

Abstract

OVERSTREET, LAURA FLINT. Boron Deficiency and Chilling Injury Interactions in Tobacco Transplants Grown in the Float System. (Under the direction of James W. Rideout.)

Decades of agricultural research have failed to determine the precise mechanisms of infliction caused by the conditions of boron deficiency and chilling injury. Both conditions affect the quality and marketability of tobacco transplants grown in the float system. Interestingly, boron deficiency and chilling injury produce strikingly similar symptoms in young tobacco transplants; so similar, in fact, that they are often confused for one another. This has led to severe boron toxicity when growers treated chilling injury as boron deficiency by applying boron to non-deficient float beds. The observation of nearly identical symptoms suggests that boron deficiency and chilling injury have interdependent effects on cell physiology and/or metabolism. Because little research has been conducted on tobacco transplants in the float system, two studies were conducted to determine general parameters for the boron deficiency threshold and effect of non-optimal temperatures and large day/night temperature differentials in this system. The boron deficiency study established that the deficiency threshold for transplants growing at 26/22° C is 10-20 $\mu\text{g B g}^{-1}$ dry matter. These tissue levels occurred with solution concentrations of 0.19-1.9 $\mu\text{M B}$. The chilling injury study determined that root and shoot growth of flue-cured cultivars is near maximum at a constant 26/26° C temperature regime. Burley

cultivars display a wider range of temperature tolerance, but in general constant day/night temperatures seem to provide the greatest shoot tissue accumulation. A reduction in night temperature resulted in decreased shoot growth in all cultivars. The chilling injury study also examined the effect of boron deficient conditions at each temperature treatment. In general, boron uptake declined at sub-optimal temperature regimes when supplied at concentrations sufficient for near-optimal temperatures. Shoot growth of flue-cured varieties at transplant stage was near maximal at a constant optimal day/night temperature regime (26/26° C) and adequate B concentrations. Sub-optimal temperatures may alter the boron deficiency threshold such that it decreases with decreasing temperatures or with stressful temperature differentials. This may be summarized in the following way: Temperature is the immediate limiting factor in tobacco transplant growth in the float system under conditions of sub-optimal temperatures and low B concentration, and B deficiency is an additional potential limiting factor.

Boron Deficiency and Chilling Injury Interactions in Tobacco Transplants Grown in the Float System

By
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Dedication:

This thesis is dedicated to my mom and dad, David and Jacqueline Flint.

The blessings and benefits I enjoy in this world are all grounded in the two individuals who raised me. Both colorful in their own ways, my parents breathe life into everything they do. They find meaning in the mundane and beauty in everyday. They are the greatest teachers I have ever studied under.

When people say I turned out alright, I say, "I'm from good stock." When people say I could have turned out better, I say, "I'm getting better all the time; someday I'll be as great as my parents."

Biography:

Laura Flint Overstreet grew up in Granite Falls, North Carolina, a small town in the Western part of the state, in the foothills of the Appalachians. She is daughter of David and Jacqueline Flint and Lucee Flint is her younger sister. Laura graduated from South Caldwell High School in 1993. She attended North Carolina State University directly out of high school with a declared major in engineering. She toiled through two years of engineering courses before she realized that her lack of spatial abilities would see to it that she never receive a grade of C or better from Dr. Eli in Statics and Mechanics, an accursed and boring subject. At said time she switched her major to botany and determined that she was much better suited for this field. She greatly enjoyed her botany courses as an undergraduate, performed an undergraduate research study in the area of allelopathy under the astute direction of Dr. Udo Blum, and was awarded the Larry A. Whitford Botany Scholarship in Fall 1997. She received her B.S. in Botany in December, 1997.

While floundering in her engineering courses, she procured a job washing lab ware in Weaver Hall for the Biological and Agricultural Engineering Environmental Analysis Laboratory under the management of Mrs. Rachel Huie. She kept this job after switching over to the botany department and remained working there for several months after graduating. In the course of time spent working in the

BAE environmental analysis lab, Laura gained responsibilities in addition to dish washing. She learned the ins and outs of a fair number of analytical equipment and, more importantly, learned to think somewhat analytically and methodically.

Shortly after graduation, while she was working full time in the laboratory, Laura accepted the marriage proposal of her high school sweetheart, Mr. Chris Overstreet. As Chris was (and still is) a musician, Laura felt it time to be moving on professionally and finding a job that would help support her and her musician husband until such time as he would “make it big” in the industry as a national recording artist.

Laura felt she would like to try her hand at something more field-oriented than the lab work the BAE labs provided and began the arduous task of sending out resumes. She was interviewed for a position as an agricultural extension agent for Davie County, but it became clear later that she was likely chosen as a candidate only to make the person already favored for the position look a little better. Not because she was inept or otherwise dumb, but certainly she was inexperienced for this position and most likely unqualified. Idealist that she is, she had hoped that she would be given a chance regardless of these sizable drawbacks, and was consequently disappointed when not hired.

Pressing on, she resorted to sending her application to several vineyards in the state when nothing more lucrative presented itself. After one unproductive and one comically disastrous interview at different vineyards, she was finally contacted by Marty York at the Biltmore Estate Vineyards in Asheville, NC, adjacent to the illustrious Biltmore Estates. She was offered a job paying \$6.00 an hour picking grapes for the remainder of the grape harvest. Laura accepted, packed up her stuff, left her fiancée in Raleigh, and moved across the state to Asheville and commenced to pickin'. After the vines were picked bare, she was fortunate enough to be hired on by the Biltmore Estates Winery to work in the wine tasting room. Laura enjoyed learning and then teaching others about wine.

Sometime in November, 1998, Laura was contacted by Dr. Greg Hoyt at the Mountain Horticultural Crops Research Extension Center in nearby Fletcher, North Carolina. She was hired to work there part time, initially, which later became full time, to grind samples, do lab work, yet more dish washing, and (finally) do some actual honest-to-God field work. She learned a lot while working at the research station, primarily that she had no idea whatsoever about farming, field production, or field research. But she had a lot of fun.

Laura and Chris were married in May, 1999. Chris moved to Asheville while Laura continued working at the field station. Laura split her time working between Dr. Hoyt and Dr. Jim Rideout, the station's two soil fertility people. Dr.

Rideout worked with, among many other things, the tobacco float system and had her working in tobacco transplant greenhouses helping with different research trials and on-farm studies. Dr. Rideout had previously received a grant from the Tobacco Research Commission which included an assistantship for a Master's student to work with boron deficiency and chilling injury in tobacco float houses. Dr. Rideout offered Laura the assistantship and she accepted. Three years later she completed the study, wrote the thesis you are holding in your hands or reading on your screen, and received a Masters of Science degree in Soil Science.

Laura and Chris Overstreet still reside in Raleigh, North Carolina, where it would appear they will remain for another three years anyway. Chris has become a computer jockey and plays in a local band destined for international fame. Laura is gearing up for her Ph.D. program under the very Dr. Greg Hoyt who initially hired her to grind samples just three years prior. She will be conducting research investigating sustainable agricultural systems and alternative cropping systems. Laura and Chris have a dog, a cat, a snake, and a chameleon.

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Introduction – Boron Deficiency Study

Tobacco Transplant Production in the Float System

Quality of tobacco (*Nicotiana tabacum* L.) transplants is a critical factor in tobacco crop production. Good transplant quality improves stand establishment, decreases stand variability, makes harvesting more efficient, and may improve the quality of the cured leaf product. The most prevalent system for production of tobacco transplants in the southeastern United States utilizes the float system in greenhouses. Ninety-three percent of all transplants grown in North Carolina in 1999 were produced in tobacco float greenhouses (Tobacco Information, 1999). These “floathouses”, as they are commonly called, vary in design and size, but generally open tanks, or “beds,” are built inside plastic-covered greenhouses. The beds sit directly on the ground and are typically lined with 0.15 mm plastic sheeting. The beds are filled with nutrient solution formulated with water-soluble horticultural fertilizers. Styrofoam Todd flats, which are divided into small cells (typically 200 to 392) that are filled with peat and vermiculite-based soil-less media, are floated on the nutrient solution. Coated tobacco seeds are sown directly onto the surface of the media. Water moves upward into the media via capillary action through holes in the bottom of each cell to support imbibition and germination of the seed and initial growth of seedlings. As growth of the seedlings continues, roots extend down through the media and directly into the nutrient solution. Seedlings reach transplantable

size in six to eight weeks after seeding, when they are 15 to 20 cm tall and have 8 to 10 leaves (Rideout et al., 1994).

Transplants in float culture are very responsive to the nutrient regime provided by the grower. Unlike traditional soil beds for transplant production, soilless float beds contain no reserve of nutrients, and plants in float beds can develop nutrient deficiencies quickly if the grower does not continuously supply sufficient nutrients in an available form to the nutrient solution. Conversely, salt injury and/or nutrient toxicity (particularly B) may occur if the grower supplies excessive nutrients.

Management of micronutrients is sometimes overlooked by growers in float systems. Boron has been the most problematic of the micronutrients. Not all fertilizers utilized in float systems contain sufficient B for tobacco seedling production. Current recommendations (Rideout and Shelton, 2001) suggest that the fertilizer contain at least 0.02 percent B. Lack of attention to micronutrient management creates a potential for B deficiencies in float systems, although in some instances trace contamination in fertilizer and water sources may provide sufficient B for acceptable plant growth (Rideout and Gooden, 1997; Abdunour et al., 2000).

Many burley tobacco growers believe that routine supplemental boron applications are required to grow good transplants. Earlier research with flue-cured seedling production showed that this was not the case (Rideout and Gooden, 1997). Burley may respond differently. If so this research should establish that fact and develop recommendations for burley. Supplemental applications of boron are potentially dangerous in that boron can be extremely toxic to seedlings if over-applied. Proper application only requires a few grams to an entire greenhouse. Entire seedling crops have been destroyed by over application of boron (J. Rideout, personal communication). There is no room for the "if some is good, then more is better" attitude in relation to B application. Boron must be recognized as a potentially harmful nutrient if not applied carefully and exactly.

Boron Nutrition in Crop Plants

Boron is a micronutrient that is frequently suspected, possibly incorrectly, to be limiting in tobacco transplant float systems. It has not been determined what concentration of B in either solution or plant tissue is sufficient to avoid deficiency in the float system. Water-soluble fertilizers commonly used in the float system contain from 0.0009 to 0.02% B as indicated by analysis on product labels (J. Rideout, personal communication). Cooke (1982) reported that most fertilizers do not contain an effective level of micronutrients, including B, but that some specialty fertilizers for susceptible crops such as beets may contain around

0.21% B. For the tobacco float system, it is important for growers to determine the B content in their irrigation water and factor that into their B fertilization regime. The average B content of ground water in North Carolina is 0.259 mg/L (North Carolina Department of Agriculture. Only three of the elements that most plant require are found in the earth's crust in concentrations lower than the 930 μM for B are copper (870 μM), cobalt (430 μM), and molybdenum (15 μM) (Lovatt, 1984). Recommendations developed for crop plants grown in soil in temperate regions generally prescribe addition of boron fertilizer when hot water soluble (hws) boron in soil is less than 0.5 mg kg^{-1} , suggest precautionary application when hws boron is 0.5-1.0 mg kg^{-1} , and consider boron deficiency unlikely when hws boron is greater than 1.0 mg kg^{-1} (Cooke, 1982). Tissue concentrations of B associated with symptoms of B deficiency vary among species. Rideout and Gooden (1997) investigated B uptake by tobacco seedlings in a float system and determined that transplant growth was acceptable at B concentrations in solution of 1-4 mg L^{-1} or lower. However, they also determined that early seedling growth was reduced at low B concentrations. At higher B concentrations in solution, they found that B accumulation by seedlings increased to values higher than those found in mature leaves, with no visual evidence of toxicity. For flue-cured tobacco production on B-deficient granitic soils in Australia, researchers recommend the addition of 0.25 to 0.3 kg B ha^{-1} in the form of Solubor (20.5% B) (Littlemore and Von Nordhelm, 1991). Hutcheson and Woltz (1956) concluded that B concentrations in unemerged leaves of flue-

cured tobacco of 15-16 mg kg⁻¹ at flowering were near the deficiency level.

Lambert et al. (1997) found that severe B deficiency symptoms in *Pinus radiata* are normally associated with B concentrations in needles of 8 mg kg⁻¹ dry wt or less and marginal deficiency in the range of 9-12 mg kg⁻¹. Roberts and Rhee (1990) determined that B deficiency symptoms occurred in greenhouse grown potato shoots with tissue B concentrations of 15, but not 19, mg kg⁻¹.

The range between threshold concentrations of B in tissues associated with deficiency and toxicity was once considered to be narrow for all species. It is now believed that considerable diversity exists among species. Blamey et al. (1997) found that the critical concentration of boron toxicity in sunflower occurred at 1.13 mg g⁻¹ dry weight in the youngest mature leaf blade (YMB) and that at B concentrations in tissue as high as 1.87 mg g⁻¹ in the YMB, dry matter yield declined by only 25%. This research would indicate that sunflower does not have a narrow threshold concentration between deficiency and toxicity. Likewise, Chapman et al. (1997) conducted a study of five temperate crop species (wheat (*Triticum* spp.) cv. Hartob, lentil (*Lens culinaris*) cv. Callisto, white lupin (*Lupinus albus*) cv. Ultra, barrel medic cv. Jemalong, and field pea (*Pisum sativum* L.) cv. Bonzer) and found that near-maximal yield (>90%) of all species except barrel medic were reached over the wide range of solution B concentrations from 0.15 or 0.625 μM to 40 or 160 μM. They concluded that the widely accepted belief of a narrow B threshold between deficiency and toxicity

for all species is false. For tobacco transplant production, however, Rideout and Gooden (1997) have observed that tobacco seedlings exhibit a narrow range between B deficiency and toxicity.

Boron as a Plant Micronutrient

Boron is perhaps the least understood of the essential mineral nutrients. The extent of its functional role in plants, the mechanisms by which it is held in the soil matrix, and the mechanism by which it is taken up by roots are not fully understood. Among the reasons for this lack of knowledge are: 1) There is no known radioactive B isotope with a half-life long enough to allow labeling of B-binding compounds and 2) B forms predominantly covalent bonds under physiological conditions, making reactions with boric acid and borate with ligands readily reversible (Findelee et al., 1997). Nevertheless, several known chemical properties of B make it unique among plant nutrients. Boron is the only plant nutrient that is present as a non-ionic molecule over the typical pH range of soil solution. In aqueous solution B most commonly occurs as boric acid (H_3BO_3 or $\text{B}(\text{OH})_3$), a very weak acid that accepts a hydroxyl rather than donating a proton (Loomis and Durst, 1992). Additionally, B is a metalloid, making it the only nonmetal among the micronutrients.

Once taken up by the root in the form of boric acid (H_3BO_3), B is transported through the xylem to the shoots. Boron is mobile in the plant via the

transpiration stream (Marschner, 1995; Shelp, 1988). Boron, however, has limited mobility in the phloem of most species and is only slowly redistributed out of the leaf (Chapman et al., 1997). Therefore, B toxicity is first noticed in the margins of older leaves at the end of the transpiration stream and deficiency is first apparent in roots and younger tissues. It is thought that B is immobilized as a result of formation of stable B complexes in the cell wall (Loomis and Durst, 1991). Because B is not readily mobile in the phloem of most species, foliar application is only effective when sprayed directly on B deficient tissue (Brown and Hu, 1996). For many years B was thought to be phloem-immobile in all species, but in 1987 Shelp and co-workers showed that B can be redistributed via the phloem to young leaves, storage roots, and inflorescences in B-starved plants (Campbell, Miller, and Loneragan, 1975; Scott and Schrader, 1947; Benson, Degman, and Chmelir, 1961; Shelp and Shattuck, 1987a; Shelp and Shattuck, 1987b; Shelp, Shattuck, and Proctor, 1987). Shelp also suggests that at toxic levels, B can be transferred laterally between xylem elements to enhance B concentration in developing sinks (Shelp, 1988). In 1996, Brown and Hu demonstrated that B is mobile in species of plants within the *Pyrus*, *Malus* and *Prunus* genera that contain high concentrations of sorbitol. Boron has been shown to form stable bonds with the *cis*-hydroxyl groups of sorbitol and other acyclic sugar alcohols, and it is thought that B is shuttled through the phloem in this complexed form. Brown and Hu found that species within the *Pyrus*, *Malus* and *Prunus* genera have highly mobile forms of B and found that B sprayed on

leaves was re-distributed to other parts of the shoot and, particularly, to the fruit tissues. It appears that B redistribution is prioritized to the sink organs (Shu et al., 1994)

Boric acid, H_3BO_3 , is the form of B most efficiently taken up by plant roots.

Boron becomes available to roots primarily by mass flow in soil solution (Gupta, 1979). Because of its predominant presence in the non-ionic form, B does not participate in the metabolic activity associated with ion uptake and accumulation in root cells (Vlamis and Williams, 1970), meaning that its uptake is passive rather than active. Boron is required in root tissues for the extension of the root from the apical meristem and is necessary for unrestricted root growth. The root tissues that are the most B-demanding are epidermal and outer cortical cells of the extension zone, xylem vessels, and root hair tips (Goldbach et al., 2001). Lovatt (1984) suggests that once B enters the root cortex, it must be utilized fairly rapidly by root cells before removal in the transpiration stream. Boron appears to be necessary for tip growth such as occurs in apical meristems, root hairs, and pollen tubes; this locally-specific high demand for B is probably the result of the need for B for the secretion of cell wall material (Goldbach et al., 2001).

Goldbach et al. (2001) report that upon removal of B from the nutrient solution there are several root responses that can be measured within minutes. These

responses include: "reduction of cell wall elasticity modulus ϵ , increase of hydraulic conductivity, reduced activity of plasmalemma-bound inducible (NADH) reductase, (smaller) changes of the membrane potential, and liberation of Ca^{2+} (apoplastic and membrane-bound)." On the other hand, some responses to B deficiency are not observed for days or even weeks after B is withheld (Goldbach et al., 2001). Individual species, and even cultivars, can have distinctive characteristic uptake rates of B. Dicotyledons characteristically have greater uptake of B than monocotyledons, possibly related to different requirements of B in cell wall composition, (Marschner, 1986) especially lignin content.

Lewis (1979) provides a compelling explanation to describe this observed discrepancy between B requirements in monocots and dicots. He speculates that when sucrose became the primary mobile carbohydrate during the origin of vascular plants, B, which does not complex strongly to sucrose since sucrose does not have *cis*-diols, was freed to develop a functional role in these species. He proposed a potential role for borate in regulating the hydroxylase and oxidase activities of the phenolases involved in the biosynthesis of the lignin precursors caffeic and hydroxyferulic acids. These acids are the precursors to the lignin components coniferyl alcohol and synapyl alcohol. He then points out that monocots, which have a lower B requirement than dicots, have a lower proportion of their lignin derived from the synapyl alcohols, which possibly requires a greater amount of B to synthesize due to increased hydroxylation and

methylation. Lewis also suggests a role for B in the polymerization process in which the three primary building blocks of lignin, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, are incorporated into lignin.

Finally, cytoskeleton components have shown prominent responses to B deficiency in the forms of increased amounts of actin and tubulin proteins observed just 20 or 40 minutes, respectively, after B withdrawal as well as denser cytoskeletal arrays in the transition and elongation zones of roots (Goldbach et al., 2001). Goldbach et al. suggest that these results "might be attributed to the up-regulation of specific actin and tubulin isoforms and/or to inhibited protein degradation." They speculate that B deficiency may impact the cytoskeletal dynamic equilibrium via the extracellular matrix-plasma membrane-cytoskeleton continuum.

How Environmental Conditions Affect Boron Availability

The balance between accumulation of and requirement for B is affected by light, temperature, and humidity, which alter rates of plant growth and transpiration (Shelp, 1988). Intensity of light has been found to be one of the chief environmental factors affecting B uptake. Any factor that causes rapid plant growth, i.e. high light intensity, has the potential to cause mineral deficiencies over a certain growth period (Gupta, 1979). The effect of temperature also has a marked affect on plant response to B due to its influence on transpiration and

plant growth rate. High humidity has been found to influence B uptake by making foliarly applied B more likely to penetrate leaf epidermal cells (Shu et al., 1994). This may be achieved due to swelling of the cuticle membrane and surface waxes under conditions of high humidity, by increasing drying time of solutions sprayed on leaves, or by increasing stomatal penetration (Shu et al., 1994). Moisture, however, is the most important of all climatic factors regarding B uptake into plants. Even in the presence of adequate soil B, the reduced mass flow and diffusion rate, as well as limited transpiration related to reduced moisture may be primary causes of B deficiency in plants.

These environmental conditions must be considered in tobacco float houses where environmental parameters are markedly different from conditions found in the field. Temperature measurements are typically greater in tobacco floathouses compared to field conditions due to the greenhouse effect. Humidity should be higher in floathouses than in the field as well, partly due to the increased surface area of water present in the greenhouse, particularly at seedling height, and partly due to the increased water-holding capacity of the warmer air mass in the greenhouse. Moisture, of course, is available to seedlings in this system provided there is not so much fertilizer in solution that there is an unfavorable osmotic flux away from plant roots.

Function of Boron in Plants

Many functions have been proposed for the effect of boron on plant growth and metabolism. Boron has been implicated in sugar translocation (Lewis, 1979; Goldbach, 1985; Blevins et al., 1998; Shelp, 1988), cell wall synthesis and structure (Loomis and Durst, 1992), auxin biosynthesis and metabolism (Lewis, 1979; Lovatt, 1984; Li et al., 1997), lignification (Lewis, 1979; Kobayashi et al., 1999), phenol metabolism (Lewis, 1979; Goldbach et al., 1991), ascorbate metabolism (Lukaszewski and Blevins, 1996), nitrogen metabolism and assimilation (Shelp, 1988; Ruiz et al., 1998), membrane function and ion uptake (Schon et al., 1991), and proton release (Goldbach et al., 1991). Boron reportedly enhances carbon mobilization to roots, nitrogen fixation in legumes, and decreases flower and pod abortion (Blevins et al., 1993, Yamagishi and Yamamoto, 1994).

The most recognized role of B in plants is production of the stable complexes with cell wall constituents required for structural integrity. The pectin gel of plant cell walls is the location for many of the reactions of B in plant cells (Matoh, et al., 1993; Hu and Brown, 1994). Pectic polysaccharides are the most abundant components of the primary cell walls of flowering plants (Jarvis, 1984). Kobayashi et al. (1999) and Matoh et al. (1993) estimate that 80% of cellular B is found in cell walls and that 80% of B in cell walls is associated with pectic polysaccharides complexes known as rhamnogalacturonan II. Breakdown of

pectic materials associated with destruction of B cross-linking results in loss of cell wall integrity (Kobayashi et al., 1999; Matoh et al., 1993) and appears to be related to symptoms of B deficiency in crops such as "black heart" in beets, turnips, and radishes, "leaky spots" in melons, and callose accumulation in bean and cotton plants (Gupta, 1979). Matoh et al. (1998) determined that the B-rhamnogalacturonan II (B-RGII) complex is located proximal to the plasma membrane, suggesting that B may form crosslinks with pectins in the plasma membrane as well as within the cell wall. Findelee et al. (1997) found that after rinsing plant roots in B-free nutrient solution for 15-20 minutes, re-hardening of the cell walls commenced. Re-addition of boric acid after three hours did not reverse this process, leading to the conclusion that a secondary process is induced by B deprivation that is not controlled by B. Goldbach et al. (2001) suggest this rehardening may be a result of the increased production of phenolic crosslinks by ester or ethers. This may explain the thickened, leathery texture of leaves of many B-deficient species, including tobacco transplants. It seems likely that many of the deleterious effects of B deficiency result from disturbances to one or more processes in the formation and maintenance of the secondary cell wall.

Another role for B in the biosynthesis of lignin has received attention. Lignin is a primary component of secondary cell walls. Lewis (1979) suggests that the primary role of B in vascular plants concerns the metabolic control of lignin

biosynthesis. He further speculates that B control of lignin biosynthesis lead to the evolution of a lignified "super-apoplast", which was an ersatz xylem in early plant species.

From her literature review, Lovatt (1984) concluded that cessation of cell division in the apical meristem is the earliest and most prevalent result of B deficiency. She further asserts that the earliest functional role of B in vascular plants was in the synthesis of lignin, which eventually resulted in the development of xylem. She supports her theory on several stands, one being that there are many reports in the literature of accumulation of phenols, which are the precursors of lignin, under conditions of B deficiency. Lignin synthesis, which is reportedly not reduced under conditions of B deficiency (McIlrath and Skok, 1964), agrees with the findings of Kirk and Loneragan (1988) and Cohen and Albert (1974) that xylem differentiation continues under conditions of B deficiency even into the terminus of the shoot and the apical meristem. This continued differentiation under B-deficient conditions leads to the lignification and eventual death of the apical meristem, although the exact mechanism is not known.

The role of B in auxin metabolism and/or transport is yet to be fully understood, but it is well recognized that under circumstances of B deficiency indole -3-acetic-acid (IAA) levels in plants decrease and IAA oxidase enzymes increase. The cause for the IAA decrease is not proven, but several theories exist.

Bohnsack and Albert (1977) found that under B deficient conditions IAA oxidase levels in roots increase, thereby potentially reducing IAA levels. Another suggestion is that, because IAA is produced predominantly in apical meristems, the cessation of apical dominance results in the decrease in IAA. Finally, since IAA is transported from the site of synthesis to the active site by means of polar transport, B deficient conditions may reduce IAA transport by impairing membrane integrity. Goldbach et al. (2001) regards this as the most probable cause of decreased measurement of IAA in plants. Indole acetic acid has been implicated in conjunction with B deficiency in increasing sugar translocation. A study by Middleton et al. (1978) revealed that B is not required for root initiation, but that actual root growth, including meristem organization, cell division and normal development of the root primordia, requires the presence of B. A study by Middleton et al. (1978) revealed that B is not required for root initiation, but that actual root growth, including meristem organization, cell division, and normal development of the root primordia, requires the presence of B. Middleton et al. (1978) found that *Phaseolus aureus* cuttings treated with indolebutyric acid (IBA) in the absence of B accumulated sugars (sucrose, glucose, and fructose) but did not form roots. This indicates that auxins initiate the mobilization of soluble carbohydrates to roots but that roots will not form in the absence of B. Middleton et al. (1978) interpret this data to suggest that, in the absence of B, roots do not form even though root initials are developed, presumably due to accumulation of IAA. Indole acetic acid also stimulates the

mobilization of soluble carbohydrates to the site of root initiation. The carbohydrates cannot be utilized to produce new roots due to the lack of B, which results in accumulation of a soluble carbohydrate pool and in part explains the translocation of sugars due to B deficiency. It appears from Middleton et al.'s work (1980) that the combination of auxin and B together effects the translocation of sugars from the leaf of young seedlings to be utilized to form roots in the hypocotyl. In another study by Middleton et al. (1980), the group excised leaves from *P. aureus* cuttings cultured in distilled water and found that cuttings without leaves produced only two roots compared to seven roots for those with leaves. Thus they determined that leaves are indeed the source of the auxin that stimulates the development of adventitious root initials and that the auxin must be transported basipetally and collect at the base of the cutting. Finally, Middleton et al. (1980) performed sugar analyses that indicated that presence of auxin alone promotes an immediate increase in free sugar in leaves but that B alone does not. They interpreted this to mean that auxin enhances the level of free sugar in the leaves and that B facilitates its transport to the hypocotyl. The higher level of sugar in the root, in conjunction with the direct effect of auxin, leads to enhanced root initiation and growth.

Two questions that have remained unanswered are 1) the physiological processes that result in apical meristem necrosis in B deficient plants and 2) cessation of root elongation due to B deficiency. A thorough search of the

literature did not find a definitive study that explains either of these phenomena fully. One study that may lay the foundation for further research into these questions is the work of Cohen and Albert (1974). By exposing the roots of squash plants that were in different stages of B deficiency to tritiated thymidine they were able to label the replicating DNA of root tip cells. Using histological sections, they prepared autoradiographs of intact root meristems. The presence of nuclei in which tritiated thymidine was incorporated into newly replicated DNA was indicated by the presence of exposed groups of black-silver grains. They were able to correlate the ability of root tip cells to incorporate label with total root elongation in the different minus-B treatments. They found that while plants were being subjected to a minus-B treatment for increasing periods of time cellular elongation and differentiation continued distally in the stele until the differentiation finally extended into the region normally occupied by the apical meristem. The meristem then became lignified and differentiated. They propose from these results that the cessation of root elongation under B deficient conditions is caused by a cessation of mitosis and cell division and not by the failure to elongate and differentiate. By examining labeled areas of the B-sufficient root, they were able to determine that DNA was synthesized in the central cylinder, cortex, and, to a lesser extent, in the root cap. The area classically considered to contain the root apical meristem showed no incorporation of label into nuclei. Autoradiographs of minus-B root sections showed no difference from the plus-B treatment for 3,6,12,15, and 18 hours

after B was withheld. This indicated that the cells had not yet become B deficient. By 20 hours after B deprivation, excised roots showed no evidence of incorporation of the label. Other experiments determined that root elongation was virtually completed during the first 6.5 hours after B deprivation. Root tips which had been without B for 20 hours were then returned to a plus-B solution, and autoradiographs were again made. No further label of DNA was found at 3 or 6 hours after B was re-supplied; however, after 9 hours some of the root tips showed a small amount of incorporation of label into cells located in the periphery of the root cap. After 9 hours cells were found to have elongated an average of 0.45 mm. After 12 hours more label was incorporated into DNA and the labeling pattern appeared similar to that of the B-sufficient root. After 12 hours of recovery, roots were found to have elongated an average of 0.72 mm. From this study we can assume that cessation of mitosis in B-deficient roots occurred as early as 6.5 hours after B was withheld while synthesis of DNA continued in some cells for as long as 20 hours after B was withheld. We can further conclude from these studies that cessation of root elongation observed in B-deficient plants is a result of biochemical changes that occur in the root meristem and that prevent the synthesis of DNA and subsequent mitosis. A recent review of reactions of roots to B deprivation written by Goldbach et al. (2001) suggests that the alteration of phenol metabolism and oxidation of phenols are responsible for the visible browning of apical meristems.

There is clearly an interaction between B deficiency, lignin synthesis, auxin metabolism, sucrose transport, and the normal functioning of the apical meristems. Any other physiological process affecting one or more of these processes could further react with the other reactions and with B allocation within the plant.

There are a number of purported interactions between B and other nutrients. Boron deficiency reportedly increases permeability of cell membranes to K^+ (Schon et al., 1991; Iikura et al., 1997). Ruiz et al. (1998) report a role for B in assimilation of plant N into NO_3^- -reduction enzymes and other nitrate-assimilation proteins. Shelp and Shattuck (1987) report decreases for re-translocation of Cu, N, and Zn to sink tissues with increasing B concentration and an overall increase in P, which suggests increased remobilization of P with increasing B concentration in tissues. There has been some evidence of B interaction with Zn. Grewal et al. (1998) demonstrated that adequate supply of Zn helps to ameliorate the symptoms of B deficiency in oilseed rape seedlings. They also showed that Zn deficiency enhanced foliar B uptake in leaves of oilseed rape and that an increased supply of Zn increases B uptake when B is in excess supply. Finally, they demonstrated that B application increased Zn uptake when Zn supply was sufficient. Some researchers have reported excess B to decrease uptake of Ca (Asad et al., 1997). Abdulnour et al. (2000) suggested that the addition of $1.08 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ may be sufficient to decrease Ca uptake by

micropropagated potato plantlets of certain cultivars, thereby contributing to disorders related to Ca deficiency. Ca has also been identified as a component of the boron-rhamnogalacturonan II complex in cell walls (Kobayashi et al., 1999). Combrink and Davies (1987) found that a low Ca:K ratio (3.5:8.0) in soil and sufficient B supply was the best combination for tobacco. This treatment gave the highest yield, delayed deterioration of leaves during senescence and ensured cured leaves with a high sugar content and good moisture-holding capacity. Goldbach et al. (2001) suggests that the enhanced callose formation under B deficient conditions indicates increased cytosolic Ca levels.

Plant Responses to Boron

A number of plant responses to addition of B also have been observed. Boron application increased the rate of fruiting of grain crops and decreased physiological disorders of sugar beet, the incidence of bare ears in maize and decreased empty pods of soybean and empty grains in rice (Li and Liang, 1997). Boron application to apple orchards generally increased fruit diameter and weight, although firmness of the apples decreased (Dong et al., 1997). The efficiency of potassium fertilizer on rapeseed and mulberry was enhanced by B additions to soils at deficient levels of B (Chen et al., 1997). Effects of foliar applications of B to soybean included increased yield and larger seed size (Gascho and McPherson, 1997). While foliar application of B to avocado trees did not affect number of fruits retained after the first fruit drop, it did increase

fruit set (Smith et al., 1997). Avocado trees fertilized with B produced less ethylene and the fruits took longer to attain eating softness compared with trees that were marginally deficient in B (Smith et al., 1997). Conversely, fertilization of apple trees with B decreased fruit drop but did not affect fruit set (Zude et al., 1997). Boron application also reduced skin cracking of apples, a condition associated with fluctuations in water potential throughout the growing season (Zude et al., 1997). This indicates that B maintains fruit texture quality in situations of physical stress caused by water potential variability.

Objectives

This study was conducted to determine the effect of solution B concentration on B uptake and growth of tobacco seedlings. Specifically the objectives of this study were 1) to determine a boron deficiency threshold for the tobacco transplant float system, and 2) to determine transplant uptake of boron in response to the boron concentration in solution.

Boron Deficiency Study:

Materials and Methods

The boron deficiency study examined the effect of reduced B availability on tobacco seedling growth and development. Plants were seeded as bare seeds in a seedling hydroponics system. The hydroponics unit was located in a temperature-controlled environmental A chamber located in the North Carolina State University phytotron (Southeastern Plant Environment Laboratory) set to a 26°/22° C ($\pm 1^\circ$ C) day/night temperature regime with a 9-hour day period. This day/night temperature regime is nearly optimal for establishment of *Nicotiana tabacum* L. seedlings (Haroon, Long, and Weybrew, 1972). Temperature was maintained at 26°/22° C ($\pm 1^\circ$ C) in the growing plane. The average photon flux density was 541 $\mu\text{mol m}^{-2} \text{sec}^{-1}$, and the relative humidity was maintained at greater than 50%. The hydroponics system used for this study was an 81.28cm X 60.96cm X 17.78cm PVC tank divided into 4 compartmentalized chambers with individual pumps to provide solution circulation and aeration, pH probes, and acid pumps. Chambers were maintained at a pH value between 5.5 and 6.5 by injecting 0.05M H₂SO₄ with an automated pH control system. Each chamber held 15 L of solution and could support 121 seedlings. Each chamber contained a separate treatment for a total of 4 solution treatments: 0, 0.19, 1.9, and 19 μM B. Treatments were replicated in time and samples were treated as a completely randomized block. Sub samples were utilized within each treatment to evaluate

the effect of B on different tobacco varieties. Four varieties were chosen; two were burley varieties (TN90 and KY14) and two were flue-cured varieties (C371 and K326). Seedlings were germinated on 0.8% agar and 0.1385 M N to provide a nitrogen source. After two weeks, the full complement of macronutrients was added in the following concentrations: 0.598 M N, 0.755 M K, 0.244 M Ca, 0.164 M Mg, 0.015 M S. Fe Sequestrene 330Fe (an iron chelate, Ciba-Geigy) was added as a source of iron at a concentration of 1.1 mL per L of solution. A micronutrient stock was added to give the following final concentrations: 1.02 μM Mn, 0.073 μM Zn, 0.033 μM Cu, and 0.027 μM Mo. Boron was added as $\text{B}(\text{OH})_3$ to provide the treatment concentrations previously mentioned. All macronutrient stocks were treated to remove trace B contamination by running solutions through an Amberlite IRA-743 resin column (Sigma Corporation). Boron concentrations in nutrient stocks after being run through a resin column were near the detection limit for all stocks except Fe Sequestrene. After being run through the resin column twice, the B concentration in Fe Sequestrene was approximately 2 ppb. Nitrate and PO_4^{3-} solution concentrations were tested weekly throughout the study using an ion chromatograph (Dionex 2110i, AS4A 4mm column) to measure concentration levels. Nutrients were added back in the form of $\text{Mg}(\text{NO}_3)_2$ and KH_2PO_4 as needed to compensate for uptake. Boron concentrations were not renewed over the course of the study. Five tobacco seedlings per sub treatment were harvested on 14, 19, 24, and 29 days after seeding to determine root and shoot fresh mass and dry mass (tissue was freeze-dried), leaf area, and tissue B concentrations. Tissue was digested as un-

ground shoot and root material in concentrated high-purity nitric acid (Fisher, Optima grade) based on procedure two described by Zarcinas et al. (1987). The Zarcinas et al. procedure was modified to use 4mL nitric acid due to the small mass of tissue samples. Samples consisted of five plants per treatment separated into roots and shoots. The samples were allowed to digest in the nitric acid overnight and then were microwaved at five-percent power for fifteen minutes in a standard food-grade microwave oven. The microwave oven was fitted with Tygon tubing leading into one side of a self-contained sample reservoir located inside of the microwave oven. Another section of tubing lead out the other side of the reservoir and continued out of the microwave oven through a custom-cut hole, and out to the back of a fume hood. Di-nitrogen gas was flushed through the reservoir to remove any nitric oxide fumes that were produced during digestion into the fume hood, where water was running to prevent corrosion of the fume hood due to acid fumes. The samples were filtered through Whatman 40 filters and brought to 15mL with deionized water. Samples were analyzed using inductively coupled plasma atomic emissions spectroscopy with a Perkin Elmer Model 2000 DV emission spectrometer. The spectrometer utilized an axial view; it ran on 1500 watts of power and had a concentric glass nebulizer with cyclonic spray chamber. The argon flow was 15 L/min, nebulizer rate was 0.85 L/min, and auxiliary flow of 0.3 L/min. As an added precaution, all samples were run low to high anticipated B concentrations to avoid B carry-over contamination. The nebulizer was flushed with DI water between B samples to remove excess B from the nebulizer.

Boron Deficiency Study
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Introduction – Chilling Injury Study

Chilling injury in young tobacco transplants is a common occurrence for plants grown in tobacco float greenhouses. Chilling injury in young transplants is characterized by cupped leaf margins, puckering of the interveinal lamina tissue, lesions, and deformities of the leaf. Most transplant growers using tobacco float greenhouses report the appearance of chilling injury in transplants. Although not typically a major threat to the health of transplants, chilling injury can stunt growth and delay the transplant-ready stage of tobacco seedlings. Apparent differences in sensitivity of various flue-cured and burley varieties have been observed (J. Rideout, personal communication). Plants typically do not suffer from long-term injuries due to non-severe chilling injury symptoms (Nessler and Wernsman, 1980?). Chilling injury does not appear to be associated with premature floral induction caused by low pre-transplant temperatures (King and Terrill, 1985). Although the conclusive cause of chilling injury at a cellular level has not been determined, there is well-documented support for the observation that chilling injury symptoms are induced by a reduction in ambient air temperature followed by a return to warmer temperatures (Lyons, 1973; Sharom, et al., 1994; Saltveit and Morris, 1990; Paull, 1990). The tobacco float system is a susceptible environment for such conditions due to the widespread use of manual, inadequate ventilation systems. In the early morning hours when the sun is first coming up these greenhouses can warm up rapidly as ultraviolet

energy is trapped inside the greenhouse. Temperature increases of seven to ten degrees C have been measured in greenhouses over the course of just 15 minutes in the early morning (Fortnum et al., 2000). This rapid increase in temperatures over a short period of time may be the key to the observable symptoms of chilling injury in young tobacco transplants produced in the greenhouse.

Chilling Injury as a Plant Condition

Chilling injury has been classified as “a physiological disorder caused by exposure of plants to low but above freezing temperatures” (Sharom et al., 1994). Saltveit and Morris (1990) further define chilling injury thus: “The physical and/or physiological changes induced by exposure to low temperatures, together with the subsequent expression of characteristic symptoms, are commonly combined in the term ‘chilling injury.’...Chilling is the act of exposing plant material to a critical or threshold low temperature, i.e., the chilling temperature, which is generally thought to cause a primary response in sensitive plants. This primary event is the initial, rapid response to the chilling temperature. The primary response causes a dysfunction, i.e., an impaired functioning of the tissue, which is reversible if the tissue is returned to a non-chilling temperature after a brief period of chilling, but which becomes irreversible after a longer period of time at the chilling temperature. The cause of the primary event and the dysfunction may be one and the same, e.g., an alteration in the fluidity of a critical

membrane, or a change in the activation energy of a critical enzymatic reaction. After some time, the dysfunction leads to the appearance of horticulturally important symptoms.”

General chilling symptoms include pitting of leaves and fruit, failure of fruit to ripen, increased occurrence of senescence and decay after chilling, as well as an increased rate of water loss both during and after chilling (Saltveit and Morris, 1990; Paull, 1990). The pitting results from the injury and collapse of subsurface cells (Lyons, 1973; Luza et al., 1992), followed by the rapid invasion of decay organisms (Lyons, 1973). The organisms that cause the decay are not normally found in healthy plant tissue (Paull, 1990). Pitting has been reduced by maintaining a high relative humidity or by waxing fruit to decrease water loss (Lyons, 1973). While the actual sequence of events that leads up to the appearance of visual symptoms is not known, common physiological phenomenon that precede the appearance of symptoms in papaya include increased ethylene synthesis, electrolyte leakage, and *Alternaria* rot (Paull, 1990).

Young tobacco seedlings are particularly susceptible to chilling injury. It is apparent that fatty acid desaturation of cell membranes is one of the factors involved in conferring chilling tolerance to young seedlings. The function of unsaturated fatty acids in chilling tolerance can be demonstrated using the FAD

mutants of *Arabidopsis*, which are unable to produce desaturation of membrane lipids. Kodama et al. (1995) found that exposure to temperatures of 15° or 20° C increased the amounts of trienolic acids (16:3 and 18:3) found in young tobacco leaves. They determined that the content of trienolic acids was markedly lower in younger leaves than older leaves, suggesting that more trienolic acids in lipid membranes, which presumably confer greater chilling tolerance, are produced in older leaves. They noted that chilling injury was only observed in younger leaves and no visible symptoms appeared in fully expanded leaves. Another important conclusion they drew from their study was that a high level of lipid polyunsaturation plays a fundamental role in chloroplast biogenesis at all temperatures and that the role is manifested more strongly at lower temperatures.

Chilling injury is a difficult condition to diagnose because there are no symptoms specific only to the condition and because it is difficult to make objective measurements of the primary changes in the plant that are direct results of the exposure to chilling temperatures. Symptoms that develop are often merely exaggerations of the effects of physical injury or other physiological stresses (Saltveit and Morris, 1990; Paull, 1990). Care must also be taken not to confuse changes in plant cells resulting from adaptations to repeated chilling episodes, also known as 'cold hardening', with symptoms of chilling injury (Kratsch and Wise, 2000). It is particularly difficult for researchers and growers to agree on

chilling injury diagnostics for plants of temperate origins because symptoms are even more subtle for these more tolerant species than for sensitive species of tropical and sub-tropical origins. Since there is no standard against which to mark chilling injury symptoms, researchers and growers must rely on qualitative visual symptoms, which may or may not be the result of chilling. These symptoms are a function of both physiological injury and the rate at which the symptoms manifest themselves visually in a particular plant tissue (Lyons, 1973).

There are two fundamental ways to view chilling injury in plants: from a fruit and vegetable perspective or from a whole plant perspective. Growers and researchers of fruit and vegetables are interested in chilling injury of the commodity at low holding temperatures. For these studies, it is primarily storage of the commodity for future sell that is of concern. Commodities such as cucumbers, persimmons, tomatoes, peaches, plums, apples, and peppers are examples of fruits and vegetables that must be observed carefully to avoid chilling injury when being held in cold storage. Conversely, there are chilling sensitive whole-plant species such as tobacco, tomato, cucumbers, corn, beans, and melons that, when grown under chilling temperatures, will suffer from chilling injury. There are some species for which both plant and fruit are susceptible to chilling injury. For this study, we are more concerned with the latter category of chilling injury to whole plants; however, we will use information from studies of fruits and vegetables, where appropriate, to draw

conclusions concerning physiological anomalies that can be applied to whole plants.

Varietal differences are commonly noted in susceptibility of a species to chilling injury (Herner, 1990). It has been commonly noted among tobacco growers that some cultivars are less cold tolerant than others. The flue-cured variety, C371, for example, has been found to be less chilling tolerant than K326, another flue-cured variety. Hakim et al. (1999) tested chilling resistance among eight Plant Introduction lines, 12 F₁ hybrids between crosses of chilling tolerant and chilling resistant lines and two commercial cultivars of cucumber. They found that severity of chilling injury varied depending on the particular symptom or physiological dysfunction being examined. The only correlations they found were that decay and weight loss were positively associated with chilling-induced visible pitting of cucumber fruits. Some researchers explain differences among cultivars by proposing differing tolerances to certain anticipated toxic compounds that result from the altered metabolism caused by changes in enzyme activation energies (Lyons, 1973). No such compound has been positively identified, however, and it is just as probable that chilling prevents the production of a necessary metabolite (Saltveit and Morris, 1990). McGlasson and Raison (1973), suggest that the differences in susceptibility among apple cultivars to low temperature breakdown is a reflection of the greater ability of some cultivars to compensate for the imbalance in metabolism caused by the change in activation

energy of critical membrane-embedded protein enzymes induced by a phase transition in the mitochondrial membrane. Regardless, results indicate that resistance to chilling injury is a heritable factor (Lyons, 1973). By obtaining a high temperature-sensitive mutant of *Nicotiana tabacum* L., Matzinger and Wernsman (1973) were able to determine that temperature sensitivity is a cytoplasmically inherited characteristic. Nessler and Wernsman (1980) provided ultrastructural evidence that suggests that the site of inheritance of extranuclear temperature-induced lethality in that mutant was the chloroplast. This was deduced partly from the fact that the first ultrastructural changes observed in temperature-sensitive mutants occurred in the chloroplasts.

Paull (1990) states that the stage of fruit ripeness and the age of the leaf tissue at the time that the temperature is decreased significantly influences the plant sensitivity to chilling injury and the appearance of visible symptoms. Bramlage and Meir (1990) theorize that due to the intricate chemical reactions involved in ripening and maturation, the effects of chilling on plants at different stages seem more likely to be associated with symptom expression than with induction of chilling damage per se. Similar symptoms are induced by conditions other than chilling injury, for example mechanical injury, contaminated handling facilities, and low relative humidity (Lyons, 1973; Saltveit and Morris, 1990; Paull, 1990). Saltveit and Morris (1990) have addressed the observation that there are several types of chilling injury over the range of chilling-sensitive plants and that these

different types of chilling injury are symptomatically distinguishable. They suggest that the differences in symptoms speaks more to the subsequent development of symptoms in the various plants than to differences in the primary event and conclude that it may not be practical to propose a single model that is applicable to all plants that show chilling injury.

Physiological Causes of Chilling Injury

Experimental evidence supports the theory that chilling injury results in damage to cellular membranes, particularly the plasmalemma, mitochondrial membrane, and chloroplast membrane. This is proposed by many researchers to be the primary event in chilling that leads to the measurable and visible symptoms identified as chilling injury. Other proposed main malfunctions leading to chilling injury are energy imbalances (ATP deficits) and altered levels of abscissic acid and calcium (Stewart and Guinn, 1971; Minorsky, 1989; Chin and Li, 2001). The most widely accepted theory, however, remains to be membrane damage, which allows the ensuing leakage of cell contents into the intercellular space (Lyons, 1973; Wright, 1974; Wright and Simon, 1973; Wilson, 1978). The water is then susceptible to loss by evaporation, which causes sunken areas on leaves and fruit and severe water deficit of young seedlings during chilling exposure. One visible manifestation of this phenomenon is the formation of water-soaked areas in chill-injured fruit. A technique used to support this notion is the measurement of electrolyte leakage as increased conductivity from chill-injured tissue (Wright,

1974; Paull, 1981, Riken and Richmond, 1979). Fluids leaked from the cell can also reduce normal gas diffusion, provide a medium for the growth of pathogens, and cause wilting, desiccation, and necrosis of the affected tissues (Saltveit and Morris, 1990).

It has often been suggested that differences in fatty acid and lipid contents in membranes make some plants more susceptible to chilling injury than other plants. Still other researchers believe that the main result of membrane damage from chilling injury is inactivation of membrane-localized proteins in the form of enzymes, thus altering cellular metabolism (McGlasson and Raison, 1973).

Finally, several researchers (Rikon and Richmond, 1979; Wright, 1974) have expressed the opinion that chilling damage, as measured by cell leakage, results from cellular dehydration as well as from the intrinsic damage affected by low temperatures on plant physiology and metabolism.

Cell wall degradation is a normal event in the process of fruit ripening (Luza et al., 1992). For Grant et al. (1992) working with persimmon fruit, there was typically solubilization of polyuronides, a net loss of arabinose and galactose and a general increase in all other monosaccharides during normal fruit ripening when levels were compared to levels at harvest. In chilling injured persimmon fruit, however, they found a marked decrease in the total amounts of every monosaccharide except for glucose when compared to values for cold-stored

fruit. Their data suggested that the hemicelluloses were degraded or solubilized more readily during chilling injury than they are during the normal course of events in ripening. Although the gross morphology of ripe fruits and chill injured fruits is similar, the degree of cell wall solubilization was greater for the chill-injured fruit and occurred more rapidly than for fruit that were allowed to ripen normally. They concluded that textural changes associated with chilling injury in persimmon were accompanied by dramatic changes in physiochemical gel properties of the cell wall and cell wall constituents.

Exogenous Factors Affecting Chilling Injury

Two interacting exogenous factors that must be addressed in the tobacco floathouse system are diurnal changes in temperature and light intensity. King et al. (1982) demonstrated that there is a significant difference in chilling sensitivity at the end of the dark period and through the first hours of the light period. In essence, tomato seedlings are most susceptible to chilling injury near the end of the dark period. Experimental evidence also reveals that tomato seedlings given high light intensity at the onset of the dark cycle are less susceptible to chilling injury (King et al., 1988). Carbohydrate content of plants is dependent on diurnal changes. It has been shown experimentally that carbohydrate content of plants affect sensitivity to chilling temperatures (King et al., 1988). They were able to demonstrate that the period of greatest chilling sensitivity (the end of the dark period) corresponded to the lowest level of plant

carbohydrate concentration. By harvesting plants at the end of the dark cycle and supplementing them with equimolar concentrations of fructose, glucose, sucrose, or mannitol, prior to chilling, they found that sucrose had a far superior ability to reduce subsequent chilling injury. Glucose and fructose also reduced chilling injury, but not to the extent that sucrose demonstrated. Mannitol actually hastened chilling injury. They determined that the reduced susceptibility to chilling injury was due to the accumulation of photosynthetic products and not through an immediate photomorphogenic response, such as light-initiated enzyme activation. They further concluded that the effect of carbohydrates in reducing chilling sensitivity is due to a metabolic change and not to osmotic effects.

Kratsch and Wise (2000) call irradiance “the most obvious confounding factor [of chilling injury in plants]”. Light intensity is an important factor in chilling injury due to the phenomenon of photo-oxidation, which occurs under conditions of low temperatures and high light intensity. Symptoms of photo-oxidation include bleaching, rapid appearance of photosynthetic dysfunction, altered chloroplast ultrastructure, and cellular lipid degeneration (Wise and Naylor, 1987). Wise and Naylor propose that the symptoms resulting from chilling of sensitive plants may not simply be due to low temperatures but may result as a secondary consequence of some processes initiated in light. This may be important to this study because the rapid temperature increase in tobacco float greenhouses is

partly a result of the increased light intensity. Wise and Naylor discovered significant ultrastructural changes in the chloroplasts of chilling sensitive cucumber after just three hours of treatment at 5° C and high light intensity ($1000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) including swelling of the chloroplasts. By 9 hours, thylakoid membranes were dilated and by 12 hours some of the cucumber tissues were completely destroyed. They did not observe any change in mitochondrial structure nor in respiration rate as a result of chilling in light vs. chilling in dark. They concluded that irradiance greatly enhances degeneration of chloroplasts but does not affect other cellular organelles. Kratsch and Wise (2000) propose that the dilation of thylakoid membranes is due to photo-oxidation. They also suggest that the swelling observed in chloroplasts is a result of chilling-stable starch degrading enzymes that produce soluble sugars, which thereby lowers the stromal water potential resulting in osmotic influx. It is also probable that photosynthate export out of the chloroplast is reduced due to lower ATP levels and chilling-induced reduction in phloem loading or phloem transport (Gamalei et al., 1994), further reducing the stromal water potential. Wright and Simon (1973) confirm that chlorophyll is degraded at low temperatures in the light, but not at low temperatures in the dark.

Relative humidity of the atmosphere surrounding plants has been shown to have a very significant effect on the expression of chilling injury symptoms (Hakim et al., 1999). One of the primary consequences of saturated relative humidity on

cells is to greatly reduce water loss from chill-injured cells (Wright and Simon, 1973; Herner, 1990). Under conditions in which the percent relative humidity is low, many symptoms of chilling injury are found to be enhanced. Membrane permeability, and therefore water loss and electrolyte leakage, as well as ethylene production and ATP reduction are all found to be decreased when the relative humidity surrounding the plant is 100% as compared to 85% at chilling temperatures (Wright, 1974; Wilson, 1978). The decline in respiration and photosynthetic rates are two characteristics resulting from chilling temperatures that are not affected by relative humidity (Wright and Simon, 1973). However, cucumber seedlings that were chilled for 3 days were capable of recovering normal respiration and photosynthesis rates after being returned to warm temperatures if they were exposed to 100% humidity, whereas plants exposed to an 85% RH were only capable of returning to normal respiration and photosynthesis rates after 1 day of chilling when returned to non-chilling temperatures (Wright and Simon, 1973). There was also no change in the quantity of phospholipids PC+PE+PI (phosphatidylcholine + phosphatidylethanolamine + phosphatidylinositol) in cucumber seedlings membranes chilled at 5° C for 3 days at 100% RH compared to a 56% decline of the phospholipids for the same treatment at 85% RH (Wright and Simon, 1973). They concluded that it was only treatments that caused a water loss that resulted in a decrease in membrane phospholipids. Wright and Simon (1973) also found that plants can endure chilling temperatures for longer periods of time

without suffering permanent damage in a saturated atmosphere. They concluded that under conditions of high humidity the symptoms of chilling damage were not only less severe, but also less variable. In a corresponding study, Rikon and Richmond (1979) found that by inducing progressively greater levels of dehydration in cucumber seedlings, a concomitant increase occurred in relative leakage of cellular solution. This was true for both chilled and non-chilled tissue, but was more severe in chilled tissue. They concluded that "as cell water content declines, it becomes an increasingly important factor in determining the extent of chilling damage." Wright (1974) proposes that ethylene synthesis, a common observation in chill-injured fruit, is not a direct result of chilling, but rather a consequence of water loss. The water loss to be due to an increase in membrane permeability in response to exposure to chilling temperatures was completely reversible when leaves were transferred to a warmer (25° C) temperature. He was able to demonstrate that ethylene synthesis did not occur at 5° C when there was 100% relative humidity, which served to prevent a water deficit in the chilled tissue. However, a water deficit of just 7.5% was sufficient to induce electrolyte leakage after only one hour at 5° C. He also found that ethylene synthesis preceded fresh weight loss and electrolyte leakage.

The two major factors that converge to determine the extent to which chilling injury is developed are time and temperature. In general, the longer the plants

are exposed and the lower the temperature they are exposed to, the greater is the injury. Below a certain threshold temperature, there is a curvilinear response between degree-hours and the extent of injury (Saltveit and Morris, 1990). The extrinsic properties of the environment that influence the degree of chilling injury are temperature, duration of temperature, light intensity, whether exposure was continuous or interrupted, relative humidity, and composition of the atmosphere (Saltveit and Morris, 1990). Light and water stress have been found to be important in determining the speed of chilling injury symptom development (Paull, 1990). One characteristic of chilling injury in post-harvest storage of most plant species is its reversibility by periodic transfer to nonchilling temperatures (Bramlage and Meir, 1990). The mechanism of this phenomenon is unclear, but it is thought to involve symptom development rather than the primary event since warming proves effective even after prolonged periods at a chilling temperature as long as symptoms have not yet developed.

Consequences of Chilling Injury

Studies investigating the extent of damage of chilling on seed germination and very young seedlings revealed extreme sensitivity and short periods required for damage to occur. Guinn (1971) found that chilling decreased RNA, protein, and lipid-soluble phosphate levels in cotton seedlings and Stewart and Guinn (1969) detected very early decline in ATP and other nucleotides.

It has also been proposed that some symptoms of chilling injury are a result of abnormal metabolism in the cell. If the activation energies of a few key enzymes are near the chilling temperature for a plant, and the enzymes' activities change at differing rates, then at some temperature threshold there should be a crossover in control and metabolism could shift to the unregulated production of compounds that could accumulate and become toxic to the plant (Saltveit and Morris, 1990). Such changes are also a possibility for those protein enzymes embedded in membranes at the time of temperature-induced phase changes which could thereby hinder the activity of the enzyme. McGlasson and Raison (1973) conducted an experiment on six apple fruit cultivars that displayed a gradient of chilling susceptibility and produced symptoms of a condition known as low temperature breakdown. By measuring the Arrhenius activation energy of respiratory enzymes of mitochondria from apple tissue, they were able to approximate the phase change temperatures of mitochondrial membranes. They found that the temperature of the phase change and the temperature at which membrane-associated enzymes exhibit a change in E_a are within the same range. Chilling has been found to decrease oxidation of certain substrates by membrane-bound enzymes of the mitochondrial membrane (Stewart and Guinn, 1971). Stewart and Guinn (1971) have also shown that certain tricarboxylic acid (TCA) cycle enzymes are more sensitive to low temperatures than others. Photosynthetic activity in chilling sensitive species also declines as a result of chilling. This has been shown to be caused in part by a decrease in the activity

of the membrane-bound Hill reactions in the chloroplasts (Smillie and Nott, 1979).

Studies have shown that the respiration rate of plant tissues of a number of chilling-sensitive plants such as apples, sweet potato roots, tomatoes, cucumbers, and peppers increase under chilling temperatures (Lyons, 1973). Leaf respiration rates of *Episcia reptans*, an extremely chilling-sensitive species, also have been found to increase during prolonged exposure to chilling temperatures (Wilson, 1978). The increased respiration rate during storage at chilling temperatures usually precedes the appearance of visible chilling symptoms (Lyons, 1973), and the peak respiration rate has been found to coincide with visible symptoms (Wilson, 1978). Some researchers have suggested that respiration rates be used as an indicator of the extent of chilling injury a plant has endured (Lyons, 1973). Wilson (1978), however, reports that the speed and degree of the respiratory increase in *E. reptans* leaves was dependent upon the physiological condition of the plant prior to exposure to chilling temperatures as well as the chilling temperature itself. Therefore, he warns against using the extent of respiratory increase as a reliable method of estimating chilling-sensitivity. Wise and Naylor (1987) did not find a change in respiration rate nor in the ultrastructure of mitochondria in cells when chilling-sensitive cucumber plants were subjected to 5° C and high light intensity (1000 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) for 12 hours. Wright and Simon (1973) found a rapid decline in both

respiration and photosynthesis rates of cucumber seedlings when they were transferred from 25° C and 7500 lx to 5° C in the dark. Similarly Stewart and Guinn (1971b) found a rapid decline in the respiration of cotton seedlings over a range of chilling temperatures. As might be expected by the reports of lowered respiration rates, there is often a corresponding decrease in ATP levels. Wilson (1978), however, found that ATP decline in chilled *Phaseolus vulgaris* leaves occurred only after the onset of visible symptoms. He further determined that the primary cause of decline in ATP levels was leaf injury and water stress and not low temperatures per se.

Subcellular abnormalities resulting from chilling have been recorded. Electron micrographs of mitochondria from chilled sweet potato roots revealed that large proportions were very swollen when compared to mitochondria from healthy tissue (Yamaki and Uritani, 1972). A separate study of mitochondria from lime and grapefruit after a chilling event revealed that the energy transfer system of the organelle had been compromised (Pantastico et al, 1969). Wright and Simon (1973) propose that mitochondrial membranes are highly susceptible to chilling damage. Kratsch and Wise (2000), however, report that mitochondria are more resistant to degradation due to chilling than are chloroplasts. Indeed, chloroplasts are generally agreed to be the first and most severely affected organelle under conditions of chilling temperatures. The dilation of thylakoid membranes and abnormalities of the shapes of chloroplast membranes due to

chilling at 5° C and high light intensity ($1000 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in chilling sensitive cucumber was mentioned previously (Wise and Naylor, 1987). Wright and Simon (1973) found a decrease in chlorophyll in the light at chilling temperatures, but not in the dark, and found no decrease in protein at chilling temperatures in either the dark or light. Herner (1990) reports that photosynthesis in chilling-sensitive species frequently declines after exposure to chilling temperatures. He suggests that this is in part due to the decreased activity of the Hill reactions, which has its proteins localized on the chloroplast membrane. The adverse affect of chilling temperatures on the chloroplast membranes may be resulting in the inactivation of the proteins in the Hill reaction. Another visible phenomenon of chilling injury on the cellular level is the effect on protoplasmic streaming. Reports of the reduction in protoplasmic streaming as a result of temperatures between 10° and 12° C in root hairs of cucumbers and tomatoes were reported in 1864 (Sachs, cited in Lyons, 1973). The streaming action requires energy and is dependent on the physical properties of the protoplasm and subcellular membrane system (Seifriz, cited in Lyons, 1973). It is likely that the reduced rate of streaming is a result of other physiological phenomenon that result from chilling temperatures, i.e. a reduced ATP supply and lipid membrane changes. Although the specific repercussions of reduced protoplasmic streaming are not known, the normal metabolism of the cell is probably greatly upset by the complete cessation of streaming (Lyons, 1973).

Several studies have attempted to correlate chilling injury with altered cellular constituents or metabolic products. McGlasson and Raison (1973) proposed that a disorganization of metabolic products occurs as a result of the change in E_a of respiratory enzymes. The accumulation of fermentation products such as ethanol and acetaldehyde noted in chilling sensitive plants stored at chilling temperatures (Murata, 1969) could result from the disproportionate decrease in respiratory enzymes compared to the products of glycolysis, which apparently are not inhibited at low temperatures (McGlasson and Raison, 1973).

Objectives

The objectives of this study were: a) to determine whether chilling injury is predominantly the result of a constant sub-optimal temperature or the result of a large differential in day/night temperatures with a sub-optimal average temperature; b) to distinguish between chilling injury symptoms resulting from average day/night temperature differentials and those due to drastic day/night temperature differentials; c) to document the visual symptoms of chilling injury at different day/night temperature regimes throughout the growth period up to transplant stage; d) to determine specific symptoms of chilling injury in tobacco transplants and to compare these symptoms with those associated with B deficiency in tobacco transplants; and e) to determine the effect of B deficiency and sufficiency on amelioration or exacerbation of chilling injury symptoms.

Chilling Injury Study

Materials and Methods

The chilling injury study examined the combined effects of temperature regime and boron concentration in solution on boron uptake by tobacco seedlings. Temperature was treated as the main treatment effect with B concentration as a split plot treatment and tobacco cultivar as a split-split plot treatment. For representative sampling, the statistical design was treated as a completely randomized block. Plants were grown in controlled-environment chambers located in the North Carolina State University Phytotron (Southeastern Plant Environment Laboratory) at four day/night temperature regimes: 26°/18° C, 32°/14° C, 21°/21° C, and 26°/26° C. Chambers were set to a 10-hour photoperiod with a 30 minute night interruption in the middle of the dark period and made use of high intensity lights to aid in the onset of visible symptoms of B deficiency/chilling injury. Average photon flux densities for each temperature regime were as follows: 26°/18° C = 931 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 32°/14° C = 920 $\mu\text{mol m}^{-2} \text{s}^{-1}$, 21°/21° C = 909 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 26°/26° C = 998 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature regimes were selected to test the hypothesis that chilling injury is induced by a large temperature differential, not by the average temperature. Each temperature regime either tested or served as a control for this hypothesis in the following manner: 26°/26° C - optimum temperature for maximum

growth; 21°/21° C - suboptimal temperature with no day/night differential; serves as a control to test the hypothesis that chilling injury is induced by a temperature differential; 26°/18° C - suboptimal temperature with an average of 21° C and an 8° C day/night difference; tests hypothesis that chilling injury is induced by a temperature differential; 32°/14° C - suboptimal temperature with an average of 21° C and an 18° C day/night difference; tests hypothesis that chilling injury is induced by a severe temperature differential.

Two B concentrations (split-treatments) were replicated twice; two high (19 µM) and two low (0.19 µM) B concentrations were located within each chamber. 12 L plastic tanks were used to hold the nutrient solution for each main treatment. Styrofoam seedling trays (Speedling Corp., Sun City, FL) were cut down to a size of 122 cells with a cell size of 2 cm² for the study and blocked into four sections for the four cultivars of tobacco. The four tobacco cultivars (split-split treatment) selected for the study were two flue-cured tobacco (C371 and K326) and two burley tobacco (TN90 and KY14).

The trays were filled with a 50/50 sand/vermiculite media which had been rinsed three times with 12 L of deionized water to leach any trace boron contamination. The sand/vermiculite media was compacted in the cells and pressed down to the bottom of each cell to insure that water would diffuse up into the media and avoid what is known as a "dry cell." The trays were then given an indentation in

the media in each cell to hold the seed. The trays were seeded with uncoated tobacco seeds by hand. Trays were thinned to two seedlings per cell after five days and then to one seedling per cell after 10 days.

All seedlings were germinated at a temperature of 26/18° C for the first two weeks after seeding. After this time temperature treatments were induced. The seedlings were started on 0.1385 M N solution with no other nutrients to avoid salt injury. After two weeks the full complement of nutrients was added to the 12 L of solution: 0.598 M N, 0.755 M K, 0.244 M Ca, 0.164 M Mg, 0.015 M S. Fe Sequestrene 330Fe (an iron chelate micronutrient from Ciba-Geigy) was added as a source of iron at a concentration of 1.1 mL per L of solution. The micronutrient stock was added to give the following final concentrations: 3.72µM MnCl₂·4H₂O, 0.32 µM ZnSO₄·7H₂O, 0.13µM CuSO₄·5H₂O, and 0.05µM H₂MoO₄·H₂O. Boron was added as B(OH)₃ to provide the treatment concentrations previously mentioned. All macronutrient stocks were treated to remove trace boron contamination by running solutions through an Amberlite IRA-743 resin column (Sigma Corporation). Nitrate and PO₄³⁻ solution concentrations were tested weekly throughout the study using an ion chromatograph (Dionex 2110i, AS4A 4mm column) to measure concentration levels. Nutrients were added back in the form of Mg(NO₃)₂ and KH₂PO₄ as needed to compensate for uptake.

Four tobacco seedlings per split-split treatment were harvested on 33, 41, 47, and 54 days after seeding to determine root and shoot fresh mass and dry mass (tissue was freeze dried) and tissue boron concentrations. At the final harvest plants were considered to be of transplant size: 15-20 cm tall with 8 to 10 leaves. Samples consisted of four plants per cultivar (split-split plot treatment) separated into roots and shoots and analyzed individually without subsampling. Tissue was digested as non-ground shoot and root material in concentrated high-purity nitric acid (Fisher, Optima grade) based on the procedure two described by Zarcinas et al., (1987). The Zarcinas et al. procedure was modified to use 4mL nitric acid for individual root and shoot samples due to the small mass of tissue samples. Each plant from the four plants per sample was divided into root and shoot tissue and individually analyzed. The samples were allowed to digest in the nitric acid overnight and then were microwaved at five-percent power for fifteen minutes in a standard food-grade microwave oven. The microwave oven was fitted with Tygon tubing leading into one side of a self-contained sample reservoir located inside the microwave oven. Another section of tubing lead out the other side of the reservoir and continued out of the microwave oven through a custom-cut hole, and out to the back of a fume hood. Di-nitrogen gas was flushed through the reservoir, to remove nitric oxide fumes that were produced during digestion, into the fume hood, where water was running to prevent corrosion of the fume hood due to acid fumes.. The samples were filtered through Whatman 40 filters and brought to 15mL with deionized water. The Whatman filters had been previously analyzed using the high purity nitric acid

used in digestions to determine the possibility of B contamination to samples. The Whatman 40 filters were not found to add any measurable amount of B to the samples. Samples were analyzed using inductively-coupled plasma atomic emissions spectroscopy with a Perkin Elmer Model 2000 DV emission spectrometer. The spectrometer utilized an axial view; it ran on 1500 watts of power and had a concentric glass nebulizer with cyclonic spray chamber. The argon flow was 15 L/min, nebulizer rate was 0.85 L/min, and auxiliary flow of 0.3 L/min. As an added precaution, all samples were run low to high anticipated B concentrations to avoid B carry-over contamination. The nebulizer was flushed with a DI water sample between B samples to remove residual B from the nebulizer.

Chilling Injury Study

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Results and Discussion:

Bare seeds were sown on an agar media under temperature and moisture conditions found to be optimal for tobacco germination in the B deficiency study. For the chilling injury study, tray cells were over-seeded and thinned twice, selecting for seedling uniformity. In spite of these techniques, there was significant variation in seedling size both between and within varieties. It is recognized that there is considerable variability in plant growth in highly controlled and favorable programmed environments due to the absence of any environmental stresses that can normalize growth rate of those seedlings that germinated first or grew faster at the beginning of the study. The damping effect of environmental stresses on the earlier-germinating or faster-growing seedlings serves to decrease variability in field studies and the absence of said effect serves to exacerbate variability under conditions of favorable growth (C.D. Raper, personal communication). Similar variability under controlled environment conditions was encountered by Haroon et al. (1972). However, because the plants that were sampled were selected at random, the results should be relatively free of bias. In an attempt to further reduce variability, seeds for each cultivar were taken from the same seed lot and therefore may not be entirely representative of all plants of that cultivar.

Boron Deficiency Study

The B deficiency study data indicated that 29-day old seedlings, when grown at a favorable 26°/22° C temperature regime, had a B deficiency threshold somewhere in the tissue B concentration range of 10-20 $\mu\text{g g}^{-1}$. This corresponded to a nutrient solution B concentration of 0.19-1.9 μM (Fig. 1a). Dry matter data indicated that root and shoot tissue mass dramatically increased between these solution B concentrations (Figs. 1b and 1c). Greater leaf area was produced by solution B levels at or above 1.9 μM compared to solution B levels at or below 0.19 μM , suggesting that sufficient B concentrations promote greater leaf area development (Fig. 2). The greatest number of leaves per plant on day 29 was observed at the highest B concentration in solution (19 μM), indicating that B stimulated leaf initiation or that B deficiency inhibited leaf initiation (Fig. 3). The 1.9 μM B concentration showed the second greatest leaf initiation in 3 of the 4 varieties (C371, K326, and KY14) at day 29 (Fig. 3). No clear differences can be detected in leaf number as a function of B concentration in solution among the different cultivars; thus there apparently was no varietal variability in sensitivity of leaf development to B concentration. The increase in leaf initiation was slightly less obvious than the increase in leaf area between the critical B concentrations of 0.19 and 1.9 μM .

Chilling Injury Study

Qualitative Results:

Visually, the 26/26° C treatment produced the most vigorous, healthy-looking transplants, especially in the high B (19 µM) treatment. This treatment produced moderate to good seedling establishment estimated at 75-80%. Germination at 26/26° C was slower than at 26/18° C, but was greater than at 21/21° or 32/14° C. Prior to day 20 after seeding, this treatment along with the 26/18° C treatment produced the largest seedlings (1 to 2 true leaves). By day 25, these seedlings were observed to be the tallest plants and appeared to have the greatest leaf area, followed closely by the 26/18° C treatment. At this stage there were no visual differences between B treatments in any temperature regime. Leaves were horizontal to the surface. The first symptoms of B deficiency were observed on day 33 after seeding and appeared in all temperature treatments. At day 38, the 26/26° C treatment had produced seedlings with healthy, large leaves. Generally there was one dominant leaf at this point. Leaves were tilted at approximately 45° from the horizontal and the stems appeared larger and sturdier than stems of other treatments, particularly the 32/14° C treatment. By day 41, leaves from both B treatments were exhibiting some lesions on leaves, indicating that this was not simply a B deficiency symptom. However, only the low B treatment exhibited leaf

deformities at this stage. The TN90 cultivar displayed some unusual leaf deformities including ruffled leaf margins and white leaf centers with green leaf margins; such coloration patterns are sometimes displayed in healthy burley varieties. By day 47, seedlings from the low B treatment showed necrosis of the apical meristem (Fig. 5c). Such symptoms were not displayed by seedlings in the high B treatment. It was also on day 47 that the first symptoms of chilling injury became distinctly noticeable in both B treatments and was apparent in all treatments. The chilling symptoms were first displayed as small round lesions, leaf deformities that made the leaf appear twisted (Fig. 6a) as opposed to leaf deformities caused by B deficiency which made the leaf appear to have grown abnormally (Fig. 6b), and as leaf discoloration, characterized by light or white areas in the middle of the lamina. This was probably not the result of N deficiency, as solution was recharged with N on a weekly basis after the solution was measured using an ion chromatograph. By day 54, over 75% of all samples taken from the low B treatment in the 26/26 °C treatment displayed symptoms of apical meristem necrosis. No plants from the high B treatment showed these symptoms. High B treatment plants were, however, somewhat bleached. Chilling injury symptoms were also evident at this stage on both low and high B treatments. This phenomenon is described and an explanation is proposed below. The 26/26° C low B treatment showed the most pronounced symptoms of B deficiency of any combination of temperature and B concentration (cupped leaves, leaf deformities and lesions, and eventual necrosis of the apical

meristem) (Fig. 5). This could be the result of the unrestricted growth rate of plants in the 26/26° C treatment, which by depleting the small amount of available B, made it unavailable to newly developing tissues with B eventually becoming limiting for plant growth. Unexpectedly, the 26/26° C treatment also showed most clearly the classic symptoms of chilling injury in young tobacco transplants. The symptoms were exhibited by those plants growing closest to the doorway of the chamber and included stunted growth, leaf cupping, and leaf deformities (Fig. 6a). It is possible that the very cool ambient temperature of the phytotron (which ranged over a single 24-hour period between 13.3° C and 16.2° C) was chilling those seedlings in the doorway of the 26/26° C treatment. These plants may have been more susceptible to the sporadic cooler ambient temperatures that occurred while the door was opened for sampling, thinning, or observations because they had never been exposed to cool temperatures and, therefore, were not cold hardened. Due to the experimental design of the study, plants from the low B treatment and high B treatment had equal exposure to the chamber doorway, and thus both B treatments showed chilling injury at this temperature regime. There was no apparent visual difference between the chilled plants of the two different B concentrations. It could be argued that symptoms observed in the B deficient treatments were only resulting from B deficiency; however, the symptoms were displayed somewhat differently. Looking at a plant displaying B deficiency symptoms and then looking at a plant displaying possible B deficiency as well as chilling injury, the symptoms of chilling

injury were subtly different. The chilling injury symptoms were manifested as twisted and distorted leaf shapes and the lesions formed on leaves had more of a light brown appearance than the darker brown lesions produced by B deficiency. The lesions were also generally smaller and looked more like a dimpling on the leaf surface than the larger lesions associated with B deficiency, which appeared to invade the lamina tissue more deeply. It should also be taken into consideration that, had the chilling temperature been more severe (lower), the chilling symptoms may have been more pronounced, making them appear even more like B deficiency symptoms. It is also possible that less-severe B deficient conditions may produce less pronounced symptoms, which may appear more like the mild chilling injury symptoms displayed. It appeared that the chilling injury symptoms may have been either masking or eliminating B deficiency symptoms. It is possible that chilling temperatures stunted growth and thereby caused plant B demand to decline and prevented the occurrence of B deficiency.

Seedlings from the 21/21° C and 26/18° C temperature treatments appeared very similar at the end of the study. Even though the 26/18° C treatment had a more rapid germination rate, seedling establishment was similar in both treatments. Prior to day 20 after seeding, the 26/18° C and 26/26° C treatments produced the largest seedlings, which had 1 to 2 true leaves. Twenty days after seeding, most of the 21/21° C treatment seedlings were at the cotyledon stage

of development. By day 25, seedlings in the 21/21° C and 26/18° C treatments had approximately the same leaf area, although the seedlings in the 26/18° C treatment appeared slightly shorter. All leaves were lying flat and approximately horizontal to the surface. Most seedlings had 2 to 3 true leaves. There were no visual differences in leaf geometry attributable to B treatments. On day 33, B deficiency symptoms (leaf cupping and interveinal puckering) were noted in both temperature treatments in the low B treatments. By day 38, most seedlings had between 3 and 5 true leaves. Leaf area appeared similar in the 21/21° C and 26/18° C treatments. At this stage, plants at the 21/21° C treatment appeared somewhat shorter than the 26/18° C treatment. By day 41, plants subjected to low B treatments displayed leaf lesions and leaf deformities that were not visible in the high B treatments. On day 47, there was no evidence of necrosis of the apical meristem in either the 21/21° C or the 26/18° C treatments, unlike the 26/26° C seedlings. The TN90 treatments showed leaf discoloration mainly consisting of white inner lamina tissue with green, ruffled margins. At this point some mild chilling injury symptoms occurred as deformed leaves and leaf discoloration. At day 54, low B treated seedlings displayed necrosis of the apical meristem (less than 25% of seedlings sampled) and lesions in the 21/21° C treatment. The 26/18° C treatment displayed lesions in the low B treatments as well as dimpling of the leaf surface and rosetting of the high B treatments.

The seedlings in the 32/14° C treatment were the most physiologically stressed of all temperature treatments. Fig. 7 visually compares differences among treatments. They exhibited low germination, and prior to day 19 seedlings still had no true leaves and were taller with smaller diameter stems than seedlings from other treatments. On day 25 after seeding, this temperature regime produced seedlings that were taller and "leggier" with a smaller leaf surface area than other treatments. Most seedlings had 2 to 3 leaves. It was not possible to discern between B sub-treatments at this stage. Leaves were judged to be at a more acute angle pointed upwards than other treatments, possibly a reaction to photo-oxidation in an effort to reduce leaf area for photosynthesis. B deficiency symptoms were first observed on day 33 in the low B treatment. By day 38, leaves were still smaller and plants were tall and "leggy" (Fig. 7). There were only sporadic indications of B deficiency among low B treatments. Plants were not well-supported by stems, and some plants would collapse on their stems in the middle of the day cycle and then straighten back up during the night cycle. Most seedlings were at the 3 to 4 leaf stage. By day 41, plants displayed a range of stress characteristics including leaf deformities and other B deficiency symptoms in both the low and high B treatments. It is evident that since B deficiency and chilling injury symptoms share many of the same morphological symptoms with other plant environmental stresses, that these symptoms cannot be used to indicate B deficiency in otherwise environmentally-stressed seedlings. At day 47, almost 75% of seedlings from the low B treatments had B deficiency

symptoms displayed as leaf deformities and leaf cupping. It was interesting to note that seedlings from both treatments with a day/night differential and the high B treatments showed some symptoms of rosetting. This did not occur in treatments with a constant day/night temperature nor in low B treatments. By day 54, over 75% of seedlings sampled from the low B treatment displayed apical meristem necrosis. Other symptoms of B deficiency were leaf lesions and deformities and cupping of leaves. There were not any distinct symptoms of chilling injury, probably due to the fact that these seedlings were highly stressed and symptoms may have been masked by the small leaf area and other evidence of environmental stress.

Quantitative Results:

Figs. 8, 9, 10, and 11 describe shoot B concentrations of flue-cured and burley varieties at different temperature treatments, B treatments, and harvest dates. Note that the previously established B deficiency threshold zone of 10 to 20 $\mu\text{g kg}^{-1}$ dry weight is shaded. Recall that this zone was established for seedlings grown at 26/22° C and an average photon flux of 541 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The 19 μM B treatments exhibited greater uptake than 0.19 μM B treatments. High B treatments had decreased uptake of B at the sub-optimal temperature treatments of 21/21°, 26/18°, and 32/14° C compared to the 26/26° C treatment (Figs 8 and 9). This reduced uptake, while not always statistically

significant, is more dramatic in flue-cured varieties than burley. This is to be expected since burley varieties are developed in and traditionally grown in cooler regions than flue-cured varieties, and therefore are acclimated, and possibly grow more optimally at lower temperatures. The low B treatments, however, show only small differences in B tissue concentration between temperature treatments or varieties. There appears to be a maximal B depletion rate at lower solution B concentrations that is not affected by the temperature treatments imposed in this study.

At the maximum day/night temperature differential of 32/14° C, B uptake was always below or within the B deficiency threshold of 10-20 $\mu\text{g g}^{-1}$ dry matter. However, this deficiency threshold was determined for plants growing at optimal temperature conditions (26/22° C), and it is possible that other, more stressful, temperature regimes may alter this B deficiency threshold, making it lower at lower temperatures. Indeed, evidence here supports that this threshold is variable depending upon environmental conditions including day/night temperature regime. Mechanisms for such a shift in deficiency thresholds at sub-optimal temperatures might include reduced transpiration, reduced mass flow, reduced uptake, and physiological chemical interactions. The reduced deficiency threshold phenomenon can be supported by data represented here describing shoot masses at the two B treatments and different temperature treatments. Shoot masses of flue-cured varieties at day 47 (Fig. 12) were

greatest for C371 at lower B than higher B treatments in the 21/21° and 26/18° C treatments. The same samples, when measured for B concentration in tissue, were found to have deficient or marginally deficient tissue B content for normal growth of seedlings at 26/22° C. More specifically, the low B treatment for the 21/21° and 26/18° C produced seedlings that were well within the deficiency range established under 26/22° C conditions and the two higher B treatments were found to be marginally deficient under the same conditions. Similarly, the K326 data (Fig. 12) show that the 26/18° and 32/14° C treatments also produced greater shoot tissue at lower B concentrations. Again, the 26/18° and 32/14° C low B treatments would be classified as B deficient while the high B treatments were found to be marginally B deficient. Shoot mass of TN90 cultivars (Fig. 13) for the 32/14° and 26/18° treatment and 21/21°, 26/18°, and 32/14° treatments have the same trend. This points strongly to the conclusion that the B deficiency threshold is reduced under sub-optimal temperature conditions. Several postulates can be developed to explain this phenomenon. One is that there is a temperature and B deficiency interaction resulting in reduced shoot growth. Another is that, if the B deficiency threshold is lowered at sub-optimal temperatures, then the B toxicity threshold could also be lowered and a marginal B toxicity thus could occur at lower temperatures even under B supply considered to be optimal at a warmer temperature.

Root mass of 47 day-old flue-cured seedlings is consistently (but non-significant) greater at the lower B values regardless of temperature regime (Fig. 14). For these flue-cured varieties sub-optimal temperatures result in decreased root growth but B deficient conditions do not. Again, it should be kept in mind that the conditions considered B deficient were based on a 26/22° C temperature regime, and may be different at different temperatures. When Figs. 14 and 15 are compared, it again appears that flue-cured varieties grow best under 26/26° C treatment and burley varieties produce most root mass under 26/18° C day/night regime. All cultivars grew best at low B treatments.

Based on this data, temperature may be the immediate limiting factor and B deficiency an additional limiting factor. It also can be proposed that the B deficiency threshold is reduced under sub-optimal temperature conditions.

Haroon et al. (1972b) studied effects of day/night temperature regimes on seedlings of a flue-cured tobacco variety "NC2326." They concluded that night temperature contributed more to the total treatment effect on dry mass than either day temperature alone or the day/night temperature interaction. It is possible to use Haroon et al.'s data to compare the results of our temperature treatments as well as examine the effects of B treatments. Table 2 represents comparisons of the 26/26° and 26/18° C treatments at low and high B levels. The most significant results from this analysis indicate that shoot growth of

transplant-stage flue-cured seedlings (Day 54, C371 and K326) was maximized by a constant optimal day/night temperature regime and by adequate B concentrations. This data is supported by the work of Haroon et al. in that the decreased night temperature regime (26/18° C) did indeed show reduced shoot mass. Other data show that root mass, in general, increased under conditions of low B and that root mass was generally greater at a 26° C day temperature. C371 shoot masses were always significantly greater under conditions of 26/26° C high B treatment. This data, ignoring the B treatments, was in agreement with Haroon et al.'s conclusion that an increase in day or night temperature resulted in increased growth and a decrease in day or night temperature resulted in decreased growth, especially, they emphasize, the effect of night temperature resulting in decreased mass.

Temperature Effects on Boron Uptake

Fig. 20 examines the effect of temperature on B uptake at sufficient solution B concentrations (19 µM only). From this graph it is evident that flue-cured varieties respond to higher temperatures and constant day/night temperatures with more growth. The C371 cultivar, known to be cold-sensitive, is clearly more responsive to the drop in average temperature than in the K326 cultivar. The burley varieties are more tolerant of cooler temperatures overall, but do reach the same tissue mass at any temperature treatment that the flue-cured varieties reach at the 26/26° C treatment. The application of this data for tobacco

transplant growers is that flue-cured varieties are very sensitive to decreases in temperature and grow optimally at a constant day/night temperature close to 26° C. Burley transplant growers have a wider range of temperatures for near optimal growth, but in general constant day/night temperatures seem to provide the greatest shoot tissue accumulation. Both varieties produced greatest tissue mass and accumulated the greatest content of B at transplant stage under constant day/night temperatures (Figs. 18, 19, and 20), indicating that reduction in night temperature and/or an increase in day/night temperature difference results in a reduction in B uptake.

Discussion: Relationship of Boron Deficiency and Chilling Injury:

There are a number of possible explanations for the similarity in symptoms between B deficiency and chilling injury observed in flue-cured and burley tobacco cultivars. It seems clear that B deficiency affects lignin synthesis, auxin synthesis and metabolism, sucrose transport, and the normal functioning of apical meristems (Lewis, 1979; Goldbach, 1985; Blevins et al., 1998; Shelp, 1988; Lovatt, 1985; Li et al., 1997; Kobayashi et al., 1999). A substantial obstacle in determining interactions involving B deficiency is the lack of understanding regarding primary versus secondary reactions of B deficiency. While there are many researchers proposing different modes of action to be the primary result of B deficiency, not many researchers are in agreement on this controversial subject. This information would greatly facilitate the ability to trace

the symptoms of B deficiency back to a primary locus and malfunction that is associated with mechanisms of chilling injury. Because chilling injury symptoms are initially and predominantly the result of damage incurred by the plasma membrane, this seems like a logical cell location to begin seeking interactions between chilling injury and the different physiological reactions to B deficiency. There is the possibility that B deficiency could produce the same symptoms as chilling injury for any of the mechanisms in which lack of B disrupts cell membranes. Several mechanisms have been suggested. Further research is needed to test these potential pathways to further elucidate the seeming myriad of plant responses to B deficiency.

One theory implicating B deficiency with decreasing membrane integrity is put forth by Jackson (1991), who conducted a study examining energy-dependent protein secretion from pollen of *Petunia hybrida*. Pollen was shaken in a culture solution of 10% sucrose with and without boric acid over a range of temperatures from approximately 10° to 35° C. Without B, protein secretion did not change markedly with temperature except over a narrow, 6° C range between 14 and 20° C. Over this narrow range there was a very rapid increase in protein secretion with a corresponding decrease in pollen tube elongation suggesting that protein secretion was uncontrolled and excessive and is therefore not available for pollen tube extension. At about 19° C the rate of secretion declines, presumably due to an improvement in plasma membrane

organization. Addition of boric acid to the culture solution was found to decrease the rate of protein secretion at 17° C and correspondingly increase the rate of pollen tube extension. The implication is that below 17° C and without B, proteins freely diffuse across the cell membranes due to the compound effects of low temperature and B deficiency, to decrease plasma membrane integrity. When boric acid is present in solution, an additional peak in protein secretion occurred at approximately 26° C followed by another plateau; however, even the highest level of protein secretion in the plus B solution was lower than the corresponding levels of protein secretion in the minus B solution. Jackson interprets the sharp discontinuities in protein secretion to be related to lipid thermotropism in membranes. Since addition of boric acid caused lipid thermotropism to occur at a different temperature, a role for B in membranes is inferred. Adding B not only gave drastically longer pollen grains, but also promoted extension of pollen tubes over a much broader temperature range. Jackson proposes that B is involved in the movement of proteins into extending pollen tube membranes from secretory vesicles and possibly in pollen tube elongation and/or protein insertion in pollen tube cell membranes.

A key to unraveling the mystery of B deficiency interactions in the plasma membrane was discovered in 1998 by Matoh et al. who determined that the stable cell wall borate-rhamnogalacturonan II (B-RGII) complex is predominantly located proximal to the plasma membrane, and that B cross-links can be

localized at the cell wall-plasma membrane interface. This implicates the plasma membrane in a variety of B interactions with the cell wall, especially with cell wall pectins and pectin synthesis.

In another study, Lovatt (1985) reported that B deprivation has been shown to decrease synthesis of nucleotide sugars or the synthesis of ribonucleotides and deoxyribonucleotides of cytidine and thymidine. This would result in decreased sucrose synthesis, increased starch accumulation, and interference with normal cell wall function. The cytidine nucleotides, in reference to membrane integrity, are of central importance in the synthesis of the major phosphoglycerides which serve as the components of membranes. This suggests a connection between B deficiency and membrane integrity as well.

A subsequent suggestion by Lovatt (1984) pertaining to the decreased synthesis of nucleotide sugars is that expression of B deficiency may actually be a case of pyrimidine starvation. Lovatt et al. (1984) cited several studies indicating that inhibiting pyrimidine biosynthesis produced symptoms identical to those of B deprivation. This raises the question of a possible relationship between chilling injury and nucleotides.

Stewart and Guinn (1971) studied the effects of chilling at 3° and 5° C on the nucleotide content of leaves and roots of cotton. They found that chilling

decreased the nucleotide concentration, especially of di- and tri- phosphates, in both the leaves and roots. There was a less dramatic decrease in NADP and NADPH induced by chilling as well. The decrease in nucleotides was concomitant with a corresponding increase in nucleosides. The authors suggested that increased phosphorolytic activity is associated with chilling injury. McGlasson and Raison (1973) showed by Arrhenius plots that the apparent energy of activation of respiration increases abruptly at low temperatures in various chilling-sensitive species. They attributed this change to reduced membrane integrity of the mitochondria under chilling stress. The decreased rate of phosphorylation indicated by the decrease in high-energy phosphates found by Stewart and Guinn could have been the result of the increased activation energy of respiration processes resulting from chilling injury.

It appears that there is sparse evidence that both B deficiency and chilling injury result in reduced levels of nucleotides. While there is not as yet enough evidence to draw definitive conclusions, there is a possibility of decreased DNA synthesis, ATP synthesis, and the resulting accumulation of precursors resulting in similar symptoms in the two conditions.

Some researchers argue that the most probable site for the primary mode of action of a condition is the site where the condition first manifests itself as an ultrastructural change. For B deficiency this would be a decrease in the nucleic

acid synthesis in apical meristems (Lovatt, 1985) and for chilling injury this would be the ultrastructural changes in the chloroplasts (Nessler and Wernsman, 1980) or the plasma membrane (Lyons, 1973; Wright, 1974; Wright and Simon, 1973).

One of the first symptoms of B deficiency is an alteration in the cytoskeleton. Similar alterations have been observed in cells during plant chilling acclimation. Cytoskeleton components have shown prominent responses to B deficiency in the forms of increased amounts of actin and tubulin proteins observed just 20 or 40 minutes, respectively, after B withdrawal as well as denser cytoskeletal arrays in the transition and elongation zones of roots (Goldbach et al., 2001).

Goldbach et al. suggest that these results "might be attributed to the up-regulation of specific actin and tubulin isoforms and/or to inhibited protein degradation." They speculate that B deficiency may be impacting the cytoskeletal dynamic equilibrium via the extracellular matrix-plasma membrane-cytoskeleton continuum. Either expression of different genes or post-translational modification of proteins could lead to the expression of multiple isoforms. Goldbach et al. correlated these findings with the results of Kerr and Carter (1990) and surmised that actin and tubulin isoform regulation by B deprivation may be similar in mode of action to similar regulation induced by chilling acclimation.

The results of the B deficiency threshold study were in agreement with Kirk and Loneragan's study (1988), despite the fact that results were analyzed in the present study by correlating tissue B content and dry matter accumulation, which was the technique they were challenging in their study. Kirk and Loneragan contended that measuring dry matter accumulation in order to determine the critical nutrient threshold may not be a sufficiently sensitive measurement in young leaf tissues. They based their argument on the fact that, because under B deficient conditions B is less available to new leaves, correlating tissue B content of young leaves with dry leaf accumulation may give a deceptively low B threshold value. They also pointed out that a reduction in dry weight of B deficient plants sometimes occurs slower than other more sensitive indicators of B deficiency, such as leaf expansion. They suggested that a measurement of leaf expansion rate would provide a more sensitive correlation for determining critical B deficiency thresholds. Data from these studies indicated a B deficiency threshold on a whole plant basis (not on individual leaves) for tobacco to be somewhere in the range of 10 to 20 $\mu\text{g B g}^{-1}$ dry matter. Compared to Kirk and Loneragan's estimate of 12 $\mu\text{g B g}^{-1}$ dry matter as an estimate of the critical value for blade elongation in the unfolding leaf of soybean plants, the estimate for a shoot threshold in tobacco seedlings appears to be in agreement.

A possible criticism of the experimental design of the chilling injury study is that the most extreme treatment, 32°/14° C, did not reach a chilling temperature and

therefore did not truly test for chilling conditions. Kodama et al. (1995) reported that "moderately low temperatures such as 14° C" may induce chilling acclimation in some plants. While there was no indication of chilling tolerance in the 32°/14° C treatment, there was also no more indication of chilling injury than in other treatments hypothesized to be more favorable.

Table 1. Solution boron concentration averaged over two replicates $\pm 1.85e-4 \mu\text{M B}$

Days After Seeding	Day/Night Temperature Treatment ($^{\circ}\text{C}$)	Avg. B Conc in Sol'n of High B Treatment (μM)	Avg. B Conc in Sol'n of Low B Treatment (μM)
20	26/18	28.5	1.02
	32/14	30.4	0.88
	21/21	28.9	0.79
	26/26	23.6	0.79
35	26/18	7.62	0.33
	32/14	5.62	0.34
	21/21	10.9	0.19
	26/26	11.21	0.19
47	26/18	5.41	0.19
	32/14	1.44	0.19
	21/21	7.22	0.33
	26/26	2.96	0.09
62	26/18	4.63	.23
	32/14	3.52	0.06
	21/21	6.76	0
	26/26	5.83	0

Table 2. Comparisons of root and shoot growth at various temperature and boron treatments. Red arrows indicate significant differences.

Day/Root or Shoot	Comparison	C371	K326	TN90	KY14
Day 54/ Shoots	26/26 Hi B vs. 26/18 Hi B	↑	↑	↓	↑
	26/26 Hi B vs. 26/18 Lo B	↑	↑	↓	↑
	26/26 Lo B vs. 26/18 Hi B	↑	↑	↑	↑
	26/26 Lo B vs. 26/18 Lo B	↑	↑	↓	↑
Day 47/ Shoots	26/26 Hi B vs. 26/18 Hi B	↑	↑	↑	↑
	26/26 Hi B vs. 26/18 Lo B	↑	↑	↑	↑
	26/26 Lo B vs. 26/18 Hi B	↑	↑	↑	↓
	26/26 Lo B vs. 26/18 Lo B	↑	↑	↑	↓
Day 54/ Roots	26/26 Hi B vs. 26/18 Hi B	↑	↑	↓	↓
	26/26 Hi B vs. 26/18 Lo B	↑	↑	↓	↔
	26/26 Lo B vs. 26/18 Hi B	↑	↑	↓	↑
	26/26 Lo B vs. 26/18 Lo B	↑	↑	↓	↑
Day 47/ Roots	26/26 Hi B vs. 26/18 Hi B	↑	↑	↑	↑
	26/26 Hi B vs. 26/18 Lo B	↑	↑	↓	↓
	26/26 Lo B vs. 26/18 Hi B	↑	↑	↑	↑
	26/26 Lo B vs. 26/18 Lo B	↑	↑	↓	↓

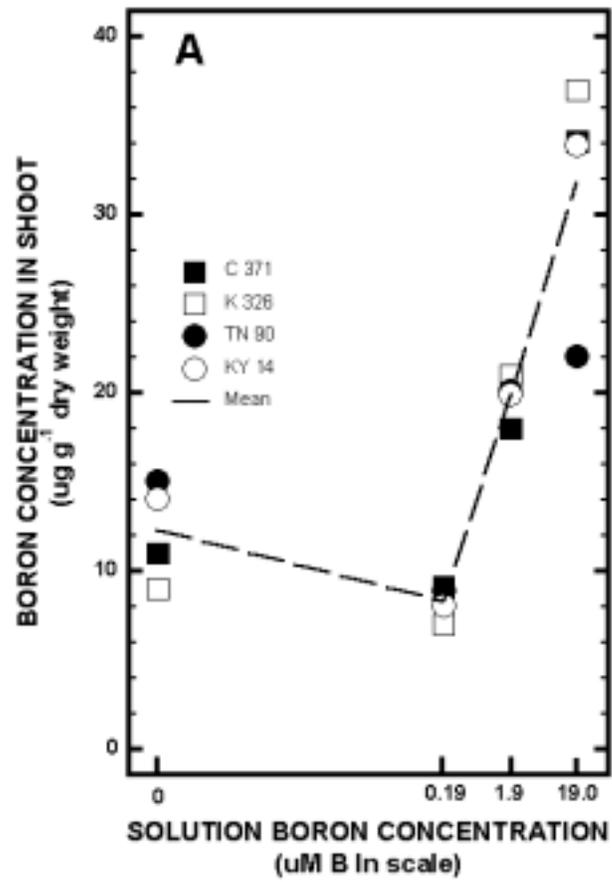


Fig. 1a. Boron concentration in shoot tissue as a function of external B concentration in nutrient solution

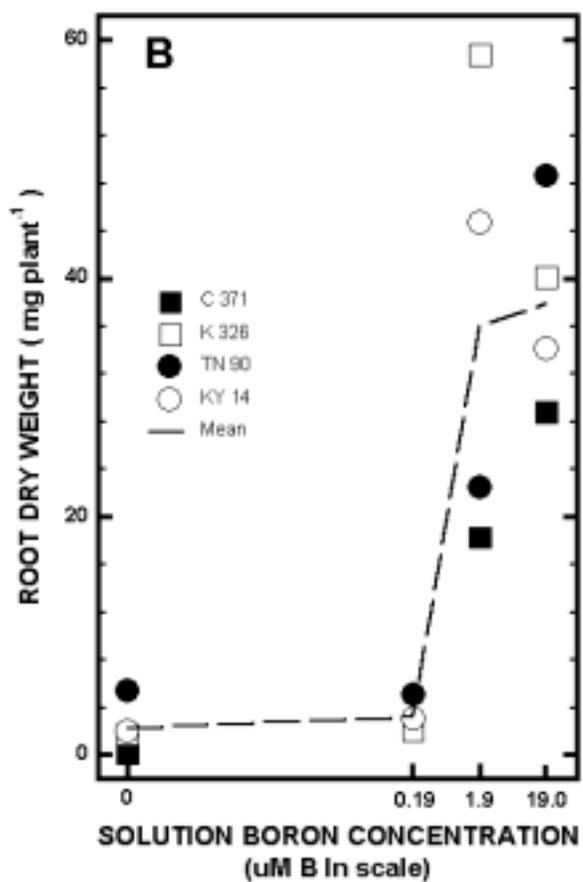


Fig. 1b. Root dry weight as a function of external B concentration

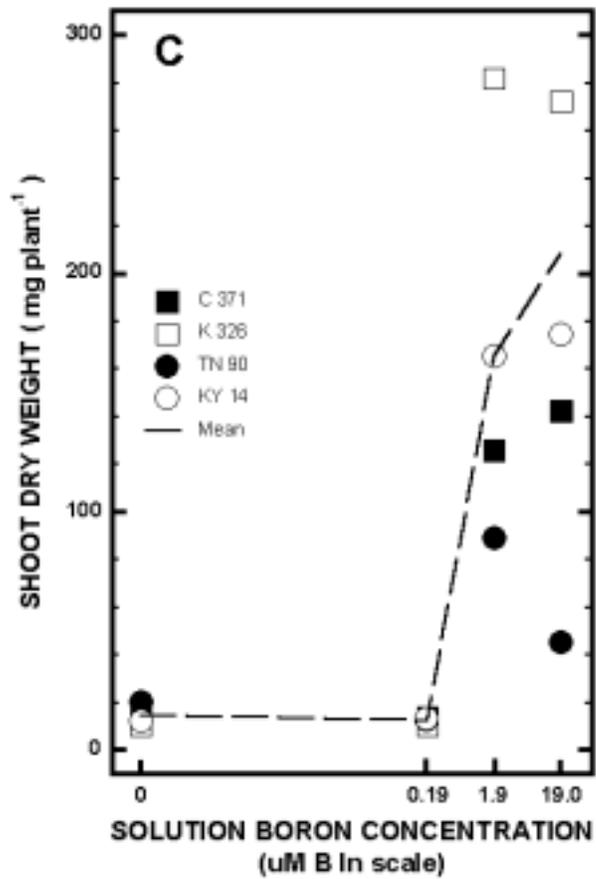


Fig. 1c. Shoot dry weight as a function of external B concentration in nutrient solution

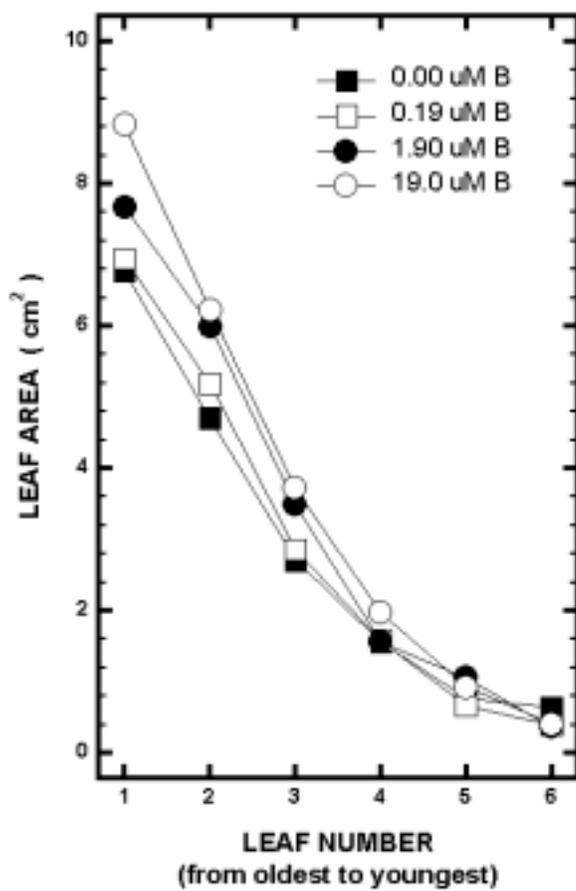


Fig. 2. Leaf area over morphological time

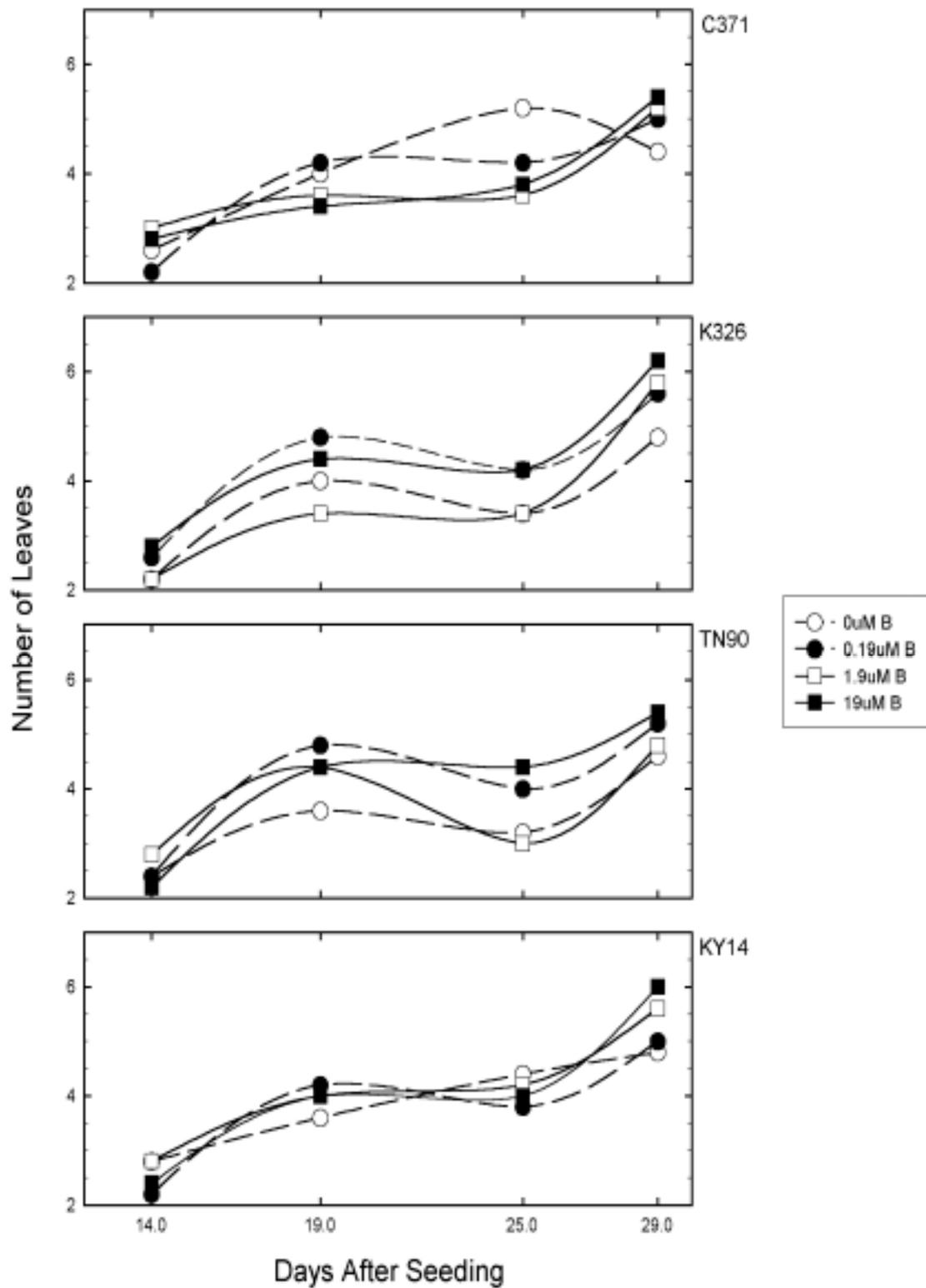


Fig. 3. Leaf initiation as a function of B concentration in solution and days after seeding

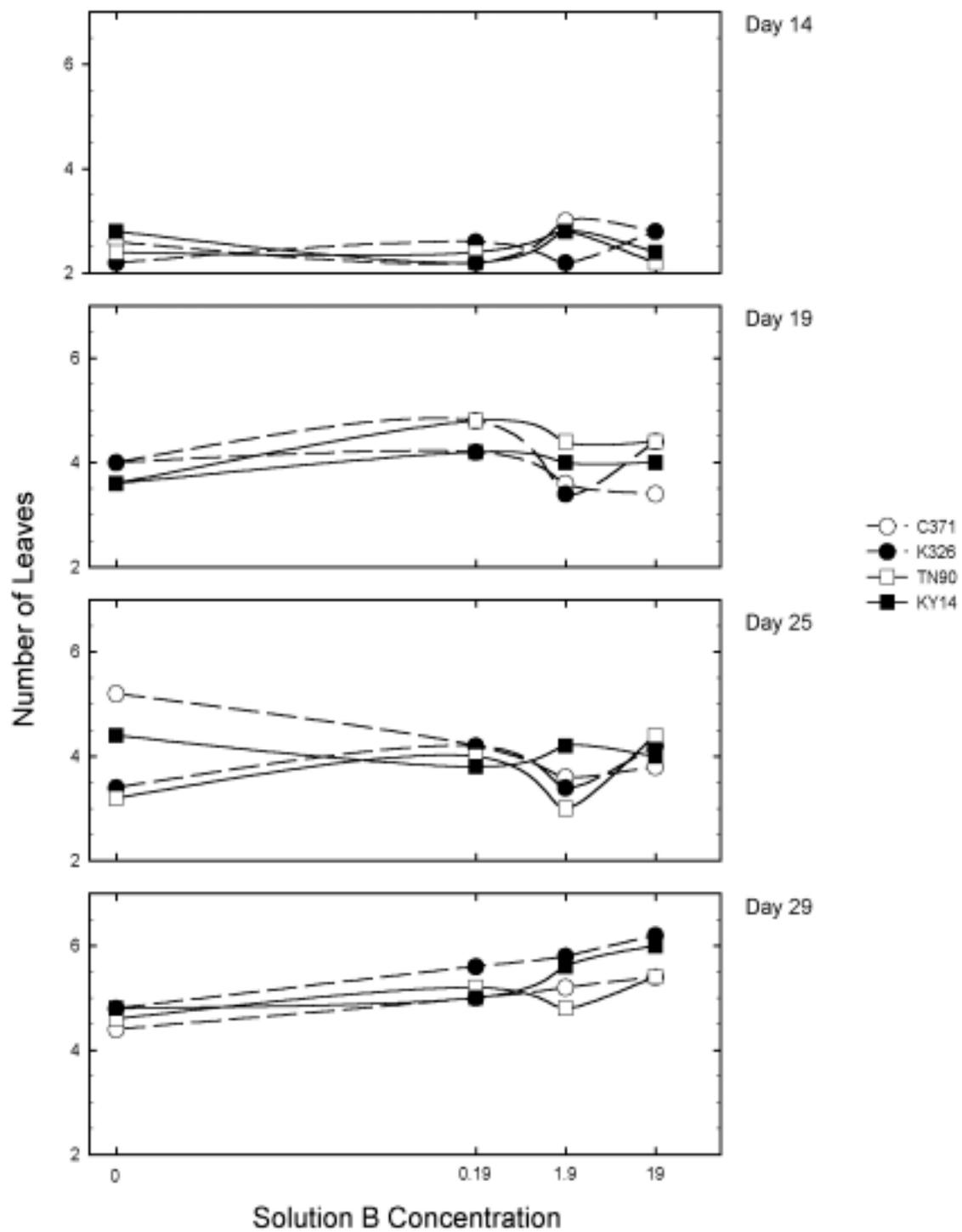


Fig. 4. Leaf Number as a function of solution B concentration over time

B Deficiency Symptoms



Fig. 5a - Symptoms of B deficiency including interveinal puckering, leaf dimpling and discoloration



Fig. 5b – Leaf discoloration among B deficient burley varieties, like this KY15 sample



Fig. 5c – Apical meristem necrosis and failure to maintain apical dominance as a result of B deficiency



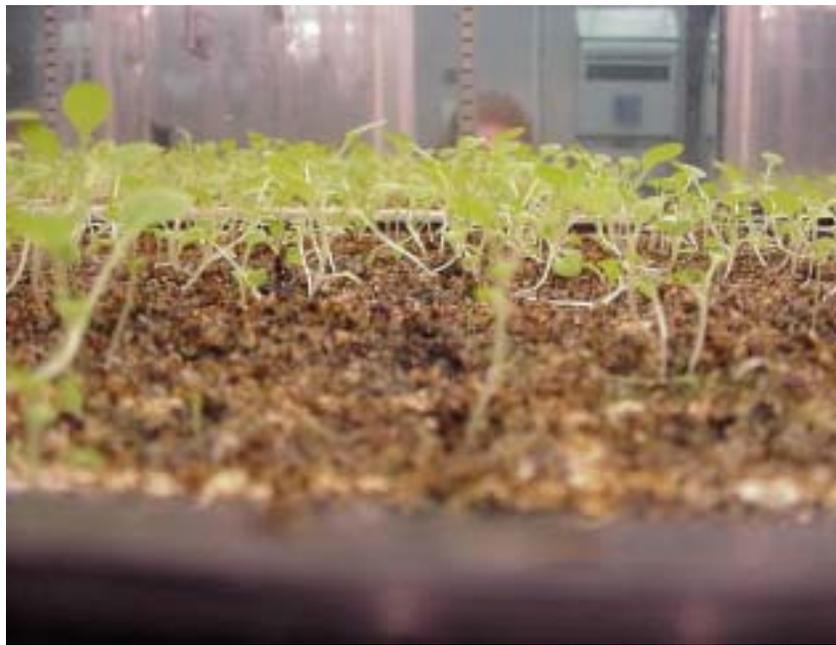
Fig. 6a – a chill-injured seedling grown at 26/26° C (in doorway of chamber) in high B treatment



Fig. 6b – a B-deficient seedling grown at 26/26° C in low B treatment



a.



b.

Fig 7a – 26/18 High B treatment
Fig 7b – 32/14 High B treatment

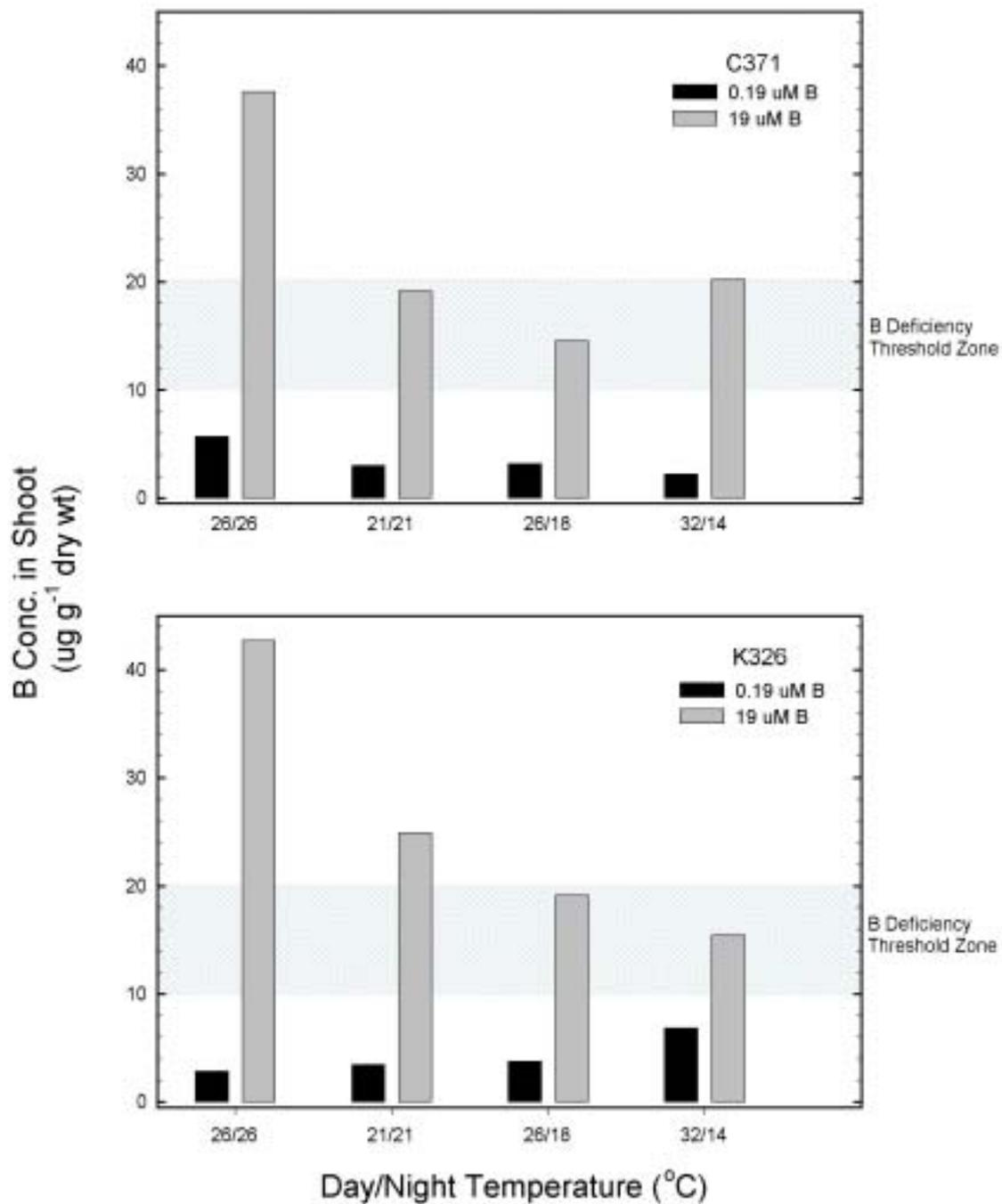


Fig. 8. Boron concentration in 47 day-old flue-cured shoot tissue at different temperature regimes

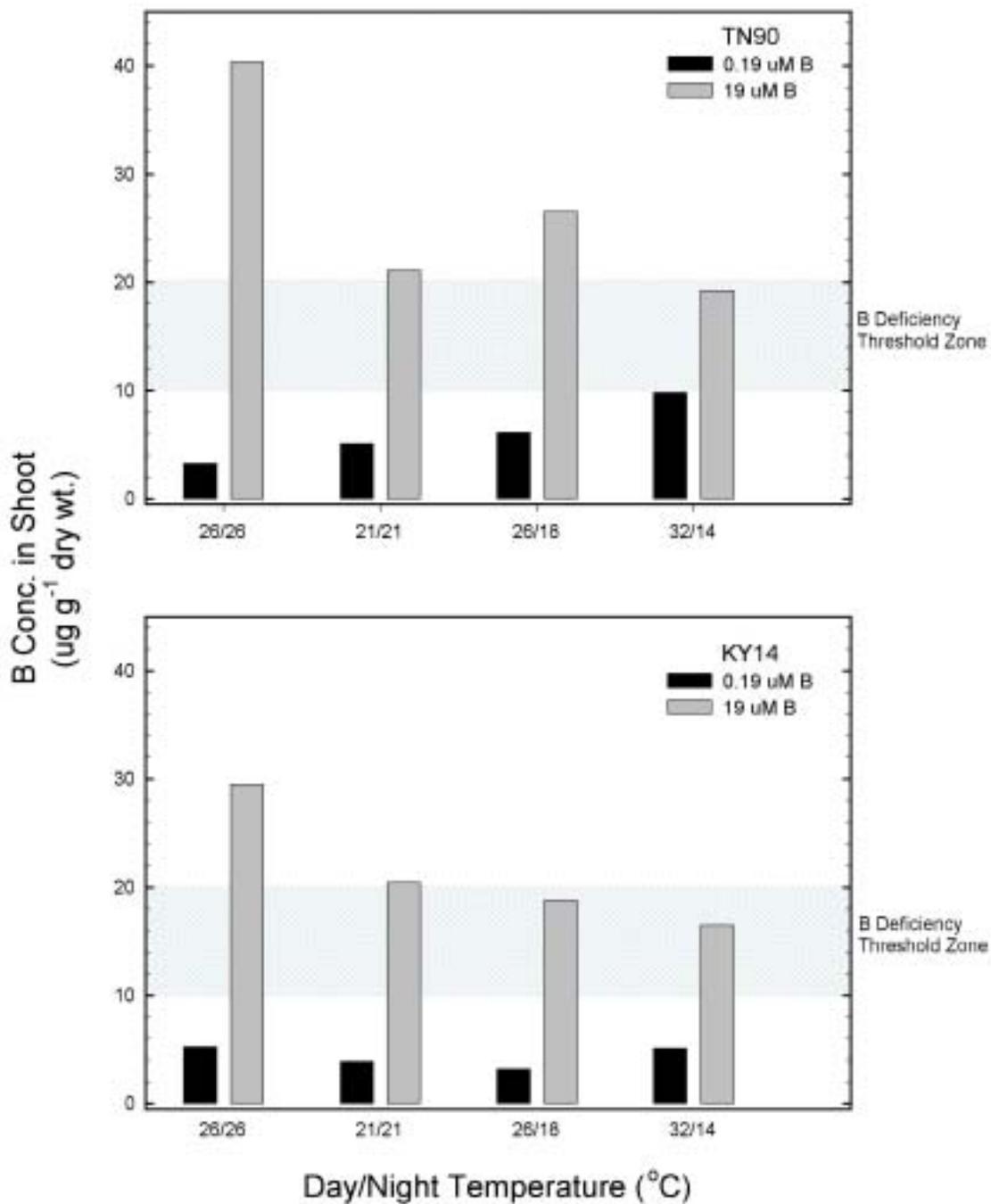


Fig. 9. B concentration in 47 day-old burley shoot tissue at different temperature regimes

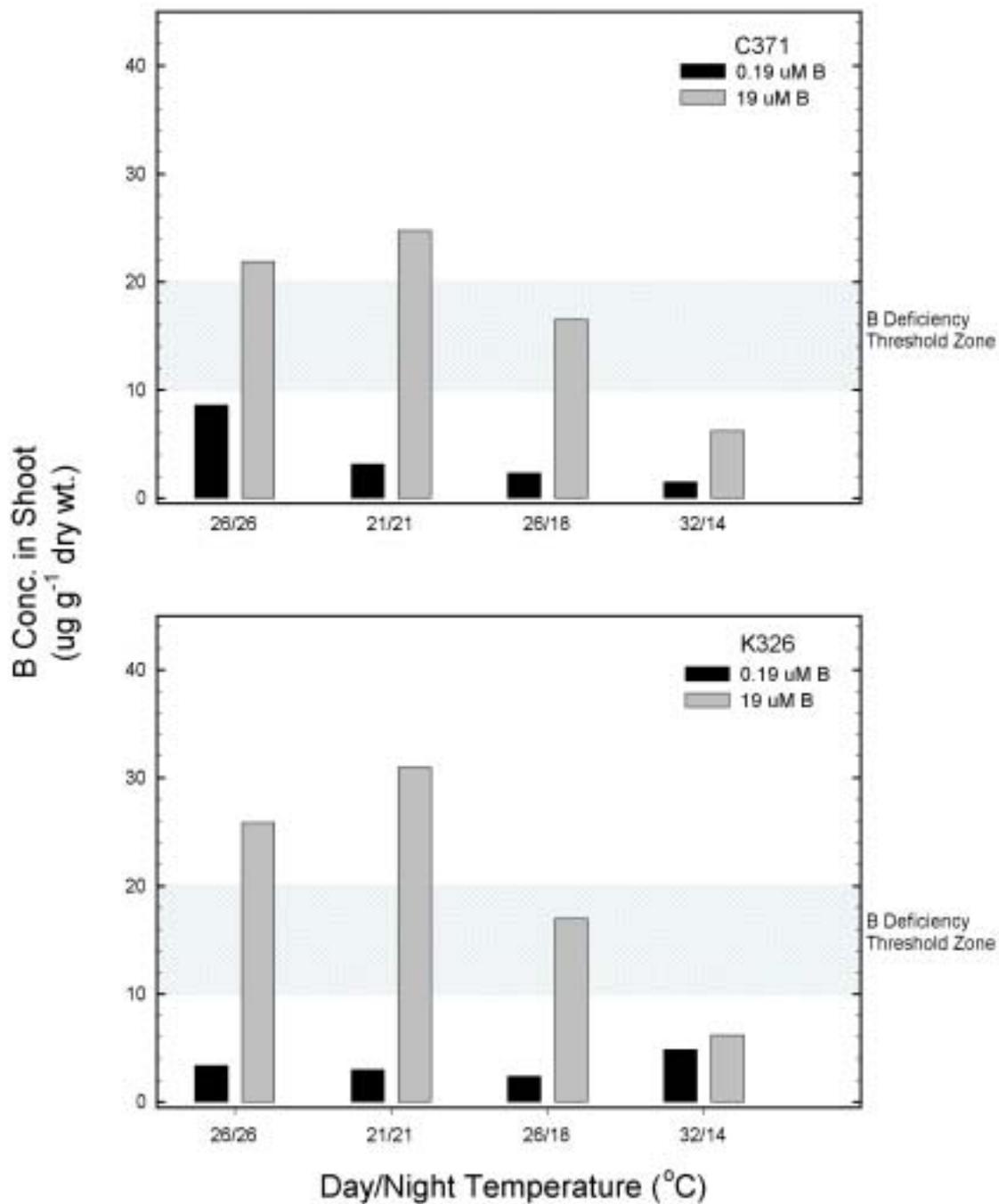


Fig. 10. Boron concentration in 54 day-old flue-cured shoot tissue at different temperature regimes.

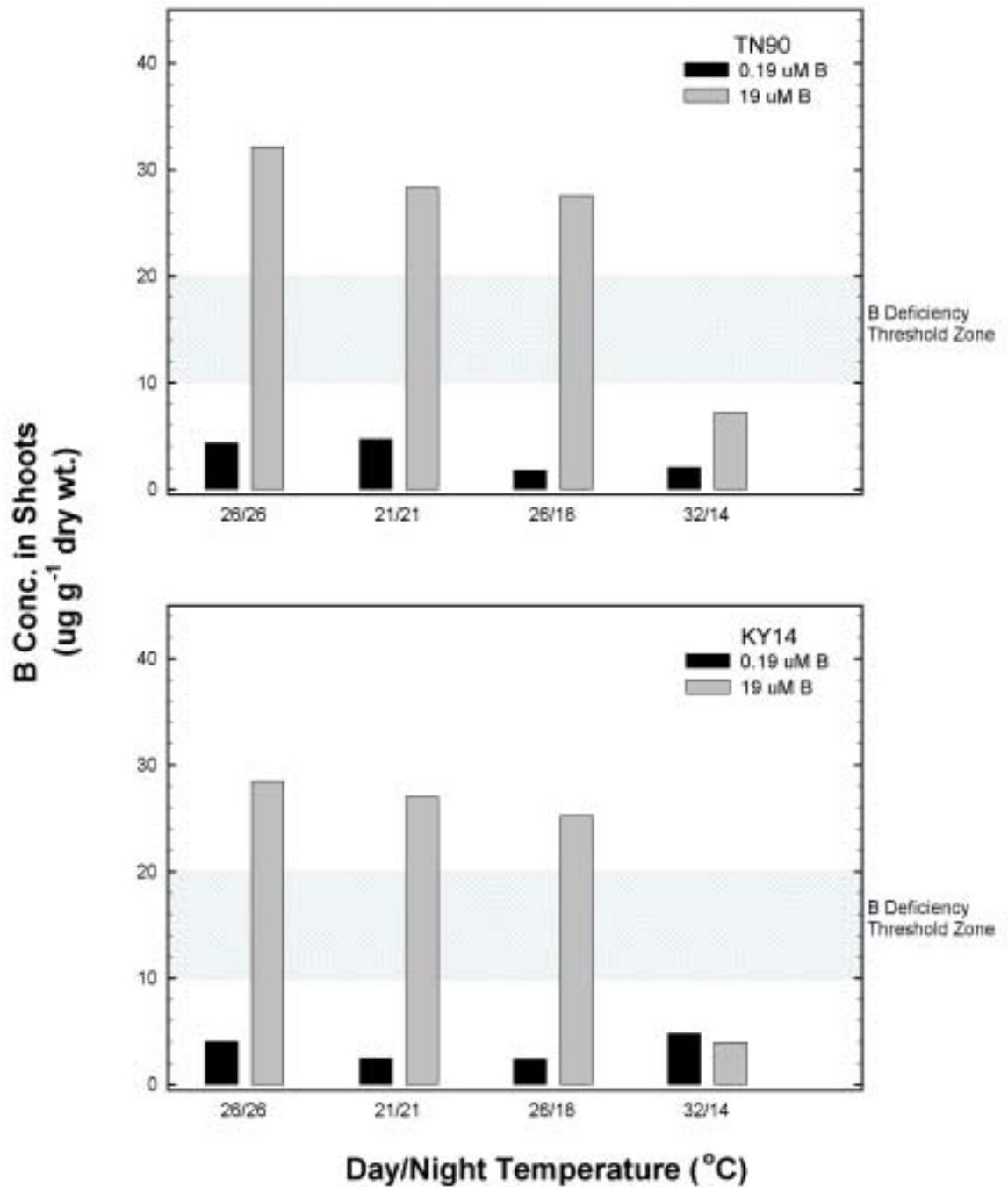


Fig. 11. Boron concentration in 54 day-old burley tissue at different temperature regimes

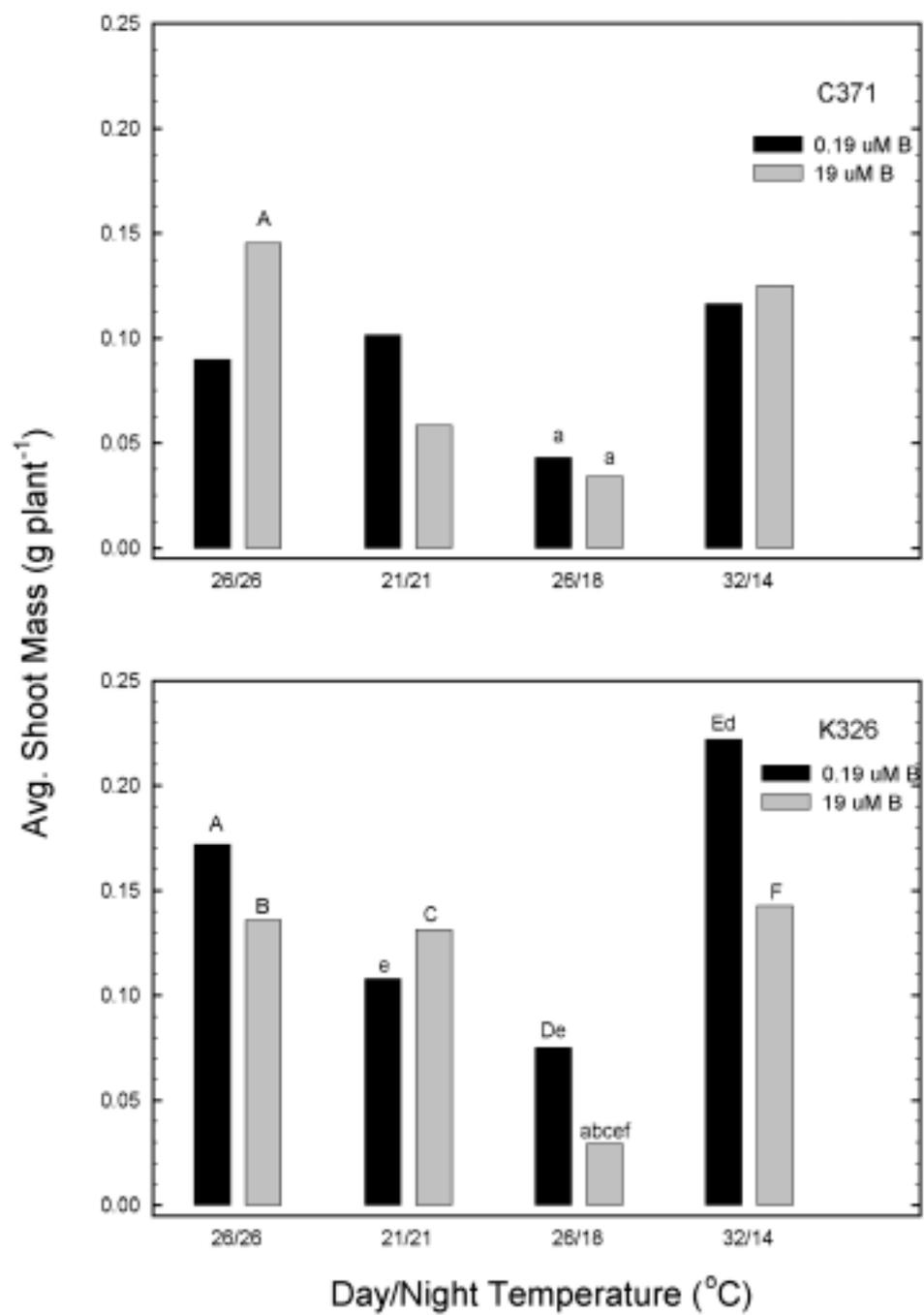


Fig.12. Shoot mass of flue-cured cultivars at day 47 in response to different day/night temperature regimes

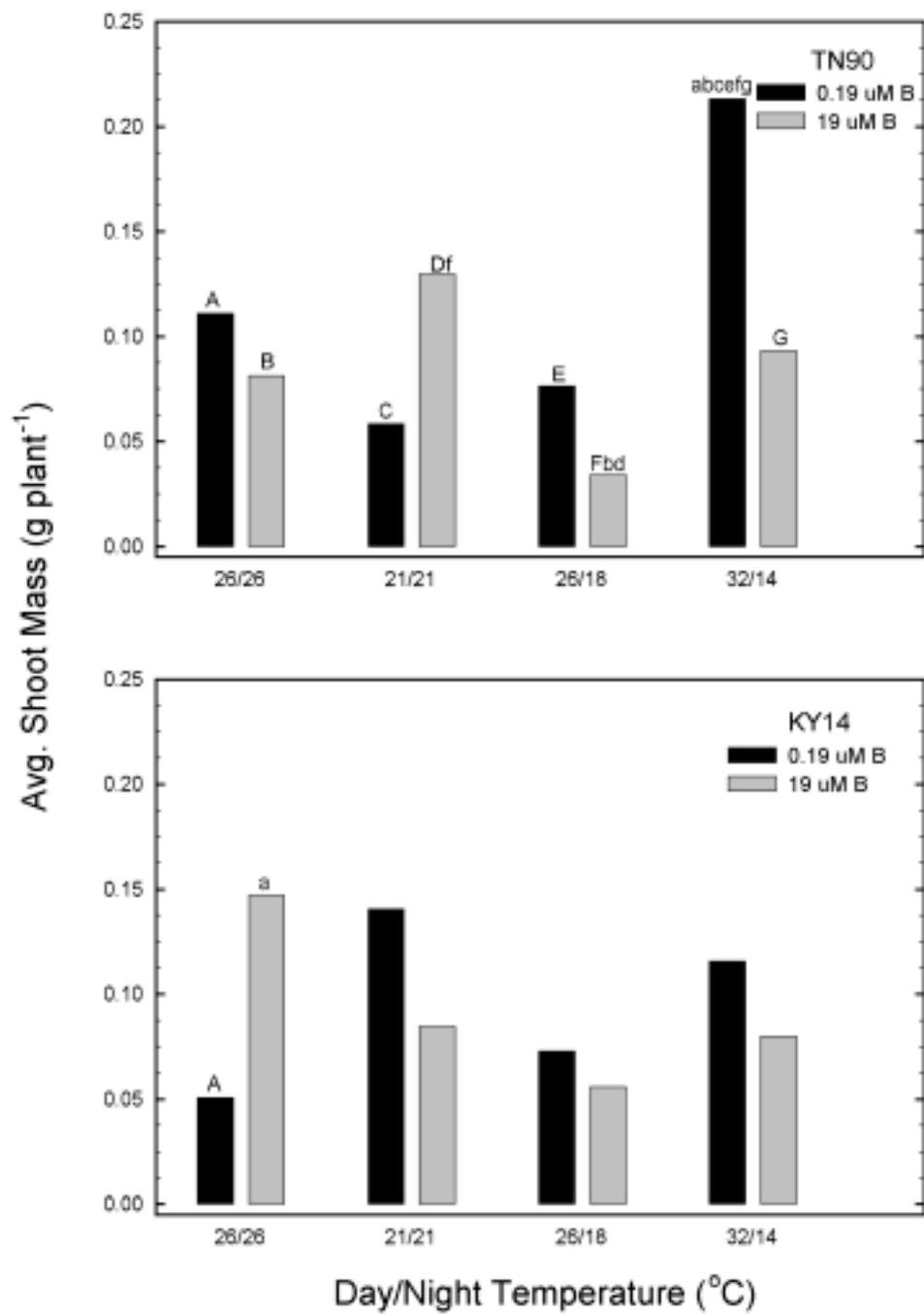


Fig.13. Shoot mass of burley cultivars at day 47 in response to different day/night temperature regimes

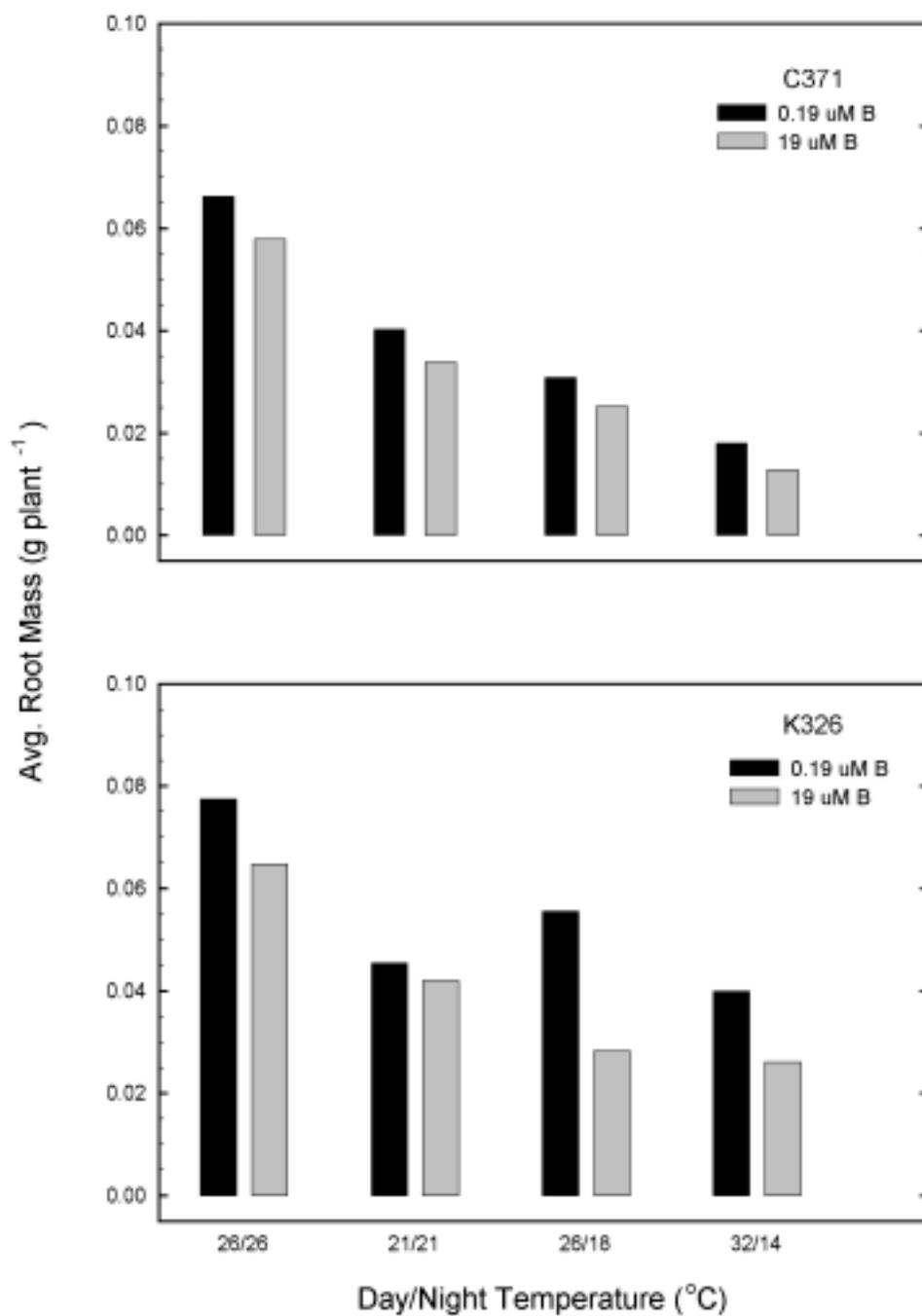


Fig. 14. Root mass of flue-cured cultivars at day 47 in response to different day/night temperature regimes.

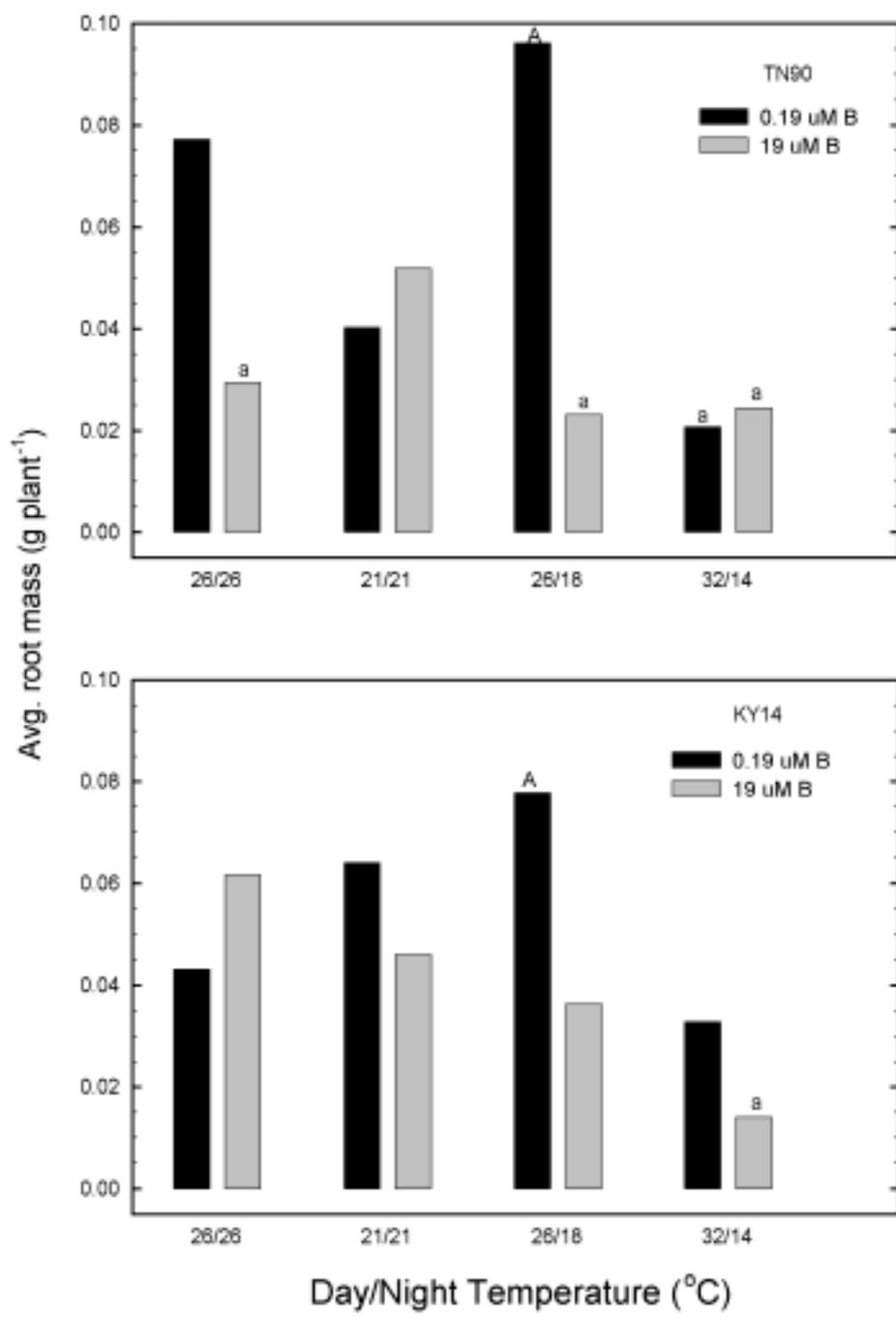


Fig. 15. Root mass of burley cultivars at day 47 in response to different day/night temperature regimes.

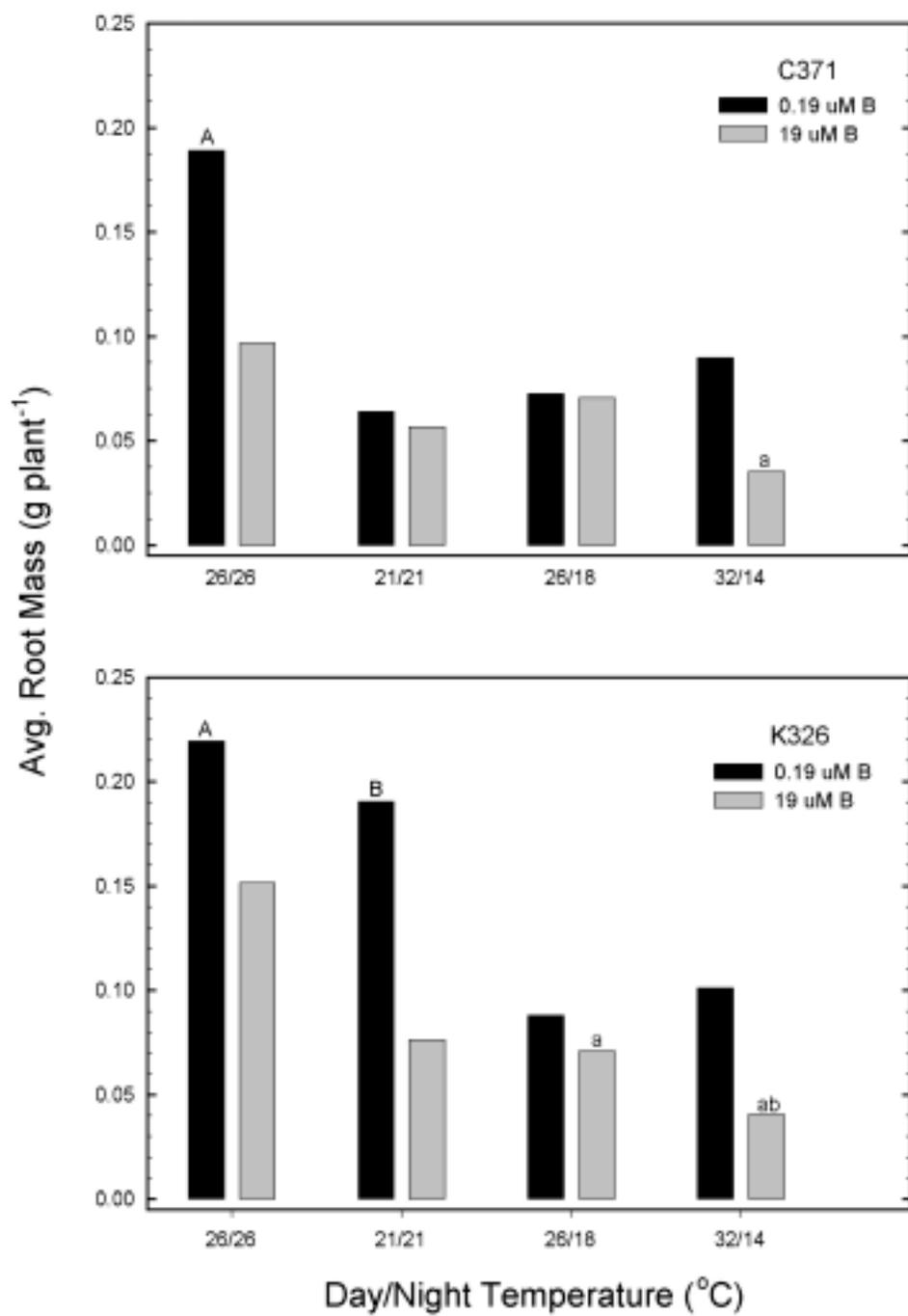


Fig. 16. Root mass of flue-cured cultivars at day 54 in response to different day/night temperature regimes

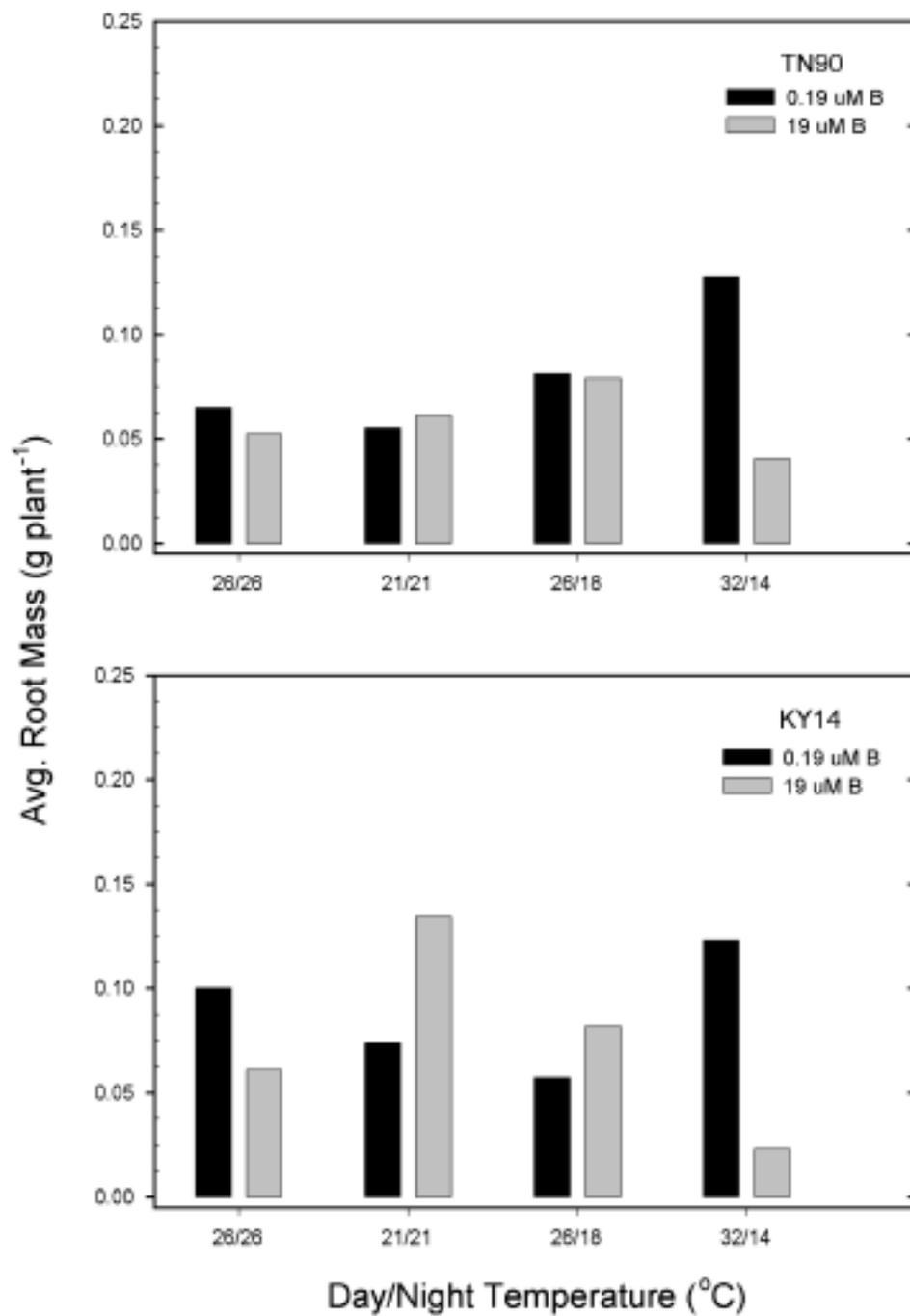


Fig. 17. Root mass of burley cultivars at day 54 in response to different day/night temperature regimes.

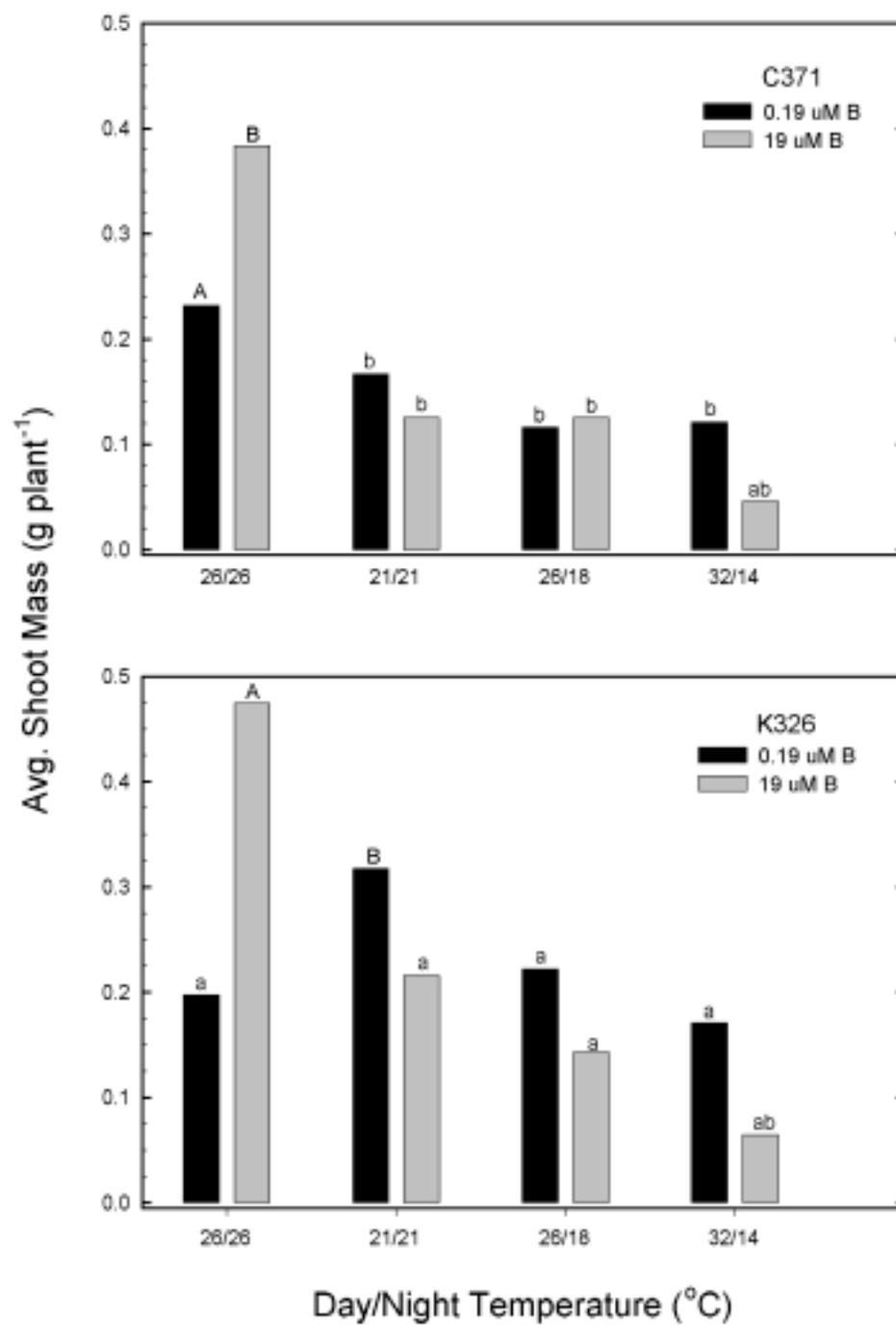


Fig. 18. Shoot mass of flue-cured cultivars at day 54 in response to different day/night temperature regimes

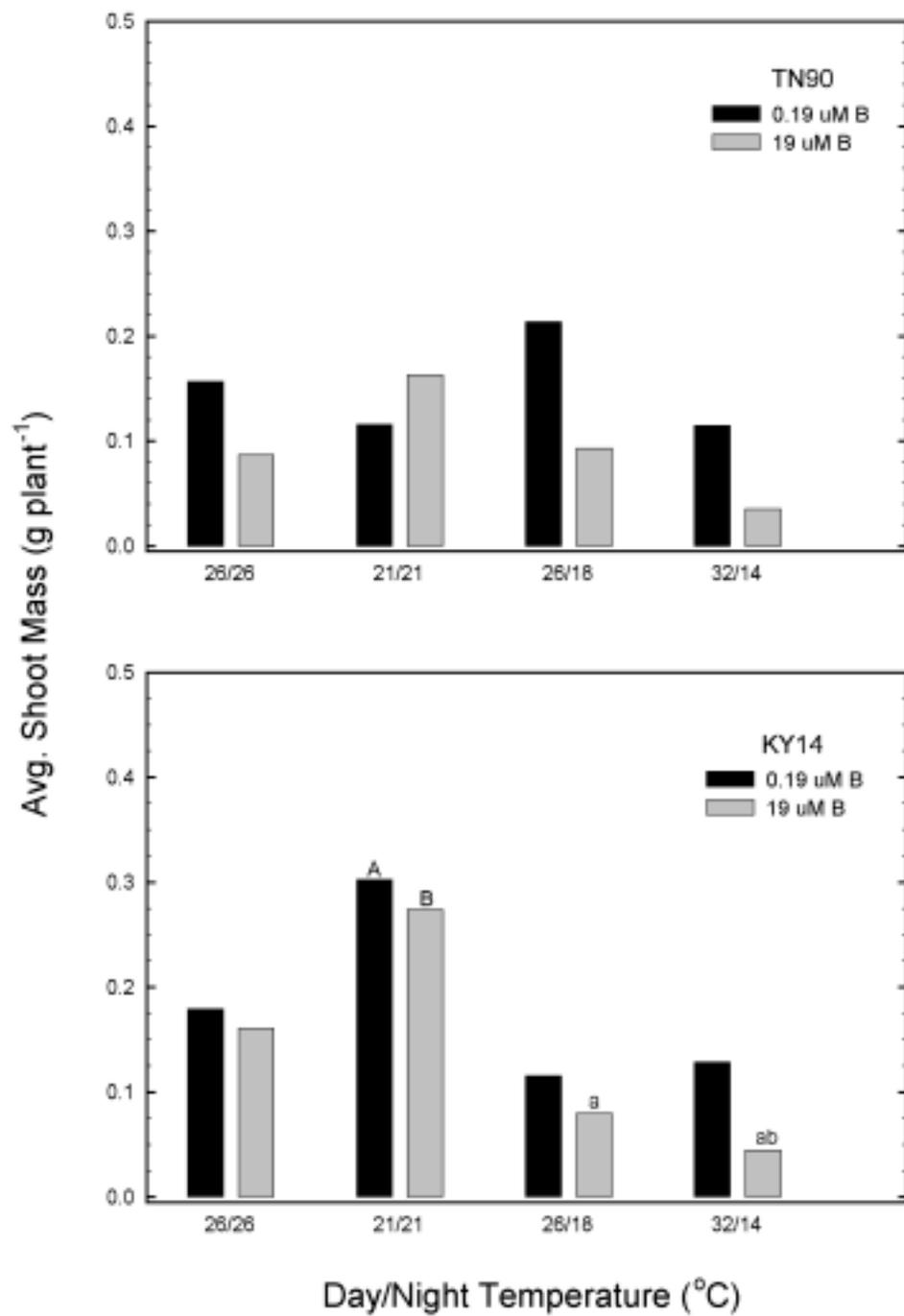


Fig. 19. Shoot mass of burley cultivars at day 54 in response to different day/night temperature regimes

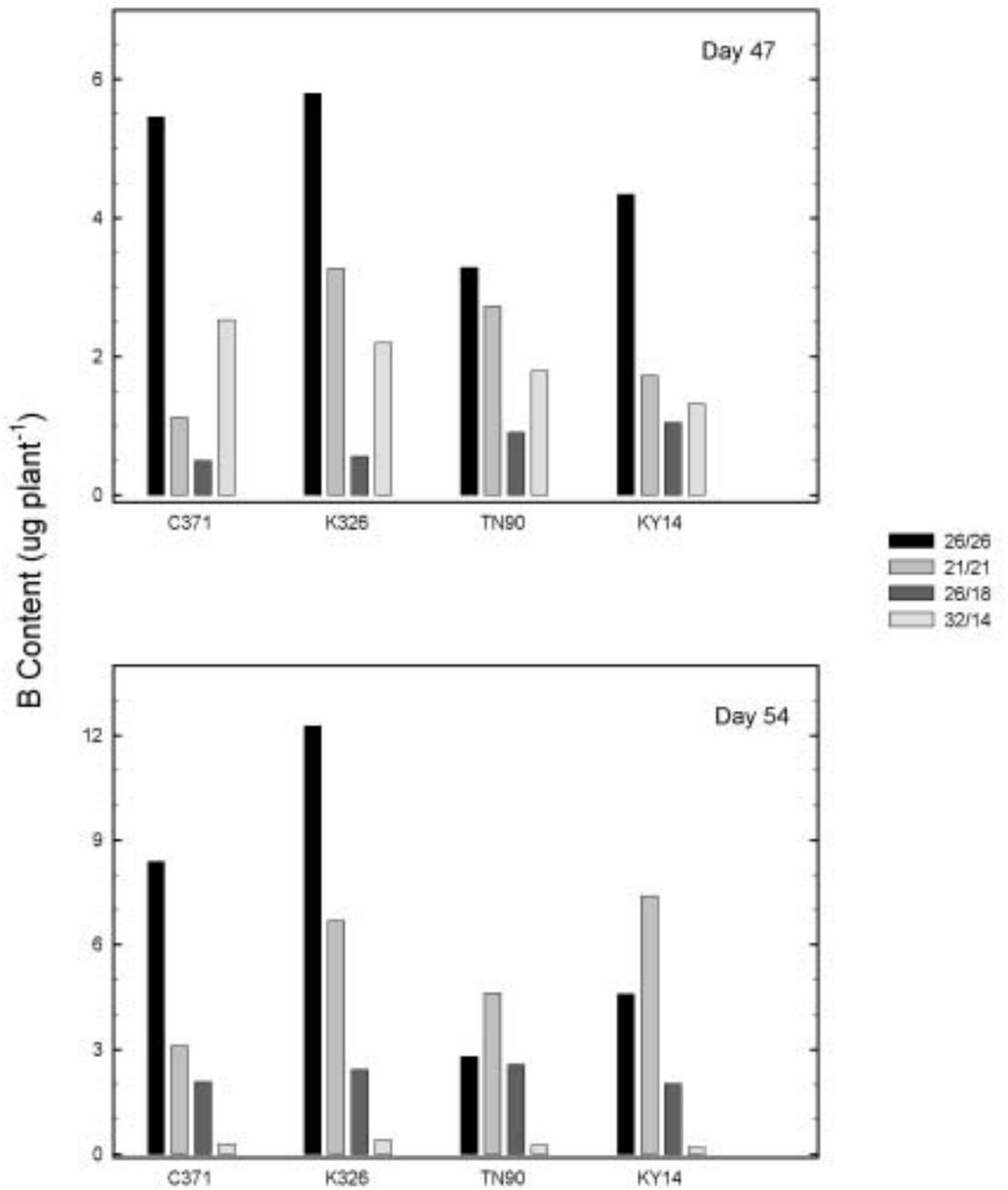


Fig. 20. Shoot tissue B content of high B treatments to disclose temperature effects

Conclusions:

These studies indicated that the boron deficiency threshold for tobacco transplants growing at a 26/22° C day/night temperature regime is in the range of 10-20 $\mu\text{g B g}^{-1}$ dry matter. This tissue B concentration corresponds to a solution B concentration of 0.19 to 1.9 $\mu\text{M B}$ (0.002 to 0.02 mg L^{-1}). The establishment of this threshold was supported by large increases in root and shoot mass, leaf area, and leaf initiation, and, to a lesser extent, number of leaves occurring between the solution B concentrations of 0.19 and 1.9 $\mu\text{M B}$.

The study investigating interactions between B deficiency and chilling injury symptoms in tobacco transplant seedlings growing in float culture indicated a distinct relationship between B content (expressed as $\mu\text{g B shoot}^{-1}$) and day/night temperature regime. Flue-cured varieties had greater cumulative uptake of B at a constant day/night temperature of 26° C at both 47 days and 54 days after seeding. Burley varieties, on the other hand, took up B in greater amounts at constant 26° C at 47 days, but then took up greater amounts at a 26/18° C day/night differential after 54 days. This study also revealed that, when supplied in adequate concentrations (19 $\mu\text{M B}$), B uptake declines at sub-optimal temperature regimes (21/21° C, 26/18° C, and 32/14° C) compared to an optimal temperature regime (26/26° C), especially for the two flue-cured varieties. Interestingly, when the B concentration in solution is low (0.19 $\mu\text{M B}$),

there is little difference in B uptake regardless of temperature, day/night differential, or variety. This suggests that there is a maximal depletion limit at low solution B concentrations that was not affected by temperature treatments imposed in this study. This study also showed that root and shoot growth of flue-cured varieties is maximized by the 26/26° C treatment and that burley varieties display a wider range of temperature tolerance.

One speculation, which cannot be confirmed without further research, is that sub-optimal temperatures may alter the functioning B deficiency threshold, which may decrease with decreasing temperatures or with stressful temperature differentials. Take, for instance, the shoot mass data of figs. 12 and 13 describing shoot growth of both flue-cured and burley varieties at 47 days after seeding as well as fig. 19 describing root mass accumulation of burley varieties at 54 days after seeding. The seedlings of the 32/14° C treatments show surprisingly good growth at the low B concentration considering the stressful day/night differential as well as the low B concentration. Under more optimal temperature conditions, the level of B measured in the tissue of these samples (figs. 10 and 11) would fall into the range considered to be B deficient. These seedlings were not suitable for transplant production owing to their small stem diameter and small leaf area (Fig 7). Despite their unsuitability for transplant production, their shoot mass accumulation at the low B concentration, most of which was stem, was close to or greater than that of the optimal 26/26° C

temperature regime. This suggests that the low B concentration was within the optimal range for growth at the most stressful temperature regime implemented in this study. Consequently, the high B treatment was likely to be at a toxic concentration for these seedlings. Therefore, it may be that environmental parameters including day/night temperature regimes affect the B deficiency threshold for a plant, in effect inducing a "sliding scale" for determination of the B deficiency concentration. This information taken together might be summarized in the following way: Temperature is the immediate limiting factor in tobacco transplant growth in the float system under conditions of sub-optimal temperatures and low B concentration, and B deficiency is a potential additional limiting factor.

It is difficult to narrow down one specific physiological interaction between boron deficiency and chilling injury for a number of reasons. One difficulty with ascribing the diagnosis of B deficiency or chilling injury symptoms to a specific condition lies in the fact that both symptoms appear similar to the symptoms of many other plant disorders and it is therefore not possible to definitively attribute either condition as the cause of a symptom when there is the possibility that it could be due to another disorder. Another difficulty is that the primary effects of chilling injury and B deficiency are as yet indistinguishable from secondary effects. There are many different opinions but no definitive conclusions concerning the primary site of symptoms origination for either of the conditions

of interest. It is thus difficult to trace back the origin of B deficiency or chilling injury symptoms to a primary locus within plant cells. Finally, there are few empirical measurements that can be made to indicate extent of symptomology for either condition. Therefore, it can only be speculated as to the cause or causes of the observed symptoms. Among the best empirical tools we can make use of in distinguishing relations between boron deficiency responses and chilling injury responses are analyses of growth as indicated by dry weight, leaf area, and leaf initiation, and tissue boron content. Analyses of growth give an overall indication of plant health but do not distinguish between boron deficiency and chilling injury and tissue B content gives concise information regarding B uptake but does not specifically indicate plant growth. The combined information however, leads to a number of possibilities.

Several possibilities for interactions have been discussed in this paper. Since the plasma membrane is one of the most commonly affected cell locations under conditions of chilling injury in plants, plasma membrane interference in which B deficiency and chilling injury induce reductions in membrane integrity is a possible initial malfunction which could explain many of the symptoms observed by the two conditions. One possible specific reaction involving the plasma membrane occurs when B deficiency mimics chilling injury by inducing breakdown of the RGII complexes of cell walls which are speculated to be involved in chemical bonds between the cell wall and the plasma membrane,

thus potentially impairing membrane integrity. Another mechanism by which B deficiency could possibly impair the plasma membrane involves the observed reduction of nucleotide sugars induced by B deficiency, which play a part in the synthesis of phosphoglyceride membrane components, eventually leading to degradation of the plasma membrane. Boron deficiency and chilling injury have both been observed to cause reduction in levels of nucleotides in affected plant cells. A final commonality of interest shared between these two conditions is alterations of cytoskeletal components, which could over time result in a number of the shared physiological symptoms induced by B deficiency and chilling injury.

Results and Discussion and Conclusions

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