RELATIONSHIP BETWEEN TRANSPIRATION AND NITROGEN UPTAKE BY PEPPER (*CAPSICUM ANNUUM)* AS MEDIATED BY VAPOR PRESSURE DEFICIT

by

KATHERINE E. BOWER

(Under the Direction of Robert O. Teskey)

ABSTRACT

As a consequence of photosynthesis, plants lose water rapidly via transpiration. However, transpiration may benefit the plant by facilitating nutrient uptake and transport through the xylem. The objective of this experiment was to study the possible relationship between transpiration and nitrogen uptake. Bell peppers (*Capsicum annuum*) were grown in growth chambers with differing vapor pressure deficits (VPD, 1.20 kPa and 1.98 kPa) to modify their transpiration rates. They were also supplied different levels of nutrient (2.23 g and 1.115 g fertilizer/week) to study the effect of transpiration on the growth of nitrogen-deficient plants. Plants were sampled every 21 days for a total of four harvests. High fertility had a positive effect on nitrogen uptake and plant growth, whereas high VPD and higher transpiration essentially had no effect, demonstrating that plants may transpire less without suffering detrimental effects towards nutrient status and growth even under low nutrient conditions.

INDEX WORDS: nitrogen, pepper, transpiration, vapor pressure deficit

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CHAPTER 1

INTRODUCTION

Plants use a copious amount of water while photosynthesizing via transpiration – the evaporation of water from plants. The amount is so large that plant biologists have stigmatized transpiration as a "necessary evil" that is inextricably linked to carbon dioxide (CO_2) absorption. As plants open their stomata to absorb CO_2, water escapes into the atmosphere. Much of the water taken up from the soil ends up leaving via the stomata. Some research has even gone into plant anti-transpirants in an effort to decrease water use, and thus the need for irrigation of food crops and greenhouse plants, a laudable effort considering the ever-increasing demand of water by the world's growing population and food needs.

However, the considerable amount of water being used by plants begs the question as to whether or not there are additional benefits to this water loss besides carbon dioxide uptake. Some research on this strongly indicates that transpiration facilitates the mass flow, uptake, and translocation of various nutrients by plants (e.g., Barber 1963, MacDonald et al. 2002, Novak and Vidovic 2003).

The original intent of this experiment was to study the effect of transpiration, as manipulated by vapor pressure deficit, on the uptake of nitrogen in a tree species as most other experiments on this topic involved non-woody plants. A tree species would have made an important study subject as its transport systems via xylem and phloem are much longer than the typical herbaceous plants used by other researchers. However, after two attempts to study poplar hybrids (*Populus deltoides x. P. nigra*) failed due to fertility and mechanical problems, a

horticulture plant (bell pepper, *Capsicum annuum*) was used instead. The first attempt with poplar did not include a fertility study, and the low nutrient fed plants received too much fertilizer and showed no sign of slower growth compared to plants fed a higher amount of nutrients. The second attempt was stopped halfway through the study when one of the growth chambers froze the plants. Pepper was chosen for the third attempt as poplar cuttings were no longer available for purchase at the time the study was restarted.

CHAPTER 2

LITERATURE REVIEW

Plants take up and transpire a great deal of water. On a per mass basis, they can take up seventeen times more water than a human (Hales 1961). While some of this water is used directly as a reactant for photosynthesis, most of it is lost during absorption of carbon dioxide (CO_2) from the atmosphere. As CO_2 enters the leaf through the stomata, water is lost to the atmosphere. This occurs because moist mesophyll cells are necessary for CO_2 to enter the leaf cells. The gas must pass through the plasma membrane, which is impervious to CO_2 unless it enters into solution as ionic bicarbonate (Raven et al. 1992). Since the absorptive surfaces must remain moist, plants must constantly take up water to compensate for evaporation from mesophyll cells.

The evaporation of water from leaf surfaces, also known as transpiration, drives the movement of water through the plant's xylem. Water flows along an increasingly negative water potential gradient from the soil-root interface, through the plant, and ultimately through the stomata of the leaf into the atmosphere. This is made possible by the cohesive and adhesive properties of water molecules, described by the cohesion-tension theory (Dixon 1914). As water molecules evaporate from the stomatal pores of the leaf into the surrounding atmosphere, they are replaced by new molecules from the leaf mesophyll cells. Consequently, water from the xylem diffuses into the cells, and due to the highly polar and cohesive nature of water, this exerts a tension throughout the water column, thus pulling water up the xylem vessels much farther than mere capillary action would have allowed. The tension and cohesive and adhesive forces of

the water allows it to overcome the gravitational forces working to pull the water column down the plant.

To counteract the problem of excessive water loss, the plant is able to control transpirational water loss and gas exchange by modulating stomatal apertures (stomatal conductance, g). The opening and closing of guard cells is controlled by rapid potassium ion (K^+) fluxes into the cells. As K^+ accumulates, the stomata open (Willmer 1983).

Various environmental factors such as light, CO_2 concentration, and water stress affect stomatal opening and closure (Raven et al. 1992). Exposure to blue light causes apertures to open in spite of internal [CO_2]. Light in general can also affect opening by virtue of photosynthesis decreasing internal [CO_2]. Though stomata are known to close in the dark except for CAM plants, they may reopen should internal CO_2 decrease enough (Mansfield et al. 1981). In addition, atmospheric [CO_2] has been found to decrease g when [CO_2] is elevated (Medlyn et al. 2001). Water stress decreases overall turgor in the leaf. As turgor subsequently drops in the guard cells, g decreases (Lösch and Tenhunen 1981).

The stomata also respond to vapor pressure deficit (VPD), which is the difference between the amount of moisture in the air and the amount the air can hold when it is saturated at a given temperature. When exposed to dry air, or high VPD, stomata close (Lange et al. 1971, Lösch 1977). This reaction is not necessarily uniform across the leaf epidermis as individual stomata can react to the humidity conditions to which they are exposed (Lange et al 1971). Closure is either a direct response of the guard cells to dry or moist air or an indirect response mediated by loss of turgor due to evaporation outstripping water supply. In general, there has been more support for the former rather than the latter (Lösch and Tenhunen 1981). Shope et al. (2008) concluded that osmosis of water into guard cells from surrounding epidermal tissues was

not a significant factor in maintaining guard cell turgidity. Instead, water vapor in the surrounding air was sufficient for guard cell hydration. As water vapor decreased, the cells dehydrated and stomatal apertures decreased. However, while g may decrease in response to drier air, transpiration may still remain high due to evaporative demand of the atmosphere (Jarvis and Morison 1981).

Plants have been known to respond positively to increased humidity (e.g. Armstrong and Kirkby 1979, Ford and Thorne 1974, Mortensen 1986). This may be due to a plant's ability to better maintain a higher water potential in higher humidity that is conducive to growth. Decreasing water potential inhibits leaf enlargement (Boyer 1970). As water content decreases within the plant due to excessive evaporation, turgor pressure decreases, therefore not allowing cells to expand. Also, if a plant closes its stomata in response to decreasing humidity, CO_2 absorption can be inhibited. This in turn can cause decreases in net photosynthesis and subsequent growth of the plant. Therefore, exposure to low VPD may be conducive to growth by keeping stomata open for gas exchange. Cunningham (2005) reported a decrease in net photosynthesis with increasing VPD in tropical tree species; however, there was no significant decrease in growth.

The apparent high cost of plant water loss may have another benefit besides CO_2 absorption – it may affect the acquisition and transport of nutrients by the plant. The mass flow of nutrients such as N, Ca, Mg, and S to root surfaces is attributed to transpirational water uptake by the plant (Havlin et al. 2005). Since mass flow of soil solution nutrients is affected by transpiration, the rate at which water evaporates from the leaves determines the rate that soil water reaches the roots. Therefore, transpiration rate affects the amount of nutrients that come into contact with the root (Barber 1962, Barber et al. 1963, Havlin et al. 2005). In a CO_2-

enrichment and humidity experiment using *Populus deltoides*, nitrogen (N) concentration in tissues was reduced when either CO_2 or humidity were high (MacDonald et al. 2002). They analyzed the nitrogen uptake on a per root mass basis and found that ^{15}N uptake was reduced for both CO_2-enriched plants and plants growing in high humidity compared to plants growing under ambient conditions. However, in the high CO_2 treatment, this [N] reduction may have been partially due to a dilution effect of carbohydrate production. A study using maize revealed a linear relationship between the rate of transpiration and the uptake rates of nitrogen, phosphorus, and potassium (Novak and Vidovic 2003).

However, while transpiration may affect the amount of minerals available for immediate absorption by the root, as previously mentioned for N, the actual transport of nutrients across the root cell membranes into the xylem is often an energy-requiring process (Raven et al 1992). Thus, the passage of ions through the cells is not controlled by transpiration. This may reduce the total effect of transpiration on nutrient uptake.

In a study by Schulze and Bloom (1984) using tomatoes (*Solanum lycopersicum*) and radishes (*Raphanus sativus*), the researchers devised a system that allowed them to monitor photosynthesis and transpiration from the shoot simultaneously with net fluxes of NO_3^- and NH_4^+ from the root. They found that the fluxes of those two molecules in the xylem were not correlated with transpiration.

In a study of sunflowers (*Helianthus annuus*) grown in hydroculture, the amount of nutrients transported through the xylem was equivalent for plants experiencing low transpiration (at >98% relative humidity) or high transpiration (Tanner and Beevers, 2001). This result was attributed to transpiration-independent forms of water flow such as growth water, phloem counterflow, and guttation. Guttation, or root pressure, would have pushed water and the

nutrients contained within up the plant. As ion concentration in roots increased, pressure developed as more water flowed into the roots to dilute the solution, and subsequently the xylem contents were transported upwards into the shoot. Phloem counterflow works in similar fashion. As water carrying sugars moves down the plant and deposits the photosynthates into roots or other carbon sinks, it moves into the xylem and pushes the xylem solution up. The study on sunflower demonstrated that transpiration may not be required for long distance transport of ions in the xylem.

Nitrogen is the most important element in plants besides carbon, hydrogen, and oxygen (Touraine et al. 2001). In terms of minerals derived from soil, N is needed in the greatest abundance, and plants contain 1-6% by weight of this particular nutrient (Crawford 1995, Havlin et al. 2005). N is taken up as NO_3^- and NH_4^+. In the case of NO_3^-, energy is required for its transport into the root cells (Crawford 1995). The electrochemical gradient within the plant is maintained by the exudation of anions such as OH^-, HCO_3^-, and inorganic anions and by the absorption of cations such as Ca^{2+}, Mg^+, and K^+ (Pilbeam and Kirkby 1990, Havlin et al. 2005). NO_3^- is eventually reduced to NH_4^+ – another energy-requiring process that uses nitrate and nitrite reductases – before it can be incorporated into the organic molecules within the plant (Crawford 1995). NH_4^+ is sometimes the preferred N source as the energy-requiring reduction steps are skipped, though high [NH_4^+] is often toxic. When it is absorbed, cation uptake is decreased, uptake of anions such as $H_2PO_4^-$, SO_4^{2-}, and Cl^- is increased, and H^+ is exuded to maintain the electrochemical gradient across the root tissues. However, NO_3^- would be more mobile than NH_4^+ in a soil that is high in organic matter due to more NH4+ being held by cation exchange sites on the organic components of the soil (Havlin et al. 2005). Thus, transpiration rate and subsequent mass flow would affect NO_3^- transport more than it would NH_4^+.

NH_4^+ is assimilated into amino acids, of which proteins are composed. Especially important are the components responsible for photosynthesis. Ribulose bisphosphate carboxylase-oxygenase for example, is considered to be the most abundant protein in the world and is the protein responsible for the first step of assimilating carbon dioxide into glucose. Chlorophyll, the pigment responsible for capturing the light energy that drives photosynthesis, also contains nitrogen in its molecular structure. As such, increasing N supply has often been associated with increasing photosynthetic activity (Havlin et al. 2005, Stulen 1990). It affects the chlorophyll concentration in mesophyll cells, and therefore an adequate supply of this element allows leaf tissues to capture more light and display a dark green color (Lawlor et al. 2001, Havlin et al. 2005).

In terms of growth, ample N increases the number of cells per leaf and can increase cell size. Leaf area increases as a result (Lawlor et al. 2001). Radin and Boyer (1982) found that leaves with low nitrogen had lower turgor and slower leaf enlargement than leaves with high nitrogen. The depressed turgor discouraged increases in cell size. In addition to affecting leaf area, N supply can also affect leaf area index (LAI, ground covered by leaf area). With increasing LAI, more solar radiation is intercepted by the plant and used for photosynthesis (Carlyle 1998, Lawlor et al. 2001). Plants, therefore, grow better (Carlyle 1998).

When plants are N deficient, they have fewer chloroplast components to invest towards photosynthesis. Their growth habits are poorer, their tissues become chlorotic, and they will often have an unthrifty, spindly appearance. Chlorosis often begins in older tissues first as proteins in these areas are broken down and converted to soluble N so the plants may translocate recycled N into newer, active meristematic tissues for use there (Havlin et al. 2005). In sunflower, a ten-fold increase in N supply increased aboveground biomass by almost four-fold

(Cechin and de Fátima Fumis 2004). This was attributed to an increase in leaf production and leaf dry matter as mediated by the N content of the plant.

If transpiration is indeed important for absorption and translocation of nutrients in the xylem, one might expect that high VPD conditions, in turn causing high rates of transpiration, would be conducive to increasing growth, at least for soils that are poor in nutrient content. This study focused on the influences of fertility and vapor pressure deficit on the uptake of N by bell pepper plants and their subsequent growth. It was hypothesized that plants exposed to high VPD would transpire more and take up more nitrogen. Subsequently, for peppers grown in limited nitrogen conditions, greater growth would be observed in those plants experiencing higher transpiration rate due to greater nitrogen uptake. N was chosen for analysis as its uptake was strongly correlated with transpiration in past experiments (Barber et. al 1963, Novak and Vidovic 2003) and for its importance to plant nutrition and physiological function.

CHAPTER 3

METHODOLOGY

Planting Materials

This experiment used Camelot Hybrid Bell Pepper seed obtained from Tomato Growers Supply Company (Fort Myers, FL). During both the fertility study and the transpiration experiment, they were planted in 1-gallon pots filled with pine bark growth media (Goodness Grows Grind, Fern Acre Farms, Washington, GA). Shredded pine bark was chosen for the study due to its low available nutrient content. The pine bark was mixed with pulverized dolomitic lime (Soil Doctor Pulverized Garden Lime, Old Castle Stone Products, Thomasville, PA) at a ratio of 10 g bark to 0.25 g lime to raise the pH to 6.9. The fertilizer used was Scotts Excel Cal-Mag 15-5-15 (The Scotts Company, LLC, Marysville, OH) dissolved in water and dosed out in 200 mL increments once weekly. The fertilizer contained 1.20% NH_4^+, 11.75% NO_3^-, and 2.05% urea. We used a fertilizer low in NH_4^+ as it was expected for that molecule to be bound more by the high cation exchange capacity of the pine bark media, while NO_3^- would have greater freedom of movement. Fertilizer concentration varied depending on fertilizing treatment regimen during both parts of the experiment. Pepper plants were grown in two plant growth chambers (Environmental Growth Chambers, Chagrin Falls, OH) that were controlled for temperature, relative humidity, and photoperiod. During the course of the transpiration experiment, plants and their respective VPD levels were switched back and forth between the two chambers after each harvest period to avoid confounding effects from the growth chambers themselves.

Fertility Study

A preliminary study was conducted to determine an appropriate high nutrient level (HN) and a low nutrient level (LN) to which to expose the plants during the transpiration study. Seeds were planted in the mixed pine bark media, initially fertilized with a small amount of nutrients (0.223 g fertilizer/pot), and allowed to germinate and grow for 18 days.

Forty peppers underwent treatment and were grown at 28°C, 50% RH (VPD = 1.89 kPa), with a 12-hour photoperiod. Eight treatment regimes with 5 plants each were tested. Treatment regimes were as follows: (g fertilizer/pot) 0.045, 0.112, 0.223, 0.334, 0.446, 0.781, 1.115, and 2.23. Plants were fertilized once weekly for 32 days; after which, it was determined that the two highest fertility treatment regimes (1.115 and 2.23) would be used for the transpiration experiment due to the 2 to 4 cm height differences demonstrated by the plants in each regime. Plants fed nutrients at lower levels tended to show much slower growth and often exhibited more severe chlorosis of the leaves.

Transpiration Study

Eighty peppers were grown in two environmental growth chambers set at different humidity levels: low VPD (LVPD) and high VPD (HVPD). Within the chambers the plants were divided into the two pre-determined nutrient treatments. Half of the peppers were fed the low treatment (LN) and half the high treatment (HN). Seeds germinated and grew for 21 days with both chambers set to 28° C, 50% RH, and 12-hour photoperiod. During the 21 days, it was observed that the actual temperature of one of the chambers was 25° C. The rest of the experiment was carried out at 25° C with periodic independent monitoring using an Omega

Temperature and RH sensor (RH 101, Omega Engineering, Inc., Stamford, CT) to ensure similar temperatures between the two chambers.

After the 21 days, plants were redistributed equally between the two chambers. Daytime chamber RH levels were set to 21% (HVPD) and 84% (LVPD); however, both chambers ultimately failed to reach their respective levels. To ease the problem, a dehumidifier was placed in the HVPD chamber, and 2 ultrasonic misting humidifiers were placed in the LVPD chamber. The LVPD chamber later included a third humidifier. The apparatuses were connected to timers so as to not interfere with the nighttime RH levels (60%). Ultimately, the HVPD plants were exposed to a RH range of 30-40%, and the LVPD plants to a range of 55-70%. The corresponding VPD ranges were 1.90 – 2.21 kPa and 0.95 – 1.43 kPa, respectively. The mean VPD during the experiment was 1.98 kPa (HVPD treatment) and 1.20 kPa (LVPD treatment).

Within each VPD treatment, plants were set into blocks of 10 and randomly assigned nutrient treatments (5 LN and 5 HN per block). They were watered with drip irrigation (Dig Corporation, Vista, CA), and each pot contained one ½-gallon/hour drip emitter. Initially, plants were irrigated for 5 minutes daily, but this gradually increased to three times daily as the plants grew. Additionally, plastic plates were placed under the pots during the last three weeks of the study to catch irrigation drippings. This enabled the pine bark to absorb water more efficiently.

A block of plants was harvested from each chamber every 21 days. A LI-6400 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE) was used to obtain net photosynthesis and stomatal conductance (g) from two mature leaves of each plant. One leaf was chosen near the top of the plant, while the second leaf was located mid-stem. A SPAD-502 Chlorophyll meter (Konica Minolta Sensing, Inc., Japan) was used to measure relative chlorophyll content from the same leaves. LiCor cuvette environmental conditions were set to approximate conditions

observed by the Omega RH sensor within each chamber prior to the onset of measurements. During the first harvest (21 days), LiCor gas exchange measurements were made at 65% RH for LVPD plants and 30% RH for HVPD plants. Temperature was 26° C, PAR_{in} was 300 µmol/s, and CO_2 ref was 400 µmol/s. For the second, third, and fourth harvests, general LiCor cuvette conditions were set to T_{leaf} = 25° C, CO_2 ref = 400 µmol, PAR_{in} = 350 µmol. RH settings varied based on Omega RH sensor readings at the time of data collection. Second harvest (42 days) RH settings were 70% for LVPD plants and 40% for HVPD plants. Third harvest (63 days) RH settings were 60% for LVPD plants and 40% for HVPD plants. Fourth harvest (84 days) RH settings were 65% and 40% for LVPD and HVPD plants, respectively.

Prior to the destructive harvest of each block, 24-hour whole plant transpiration (by weight) was measured in all of the plants in the block. Drip emitters were removed, the plants were watered by hand, and they were allowed to drain for a few hours before the first mass measurement. They were weighed again 24 hours later to determine water lost to transpiration. Two control pots per chamber without plants were also treated in this manner to determine evaporation from the potting media. The amount of water that evaporated was averaged between the two control pots in each chamber and subtracted from measurements obtained from planted pots to remove soil evaporation from the calculations of whole-plant transpiration.

Growth data were collected during the destructive harvest of each block. Diameter measurements were taken below the cotyledons with digital calipers. Height was measured with a meter stick from ground level to the apical bud of the tallest branch. Leaves were stripped from the peppers and leaf area was determined using a LI-3100 Leaf Area Meter (LI-COR, Inc., Lincoln, NE). Stems were cut at ground level, and the aboveground tissues were placed in a drying oven at 60° C to dry. Pots were stored in a cooler at 38° F until they could be washed and

dried. They were weighed for dry mass data. Stem, leaf, and flower bud tissues were ground for nitrogen analysis. Stems and flower buds were ground with a Wiley Mini-Mill (Thomas Scientific, Swedesboro, NJ); leaves were ground with a SPEX CertiPrep 8000-D Mixer Mill (SPEX CertiPrep, Inc., Metuchen, NJ). Tissue samples were placed in elemental reagent tins and combusted and analyzed for N and C content with an NC2100 elemental analyzer.

Data were analyzed using two-way ANOVA via the Proc GLM procedure in SAS 9.1. The first treatment factor was VPD, and the second factor was fertility treatment. Significance was determined at $p<0.05$ and means were separated using the Tukey test.

CHAPTER 4

RESULTS

In general, differences in growth observed among the four treatment regimes (high VPD/high N, high VPD/low N, low VPD/high N, and low VPD/low N) were significant for fertility treatment but not VPD. Plants exposed to LVPD/HN conditions had greater height growth, but the difference from HVPD/HN was negligible, and not statistically different (Fig. 1). Analysis of fertility and VPD indicated that height differences between the four plant groups were generally due to nitrogen level rather than VPD. Diameter was not significantly different during harvests 1 and 2 (Fig. 2). During harvests 3 and 4, diameter was greatest for plants subject to high fertility regimen. There were no differences between the VPD treatments. Leaf area was affected by nutrient level during harvests 2, 3, and 4 (Fig. 3). Again, VPD had no effect.

Differences in whole-plant dry weight observed during harvests 3 and 4 were attributed to fertility treatment rather than effects from VPD (Fig. 4). Aboveground dry weight was affected by fertility treatment in harvests 3 and 4 (Fig. 5). Plants studied during harvest 4 indicated that the plants grew best when exposed to both low VPD and high nutrient levels; however, further analysis (not shown) did not indicate an overall humidity effect on the plants except during harvest 2. During harvest 3, plants exposed to LVPD/HN conditions had a greater percent aboveground dry weight than plants grown in HVPD/LN conditions, indicating that fewer resources were allocated to the root system. For the other two treatments, differences were negligible compared to the LVPD/HN and HVPD/LN conditions. During harvest 4, plants

exposed to LVPD/HN again had the greatest percent aboveground biomass (Fig. 6). The differences were attributed to fertility treatment rather than VPD.

Water lost via transpiration was affected by VPD and fertilizer. Water loss during harvest 3 was negatively affected by low fertility only, whereas water loss during harvest 4 was negatively affected by both low VPD and low fertility (Fig. 7). Total water loss was greatest for plants in the high nutrient and high VPD regime during harvest 4. High nutrient fed plants transpired 27% less water and low nutrient fed plants transpired 34% less under LVPD conditions. The results of harvest 3 indicate the possibility that VPD was not different between the two chambers during that particular 24-hour observation period (Fig. 7 and 8). Transpiration per leaf area was greater for plants exposed to HVPD during harvests 2 and 4. There was no effect from fertility regime (Fig. 8).

SPAD levels were not different among the four treatments during harvests 1 and 2 (Fig. 9). Differences noted during the third and final harvests were attributed to fertility. Harvest 4 plants indicated a VPD effect on chlorophyll concentration, but this was not confirmed by a separate VPD analysis (not shown). Photosynthesis measurements were never consistent across the four harvests. A possible trend, however, was noted for plants exposed to HVPD/HN conditions, as plants in that regime tended to have the highest photosynthetic rate, though negligibly so (Fig. 10). There was a general decrease in photosynthesis as the experiment progressed. Analyses of fertility or VPD effects were either insignificant or inconsistent. Like photosynthesis, stomatal conductance was also inconsistent across the four treatment regimes, and differences could not be attributed to any single factor (Fig. 11).

Analyses of nitrogen content in the aboveground portions of the plants showed that differences in nitrogen absorbed were due to fertility conditions (Fig. 12). VPD did not have a statistically

significant effect on total N uptake during any of the harvest periods. The ratio of nitrogen allocated into the leaves verses the stems showed no differences among the four treatment regimes except during harvest 3 (Fig. 13).

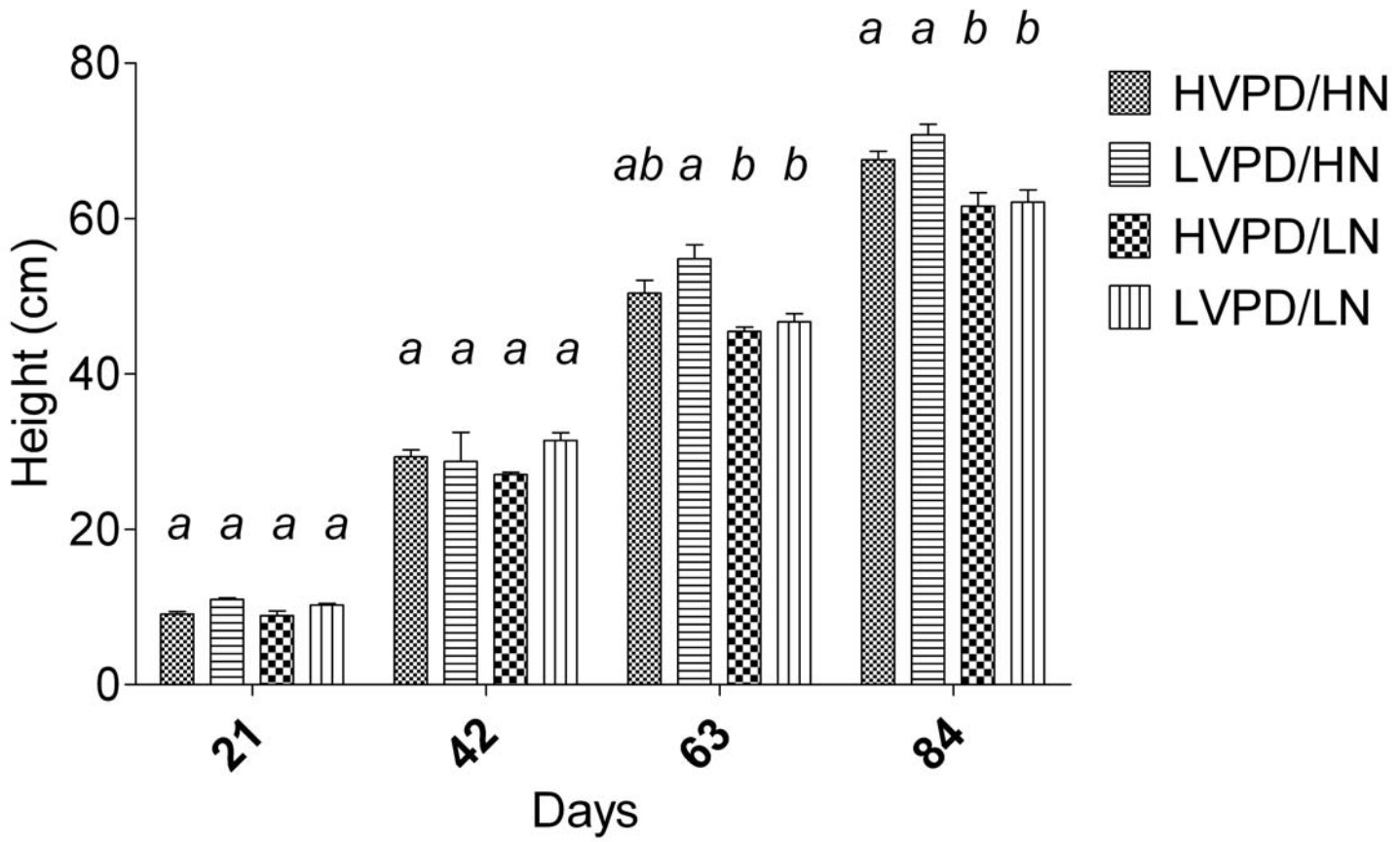

Figure 1: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and height growth of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

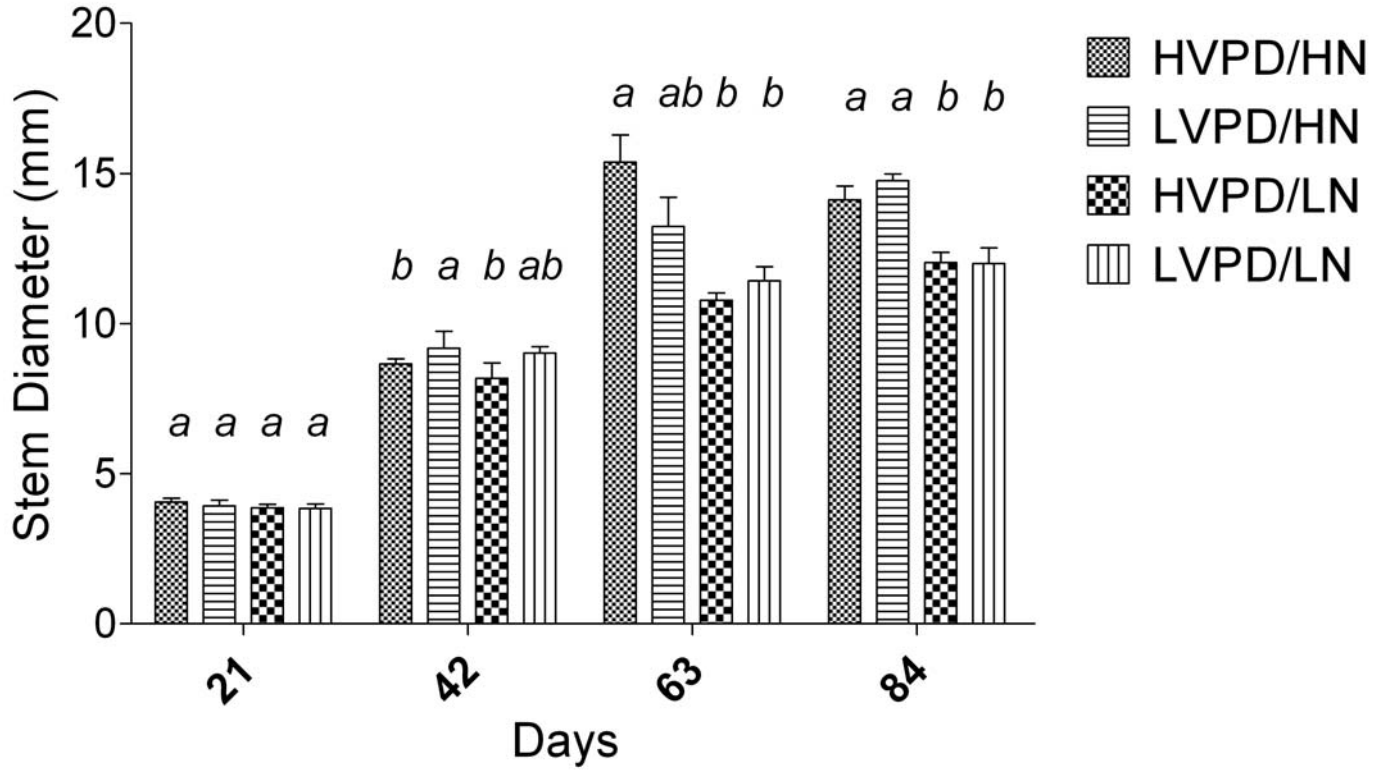

Figure 2: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and diameter growth of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

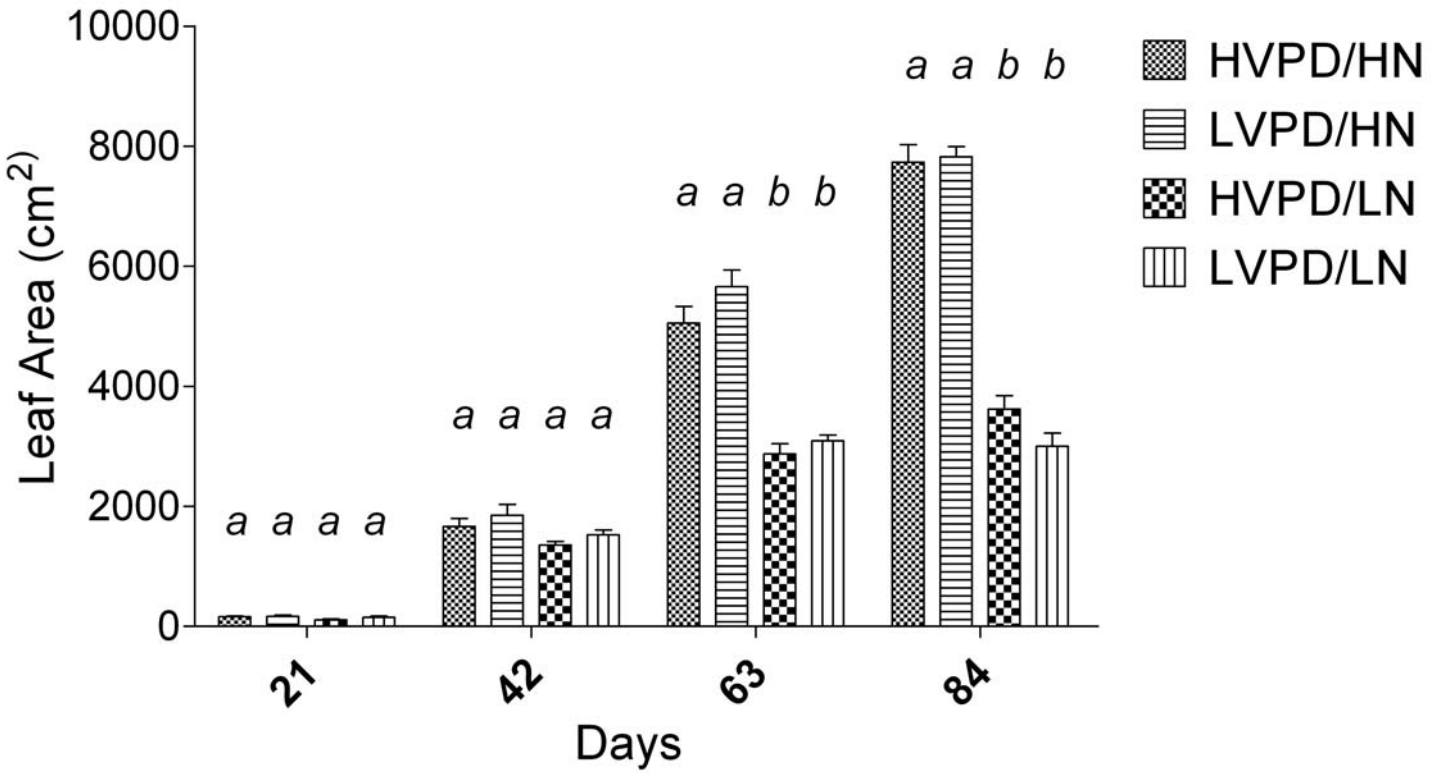

Figure 3: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and leaf area of pepper during four different harvest periods. Means with same letters are insignificant among harvest periods ($p<0.05$).

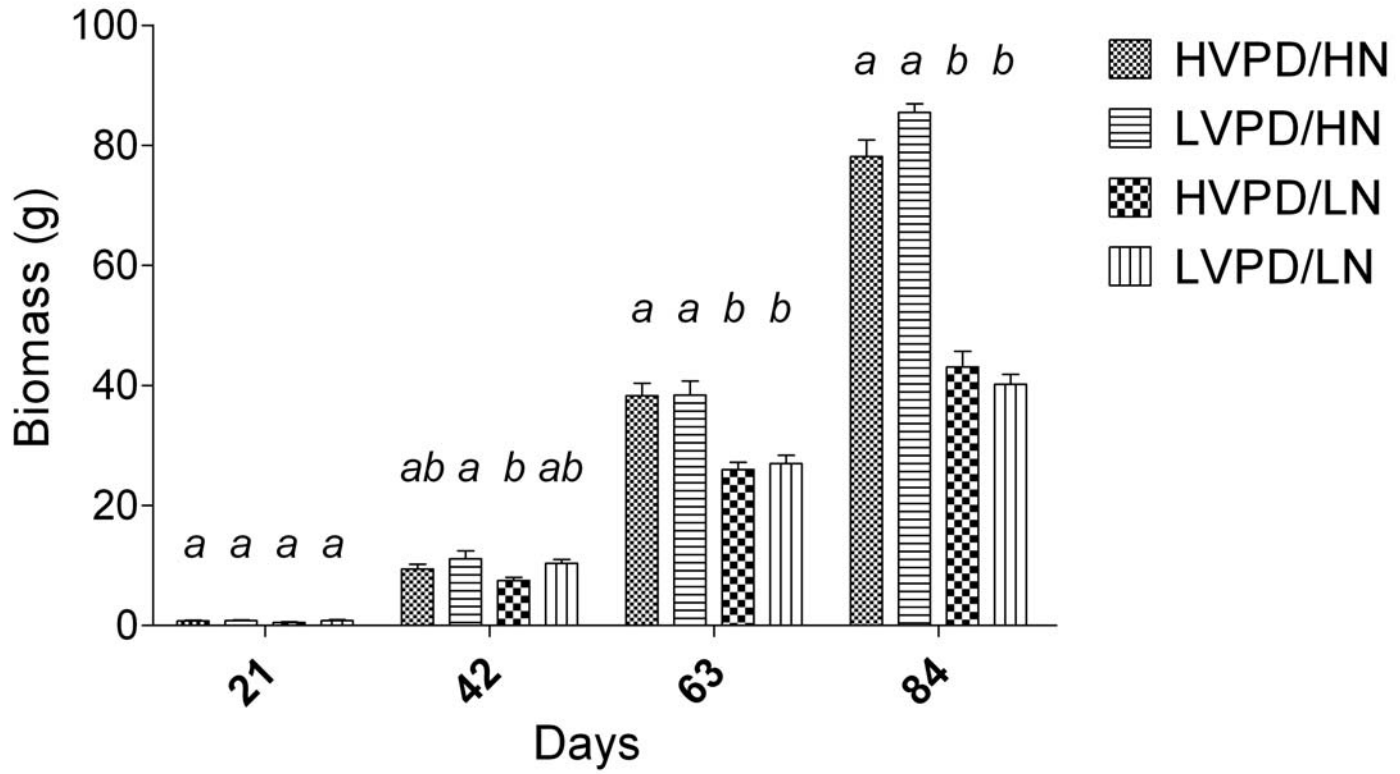

Figure 4: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and total dry weight of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

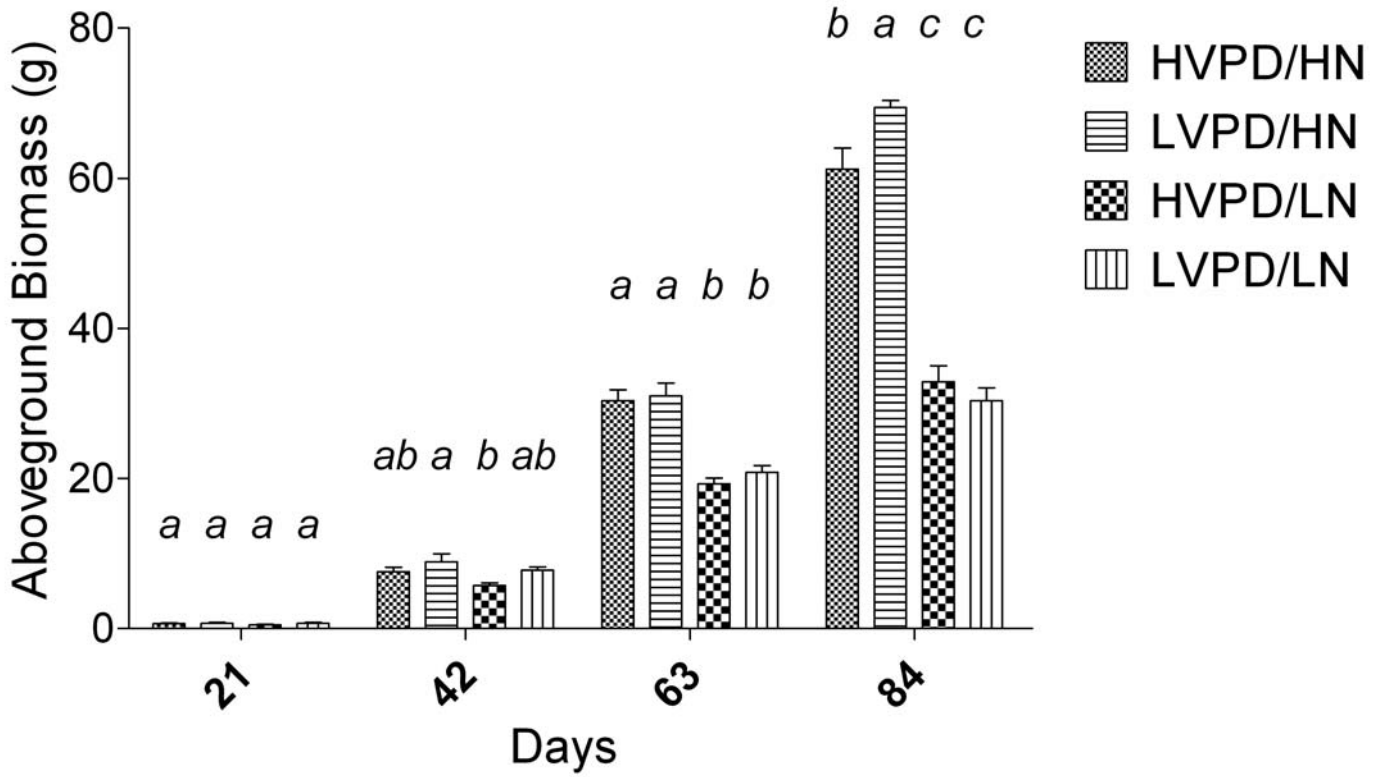

Figure 5: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and aboveground dry weight of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

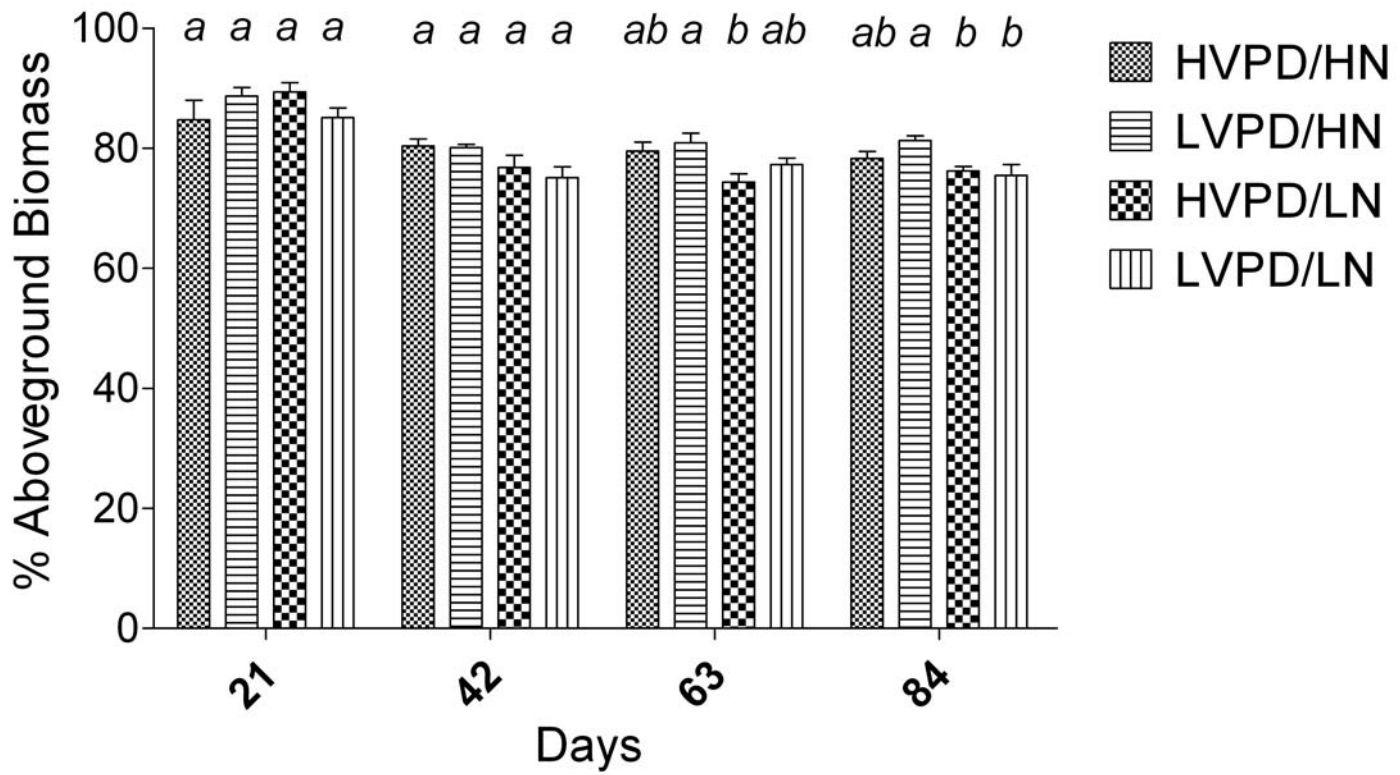

Figure 6: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and % aboveground dry weight of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

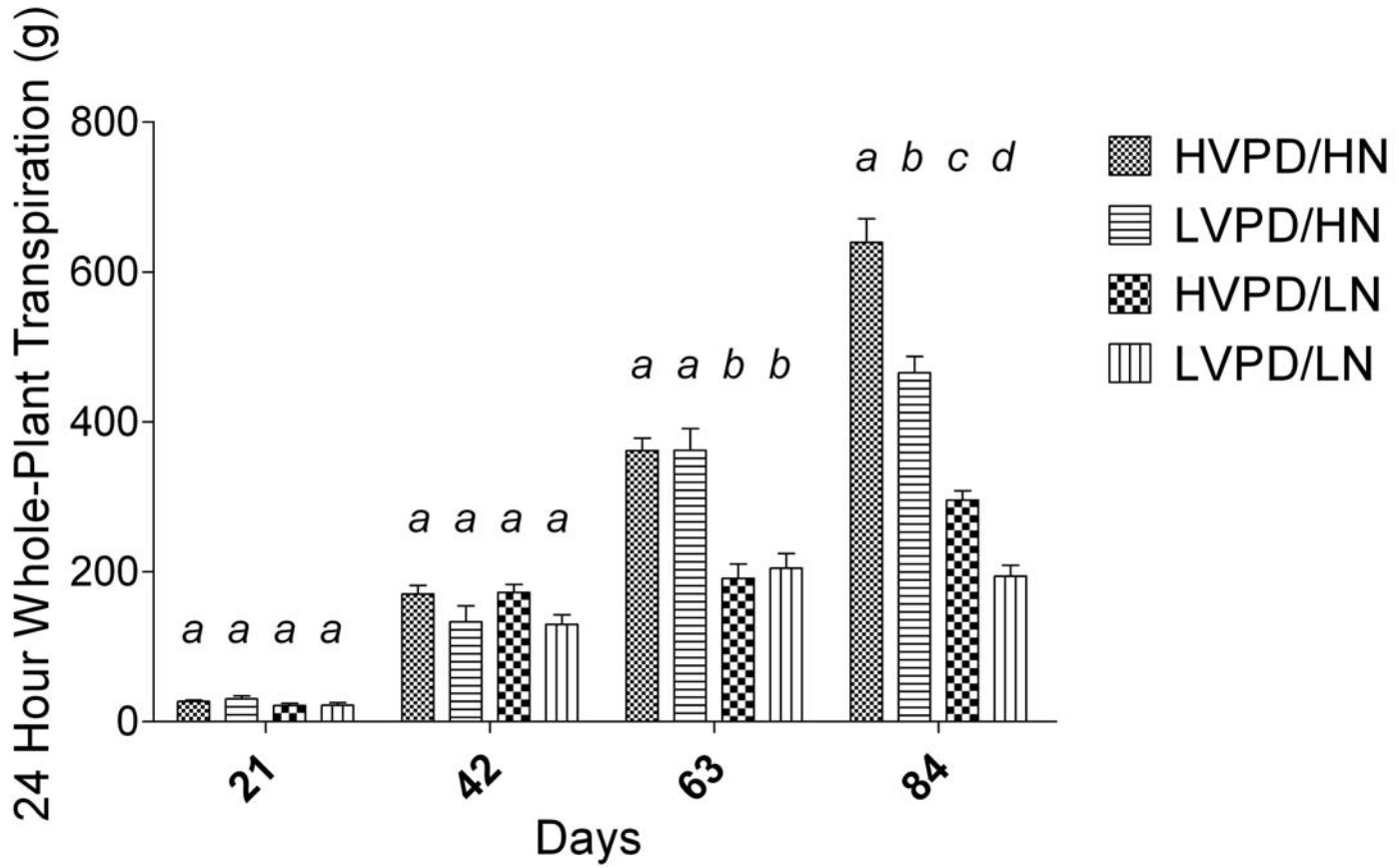

Figure 7: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and whole-plant transpiration of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

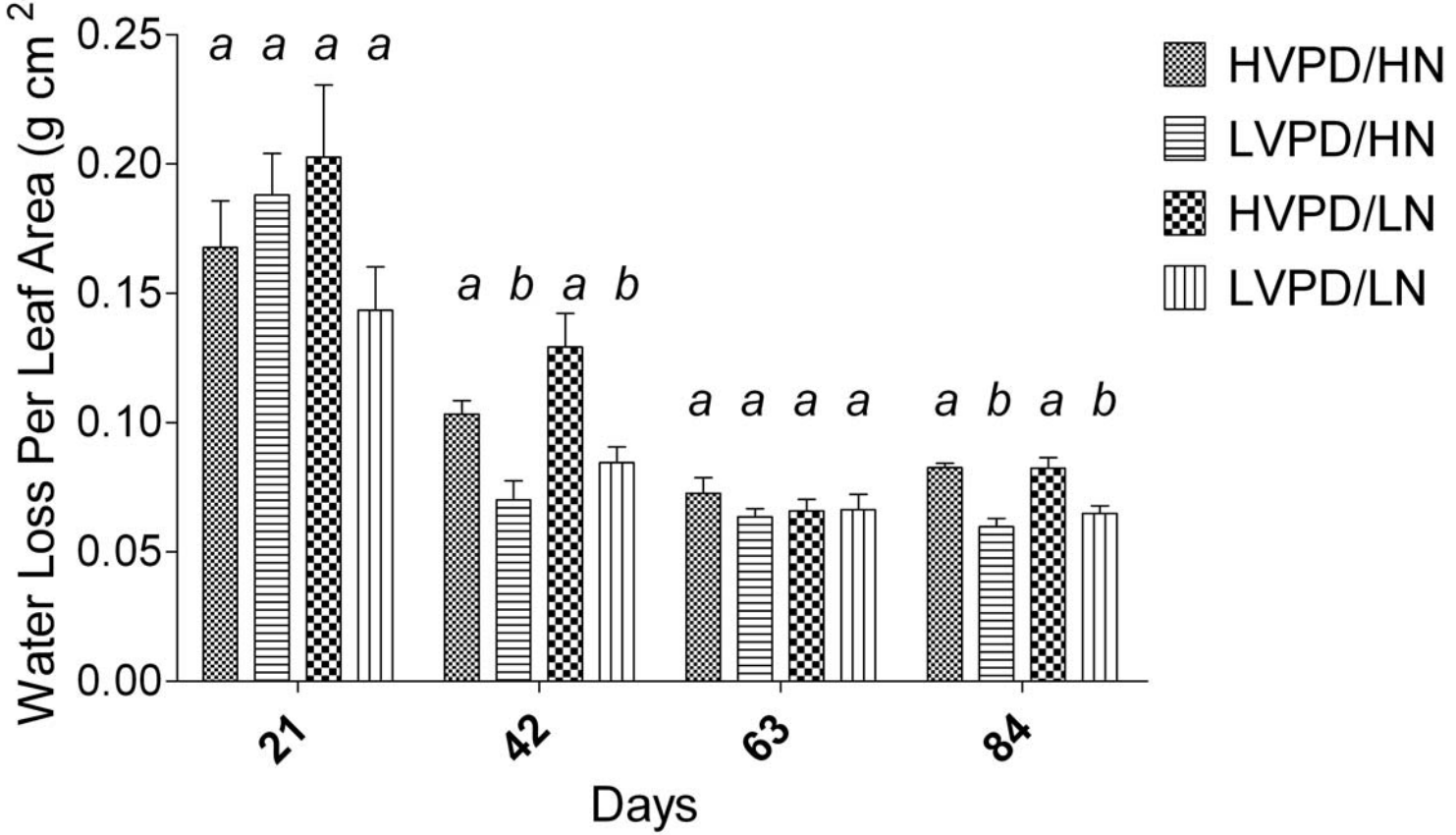

Figure 8: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and transpiration per leaf area of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

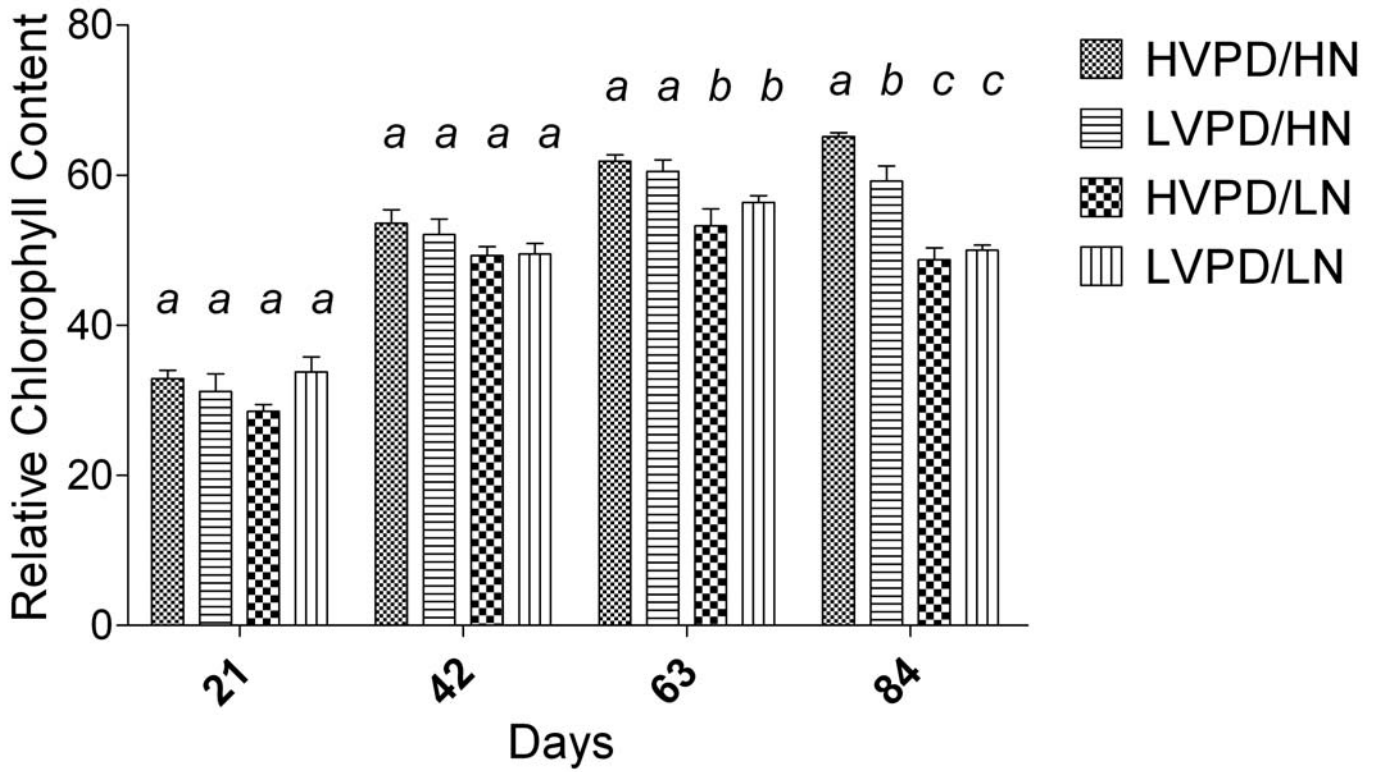

Figure 9: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and SPAD levels of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

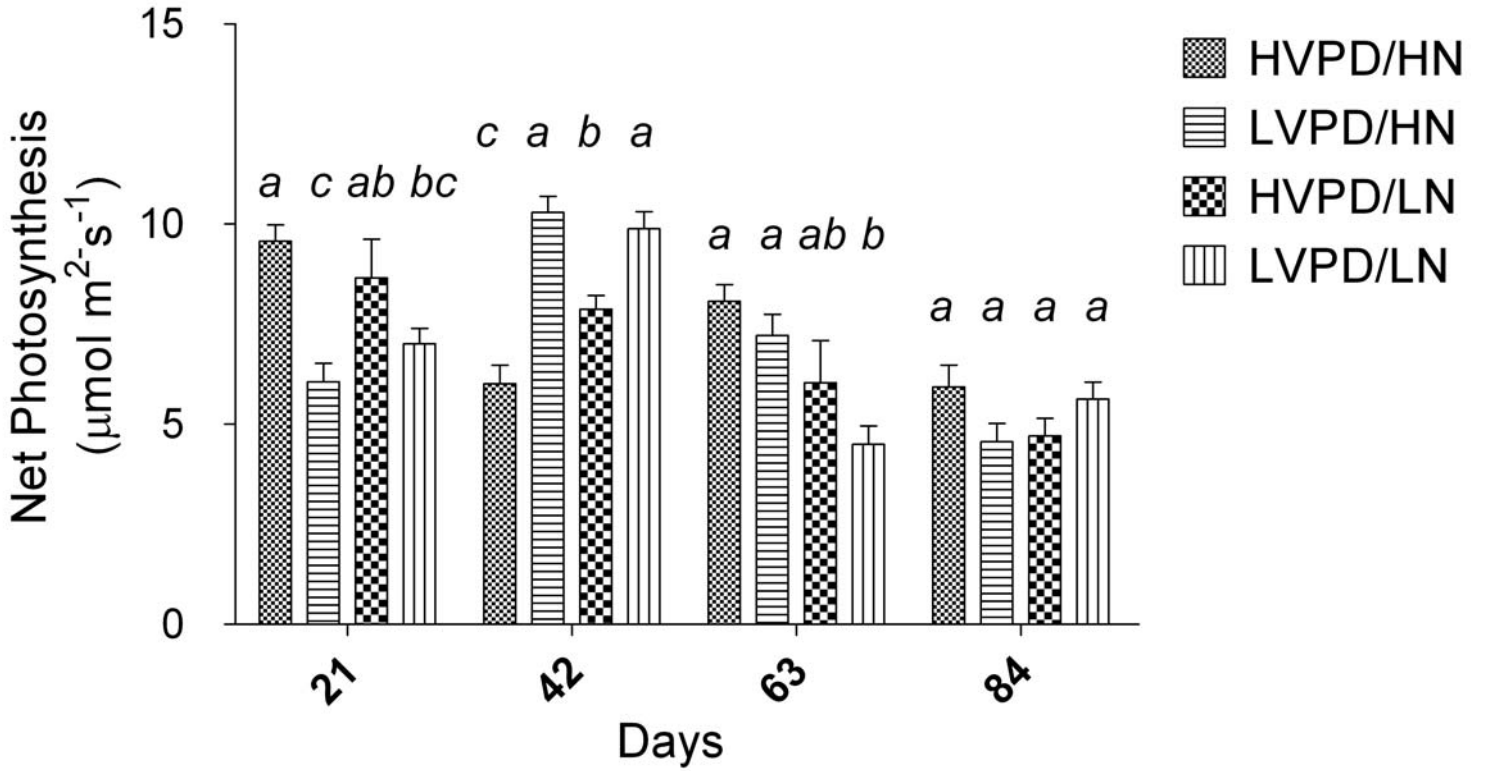

Figure 10: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and net photosynthesis of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

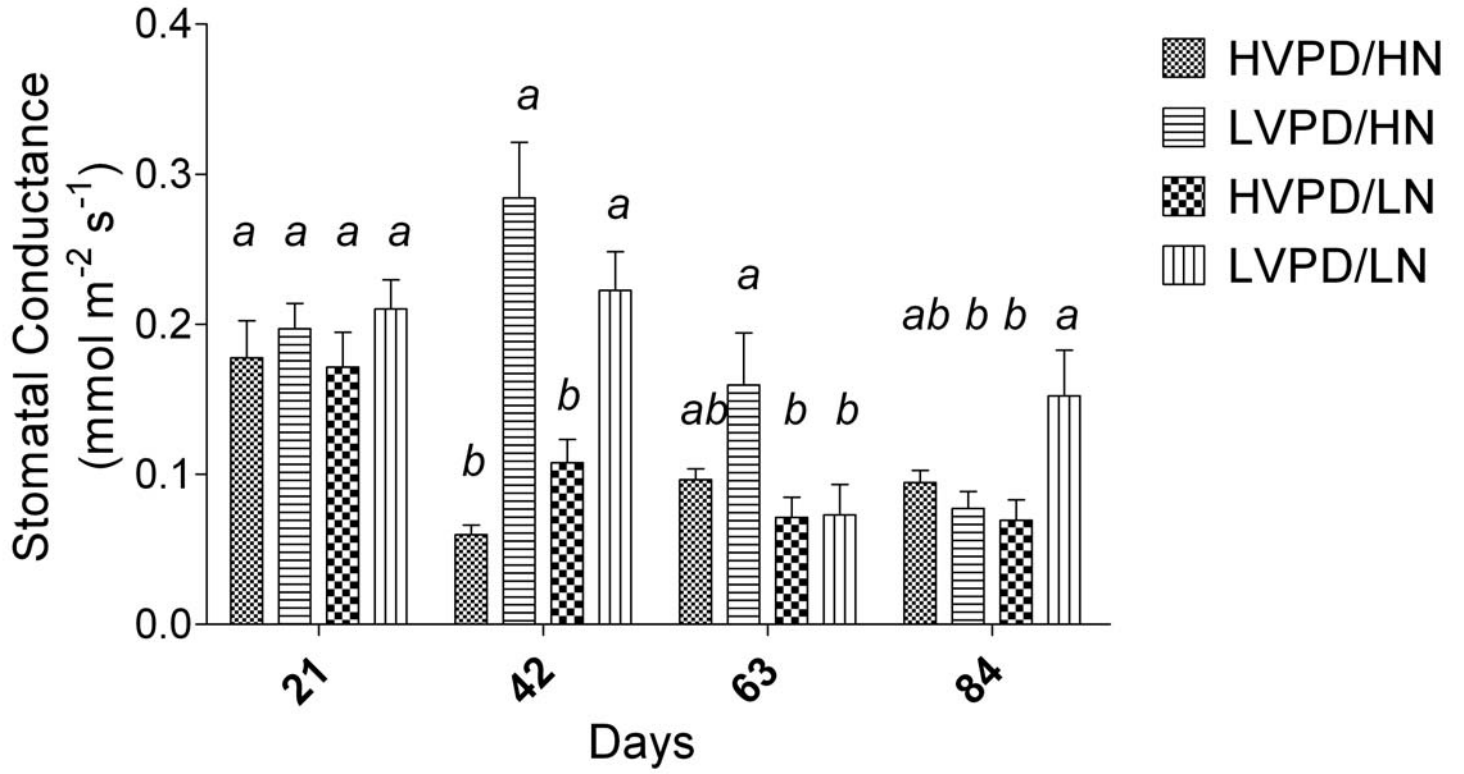

Figure 11: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and stomatal conductance of pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

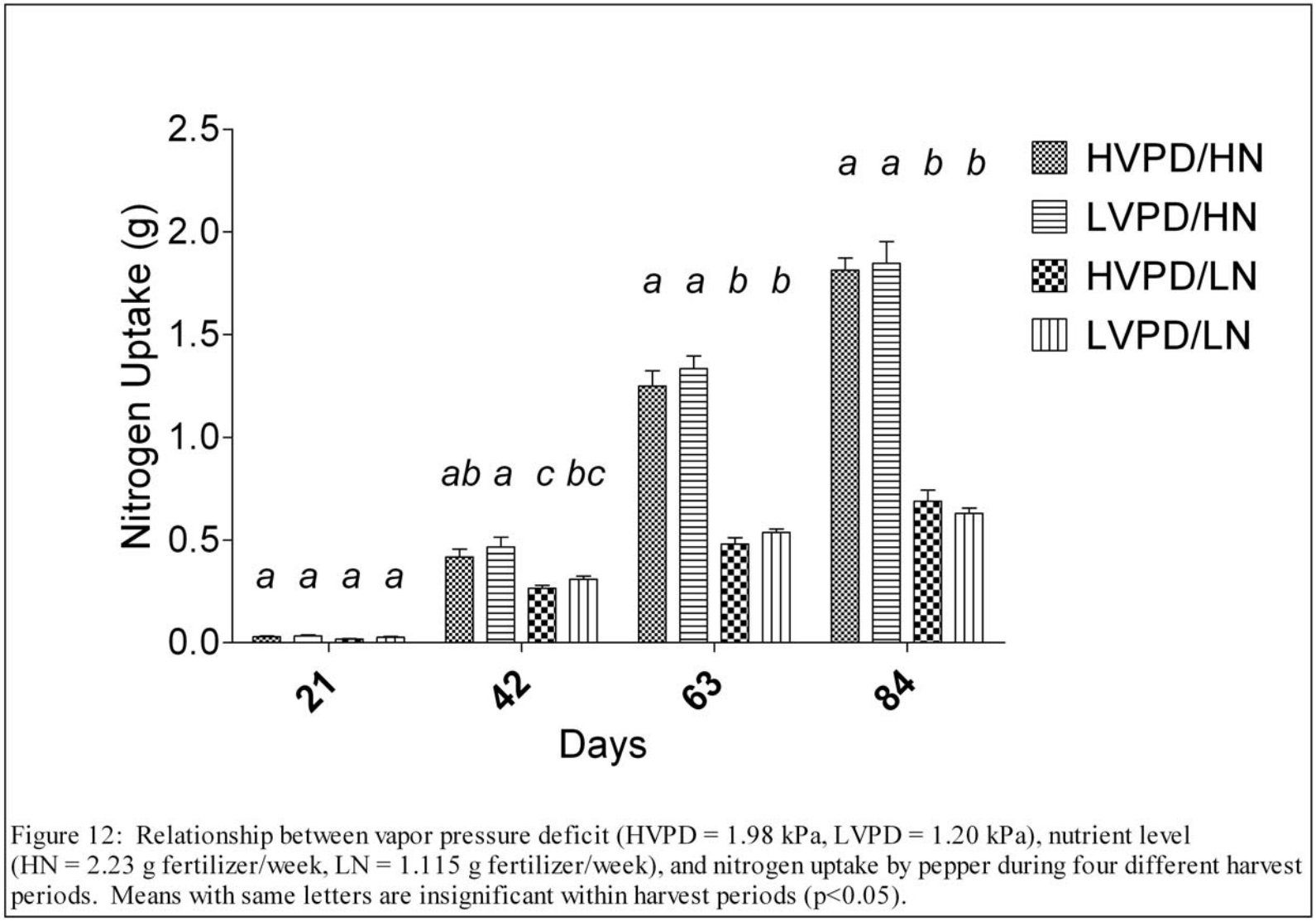

Figure 12: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and nitrogen uptake by pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

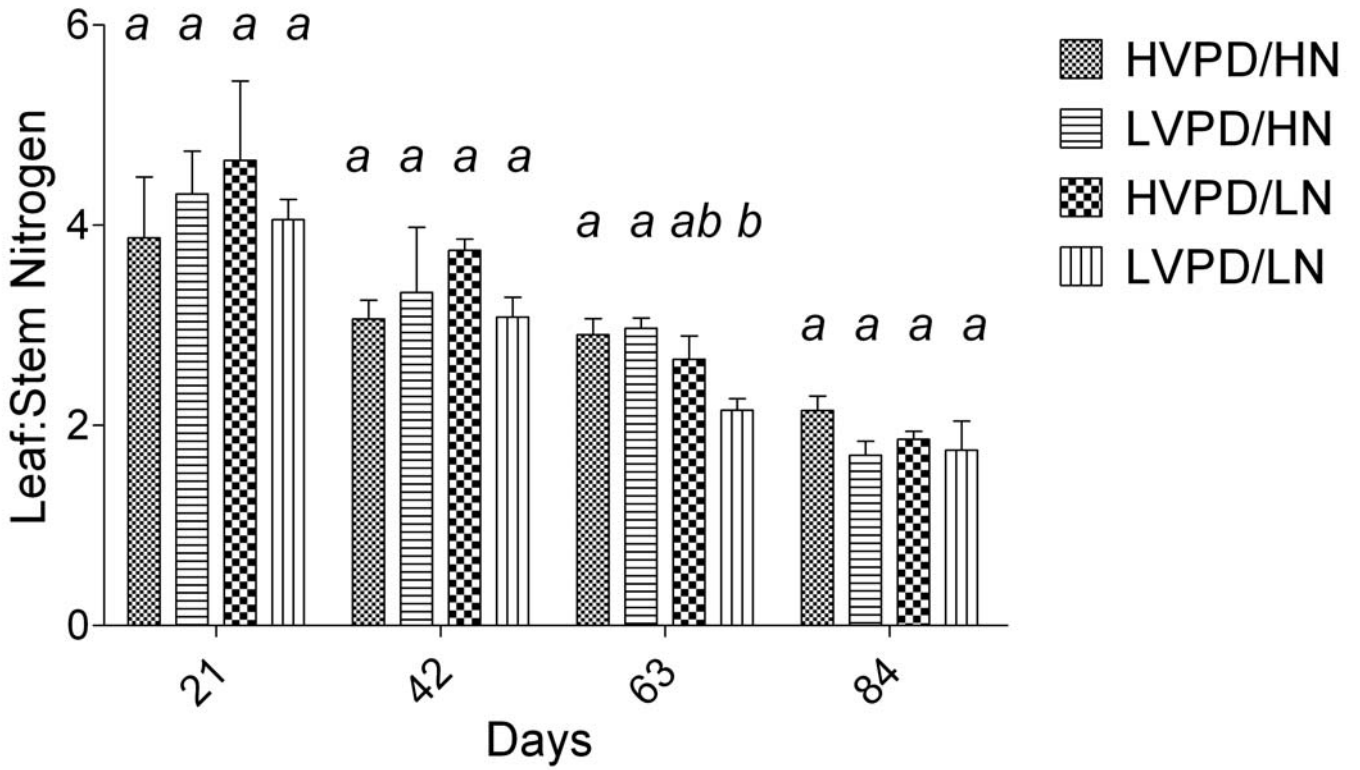

Figure 13: Relationship between vapor pressure deficit (HVPD = 1.98 kPa, LVPD = 1.20 kPa), nutrient level (HN = 2.23 g fertilizer/week, LN = 1.115 g fertilizer/week), and leaf to stem nitrogen ratio in pepper during four different harvest periods. Means with same letters are insignificant within harvest periods ($p<0.05$).

CHAPTER 5

DISCUSSION

The growth results obtained from the experiment did not support the hypothesis that higher VPD and transpiration would positively affect growth. Height, diameter, leaf area, and dry weight were influenced by fertility rather than by VPD. Peppers grown in limited nutrient conditions did not benefit from high VPD. For plants exposed to non-limiting nutrient conditions, low VPD appeared to have a slight positive affect on biomass growth during the final harvest period. In contrast to most of the results, however, Armstrong and Kirkby (1979) demonstrated faster growth by tomatoes when grown at 95% RH, though ultimately, dry weight was the same between the humidity treatments by the end of the experiment. Ford and Thorne (1974) also showed positive effects on growth by increasing air humidity for sugar beet (*Beta vulgaris*), kale (*Brassica oleracea),* and wheat (*Triticum aestiva*). The growth effects were variable. Leaf area and dry weight were influenced to varying degrees, but overall, they were positively impacted by increasing RH in sugar beet and kale. For 3 of the 6 experiments on wheat, increasing RH increased dry weight and leaf area. In the case of increasing leaf area, Ford and Thorne (1974) found that increasing the RH increased the leaf area of individual leaves rather than encouraging an increase in the number of leaves. Mortensen (1986) found that in different species of greenhouse plants, the response to increasing RH (55-60% to 70-75% or 90-95%) was not uniform across species. Six species exhibited a positive effect on dry weight, whereas, 4 species remained unaffected. The species unaffected were bellflower (*Campanula*

isophylla), rose (*Rosa* 'Mercedes'), cucumber (*Cucumis* 'Farbio'), and lettuce (*Lactuca* 'Salina'). An herb, *Soleirolia soleirolii*, was negatively affected by increased RH.

As previously mentioned, high humidity had a positive effect on aboveground biomass during the 4th harvest when plants were grown at high nutrient levels. However, del Amor and Marcelis (2004 and 2006) found that tomatoes exhibited a decrease in dry weight and leaf area as RH was increased from 70% to 95%. There was no change in those two growth parameters as RH increased from 50 to 70%. This contradicts the results of Armstrong and Kirkby (1979), thus indicating that even within the same species, VPD can have a variable effect on growth, or that experimental conditions played a role in the outcome of the studies.

Percent aboveground dry weight results indicated that more of the pepper plants' internal resources were directed to the roots when media fertility was low, but VPD had an insignificant effect on allocation. MacDonald et al. (2002) postulated that plants would compensate for low transpiration-mediated N supply by changing their root morphological, carbon allocation, and physiological traits. From an allocation standpoint, this does not appear to be the case with the peppers in this experiment.

SPAD measurements – an indicator of N content in the leaves – were not significant between VPD treatments. While the assumption that transpiration mediates translocation of N from the root to the shoot and leaves is reasonable, it was not supported by the results in this experiment. Instead, nutrient supply affected SPAD. Many studies prior to this one found different results. MacDonald et al. (2002) reported that increasing RH decreased total N and ^{15}N accumulation by cottonwood. Novak and Vidovic (2003) found that maize (*Zea mays*) took up more nitrogen, phosphorus, and potassium as canopy transpiration rate increased. A study on the uptake of copper and zinc by buckwheat (*Fagopyrum esculentum*) showed that Cu and Zn

increased with increasing transpiration rate (Tani and Barrington, 2005). On the other hand, a few other studies found no relationship between various nutrients and transpiration. del Amor and Marcelis (2006) also found no relationship between transpiration and calcium uptake in tomato. Armstrong and Kirkby (1979) found that though tomato leaves appeared to have a boron or calcium deficiency when exposed to low VPD, the overall cation content of the plants was not influenced by humidity. Low VPD did, however, restrict distribution of calcium to the stem tissue. Ford and Thorne (1974) found that decreasing VPD did not appear to restrict nutrient uptake by plants, even when nutrient supply was low. There were no differences in leaf to stem N ratios, indicating that transpiration has little translocational power despite significant differences in transpiration rate. Tanner and Beevers (2001) postulated that mineral ions could be transported long-distance within the plant by guttation and phloem counterflow, which could be possible explanations for the lack of response to differences in rates of transpiration.

The variability in measurements of net photosynthesis across sampling dates and treatments was inexplicable. In the case of this experiment, photosynthesis did not follow the trends observed in leaf chlorophyll content or in the overall plant N uptake in which [N] in leaves is often positively correlated with increasing photosynthetic rate (Reich et al. 1995, Cechin and de Fátima Fumis. 2004).

Stomatal conductance, like photosynthesis, was highly variable. At times, it appeared to be affected by VPD, with high VPD correlating with a lower g as expected. However, the effect of VPD was negligible at best during most of the harvest periods. This observation disagrees with those of Lange et al. (1971) and Lösch (1977) who found marked responses of stomata to dry air. In the case of this experiment, it is possible that VPD was not high enough in the low humidity chamber for us to see a significant negative response of g to increased VPD. The

plants may have been able to maintain sufficient water potential to keep leaf cells turgid despite higher transpiration in HVPD conditions. Stomata would have remained open, and leaf cells would have expanded at the same rates between the two VPD conditions if turgidity and water potentials were similar enough. If plants maintained similar turgidity, that may explain the similar leaf areas between the two VPD levels. Along the same line, light interception would have been similar and possibly would have translated to similar growth rates (Carlyle 1998).

Whole-plant transpiration was affected by both fertility and VPD. Increasing VPD increased the evaporative demand experienced by the plants. Thus, they lost more water from their stomata. Increasing fertility also increased transpiration by increasing leaf area from which water transpired. Transpiration per unit leaf area also showed a higher rate of water loss when plants were exposed to high VPD. Since high VPD affected transpiration without affecting N uptake, one could conceivably lower the transpiration rate of plants without detrimentally affecting absorption or translocation of nutrients (Gale and Hagan 1966, Tanner and Beevers 2001). In fact, under non-limiting nutrient conditions, high transpiration mediated by high VPD appeared to have a negative impact on aboveground biomass by the end of the experiment, though this was not reflected in total biomass. In limiting nutrient conditions, VPD had no effect on biomass. Overall, other growth parameters such as height, diameter, and leaf area were not impacted by VPD or transpiration. This further indicates that peppers may be neither detrimentally nor beneficially affected by humidity, at least for the two VPD levels studied.

The mass flow mediated by transpiration in the low VPD treatment may have been sufficient for bringing nitrogen into contact with the rhizosphere. Transpiration in and of itself does not pull N into the plant. Rather, transport of NO_3^- into the root xylem is an energy–requiring process. Additionally, nitrogenous compounds are often more abundant within the

xylem than in the soil, therefore, they have to be transported against the gradient. Since N will not diffuse into the plant with the transpiration stream, the ability of transpiration to translocate N from the soil is reduced.

Overall, the lack of effect of VPD on growth in this experiment was surprising as many of aforementioned studies have indicated some sort of effect, whether positive or negative. Armstrong and Kirkby (1979), Ford and Thorne (1974), and Mortensen (1986) all showed positive responses of plant growth to humidity. Armstrong and Kirkby (1979) found that an increase of RH from 50 to 95% gave plants an initial boost in growth, but by the end of the experiment, dry weight was not affected. Mortensen reported a dry weight increase from 17-68% when RH was raised from 50-55% to 70-75 or 90-95% in six species.

del Amor and Marcelis (2005 and 2006) in two studies on tomato reported a negative effect of increasing humidity on growth. They found an 11.4% decline in dry weight when RH was increased from 70% to 95% (2005). In 2006, they found an 11.3% decline in dry weight and 15.9% decline in leaf area between the same RH levels. The current experiment did not reach 95% RH, thus a comparison between the results found here and in del Amor and Marcelis' research may be weak. Had the peppers experienced such a high humidity, the results may have been similar. Mortensen (1986) found a negative response of growth in high humidity in one greenhouse plant studied. *S. soleirolii* dry weight declined by 19% when RH was increased from 50-55% to 90-95%. However, four other species studied by Mortensen showed no response.

The clearest positive influence on growth during the experiment was mediated by fertilizer supply. As nitrogen and other nutrients was increased in the soil solution, growth increased for height, diameter, leaf area, and biomass. This was expected based on previous research and knowledge (Cechin and de Fatima Fumis 2004, Lawlor et al. 2001, Radin and

Boyer 1982). The lack of effect of transpiration on N uptake may be the reason why there was no concurrent effect on growth. This is especially clear in limited nutrient conditions. If transpiration had a transport effect on N, increased N in the deficient plants would have increased growth and health of those plants.

CHAPTER 6

CONCLUSION

The objective of this paper was to investigate the influence of transpiration on the uptake of nitrogen by pepper plants. Nitrogen was chosen as the nutrient of interest because it had been highly correlated with transpiration rate in previous research (Barber et al. 1963, Novak and Vidovic 2003). Though higher transpiration rate as mediated by high VPD was expected to increase N uptake and therefore encourage growth of the plants, this proved not to be the in this study. The peppers showed little response to the two humidity levels to which they were exposed, whether from a N uptake standpoint or growth standpoint.

This may be a case of VPD not being different enough in the two growth chambers, as daily whole-plant transpiration was not always significantly affected by humidity levels. Transpiration per unit leaf area was generally significant between humidity levels, though, indicating that water loss from plants can be reduced without detrimental effects to growth and nitrogen uptake.

Gale and Hagan (1966) supposed that transpiration could be reduced by half without negatively affecting growth. This study brings into consideration their research on anti-transpirants. If transpiration can indeed be reduced without harming the plants, anti-transpirants could prove important for crop and greenhouse plant cultivation. There are anti-transpirants available on the market that supposedly reduce plant demand for water, thus decreasing the need for irrigation. As the world's population grows along with food demand, the need for more efficient use of water resources also grows. Either by anti-transpirant uses or by breeding and genetically

modifying crop water use efficiency even further, the idea that a plant is not entirely dependent on transpiration-mediated nutrient uptake could prove very useful to farmers and horticulturists for keeping their water demands relatively low.

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