APPLICATION OF CALCIUM AND NITROGEN FOR MITIGATING HEAT STRESS EFFECTS ON POTATOES

Ahmed A. Tawfik[1], Matthew D. Kleinhenz[2], and Jiwan P. Palta[3]

Abstract

This study was designed to investigate the effect of calcium and nitrogen application during heat stress on leaf calcium concentration, transpiration rate, membrane thermostability, and biomass accumulation and partitioning. Micropropagated Russet Burbank potato (*Solanum tuberosum* L.) plants were transplanted into 20 L pots containing 1:1 (v/v) soil: perlite and exposed to 30/20C (D/N) temperatures for four weeks (weeks 9-12 after transplanting) in a controlled-environment growth room. The maximum temperature was maintained for 6 hr during the middle of the 14 hr photoperiod. The nutrition treatments were N before stress (NBS), N during stress (NDS) and Ca and N during stress (Ca+NDS). Calcium was supplied as $Ca(NO_3)_2$. All treatments received the same total amount of nitrogen. Native soil Ca level without amendment (550 mg Ca/kg soil) was sufficient for potato plant growth under normal temperatures.

Plants given Ca and N during heat stress had the highest leaf Ca concentration and transpiration rate during and 2 weeks after conclusion of the heat stress period. When measured after 4 weeks of heat stress, area and fresh and dry weight of the most recently mature leaf was significantly greater in NDS and Ca+NDS plants compared to NBS plants. Cellular membrane thermostability (measured as ion leakage from heat-treated leaf disks) was not affected by any treatment prior to heat stress. However, leaf tissue from Ca+NDS plants exhibited significantly higher membrane thermostability compared to NBS plants after 2 and 4 weeks of heat stress. At harvest, NDS and Ca+NDS plants had significantly higher leaf/stem (fresh weight ratio) values compared to NBS plants. Also, Ca+NDS plants had significantly greater total tuber and biomass values than NBS and NDS plants. Results of this study suggest that some detrimental effects of heat stress on plant growth and stomatal function may be alleviated by Ca and N application during heat stress. The data also suggest that mitigation of heat stress by Ca and N application during heat stress may maintain plant productivity when optimum growing temperatures are restored.

[1]Former graduate student, Department of Horticulture, University of Wisconsin-Madison. At present, Researcher, Horticulture Research Institute, Vegetable Research Department, Potato Section, Dokki, Giza, Egypt.
[2,3]Graduate student and Professor, respectively, Department of Horticulture, University of Wisconsin-Madison, Madison, WI 53706-1590.

Compendio

El presente estudio fue diseñado para investigar el efecto de la aplicación de calcio y nitrógeno en la concentración de calcio en las hojas, tasa de transpiración, termo-estabilidad de membrana, y acumulación y distribución de biomasa durante estrés producido por altas temperaturas. Plantas de papa de la variedad Russet Burbank (*Solanum tuberosum* L.) fueron micropropagadas y luego transplantadas en macetas de 1 litro de capacidad conteniendo suelo y perlita (1:1 volumen). Las plantas fueron expuestas a tempeaturas de 30/20C (D/N) durante 4 semanas (9-12 semanas después de haber sido transplantadas) en una cámara de crecimiento con todos los factores medioambientales controlados. La temperatura máxima fue mantenida por 6 horas a la mitad del fotoperíodo de 14 horas. Los tratamientos fueron nitrógeno antes del estrés (NBS), durante el estrés (NDS) y calcio y nitrógeno durante el estrés (Ca+NDS). El calcio fue aplicado en forma de nitrato de calcio (Ca(NO$_3$)$_2$). Todos los tratamientos tuvieron la misma cantidad total de nitrógeno. Los niveles de calcio del suelo sin alteración (550 mg/Kg suelo) fueron suficientes para el crecimiento de las plantas de papa bajo temperaturas normales.

Las plantas tratadas con calcio y nitrógeno tuvieron la concentración más alta de calcio en las hojas así como también la tasa más alta de transpiración durante y hasta dos semanas después del período de estrés causado por altas temperaturas. Después de 4 semanas del período de estrés, el área total así como los pesos seco y fresco fueron medidos en hojas de reciente maduración. Los resultados indicaron que éstos fueron significativamente mayores en plantas NDS y Ca+NDS que en plantas NBS. La termo-estabilidad de la membrana celular (cuya medida es basada en la pérdida de iones de discos de hojas sometidas a altas temperaturas) no fue afectada por ningún tratamiento antes del estrés. Sin embargo, el tejido foliar de las plantas Ca+NDS exhibió una mayor termo-estabilidad de membrana comparada con la de las plantas NBS después de 2 y 4 semanas de estrés causado por altas temperaturas. En la cosecha, las plantas NDS y Ca+NDS tuvieron valores de hoja/tallo (tasa de peso fresco) significativamente mayores comparados con los de las plantas NBS. Asímismo, las plantas Ca+NDS tuvieron valores de biomasa y número de tubérculos significativamente mayores que los de las plantas NBS y NDS. Los resultados del presente estudio sugieren que algunos efectos negativos del estrés causado por altas temperaturas en el crecimiento de la planta y en la función de los estomas pueden ser aliviados mediante la aplicación de calcio y nitrógeno durante el estrés. Los datos también sugieren que dicho alivio puede mantener la productividad de la planta cuando las temperaturas óptimas para el crecimiento son restauradas.

Introduction

The cultivated potato (*Solanum tuberosum* L.) is adapted to regions with moderate climates. High temperature (> 28/18C, day/night) is con-

sidered a major physiological constraint resulting in reduced plant growth (13) and limited tuberization (6, 15). High temperature also alters photosynthesis, respiration, membrane permeability (1, 18, 20) and photosynthate partitioning to tubers (7).

Potato plants grown under high temperatures often produce leaves with reduced area compared to cool-grown plants (7, 19). Reduced potato leaf size may be due to reduced cell division such as observed in other crops (14, 24, 25), alteration in cell membrane permeability (5, 11), or lowered stomatal conductance and reduced CO_2 supply for assimilate production (7). The impact of calcium and nitrogen nutrition on heat stress responses of potato has not been investigated.

In this study, measures of leaflet Ca concentration, transpiration rate, membrane thermostability, and plant biomass accumulation and partitioning were taken on plants simultaneously exposed to differential Ca and N nutrition and high temperature.

Materials and Methods

Plant Material and Growing Media

Micropropagated Russet Burbank (*Solanum tuberosum* L.) plantlets were transplanted to 10 cm (diameter) pots containing Jiffy Mix (JPA, East Chicago, IL) and acclimated to growth room conditions. One week after transplanting, plants were transferred to 20-liter pots containing 1:1 (v:v) loamy-sand soil (Typic Udipsamment) and perlite. Soil was collected from the top 25-30 cm layer at the University of Wisconsin-Madison Hancock Agricultural Research Station. Soil analyses of CEC (meq/100 g), organic matter (%), and nutrients (mg/kg soil) P, K, Ca, and Mg produced values of 3, 1, 35, 130, 550 and 130, respectively. It is important to note that the soil used in the present study contained 550 mg/kg total extractable calcium. This Ca level has been shown to be sufficient for potato production on this soil (23). Also, in our previous studies conducted on the same soil type containing native total extractable Ca levels of 350-550 mg/kg, we have found no significant yield differences among Ca treatments (10, 28). Required amounts of N, P and K per plant were calculated as 1, 1.5 and 5.2 g, respectively (9) and added to the growing mix at transplanting.

Environmental Conditions and Nutrient Applications

The experiment was conducted in a controlled environment growth room (3.7m x 2.6 m) at the University of Wisconsin-Madison Biotron. A randomized complete block design with three nutrition treatments and four replicates was employed. Replicates consisted of a single plant. Data were analyzed using General Linear Model procedures (21) and Duncan's Multiple Range test ($\alpha = 0.05$) was used to separate treatment means. Daily temperature minima and maxima were 20/15C weeks 1-3, 25/15C weeks 4-8, 30/20C weeks 9-12 (heat stress period), 25/15C weeks–13-14,

20/15C weeks 15-16. Temperature minima and maxima were reached after gradual temperature changes each day. Maximum temperatures were maintained for 6 hr during the middle of the light period and the relative humidity was maintained at ca. 60% throughout the experimental period. Vapor pressure deficit during the heat stress period (30/20C, day/night) was 1.6/0.9 kPa during day/night. The photoperiod was 14 hr (460-480 μmol m^{-2} s^{-1}, PPF) under cool-white fluorescent lamps.

Nutrition treatments included 3 combinations of Ca and N supplementation before and during the heat stress period (Table 1). The total amount of applied N was identical in all treatments while one treatment received additional Ca during the heat stress period (Table 1). Nutrient sources were dissolved in distilled water (ca. 400 ml) and applied between irrigations. Plants were drip-irrigated with distilled water 4 times per day and 50% of the leached water from each pot was pumped back once per week to the same pot using an attached pump. Plants were irrigated one day per week with a complete nutrient solution (excluding N, P, K, Ca) to ensure an adequate supply of other nutrients during plant growth.

Leaf Area, Weight, Transpiration Rate, and Calcium Concentration; Total Plant Fresh Weight

After 0, 2, and 4 weeks of heat stress, measures of area, fresh weight, dry weight, and transpiration rate were taken on leaf 5 or 6 of all plants. Prior to leaf removal for destructive measures, transpiration rate was recorded on the fully-expanded terminal leaflet using a LI-COR steady state porometer (model LI-1600; LI-COR, Inc., Lincoln, Nebraska, USA). Transpiration rate readings were repeated 2 weeks after the conclusion of heat stress. Total leaf area (cm^2) was measured with an area meter (model LI-1300; LI-COR, Inc., Lincoln, Nebraska, USA). After fresh weight readings, all leaflets were removed from each leaf and prepared for Ca analysis as one sample. Samples were dried (70C, 48-72 hr), weighed, ground to pass a 40-mesh screen, ashed (450C, 8 hr), dissolved in 2N HCl, and diluted with a lanthanum chloride solution and distilled-deionized (dd) water to obtain samples in 0.2N HCl and 2000 mg L^{-1} LaCl$_3$. Calcium concentration was determined by atomic absorption spectrophotometry (Varian model SpectrAA-20, Varian Associates, Inc., Sunnyvale, California, USA).

To determine the impact of Ca and N nutrition on biomass accumulation and partitioning, fresh weight (g/plant) of leaves, stems, and tubers were determined at harvest (16 weeks after transplanting).

Membrane Thermostability

Cellular membrane thermostability (MT) of leaf tissue was estimated after 0, 2, and 4 weeks of heat stress. Membrane thermostability was estimated using fully expanded terminal leaflets of recently mature leaves (leaves 2, 3) and measures of electrical conductivity as described previously

TABLE 1.—*Nutrient application schedule and temperature regimen.*

	Amount of Nutrient Applied (gram/plant)								
	Calcium[1]				Nitrogen				
	weeks after transplanting[2]								
Treatment	8	10	12	Total	3	8	10	12	Total
N Before Stress (NBS)	0	0	0	0	3	3	0	0	6
N During Stress (NDS)	0	0	0	0	3	1	1	1	6
Ca and N During Stress (Ca+NDS)	1.1	1.1	1.1	3.3	3	1	1	1	6

[1]In Ca+NDS, N supplied as NH_4NO_3 + $Ca(NO_3)_2$ and Ca supplied as $Ca(NO_3)_2$.
[2]Heat stress was initiated at the beginning of week 9 and ended at the conclusion of week 12. Daily temperature maxima/minima for the experimental period beginning at transplanting: 20/15C (weeks 1-3), 25/15C (weeks 4-8), 30/20C (weeks 9-12, heat stress), 25/15C (weeks 13-14), 20/15C (weeks 15-16).

(3, 18). Leaflets were placed in open plastic bags and on ice for 15 min prior to sample preparation. Leaf disks (10 mm diameter) were obtained using a cork borer from the center of the leaflet but avoiding heat-injured tissue and the midvein. For each treatment, four disks were placed in a stoppered culture tube (25 x 200 mm). Disks were washed thoroughly with three changes of dd H_2O to remove electrolytes adhering to the tissue and released from cut cells at the periphery of leaf discs. After rinsing, all tubes were drained, 2 ml of dd H_2O were added (to prevent desiccation of tissue during heat treatment) and tubes were covered with plastic wrap. Heat treatment (T) tubes were incubated in a temperature regulated water bath for 1 hr at 45C, whereas control (C) tubes were placed at room temperature (ca. 25C) for the same period. As suggested previously (2, 3), the treatment temperature was chosen after preliminary experiments to determine the temperature producing the greatest effect. After heat treatment, 20 ml of dd H_2O were added to both control and heat-treated tubes. All tubes (T and C) were incubated for 24 hr at ca. 10C to allow diffusion of electrolytes from leaf disks in proportion to heat damage. Tubes were then transferred to a water bath at 25C and shaken for 15 min to mix tube contents prior to conductivity measurement. The initial conductivity of tube contents (C_1 or T_1) was determined with an electrical conductivity meter (YSI Model 32, Yellow Springs, OH). Tubes were autoclaved (121C, 15 min), equilibrated to 25C in a water bath, and shaken before the final

TABLE 2.—*Effect of calcium and nitrogen application before or during heat stress on fresh weight of different plant parts 16 week after transplanting. Measurements included all leaves, stems and tubers per plant. Nutrient applications were at 3, 8, 10 and 12 weeks after transplanting (Table 1). Treatments NBS, NDS and Ca+NDS described in Methods and Table 1.*

Treatment	Leaves	Stems	Leaf/stem	Tubers	Total foliage[1]	Tubers+ Total foliage	Tuber/Total[2]
	---------------------- g/plant --------------------------						%
N B S	173.4 b	144.9 a	1.2 b	611.7 b	318.3 a	930.1 b	65.8 b
N D S	202.1 ab	121.8 b	1.7 a	699.8 b	323.9 a	1023.7 b	68.4 ab
Ca+NDS	248.7 a	132.3 ab	1.9 a	1000.3 a	381.0 a	1381.3 a	72.4 a

Means within the same column followed by the same letter are not significantly different (α=0.05; Duncan's Multiple Range test).
[1]Fresh weight of leaves and stems.
[2]Fresh weight of tubers and foliage.

conductivity readings (C_2 or T_2) were recorded. The level of injury was calculated as relative injury (RI) as described previously (3):

RI (%) = 1- {[1- (T_1/T_2)] / [1- (C_1/C_2)]} * 100

where T and C refer to conductance values of heat-treated and control tubes, respectively, and subscripts 1 and 2 refer to initial and final (autoclaved) conductance readings, respectively.

Results

Visual Observations

All experimental plants were similar in size at initiation of the heat stress period. During the heat stress period only, new emerging leaves in the NBS treatment, but not in the NDS or Ca+NDS treatments, showed symptoms of Ca deficiency such as tip burn and leaflet curling and rippling. In contrast, none of the plants showed symptoms of nitrogen deficiency.

Fresh Weight of Leaves, Stems and Tubers at Harvest

Dramatic differences among treatments in biomass accumulation and partitioning were observed at harvest (Table 2). Leaf fresh weight of Ca+NDS plants was 30% and 19% greater than NBS and NDS plants, respectively, but the difference between Ca+NDS and NDS treatments was not significant. Similarly, the leaf/stem fresh weight ratio was significantly increased in NDS and Ca+NDS plants compared to NBS plants. Fresh weight of tubers and total biomass (foliage + tubers) was significantly greater in the Ca+NDS treatment compared to the other treatments. None of the treatments affected total foliage (leaves + stems) fresh weight.

TABLE 3.—*Influence of calcium and nitrogen application on cellular membrane thermostability as determined by ion leakage (electrical conductivity test) following exposure of leaf disks to 45 C for 1 hr (details in Methods).*

Nutrition treatment	Cellular membrane thermostability		
	Before heat stress	2 weeks of heat stress	4 weeks of heat stress
	--------- Relative injury (%) ---------		
NBS	61.4 a	58.6 a	52.2 a
NDS	65.2 a	49.3 b	47.0 ab
Ca+NDS	61.8 a	47.8 b	44.2 b

Means within the same column followed by the same letter are not significantly different ($\alpha=0.05$; Duncan's Multiple Range test).

Also, tubers comprised a significantly higher percent of total biomass in Ca+NDS plants compared to NBS, but not NDS, plants (Table 2).

Leaf Ca Concentration and Transpiration Rate

At all points of measure, leaf Ca concentration and transpiration rate were greatest in Ca+NDS plants (Fig. 1). At the conclusion of heat stress, NDS leaves had higher Ca levels than NBS leaves (Fig. 1A). When measured two weeks after the conclusion of the heat stress period, leaf Ca concentration had declined further in NBS and NDS plants but concentrations in Ca+NDS plants had increased (Fig. 1A). Ca+NDS plants maintained nearly constant transpiration rates which were significantly greater at all points of measure than rates of NBS and NDS plants (Fig. 1B). However, transpiration rates of NBS and NDS plants declined dramatically but were comparable during the heat stress period (Fig. 1B). But, when measured two weeks after conclusion of the stress period, the transpiration rate of NDS plants had increased and was significantly greater than NBS plants (Fig. 1B).

Area, Fresh and Dry Weight of Recently Mature Leaves

None of the treatments affected leaf area or weight prior to heat stress (Fig. 2). However, NDS and Ca+NDS plants displayed ca. 35% greater leaf area than NBS plants after exposure to heat stress (Fig. 2A). The difference between NDS and Ca+NDS treatments in leaf area was not significant during heat stress (Fig. 2A). Fresh (Fig. 2B) and dry (Fig. 2C) weights of the same leaves (leaf used for area measurement) showed trends similar to leaf area (Fig. 2A) throughout the heat stress period.
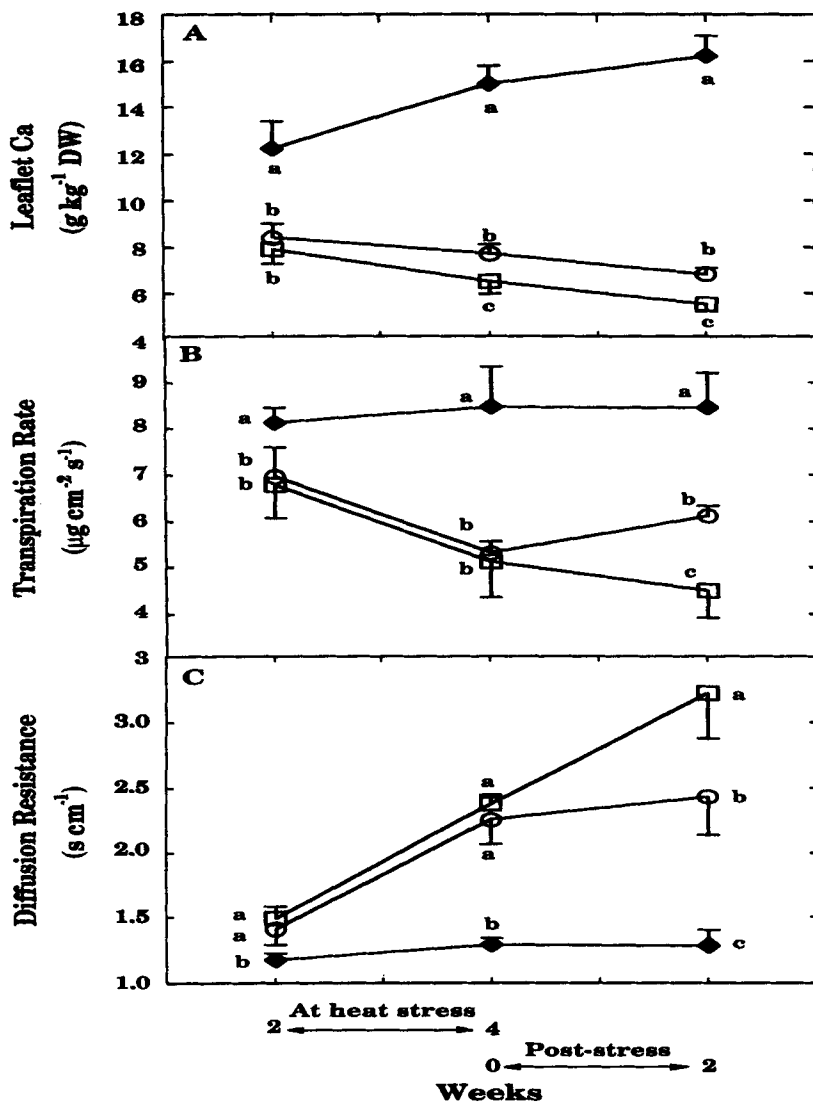
FIG. 1. Nutrient application effect on leaflet Ca concentration and transpiration rate measured during and after a heat stress period. The same leaflet was used for both measures. Values are the mean of 4 single-leaf replicates ± S.D. Values within the same time and labelled with the same letter are not significantly different by Duncan's Multiple Range test (α=0.05). NBS (□), NDS (O), Ca+NDS (◆).
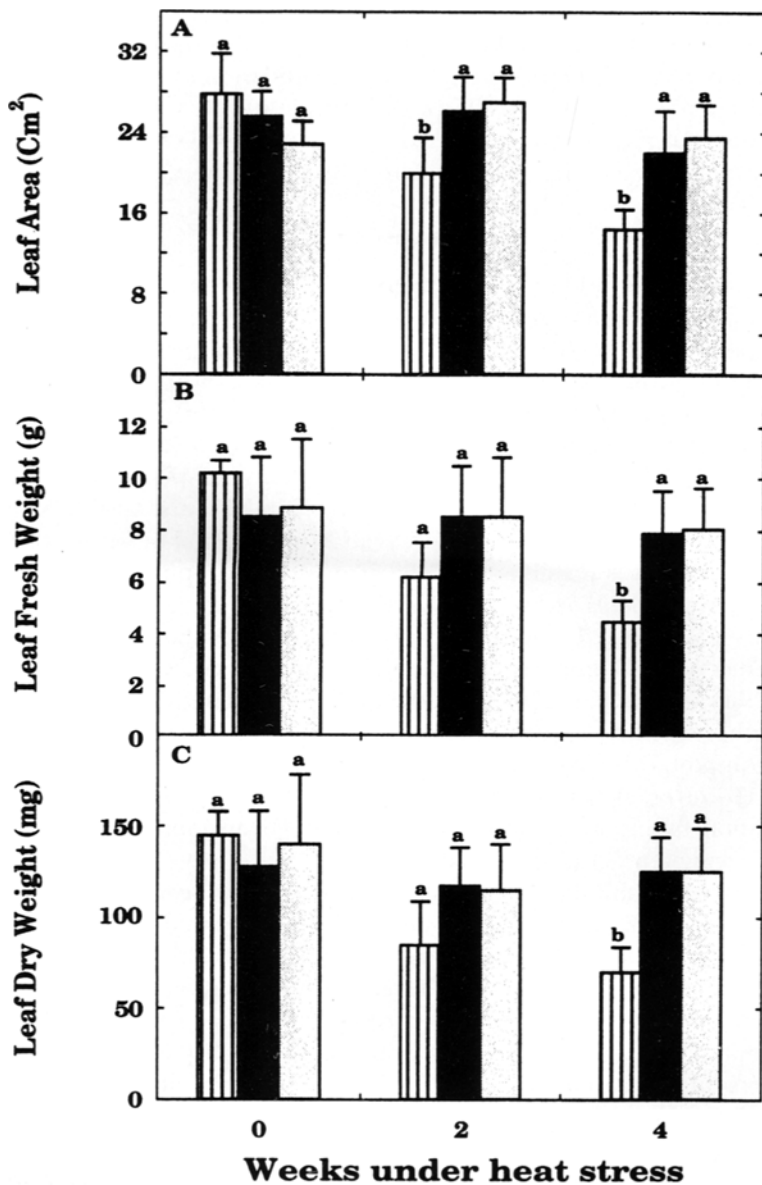
FIG. 2. Nutrient application effect on leaf area and fresh and dry weight measured during heat stress. The same leaf was used for all measures. Values are the mean of 4 single-leaf replicates ± S.D. Values within the same time and labelled with the same letter are not significantly different by Duncan's Multiple Range test (α=0.05). NBS (▥), NDS (■), Ca+NDS (▨).

Cellular Membrane Thermostability

Membrane thermostability, expressed as relative injury (RI), was not influenced by nutrition treatment before the initiation of heat stress (Table 3). Although NDS and Ca+NDS plants had similar RI values during heat stress, Ca+NDS plants consistently had the lowest RI values and significantly greater membrane thermostability than NBS plants after four weeks of heat stress (Table 3).

Discussion

Results of the present study suggest that the impact of heat stress on some aspects of potato growth can be mitigated by Ca and N application during stress. Although we do not know the mechanisms by which Ca and N nutrition during heat stress might benefit plant growth, our results provide some insight into these mechanisms. For example, the N application method may have contributed to the greater leaf area values observed in NDS and Ca+NDS plants compared to NBS plants. All plants received the same total amount of N but 33% of the total N in NDS and Ca+NDS plants was applied during heat stress, whereas all N was applied before heat stress in NBS plants. It is possible that NBS plants used proportionally more N than other plants before and during the early part of the heat stress period. This may have led to lower N in the soil for the NBS plants later in the heat stress period compared to N available for NDS and Ca+NDS plants. It is possible, then, that NDS and Ca+NDS plants benefitted from greater rhizospheric N levels during heat stress. Of course, measures of soil N (not completed in the present work) would be required to clarify this issue. However, it is important to note that no systematic effects of N application timing on potato tuber yield have been found under normal field growing temperatures (8, 10, 16, 28).

Our results show that Ca and N application during heat stress resulted in greater membrane thermostability in leaf tissue compared to N application before stress (Table 3). Membrane thermostability was affected by treatment only during the heat stress period (Table 3). Cell membranes are thought to be a site of cellular and sub-cellular response to heat stress (2, 17) since maintenance of differential membrane permeability is a fundamental requirement for normal metabolism and plant survival. Calcium may reduce heat-induced ion leakage by stabilizing cell membranes and allowing normal function of ion transport mechanisms (5, 17, 29). Since young leaves of NBS plants developed advanced calcium deficiency symptoms during heat stress, higher Ca levels may be beneficial under these stress conditions.

In this study, greater leaf Ca levels and transpiration rates were recorded in plants supplied with additional Ca during heat stress. A mechanistic relationship among these variables is unknown but accepted aspects of Ca nutrition may be relevant in this regard. For example, Ca transport in the xylem is primarily by mass flow and the delivery rate to

leaves is influenced by transpiration rate (4). Our data support the hypothesis that plants with higher transpiration rates will accumulate comparatively higher levels of calcium. Low levels of Ca may hinder stomatal function since the selectivity of potassium uptake by guard cells has been found to be enhanced by Ca (30). Also, there is evidence that the plasma membrane H^+-ATPase, thought to be involved in stomatal opening, is regulated through phosphorylation by a protein kinase shown to be stimulated by Ca in vitro (22). Similar activity of a protein kinase was detected in guard cell protoplasts (12). It is possible that elevated leaf Ca levels are required early in a heat stress period to reduce the likelihood that low Ca-related disruptions of cell function will occur. Sufficient Ca during heat stress may assist in stabilizing stomatal function, promote additional Ca accumulation, and allow for greater transpirational cooling and carbon fixation.

Our data demonstrate a significant increase in tuber yield in plants supplemented with Ca and N during heat stress (Table 2). Exposure to heat stress after tuber initiation likely minimized the inhibitory effect of heat stress on tuberization (6) in this study. Therefore, differences in tuber yield may have resulted from plants' differential ability to fix carbon and partition photosynthate to tubers. Greater tuber yield in the Ca+NDS, but not NDS, treatment compared to the NBS treatment demonstrates that N application alone during heat stress was insufficient to maintain tuber yield in this study. These results suggest that the combination of Ca and N nutrition may have a specific role in maintaining tuber yield under heat stress. The proposed effect of calcium on stomatal function (30) may be important in this regard.

We have conducted parallel studies in the Biotron facility under heat stress and non-stress conditions using the same soil and plant material as used in the present study. In previous experiments, neither Ca nutrition nor nitrogen application timing significantly affected biomass accumulation or partitioning under non-stress conditions (26, 27). Furthermore, in our field studies, tuber yield was significantly affected by Ca and N application during tuber bulking only in the hot and dry 1988 season (17). No yield differences were apparent in normal seasons (10, 28).

In conclusion, results of this study suggest that the negative effects of heat stress: (i) on potato leaf growth can be mitigated by application of Ca or N during the stress period; and (ii) on stomatal function can be mitigated by Ca and N application during the stress period. In addition, plants given supplemental Ca and N during heat stress had significantly greater tuber yield at the completion of the study.

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