Incidence of Boron Deficiency in Bedding Plants Caused by Drought Stress or Abscicic Acid Application

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Abstract

Growers have reported boron (B) deficiency in pansy (*Viola ×wittrockiana)* plug production, specifically in plugs grown in the high heat and humidity conditions of summer. Past studies have reported that soil moisture has an impact on boron availability. To simulate drought conditions, 'Dynamite Yellow' pansy plants were grown in a peat-based substrate which was allowed to dry down to 40, 30 or 20% container capacity (CC), 10 or 20 days after sowing (DAS) or on a continual basis. Boron tissue concentration was not affected by any induced drought stress, with the exception of those allowed to dry to 40% CC 20 DAS, which had higher levels of boron than the control. To simulate a physiological response to drought stress, exogenous abscisic acid (ABA) was applied as a substrate drench or foliar spray at concentrations of 150 or 300 mg L^{-1} to 'Dynamite Yellow' pansy plants grown in a peat-based substrate 10 or 20 DAS. All treatments resulted in lower tissue concentrations of B compared to an untreated control. Plants treated 10 DAS with a substrate drench (300 mg L^{-1}) or a foliar spray (150 mg L^{-1}) and plants treated 20 DAS with a foliar spray (150 or 300 mg L^{-1}) of ABA showed a reduction in transpiration. Plants had lower ratios of transpiration/leaf area when ABA was applied as a 300 mg L^{-1} substrate drench 10 DAS and as foliar spray at both concentrations at either application time. The lower B tissue concentrations coupled with lower transpiration rates were similar to circumstances in greenhouse production of fall pansy crops. Boron deficiency is most common in August when high temperatures and relative humidity cause the plants to transpire less.

INTRODUCTION

Moisture levels affect boron (B) availability more than any other micronutrient. It is generally accepted that B availability decreases under dry soil conditions (Fleming, 1980; Gupta, 1993). Under drought conditions mass flow is reduced, resulting in reduced B in contact with the roots available to be taken up by diffusion (Kluge, 1971). Gupta (1993) found that even when B is adequately available in the soil, concentrations in barley plants were lower when soil moisture was low. Mortvedt and Osborn (1965) reported that increasing soil moisture from 12 to 20% increased B dispersal and movement in the soil.

Drought stress can commonly occur in field production. However, in greenhouse production the grower has greater control of moisture levels and water stress is rarely allowed. It is unlikely that B deficiency is being induced by dry conditions during seed germination of pansy plugs. Nevertheless, Hobbs and Bertramson (1949) reported that tomato plants grown in greenhouse culture became deficient in B with inadequate moisture in the topsoil. With the small volume of soil contained in a plug container, it is possible that the soil surface dries enough to restrict B movement into the plant.

The objectives of these studies were to investigate how single or repeated water

stresses and the application of ABA affect B concentrations in pansy seedling tissue.

MATERIALS AND METHODS

Experiment 1

'Dynamite Yellow' pansy seeds were sown in 288-plug trays cut into 6×6 cell flats (each cell: 2×2×3 cm deep), with one 6×6 flat being considered a replication. 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 density (PPFD) of 24 to 75 μmol m^{-2} s^{-1} for 12 h per d. The substrate was kept moist using tap water until seeds germinated. After seeds had germinated, plants were moved into a greenhouse with day/night temperature set points of 23.9/17.8°C. Plants were fertilized at each watering after germination with 50 mg L^{-1} N from Champion 13-2-13 Plug Special (Scotts, Marysville, Ohio) (13N-0.86P-10.79K).

Using methods described by Milks et al. (1989) and Van Genuchten (1980) water content was determined for the rooting substrate (Berger BM 2) at a range of pressures. The substrate was allowed to dry to 40, 30 or 20% of container capacity (CC) (-2, -5 or -20 kPa osmotic pressure, respectively) to obtain the desired drought stress. Plants were exposed to this stress at 10 or 20 days after sowing (DAS) or after every irrigation. An untreated control was also included. The experiment was a completely randomized design with 4 replications (6×6-cell flats) of 10 treatments. Plants were harvested 33 DAS. Tissue samples were taken by removing the entire shoot.

Experiment 2

'Dynamite Yellow' pansy seeds were grown as in Experiment 1. Abscisic acid was applied 10 or 20 DAS as either a drench or a foliar spray at a concentration of 150 or 300 mg L^{-1}. Drenches were pipetted onto each individual plug cell at a volume of 0.35 ml/cell (5% of the substrate volume). Foliar sprays were applied at a volume of 306 ml m^{-2}. Untreated controls were also included. The experiment was a completely randomized design with 4 replications (6×6-cell flats) of 9 treatments. Plants were harvested 33 DAS. Tissue samples were taken by removing the entire shoot.

Tissue Analysis

The tissue samples for all experiments were 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, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Tissue was analyzed for total P, K, Ca, S, Mg, B, Cu, Fe, Mn and Zn with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples.

Transpiration

Transpiration was quantified gravimetrically for both experiments 3 times/week for the duration of the experiments. Values were averaged over 10 or 11 d, for experiments 1 and 2, respectively. Flats containing plants and those containing only substrate were weighed at dawn and at dusk (7:00 to 8:30 and 17:30 to 19:00, depending on day length). The difference between the two weights was the amount of water loss to evapotranspiration. 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 lost

due to transpiration alone.

The area of the plant canopy was determined using digital photography and PixelCounter 1.0 (North Carolina State University, Raleigh, N.C.) as described by Steward et al. (2007). The plant canopy area values were used along with the amount of water loss due to transpiration to calculate the amount of water loss/cm^2 of plant canopy.

Data Analysis

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

RESULTS AND DISCUSSION

Experiment 1

When a drought stress was applied 10 DAS, flats allowed to dry to 20 or 30% CC lost significantly ($P \leq 0.001$) less water due to transpiration (0.64 and 1.32 ml, respectively) compared to the untreated control (4.50 ml) (Table 1). Flats dried to 20% CC 20 DAS lost significantly ($P \leq 0.001$) less water due to transpiration (1.88 ml) compared to the untreated control (5.94 ml) (Table 1). When the drought stress was applied on a continuous basis, flats dried to 20 or 30% CC lost significantly ($P \leq 0.001$) less water due to transpiration (1.00 and 0.25 ml, respectively) compared to the untreated control (4.55 ml) (Table 1). Although there were differences in the total amount of water loss due to transpiration among treatments, the amount of water loss due to transpiration/canopy area was not different from the controls for any of the treatments with the exceptions of plants dried to 20 or 30% CC 10 DAS (Table 1). As plants experienced harsher drought conditions some fatalities occurred as well as smaller leaf areas on surviving plants. The smaller leaf canopies explain why there was significantly less transpiration, but no differences in transpiration/area ratios for many of the treatments.

The only treatment that had significantly ($P \leq 0.05$) different concentrations of B was that which was allowed to dry to 40% CC on 20 DAS. The B concentration (45.09 mg L^{-1}) was greater than the untreated control (24.52 mg L^{-1}) (Table 1). The concentrations of B were above those determined to cause deficiency symptoms on fully expanded mature leaves from transplanted pansies (72 DAS) by Pitchay (2002). Adequate tissue concentration values were expected as there were no symptoms of nutrient deficiency.

Experiment 2

When ABA was applied 10 DAS as a drench of 300 mg L^{-1} or a foliar spray of 150 or 300 mg L^{-1} there was significantly ($P \leq 0.001$) less water loss due to transpiration (-2.42, -0.90 and -0.08 ml, respectively) compared to the untreated control (1.91 ml) (Table 2). When applied 20 DAS, only the foliar sprays (150 and 300 mg L^{-1}) lost significantly ($P \leq 0.05$) less water due to transpiration (-0.25 and -1.20 ml, respectively) than the untreated control (1.83 ml) (Table 2). The negative value for transpiration indicates that transpiration was effectively stopped; with no canopy cover, the fallow flats lost more water due to evaporation than the flats with plants lost to evaporation and transpiration (Table 3).

Transpiration/area ratios were significantly lower ($P \leq 0.01$) for plants treated on 10 DAS with an ABA substrate drench of 300 mg L^{-1} and both foliar spray concentrations (150 and 300 mg L^{-1}) all other treatments. Only plants treated with ABA foliar sprays (150 or 300 mg L^{-1}) on 20 DAS had significantly ($P \leq 0.05$) lower ratios of transpiration/area than the untreated control (Table 2).

None of the plants displayed symptoms of B deficiency, however, B tissue concentrations from all treatments were significantly ($P \leq 0.05$) lower than that of the

untreated control (Table 2); they remained above the concentrations reported to cause deficiency symptoms by Pitchay (2002).

CONCLUSION

Drought stress alone appears to have less effect on the tissue concentration of B than does the rate of transpiration. Increasing osmotic potentials to simulate water stress had no effect on the concentration of B in tissue. Uptake rates (mg g^{-1} root) may be influenced, but smaller plants effectively concentrated the boron taken up. Unlike previous research by Hobbs and Bertramson (1949) and Gupta (1993) on tomatoes and barley, respectively, there was no evidence that dry substrate conditions had any effect on boron tissue concentrations in pansy seedlings. Drought conditions did affect the amount of water loss due to transpiration by the plants but did not affect transpiration per leaf area. The application of ABA on pansy seedlings produced more conclusive results, with a reduction of transpiration in plants. Transpiration and transpiration/leaf area ratios were both reduced when ABA was applied, which also resulted in lower concentrations of B in leaves.

These studies indicate that rather than water stress, lower transpiration and more specifically lower ratios of transpiration/leaf area might cause B deficiency that is observed during pansy production.

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Tables

Table 1. Boron concentration of 'Dynamite Yellow' pansy shoots, water loss (ml per rep.) due to transpiration (average of 10 d), and transpiration/area of canopy (ml cm^{-2}) from plants 36 DAS allowed to dry to 40, 30 or 20% container capacity (CC); treatments were imposed at 10 or 20 DAS or on a continual basis and transpiration values are averages of 10 days.

Treatment	B (mg L^{-1} dry wt.)	Trans	Trans/area[z]
Treated 10 DAS			
Control	24.52	4.42a	0.066a
40% CC	23.42	3.77a	0.054ab
30% CC	22.20	1.18b	0.009bc
20% CC	23.29	0.48b	-0.018c
P-value[y]	NS	***	*
Treated 20 DAS			
Control	24.52b	5.94a	0.059
40% CC	45.09a	6.83a	0.039
30% CC	22.46b	5.32a	0.050
20% CC	24.40b	1.88b	0.056
P-value[y]	**	***	NS
Treated continuously			
Control	24.52	4.42a	0.067
40% CC	23.37	3.61a	0.076
30% CC	20.87	0.28b	-0.053
20% CC	21.39	0.95b	0.099
P-value[y]	NS	***	NS

[z] Values with negative numbers indicate the value used for evaporation from the substrate surface of unplanted vessel was greater than total water loss of planted vessel in that treatment.

[y] NS, *, ***, Not significant, significant at $P \leq 0.05$ or $P \leq 0.001$. Mean separations are shown by day of treatment in columns.

Table 2. Nutrient concentration of 'Dynamite Yellow' pansy shoots, water loss (ml per rep.) due to transpiration (average of 11 d), and transpiration/area of canopy (ml cm^{-2}) from plants 36 DAS treated with abscisic acid as a drench (150 or 300 mg L^{-1}) or a foliar spray (150 or 300 mg L^{-1}) 10 or 20 DAS; transpiration values are averages of 11 days.

Treatment	B (mg L^{-1} dry wt.)	Trans	Trans/area[z]
Treated 10 DAS			
Control	32.75a	1.91a	0.038a
Drench (mg L^{-1})			
150	22.71b	0.76ab	0.018a
300	23.58b	-2.42d	-0.048c
Foliar spray (mg L^{-1})			
150	24.65b	-0.90c	-0.028bc
300	24.95b	-0.08bc	-0.015b
P-value[y]	*	***	***
Treated 20 DAS			
Control	32.75a	1.83a	0.018a
Drench (mg L^{-1})			
150	24.68b	0.72ab	0.001ab
300	24.78b	0.32ab	-0.001ab
Foliar spray (mg L^{-1})			
150	24.77b	-0.25b	-0.012b
300	23.39b	-1.20b	-0.020b
P-value[y]	*	*	*

[z] Values with negative numbers indicate the value used for evaporation from the substrate surface of unplanted vessel was greater than total water loss of planted vessel in that treatment.

[y] NS, *, ** Not significant, significant at $P \leq 0.05$ or $P \leq 0.01$. Mean separations are shown by day of treatment in columns under each element.

Table 3. Differences in weight (g) from sunrise to sunset for flats with plants or flats without plants when flats were treated with abscisic acid as a substrate drench (150 or 300 mg L^{-1}) or a foliar spray (150 or 300 mg L^{-1}) 10 or 20 DAS.

	19 Feb.	21 Feb.	26 Feb.	28 Feb.	2 Mar.	5 Mar.	7 Mar.	9 Mar.	12 Mar.	14 Mar.
Control										
Plants	34.2	27.4	26.3	33.6	42.3	40.1	39.9	39.9	39.5	46.2
Blanks	31.9	15.1	24.4	32.9	41.4	38.1	38.4	40.6	37.6	39.6
Treated 10 DAS										
Drench 150 (mg L^{-1})										
Plants	33.6	27.6	26.1	32.5	39.7	37.7	37.8	39.7	39.4	44.5
Drench 300 (mg L^{-1})										
Plants	30.8	26.5	24.5	30.1	37.1	34.9	33.6	35.5	35.4	40.1
Foliar spray 150 (mg L^{-1})										
Plants	29.4	25.2	24.8	31.6	38.7	36.8	37.6	37.3	36.3	46.2
Foliar spray 300 (mg L^{-1})										
Plants	30.5	26.8	25.2	31.6	39.2	37.6	38.1	39.2	39.3	45.2
Treated 20 DAS										
Drench 150 (mg L^{-1})										
Plants	No data taken before treatment			31.1	37.3	38.2	39.9	40.9	39.5	46.8
Drench 300 (mg L^{-1})										
Plants	No data taken before treatment			31.9	37.9	36.9	38.3	40.9	39.2	45.9
Foliar spray 150 (mg L^{-1})										
Plants	No data taken before treatment			30.1	36.4	36.6	39.3	39.2	39.7	45.5
Foliar spray 300 (mg L^{-1})										
Plants	No data taken before treatment			30.2	36.7	36.0	35.0	39.7	38.4	44.3