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Elevated Relative Humidity Increases the Incidence of Distorted Growth and Boron Deficiency in Bedding Plant Plugs

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Abstract**. High relative humidity (RH) can cause lower concentrations of boron (B) accumulating in plants. The common greenhouse practice of controlling excess temperatures by applying mist irrigation to young plants (plugs) can result in elevated RH levels, especially with plugs grown in high heat and humidity conditions of summer. 'Dynamite Yellow' pansy (*****Viola* ×*wittrockiana*** **Gams.), 'White Storm' petunia (*****Petunia* ×*hybrida*** **Vilm.), and 'Festival Apricot' gerbera (*****Gerbera jamesonii*** **Bolus) plugs were grown in high or ambient RH conditions to determine the effect RH had on B uptake. Results indicate that an increase in RH decreased the amount of water the plant lost as a result of transpiration resulting in lower concentrations of B in shoot tissue. Boron concentrations in leaf tissue were 9.43, 10.56, and 17.81 mg·L^{-1} in pansy, petunia, and gerbera plants, respectively, grown in high RH conditions. These values were significantly lower than pansy, petunia, and gerbera plants grown in ambient RH conditions (19.94, 25.49, and 42.71 mg·L^{-1}, respectively). Leaf distortion, consistent with B deficiency symptoms, was present in petunia and gerbera plants. Similar trends were observed when the experiment was repeated and leaf distortion was present in all species. This provides convincing evidence that the distorted growth observed in pansy, petunia, and gerbera plug production is the result of limited B caused by excessive humidity.**

Boron moves passively into plants from the roots to the shoots through the transpiration stream through the xylem (Jones, 1991; Kochian, 1991; Kohl and Oertli, 1961; Raven, 1980). Relative humidity can be a major factor influencing the rate of plant transpiration and, therefore, the amount of B accumulated. The uptake of B has been reported to be negatively affected by elevated RH (Bowen, 1972; Halbrooks et al., 1986). Oertli (1963) reported lower concentrations of B in leaves of barley when plants were grown in higher RH conditions but also concluded that the lower B concentration may be the result of loss through guttation in high RH conditions. Plant uptake of calcium (Ca) has also been extensively studied and it is known that Ca is taken up passively with water and is closely linked to transpiration (Clarkson, 1984; Marschner, 1995). Chang and Miller (2004) reported that Ca uptake increases with higher transpiration rates. Furthermore, Frantz et al. (2004) eliminated Ca deficiency in lettuce through enhanced transpiration by increasing the flow of low-humidity air directly over the meristem.

During germination, seeds of bedding plants are maintained in growing conditions near 100% RH. Once the plants have germinated, they are moved into a greenhouse and often held under mist to maintain high RH, particularly with seedlings grown during hot conditions. Plants can develop distorted growth of the youngest leaves, which is characteristic of B deficiency (Krug et al., 2009). Thus, it has been reported that high RH environments could be the cause of the B-deficient symptoms occurring in commercial production. Therefore, the objective of this study was to determine if elevated RH levels could cause lower tissue concentrations of B and lead to the development of visual symptoms of B deficiency.

Materials and Methods

'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera seeds were sown in 288-plug trays cut into 2 × 2-cell flats (each cell: 2 cm × 2 cm × 3 cm deep) on 5 Jan. 2007, referred to as "Expt. 1" from this point forward. The germination substrate was Berger BM 2 (Berger Peat Moss; St. Modestede, Quebec, Canada). Once sown, seeds were placed in a germination chamber with a temperature set point of 20 °C. Light was provided by fluorescent bulbs with a photosynthetic photon flux (*PPF*) of 24 to 75 µmol·m^{-2}·s^{-1} at plant canopy for 12 h·d^{-1}. The substrate was kept moist using tap water until seeds germinated. When the first true leaves began to emerge, the plants were moved to one of two environments in the greenhouse: ambient relative humidity (AH) or high relative humidity (HH) with day/night temperature set points of 23.9/17.8 °C. A 122-cm high plastic curtain was used to create the two environment chambers, each 152 cm × 267 cm. Plastic lined the bench to prevent airflow from below, but the top of the chamber was open. On average, the RH of the AH chamber was 65% and the RH was raised to 100% in the HH chamber using humidifiers (Model 707; Fedders Corp., Liberty Corner, NJ). RH and temperature were monitored using Hobo H8 data loggers (Onset Computer Corp, Bourne, MA). The experiment was a completely randomized design with 36 flats (2 × 2-cell flat) of each species for each treatment. An additional 36 unplanted flats (2 × 2-cell flat) were included in each treatment to use for transpiration data. Plants were fertilized at each irrigation after germination with 50 mg·L^{-1} nitrogen (N) from Champion 13N–0.86P–10.79K Plug Special (Scotts, Marysville, OH). Plants were harvested 35 d after sowing (DAS). Tissue samples were taken by removing the entire shoot. To ensure sufficient tissue was available for tissue analysis, six 2 × 2-cell flats were combined and used as one replication for a total of six replications per treatment. The experiment was repeated with an initiation date of 10 May 2007, referred to as "Expt. 2" from this point forward, and harvest occurring 41 DAS. The RH was 81% and 100%, on average, for the AH and HH treatments, respectively, in Expt. 2.

Transpiration. Transpiration was quantified gravimetrically three times/week for the duration of the experiments. Values were averaged over 10 or 8 d for Expts. 1 and 2, respectively. Flats were weighed at dawn and dusk (0700 to 0830 and 1730 to 1900 HR, depending on daylength) to determine evapotranspiration (flats with plants) and evaporation (flats with substrate only). The average difference of the flats with only substrate was subtracted from the individual differences of the flats with plants, leaving the amount of water loss resulting from transpiration alone. Some reported values appear as negative numbers; these indicate when plants gained water over the course of the day.

Plant canopy area was recorded to base transpiration on leaf area. Plant canopy area was determined using digital photography

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Table 1. Nutrient concentration of tissue, water loss (mL) resulting from transpiration (average of 10 d), and water loss (mL) resulting from transpiration/area of substrate covered by leaves (cm^2) (average of 10 d) from 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants 35 d after sowing, grown in ambient (65%) humidity (AH) or high (100%) humidity (HH) (Expt. 1).

	Percent dry wt					mg·L^{-1} dry wt						
Treatment	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Trans[z,y]	Trans[z]/area[y]
							Pansy					
AH	0.29	2.55	0.49	0.46	0.28	19.94	9.76	61.66	121.54	45.15	1.63	0.121
HH	0.24	1.82	0.35	0.32	0.27	9.43	12.34	120.26	137.24	32.69	–0.008	–0.152
P value[x]	**	***	***	***	NS	***	***	***	*	NS	***	***
							Petunia					
AH	0.23	3.12	0.63	0.45	0.33	25.49	9.23	67.47	48.01	28.82	1.43	0.081
HH	0.26	3.35	0.61	0.35	0.43	10.56	18.12	96.98	59.92	68.68	0.05	0.013
P value[x]	*	NS	NS	***	***	***	***	*	**	***	***	*
							Gerbera					
AH	0.39	3.86	0.72	0.50	0.35	42.71	13.41	123.00	76.97	50.32	0.46	0.040
HH	0.28	2.77	0.61	0.35	0.35	17.81	18.31	161.09	64.53	53.29	0.03	–0.002
P value[x]	***	***	***	***	NS	**	***	**	**	NS	***	***

[z]Transpiration.

[y]Values with negative numbers indicate a net gain of water.

[x]NS, *, **, *** Not significant, significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$.

P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

and PixelCounter 1.0 (North Carolina State University, Raleigh, NC) as described by Steward et al. (2007) to avoid destructive harvest. Total canopy area and water loss resulting from transpiration were used to calculate water loss/cm^2 plant canopy.

Tissue analysis. The tissue samples were first rinsed in deionized water, then washed in 0.2 N HCl for 30 s, re-rinsed with deionized water, and dried at 70 °C for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1-mm screen (20-mesh) and 0.15 g was digested in a microwave digester (MARS; CEM Corp., Matthews, NC) using a modified U.S. Environmental Protection Agency (EPA) method (EPA method 3051 with additional peroxide step). Nutrient concentrations, except N, were determined with inductively coupled plasma optical emission spectroscopy (Model IRIS Intrepid II; Thermo Corp., Waltham, MA). The lower detection limit for B was 1.70 mg·L^{-1}. A quality control was run every 10 samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato (*Solanum lycopersicum*) standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach (*Spinacia oleracea*) standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for phosphorus (P), potassium (K), Ca, magnesium (Mg), sulfur (S), B, copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn).

Data analysis. Data were tested by analysis of variance using the general linear model procedure (SAS Institute, Cary, NC) and means were separated by least significant differences at $P \leq 0.05$.

Results and Discussion

The amount of water loss resulting from transpiration as well as the relative amount of transpiration/canopy area (trans/area) were significantly less in the HH treatments as compared with the AH for both experiments and for all three species (Table 1). In some cases the value for transpiration and transpiration/area ratio for pansies in the HH treatment in Expt. 1 were negative numbers. As stated earlier, a negative number indicates a gain in water by the plant. No watering occurred between sunrise and sunset on days when transpiration data were taken; therefore, the gain was not the result of irrigation. However, it is possible that the plants and/or the substrate absorbed moisture from the surrounding air. The substrate of the HH treatments never dried and condensation was observed on a regular basis on the leaves of the plants; therefore, it is reasonable to assume, with a RH of 100%, that water also condensed on the substrate surface and resulted in the negative numbers.

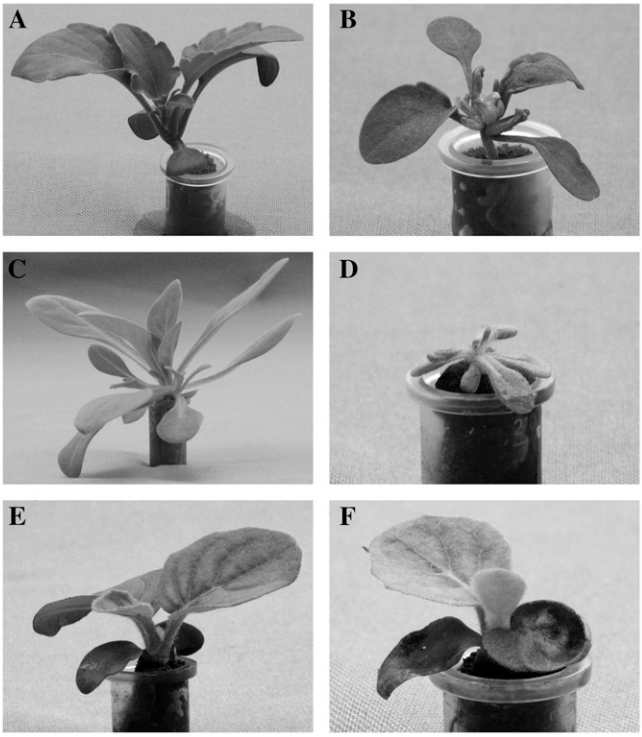

Fig. 1. Normal growth on 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera (**A, C,** and **E,** respectively) compared with boron deficient plants: 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera (**B, D**, and **F**, respectively).

Pansy. No symptoms of B deficiency were observed in Expt. 1, but B deficiency symptoms consistent with those reported for B deficiency were observed in the HH treatment during Expt. 2. Symptoms included distorted leaves and meristems and the proliferation of axillary shoots (Fig. 1B). The shoot tissue concentrations of B in both Expts. 1 and 2 were significantly ($P < 0.001$ and $P < 0.01$, respectively) less in the HH treatments as compared with the AH treatments (Tables 1 and 2). The B concentration in HH treatment in Expt. 1 was lower that those reported to result in B deficiency on fully expanded mature leaves from transplanted pansies (72 DAS; Pitchay, 2002).

Table 2. Nutrient concentration of tissue, water loss (mL) resulting from transpiration (average of 8 d), and water loss (mL) resulting from transpiration/area of substrate covered by leaves (cm^2) (average of 8 d) from 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants 41 d after sowing, grown in ambient (81%) humidity (AH) or high (100%) humidity (HH) (Expt. 2).

	Percent dry wt					mg·L^{-1} dry wt						
Treatment	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn	Trans^z	Trans^z/area
							Pansy					
AH	0.21	3.99	0.49	0.45	0.26	19.66	3.94	34.78	36.66	47.03	1.29	0.108
HH	0.23	3.05	0.46	0.41	0.27	12.66	3.97	95.59	36.52	56.34	0.384	0.055
P value^y	NS	***	NS	*	NS	**	NS	NS	NS	NS	***	***
							Petunia					
AH	0.11	3.75	0.49	0.26	0.25	20.89	5.68	45.42	22.96	34.88	2.05	0.167
HH	0.17	3.98	0.70	0.32	0.32	14.82	6.99	82.19	25.90	53.44	0.52	0.083
P value^y	***	NS	***	*	***	**	NS	NS	NS	***	***	***
							Gerbera					
AH	0.16	3.16	0.60	0.35	0.21	24.37	3.34	88.76	31.95	31.83	0.79	0.049
HH	0.14	3.52	0.84	0.34	0.21	13.33	3.63	135.25	35.05	38.16	0.165	0.010
P value^y	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	***	***

^{z}Transpiration.

^{y}NS, *** Not significant, significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$.

P = phosphorus; K = potassium; Ca = calcium; Mg = magnesium; S = sulfur; B = boron; Cu = copper; Fe = iron; Mn = manganese; Zn = zinc.

Plants from both experiments in the HH treatment had significantly lower concentrations of K and Mg when compared with the AH treatment (Tables 1 and 2). In Expt. 1, tissue concentrations of P and Ca were also significantly lower in the HH treatment as compared with the AH treatment, but the concentrations of Cu, Fe, and Mn were significantly higher in the HH treatment as compared with the AH treatment (Table 1). With the exception of Ca in the HH treatment in Expt. 1, these nutrients were above the levels in which deficiencies were observed on fully expanded mature leaves from pansy transplants (46 DAS; Pitchay, 2002).

Petunia. Symptoms of B deficiency, including thickened, distorted, and upward curled leaves (Fig. 1D), were observed in the HH treatment in both experiments. Shoot tissue concentrations of B in the HH treatments for Expts. 1 and 2 (10.56 and 14.82 mg·L^{-1}, respectively) were significantly lower than the AH treatments; values were approaching levels at which B deficiency (10.3 mg·L^{-1}) was observed in fully expanded mature leaves from petunia transplants (102 DAS; Pitchay, 2002). Shoot tissue concentrations of P, Mg, S, Cu, Fe, Mn, and Zn in Expt. 1 and P, Ca, Mg, S, and Zn in Expt. 2 were significantly different (Tables 1 and 2). None of these values, however, were less than those reported by Pitchay (2002) to cause the respective deficiency in fully expanded mature leaves of mature petunia transplants.

Gerbera. Symptoms of B deficiency were observed in the HH treatment for both experiments. Symptoms included thickened, distorted, and upward curled leaves (Fig. 1F). Shoot tissue concentrations of B for both experiments were significantly lower ($P \leq$ 0.01) in HH (Tables 1 and 2). In Expt. 1, shoot tissue concentrations of P, K, Ca, Mg, and Mn were significantly lower in HH than AH. Copper and Fe were significantly higher in HH than in AH (Table 1). Calcium concentrations for both treatments in both experiment. were lower than optimal levels for gerbera 2 weeks after transplant when fertilized with 50 to 75 mg·L^{-1} N (Jeong et al., 2009). Concentrations of B for the HH treatment in Expt. 1 and both treatments in Expt. 2 were below those reported by Jeong et al. (2009). However, the B value for the AH treatment in Expt. 2 (24.37 mg·L^{-1}) was only slightly lower than the published value (26.60 mg·L^{-1}).

The results presented here support the findings of Bowen (1972), Halbrooks et al. (1986), and Oertli (1963) that B concentrations were lower in plants in high RH conditions. Furthermore, with the exception of petunia and gerbera in Expt. 2, Ca levels were also lower in the HH treatments, although not always significantly lower. As mentioned previously, both Chang and Miller (2004) and Frantz et al. (2004) reported that when transpiration was hindered, leaf Ca concentrations were decreased when plants were grown in HH environments. Uptake of both elements is negatively affected by HH growing conditions.

Results indicate that an increase in RH decreased the amount of water transpired resulting in lower concentrations of B in shoot tissue. The symptoms of B deficiency reported by Krug et al. (2009) were associated with the decrease of B concentrations in foliage and symptoms observed in this study were consistent with those described by Krug et al. This provides evidence that the distorted growth observed in pansy, petunia, and gerbera plug production is the result of limited B uptake caused by reduced transpiration.

To aid in the uptake of B by pansy, petunia, and gerbera plugs, growers should maintain a lower RH during germination and immediately afterward but ensure that RH is sufficiently high enough to avoid compromising germination rates or plant quality. This could be accomplished by limiting the amount of moisture added, use of horizontal air flow fans, or proper ventilation.

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