 cycle lengths.

NUTRIENT SOLUTION POTENTIAL: The nutrient concentration was reduced to promote greater root pressure during the dark period and thus obtain greater calcium transport into the developing leaves. This technique has effectively reduced tipburn injury in strawberries (Bradfield and Guttridge, 1979).

Seedling plants of Buttercrunch lettuce were grown for 14 days from seeding in the solution culture system at the normal nutrient strength (1.170 decisiemans m^{-1} or 1.170 mmhos cm^{-2}). They were then transferred to a growing room into separate plexiglass treatment chambers as described in the Humidity and Root Temperature studies. Two separate solution systems were provided, one maintained at one-fourth concentration (.305 decisiemans m^{-1}) and one maintained at the same concentration as used for the seedling plants (1.170 decisiemans m^{-1}). Four plants were maintained in separate chambers at each solution concentration. The humidity during the dark period was maintained near saturation by directing moist air from atomizing humidifiers into the plexiglass chambers surrounding the plants. The humidity during the

light period was maintained at 50% by drawing 20 l/min of room air through the chambers with squirrel-cage fans. Plants were harvested after 14 days of growth and dry weight and calcium concentration determined.

Plant Shaking: This study was performed to test whether vibration caused by plant shaking would cause morphological or anatomical changes which would allow increased calcium to be transported to the tipburn-sensitive leaves at the growing point or provide structual strengthening to decrease or eliminate tipburn injury.

Plants of cultivar, Buttercrunch, were grown in peat-vermiculite for these studies. Fifteen days after seeding, 5 plants were selected for shaking treatment. Beginning on this day and continuing each day until the plants were 35 days old, each of these plants was shaken for one minute, 3 to 4 hours after the start of the light period, by rocking the base of the pot back and forth on a hard surface four times per second. Five other plants were maintained in the same chamber as controls. All plants were checked daily for the occurance of tipburn. After 20 days of treatment the top of each plant was harvested.

Studies of Calcium Accumulation in Developing Leaves

Effect of Leaf Enclosure: Plants of cultivar, Butter Crunch, were transplanted from the seedling system into the solution system within a growing room. After 6 days of growth, thirty-two uniform size plants were selected. The growing points of one-half of the plants were sheathed with an enclosure made from aluminized polyethylene film (Figure 2). This sheath was formed by heat-sealing the edge of a folded piece of the polyethylene. It measured 5 cm long and 3 cm wide, and tapered toward the base for a snug fit

on the plant. The sheaths were fitted over the growing point beginning with leaf 7, as numbered from the base, and included all younger leaves. After 2 and 3 days of enclosure, the sheaths were removed and repositioned to expose the 7th and 8th leaves, respectively, which had become too large to be enclosed. All younger leaves were constantly covered by the enclosures over the 4 day treatment period. Sixteen plants were never covered and were left as controls.

After 4 days of the enclosure treatment, the sheathed and non-sheathed plants were cut at the cotyledonary node and fresh weight was determined. Leaves from 1 to 3 cm in length, designated as 'inner leaves', were sampled and assessed for tipburn injury. This included leaves 9 through 14 as counted from the base. Leaves 5 and 6, designated as the 'outer leaves', were also sampled. These outer leaves were nearly fully developed at harvest and were not covered by the enclosure treatment at any time. In addition to calcium analysis, a magnesium analysis was also undertaken on the samples through use of atomic absorption spectrophotometric procedures.

The experiment was repeated. As no unusual differences were present between the two experiments, the data were combined and are reported together. The experiments were of a completely randomized design.

Diurnal Fluctuations: The calcium concentration of the inner tipburn-susceptable leaves was determined at different times over the day to find if low calcium concentrations tend to occur during a particular part of the day and thus give information indicating when and how calcium moves to the developing tissues.

The plants analyzed for this study were those maintained under constant conditions in the Environmental Modulation study. Plants were of Butter-crunch lettuce and maintained in a solution culture system. Four

sheathed plants and four nonsheathed plants were analyzed at each of four different times during the day. The harvests were timed so that they were made two hours into the light period, at the middle of the light period, two hours before the end of the light period and during the middle of the dark period. The light period was of 16 hr and the dark period of 8 hr.

Localization in Small Leaves: The x-ray diffraction microprobe, utilizing a wavelength dispersion detection system, has been found to be useful for monitoring low levels of calcium in lettuce tissue (Gupta and Hall, 1978; Chino, 1978). The beam width has been set at 50 micrometer so that it has been possible to monitor localized areas of leaves. Leaf samples were obtained from lettuce plants, cv. Buttercrunch, growing in the liquid culture system. When plants were 25 days from seeding, the 5th and 14th leaves were removed from plants, quick frozen in liquid nitrogen and dried in a VirTis lyophilizer. The dried samples were mounted on stubs and then coated with carbon.

RESULTS AND DISCUSSION

Studies on Injury Prevention

None of the different studies undertaken provided effective control of tipburn injury. Of the different treatments investigated, regulation of relative humidity levels was found to have the greatest controlling effect. This control was found to be a few days delay in tipburn development and was associated with an increase in calcium concentrations in the young enlarging leaves. Certain other treatments caused small increases in calcium concentrations in the developing leaves but provided no evidence for a

decrease or delay in tipburn injury development unless growth was significantly slowed by the treatment.

Relative Humidity and Root Temperature: Tipburn was delayed by reducing the RH during the light period (Table 1) and by increasing it above 95% during the dark period (Table 2). Tipburn developed later at the lower root temperature in most cases (Tables 1 and 2). Plants maintained at 51% RH during the light period, and with cool root temperatures, did not develop tipburn by harvest at 28 days; but on the basis of other unreported studies it can be assumed that the plants would have developed tipburn if grown for a longer period.

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

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

Ca concentration of the remainder of the plant was between 9.5 and 10.6 mg Ca g^{-1} DW in the different humidity and root temperature treatments.

The delay in tipburn development with low humidity levels in the light period was associated with slower growth and increased Ca concentrations in the leaf tissue. The time taken to the onset of tipburn was correlated negatively with dry weight but was unrelated to ontogenetic age. It is reported that slow-growing plants are resistant to tipburn (Cox et al., 1976; Tibbitts and Rao, 1968), apparently because the demand for Ca is reduced. It also has been suggested that the increased Ca level which occurs with slower growth at reduced humidities results from increased transpiration and thus increased water transport to the developing leaves (Palzkill et al., 1980; Wiebe et al., 1977). Although saturated humidities at night increased the rate of growth, tipburn was reduced probably as a result of root pressures that developed during the dark period which promoted Ca transport to the young, expanding leaves. It has been shown in studies with cabbage (Palzkill and Tibbitts, 1977) that substantial quantities of Ca are moved to young tissues when environmental conditions encourage the development of root pressure and subsequent guttation from the leaves. The results therefore indicate that humidity has distinctly different regulatory effects on tipburn during the light and the dark period. Nonetheless, the combination of low humidity during the day and saturated moisture conditions at night would act together to provide a large fluctuation in plant water potential which would encourage Ca movement to the young leaves and delay tipburn as shown for cabbage (Wiebe et al., 1977).

It was anticipated that the higher root temperature, in combination with saturated humidity during the dark period, would increase root pressure flow and thereby increase the Ca concentration in young leaves above those found in similar tissues of plants grown at the lower root temperature. The small

increase in Ca concentration observed with the higher root temperature was not great enough to have a significant effect in reducing tipburn injury. In other unreported studies, tipburn developed readily when the root temperature was 20 C, thus it appears that root temperature regulation between 15 and 25 will not be effective in preventing tipburn.

The Ca concentration of 1 mg g^{-1} found in the inner leaves was considerably less than the level of 3 mg g^{-1} which has been reported in other tipburn-injured tissue (Krug et al., 1972). Thus, even though there were increases of 0.3 to 0.5 mg g^{-1} of Ca under particular growing conditions, significantly greater increases appear to be required to avoid Ca-deficiency injuries.

Environmental Modulation: Although this study was developed to modulate the environment so that a greater fluctuation in plant water stress would be produced in plants than under constant conditions, no difference in water stress was produced between the modulating and constant environments. This is seen in Table 7 which details the water content of the tissue at four different times during the day. In both environments the water content decreased 0.5 to 0.7% during the light period and regained this during the dark period. Consequently, there was little difference in tipburn development under the two environments. Tipburn developed in 25 days and on the 11th or 12th leaf in both environments (Table 8). Only small differences in calcium concentrations and dry weight were found between the two treatments with slightly less calcium and dry weight in the plants under the modulating environment than under the constant environment.

This study indicates that more drastic environmental modulations must be undertaken to have a significant effect on plant water stress. This probably could be accomplished by utilizing growth chambers fitted with HID lamps to

increase irradiance significantly and installing fans to direct a large air movement over the plants during the light period.

Light:Dark Cycle Length: The use of a 8:4 light dark cycle instead of a 16:8 cycle provided no reduction in tipburn nor caused any increase in calcium concentration in the leaves. Tipburn occurred between 23 and 24 days after planting and at a calcium concentration between 0.07 and 0.08% in both light cycles (Table 9). Thus there was no indication that more frequent periods of dark, with saturated humidity and plant guttation, would minimize tipburn damage.

It was interesting to note that the shoot dry weight of the plants under the two cycles was similar, suggesting that there is no exacting requirement of a 24 hour photoperiod for lettuce growth.

Nutrient Solution Potential: The use of low concentrations of nutrient solution delayed tipburn. Tipburn was delayed from 23 to 27 days after seeding and occurred first on the 13th leaf with nutrient concentrations having a conductivity of .305 dSm^{-1} (1 decisieman m^{-1} = 1 mmhos cm^{-1}) compared to occurring first on the 10th leaf with a conductivity of 1.170 dSm^{-1} (Table 10). The common solution utilized for nutrient culture studies has a conductivity of approximately 1.200 dSm^{-1} (Hammer et al., 1978) and is approximately one-half strength of the original Hoaglands solution.

There was also an increase in calcium in both the inner and outer leaves, indicating that there may have been a greater calcium transport in plants maintained with the lower nutrient concentration. The increase in the inner leaves is of questionable significance, however, because of the wide variability in levels found in the four plants. Two plants had nearly the

same levels of calcium as found in the plants maintained at the higher nutrient concentrations.

Of particular significance is the fact that the reduced strength solution produced significantly smaller plants (Table 10) and thus the tipburn delay may be the result of slowed growth rather than the increased calcium transport to the inner leaves. The smaller size of the low nutrient plants may also have contributed to the large variability in calcium levels in the inner leaves for there could have been less enclosure of the inner leaves in these smaller plants and thus increased transpiration and greater calcium accumulation.

Plant Shaking: The shaking of plants did not prevent tipburn although there was an indication that tipburn was delayed. Shaking delayed tipburn from 26.6 to 27.8 days after seeding and the first leaf tipburning was the 12th rather than the 10th leaf (Table 11). Interestingly, there was also indication that the shaking treatment increased the shoot dry weight.

Thus, although the benefit of shaking was minimal, additional research should be undertaken to investigate this further. Study should be made utilizing several intervals of shaking over each day and use of wind to induce leaf flutter.

Studies on Calcium Accumulation in Developing Leaves

These studies have documented that there are very low levels of calcium present in developing leaves and significantly lower levels toward the margins of leaves than in midveins. These studies also document that when developing leaves are enclosed, so that transpiration is slowed, calcium accumulation is significantly reduced in these enclosed leaves.

Effect of Leaf Enclosure: At harvest, tipburn injury was present on all plants included in the enclosure treatment, but was present on only one of the untreated control plants (Table 12). On the average, 53.4% of the enclosed inner leaves tipburned on covered plants compared to less than 1% (only one leaf on one plant) on uncovered control plants. Enclosure of the growing point thus substantially enhanced tipburn development.

The injury seen on the plants was characterized by tissue collapse at the apex and margin of the affected leaves, usually accompanied by veinal necrosis. Laticifer ruptures were occasionally seen as evidenced by latex exudate at the laminal surface. These symptoms are characteristic of lettuce tipburn (Collier and Tibbitts, 1982).

The amount of calcium in the inner leaves was significantly affected by the enclosure treatment (Table 13). The calcium concentration was only .63 mg/g dry weight in the covered inner leaves, 57% lower than the uncovered controls. This suggests that these young developing leaves are greatly dependent on transpiration-driven calcium transport. These results are similar to those of earlier workers using cabbage, where the artificially covered inner leaves had 90% less radiolabeled $^{45}Ca^{++}$ when compared to exposed controls following a 4-hr uptake period (Palzkill and Tibbitts, 1977; Palzkill et al., 1976). Field studies of heading butterhead lettuce have shown an abrupt difference in calcium concentration between covered inner leaves and outer wrapper leaves (Collier and Huntington, 1983) indicating that the natural heading response suppresses calcium transport to the inner developing leaves.

The calcium levels within the outer leaves were not affected by the enclosure treatment. These leaves were never covered by the polyethylene enclosures during the treatment period, and transpired freely. The calcium concentration in these leaves was 9.9 mg/g, about an order of magnitude higher

than that of the inner leaves. These higher values reflect leaf maturity and the several days of growth during which transpiration-driven transport of calcium occurred (Bangerth, 1979).

The relationship between the number of inner leaves with tipburn and the calcium concentration in these leaves is illustrated in Figure 3. As the severity of tipburn increased, the calcium concentration decreased. Tipburn was first evident on the inner leaf tissue when the concentration of calcium was below about 1 mg/g. This data suggests that the leaf enclosure treatment induced tipburn by inhibiting calcium movement to the covered leaves and further supports calcium's involvement in the disorder. The critical concentrations seen here are similar to those seen in studies of field grown butterhead (Collier and Huntington, 1983). Since these calcium determinations included both injured and uninjured leaves and used whole leaves (including the midrib), the actual critical concentration for injury at the leaf margin may be much lower. The midrib of young developing leaves of lettuce is about 4-fold higher in calcium concentration than the injury susceptable margin (Ashkar and Ries, 1971; and Collier and Huntington, 1983).

The magnesium concentration of the inner leaf tissue was only slightly affected by the enclosure treatment (Table 14). The magnesium concentration of the covered leaves was only about 3% lower than the uncovered controls. This difference reflects that only a small part of the total magnesium being transported to the inner developing leaves is due to transpirational mass flow. As would be expected, the magnesium concentration of the outer leaves was not affected by inner leaf enclosure. Magnesium data is included because magnesium is a competitive cation with calcium and has been implicated in tipburn development (Sonneveld and Mook, 1983). Because its level in the tissue is not physiologically abnormal, this data would suggest that it is not involved in tipburn development.

The shoot fresh weight at harvest averaged between 12.5 to 13 grams (Table 15). The shoot dry weight was 0.85 grams. No significant differences between treated and untreated plants were apparent, indicating that the enclosure treatment did not affect overall plant growth or vigor.

It is unlikely that the plastic sheaths brought on the injury by trapping heat or preventing exchange of oxygen, carbon dioxide or ethylene. The sheaths were made from reflective plastic to limit any possible heat buildup, and temperatures within them were never more than 1°C above ambient during the experiments. Elevated temperatures which might have promoted tipburn (Misagi and Grogan, 1978a) did not occur. Any buildup of carbon dioxide or ethylene or depletion of oxygen under the sheaths would likely not have been responsible for initiating the injury for abberent levels of these gases within mature heads have been found to be negatively correlated with tipburn (Misagi and Grogan, 1978b).

This research documents that enclosure of leaves at the growing point, as would occur with normal head development, is sufficient to create a limiting condition of calcium in the tissue capable of initiating tipburn injury. The effects of leaf enclosure may be more critical in greenhouses and growth rooms where growth rates and demand for calcium appear to be magnified (Collier and Tibbitts, 1982). Under conditions of rapid growth, leaf enclosure may be a primary controlling factor in tipburn initiation and development.

Diurnal Fluctuations: The monitoring of calcium over the day at four different times indicated a reduction of calcium concentrations during the day and an increase at night. A low level of 0.047% Ca was seen in the inner tipburn sensitive leaves near the end of the light period and a high of 0.059% Ca at the start of the light period (Table 16). There was a similar pattern

of decrease and increase in the outer enlarging leaves but concentrations were approximately 15 times greater.

The magnesium concentration of inner leaves varied little over the diurnal period; however, the concentration in outer leaves decreased during the light and increased during the dark.

This data documents that there is a measurable dilution of calcium in the inner leaves during the light period; indicating that plants would be most susceptible to calcium-related injuries toward the end of the light period. It can be assumed that during the light, there would be very little transport of calcium to the non-transpiring leaves because xylem water transport would be low. In contrast, transport of carbohydrates and other nutrients would be maintained through phloem transport into these young leaves. During the night, as the tissue regained turgor and developed root pressure flow, water would move through xylem to these leaves and transport certain amounts of calcium to increase the calcium levels.

Localization in Leaves: The studies with the x-ray probe have shown that there are large gradients in calcium from the midrib to the margin of young developing lettuce leaves. This was shown in 2 cm long leaves, with both the tip and side margin having only one-tenth the calcium found in the midrib near the base (Table 17). This small leaf was partially enclosed by older leaves around the growing point and thus would have had a reduced rate of transpiration. In contrast, the larger exposed leaf had no significant calcium gradient from the center toward the tip and margins. The 8 cm long leaf had nearly equal calcium concentrations in all areas of the leaf (Table 17).

A more detailed evaluation of the gradient from the midrib to the leaf margin documented a similar gradient from the midrib to the margin (Table

18). However, the highest concentration was found midway between the midrib and margin apparently as a result of a major vein at this location. In contrast, magnesium and potassium concentrations did not decrease from the midrib to the margin and potassium was seen to increase significantly toward the margin.

This x-ray probe data documents the very low concentrations found in the tip and marginal areas of leaves that are the locations of tipburn injury in lettuce.

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

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

[z]Dark period RH >95%.

[y]Four plants in each treatment.

[x]Mean days derived from only those plants that developed tipburn.

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

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

[z]Light period RH = 62%.

[y]Four plants in each treatment.

[x]Mean derived from only those plants that developed tipburn.

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

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

[z]Dark period RH >95%.

[y]Mean of 3 plants per treatment.

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

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

[z]Light period RH = 62%.

[y]Mean of 8 plants per treatment.

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

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

[y]Mean of 12 plants per treatment.

**Significantly different from 15°C treatment at 1% level.

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

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

[y]Mean of 16 plants per treatment.

*,**Significantly different from 15°C treatment at 5% and 1% level, respectively.

Table 7. Effect of environmental modulations on water content of shoots of lettuce.

Time Of Day[z]	Water Content (%)	
	Modulating Environment	Constant Environment
2 hrs after lights on	93.33 ± 0.10	93.29 ± 0.13
8 hrs after lights on	93.21 ± 0.12	92.94 ± 0.18
14 hrs after lights on	92.73 ± 0.26	92.58 ± 0.20
4 hrs after lights off	93.42 ± 0.14	93.19 ± 0.10

[z] 16 hr light period and 8 hr dark period.

Table 8. Effect of environmental modulations on tipburn injury and calcium concentrations in lettuce leaves.

Environmental Control	Tipburn		Calcium Concentration (% dry wt)		Shoot Dry Weight
	First Leaf With Tipburn[z]	Days From Planting[y]	Inner Leaves	Outer Leaves	(g)
Modulating	11.0	24.5	0.05±0.01	0.87±0.07	1.00±0.11
Constant	12.3	25.1	0.06±0.01	1.04±0.09	0.94±0.12

[z] Leaves numbered from cotyledons

[y] For 50% of plants injured

Table 9. Effect of light:dark cycle length on tipburn and calcium concentrations in lettuce leaves.

Light:Dark Cycle (hrs)	Tipburn		Calcium Concentration (% dry wt)		Shoot Dry Weight
	First Leaf With Injury[z]	Days From Planting	Inner Leaves	Outer Leaves	(g)
16:8	10.5±1.2	23.9±1.3	0.08±0.01	1.36±.06	2.40±.29
8:4	9.5±0.7	23.0±1.4	0.07±0.01	1.34±.17	2.39±.09

[z] Leaves numbered from cotyledons.

Table 10. Effect of nutrient solution concentration on tipburn and calcium concentration in lettuce leaves.

Nutrient Solution Conductivity	Tipburn		Calcium Concentration (% Dry Wt)		Shoot Dry Weight
$(dSm^{-1})^z$	First Leaf With Tipburn[y]	Days From Planting	Inner Leaves	Outer Leaves	(g)
.305	12.8±1.5	26.8±1.0	.101±.027	1.91±0.22	1.85±0.11
1.170	10.0±2.0	22.8±1.5	.071±.003	1.30±0.05	2.39±0.29

[z] decisiemans m^{-1} = mmhos cm^{-1}

[y] Numbered from cotyledons

Table 11. Effect of plant shaking on tipburn development and shoot dry weight.

Shaking Treatment	Tipburn Development		Shoot Dry Weight
	Days From Planting	First Leaf With Injury[z]	
Yes	27.8±0.8	12.2±0.8	4.56±0.26
None	26.6±2.4	10.0±1.0	4.11±0.69

[z] Numbered from cotyledons

Table 12. The effects of leaf enclosure on the incidence of tipburn on the 1-3 cm inner leaves.

	Inner leaf treatment		
	Enclosed	Control	Significance
Percent of plants tipburned	100	3	---
Percent of inner leaves tipburned	53.4	0.8	***

***,--- Significant at 0.1% (***) or not applicable (---).

Table 13. Effects of enclosure of the inner leaves on the calcium concentration of the inner and outer leaves.

Calcium concentration (mg/g dry wt)

	Inner leaf treatment		
Leaf sample	Enclosed	Control	Significance
Inner	0.63	1.48	***
Outer	9.87	9.90	NS

NS,*** Not significant at 5% (NS) or significant at 0.1% (***) level.

Table 14. The effects of enclosure of the inner leaves on the magnesium concentration of the inner and outer leaves.

Magnesium concentration (mg/g dry wt)

	Inner leaf treatment		
Leaf sample	Enclosed	Control	Significance
Inner	2.25	2.34	***
Outer	3.59	3.64	NS

NS,*** Not Significant at 5% (NS) or significant at 0.1% (***) level.

Table 15. Effects of inner leaf enclosure on shoot fresh and dry weight.

	Shoot weight (g)		
	Inner leaf treatment		
Type	Enclosed	Control	Significance
Fresh	12.51	12.93	NS
Dry	0.853	0.854	NS

NS Not significant at 5% level.

Table 16. Diurnal fluctuations in calcium and magnesium concentration in lettuce leaves.

	Nutrient Concentration (% Dry Wt)			
	Calcium		Magnesium	
Time of Day[z]	Inner Leaves	Outer Leaves	Inner Leaves	Outer Leaves
2 hrs after lights on	0.059	0.90	0.22	0.35
8 hrs after lights on	0.050	0.86	0.21	0.33
14 hrs after lights on	0.047	0.84	0.21	0.31
4 hrs after lights off	0.051	0.88	0.22	0.32

[z] 16 hr light period and 8 hr dark period

Table 17. Relative calcium concentrations at separate positions on different size lettuce leaves.

Measurement Position	Leaf Length (cm) 2	8
Tip	6.5	385
Side at Margin	9.8	289
Base in Midrib	90.3	342

[z] Counts per second in 150 μm^2 area with 20 kv beam energy.

Table 18. Calcium, magnesium and potassium concentrations across leaf blade of lettuce leaf.[z]

Distance From Midrib (mm)	Nutrient Concentration (% dry wt) Calcium	Magnesium	Potassium
0 (midrib)	0.075	0.18	4.35
1	0.065	0.21	4.27
2	0.060	0.24	6.19
3	0.110	0.45	6.60
4	0.060	0.40	4.60
5	0.045	0.19	6.53
6 (margin)	0.035	0.24	7.96

[z] leaf 2 cm in length and 2.4 cm in width at widest point.

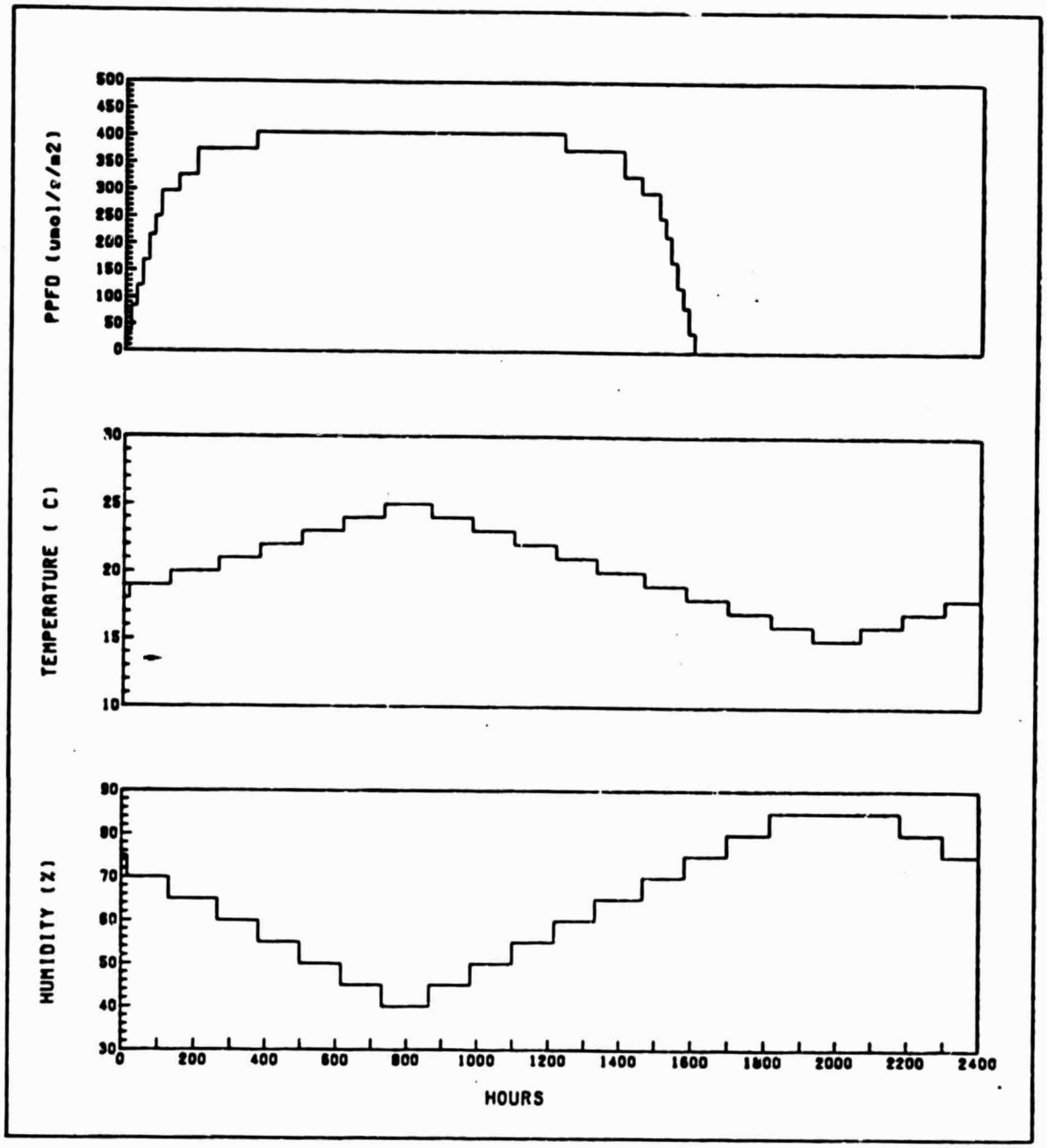

Figure 1. Diurnal changes in environmental conditions that provided day - night environmental modulations.

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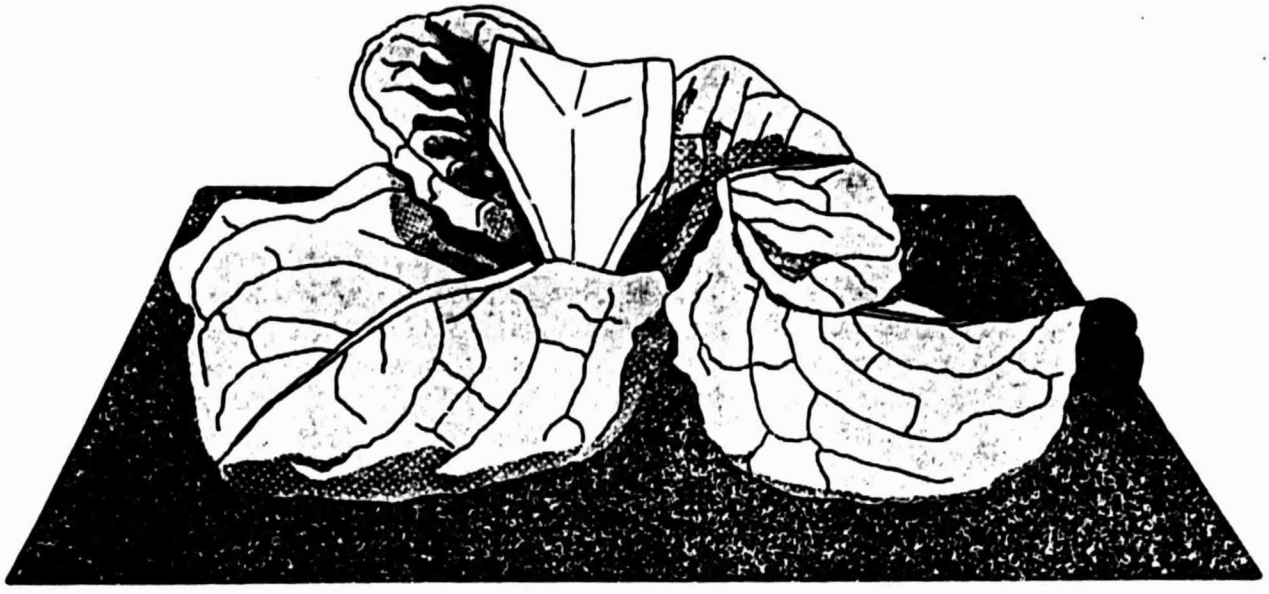

Figure 2. 'Buttercrunch' lettuce with an aluminized polyethylene sheath placed over the growing point to restrict transpiration.

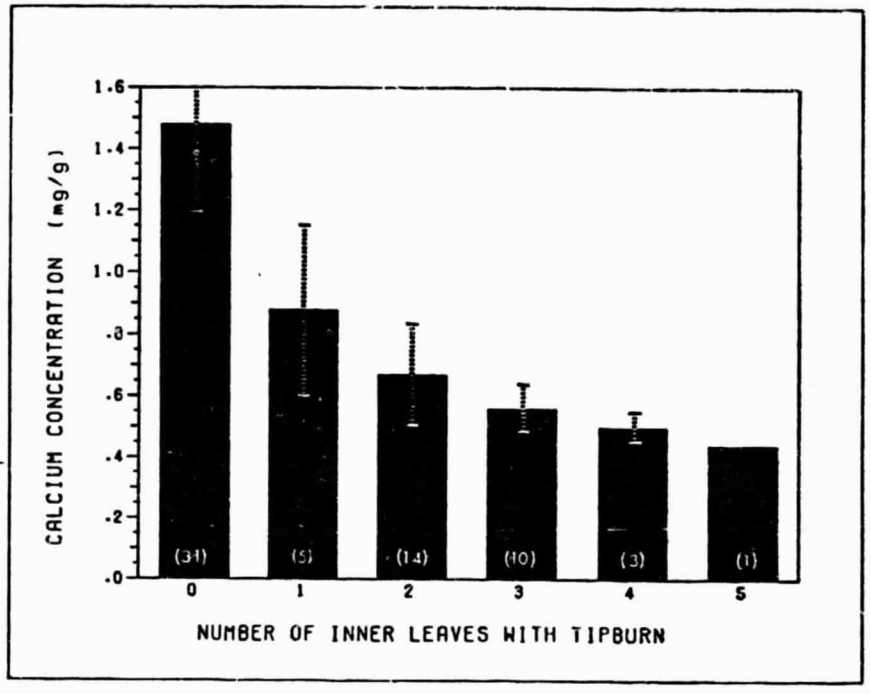

Figure 3. Relationship between the calcium concentration of the 1-3 cm inner leaves and number of leaves tipburned. The number of plants in each sample is given in parentheses. The bars indicate the standard deviation of calcium levels. No standard deviation can be determined for n=1.

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