

Reduction of leaf tip burns of *Ornithogalum dubium* by controlling the temperature during bulb storage and greenhouse forcing to produce quality plants

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Both authors, X. Wu and L. Wang, contributed equally in preparing the manuscript.

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Abstract: Production of quality potted *Ornithogalum dubium* Houtt. plants were investigated under multiple conditions: pre-planting treatment at 10, 16, and 22°C for 40 days from Sept. 21 (stage A; ST-A) during bulb storage and then bulbs were potted. After potting, post-planting treatment at 15/12, 18/15, and 21/18°C (day/night) during stage B for 35 days from Nov. 2 (stage B; ST-B), and at 15/12 and 21/18°C during stage C for 30 days from Dec. 7 (stage C; ST-C) during greenhouse forcing was applied. Leaf tissue analyses for macro- and micro-nutrients were performed to investigate the cause of leaf tip burn symptom (LTB). Three criteria for quality of the plants at flowering were established: (1) LTB occurs on less than 1.5 leaves per plant. (2) the number of days to flower is less than 115 days, the length of the third leaf counted from the crown (the junction of the shoot and roots) is shorter than 11.5 cm, and the width is narrower than 2.5 cm; the scape length is shorter than 15 cm, and there are more than 45 flowers. (3) the leaf spread and morphology (leaf spread) and the pattern of the scape curvature (scape growth) have a score of less than 1.5. The following conditions are optimal to produce quality plants based on these three criteria: (1) Pre-planting bulbs treatment was applied at 10 or 16°C during ST-A, and forcing was performed at 15/12°C during ST-B and 21/18°C during ST-C. These conditions accelerated flowering, produced straight scape growth and upward (erect) growing leaves, and yielded acceptable leaf length and width. (2) The incidence of LTB was minimal at 10°C or possibly 16°C during ST-A, and at 15/12°C in ST-B and at 21/18°C in ST-C during greenhouse forcing. Leaf tip burn symptom was observed in both young and old leaves and was caused by a high boron (B) concentration (218-230 ppm) and possibly a high zinc (Zn) concentration (155-159 ppm) in *O. dubium*. A low calcium (Ca) concentration was not the cause of LTB. Although LTB cannot be avoided, it can be minimized by temperature manipulation during pre- and post-planting phase to produce high quality potted plants.

1. Introduction

Ornithogalum dubium Houtt. is a geophyte native to South Africa with yellow or orange flowers with a dark greenish-brown center (Du Plessis *et al.*, 1989; Littlejohn and Blomerus, 1997). Temperature treatments during the pre-planting phase before potting the bulbs and the greenhouse forcing phase after planting the bulbs should be optimized to produce quality *O. dubium* potted plants. "Leaf tip scorch" was believed to be caused either by genetics or by high salt levels in the growing medium after treatment with 5.5 grams of a slow-release fertilizer. However, no information on the salt levels in the potting medium or leaf tissue analyses was presented (De Hertogh and Gallitano, 1997).

The boron (B) concentration of healthy and necrosed leaves of the Oriental hybrid lily 'Star Gazer' did not significantly differ, although those of Ca, magnesium (Mg), manganese (Mn), and copper (Cu) were higher in necrosed than in healthy leaves (Chang, 2002). In the older leaves of *Curcuma* 'Chiangmai University Pride' ('CMU Pride'), a high B concentration (122 ppm) at the margin of leaves may have caused the marginal leaf burn (Roh *et al.*, 2006). It is unclear whether LTB in *O. dubium* is similar to marginal leaf burn observed in the older leaves of *Curcuma* 'CMU Pride'.

Leaf scorch symptoms were also reported in *Lilium* (Berghoef, 1986; Chang *et al.*, 2008). In *Lilium xelegans*, 'Red Carpet' and 'Sterling Star,' the scorch symptoms were caused by a low calcium (Ca) concentration resulting from an inefficient translocation of radioactive calcium (⁴⁵Ca) to the tip of the leaves (Roh, unpublished data). The critical Ca concentration associated with upper leaf necrosis of *Lilium* 'Star Gazer' was 0.3 to 0.4%, and upper leaf necrosis was caused by low Ca concentration (Chang, 2002) although "it is difficult to isolate a single characteristic to explain the observed cultivar variation to upper leaf necrosis in Oriental hybrid lilies" (Chang *et al.*, 2008).

Although forcing techniques in geophytes, such as tulip and lily, have been well documented (De Hertogh, 1974), forcing techniques for *O. thyrsoides* and *O. dubium* to produce quality potted plants are still lacking, especially regarding the incidence of LTB (Jansen van Vuuren and Holtzhausen, 1992; Littlejohn and Blomerus, 2000; Suh *et al.*, 2000; Luria *et al.*, 2002; Reinten *et al.*, 2011; Lee and Miller, 2015).

Flowering of *O. dubium* was accelerated when the plants were forced at 27/22°C (Luria *et al.*, 2002) or 19/13°C (Suh *et al.*, 2000) compared with 17/12°C or 13/10°C, respectively. Flowering was accelerated in several new cultivars forcing at 22/18°C during a short day between the visible bud stage to flowering (Lee and Miller, 2015). The highest (visual) quality *O. dubium* cultivars were produced when forcing occurred at constant 17 to 19°C (Lee and Miller, 2015), which is comparable to 22/18°C for forcing seed-raised bulbs (De Hertogh and Gallitano, 1997). However, the criteria used to assess the visual quality were not specified (Lee and Miller, 2015).

The objectives of this research were to produce quality potted *O. dubium* plants by reducing the incidence of LTB, accelerating flowering while ensuring the maximum number of flower buds, and desirable morphologies as influenced by temperature treatment during the bulb handling and greenhouse forcing stages to establish the optimum temperature regimes that reduce the incidence of LTB, and accelerate flowering with desirable morphologies. The number of leaves showing LTB, the time of flowering, and the leaf and floral morphology were used to develop criteria for quality *O. dubium* potted plants. The macro- and micro-elements of the leaf tips showing LTB/no LTB during growth and development were analyzed to determine the cause of LTB.

2. Materials and Methods

General cultural practices

Ornithogalum dubium bulbs (5-6 cm in circumference) purchased from Agrexco (Jamaica Plain, NY, USA) were used in the experiment conducted between 1999 and 2002. The bulbs were stored dry or potted, with one bulb per 6.3 or 10 cm pot, filled with ProMix (Pro-Mix BX mycorrhizae, Quakertown, PA, USA). Temperature treatments were performed in growth chambers as specified in each experiment with a 12 h day (29 W·m⁻²)/night (day: 08:00-18:00 hr; night: 18:00-08:00 hr). At planting, 0.8 g of a slow-release fertilizer (14 N - 6.2 P - 11.6 K, Osmocote, Scotts Co., Marysville, OH, USA) was applied to the surface of the growing medium. In addition, the plants received 200 ppm N from 15 N - 16 P - 18 K water-soluble fertilizer (JR Peter's Laboratory, Allentown, PA, USA) once a month during culture in the greenhouse.

The effect of the pre-plant bulb (ST-A) and post-plant forcing (ST-B and ST-C) temperatures on growth, flowering, and plant quality

Ornithogalum dubium bulbs (7-8 cm in circumference) from the previous experiment were harvested and stored dry at 20°C until potting on June 1 and then potted per 10 cm pot filled with ProMix and grown at 18/15°C. Leaf samples were collected for tissue analysis on July 12, 2000 from leaves without any LTB.

The tips and basal portions of the leaves were separated, and the width at mid-leaf was 2 cm. Samples were collected from the third and fourth leaves of each plant, counted from the tip of the bulbs, and were combined into a sample. The third leaf was collected from 15 plants, and the leaves were divided into three segments. The two-cm long distal end (tip) did not show any incidence of LTB, the proximal end (base) of each leaf, and mid portion between the tip and base was used for macro- and micro-element tissue analysis.

In another experiment for macro- and micro-element analysis, bulbs were harvested after forcing in 2000, and were potted in 2001. On Jan. 13, 2002, flowers began to develop and the flowers had fully developed color and were ready for anthesis. Leaves from the distal end (tip) with and without LTB (Fig. 1) and the proximal end (base) were collected from 15 plants during forcing on Jan. 14 and 26, Feb. 3 and 10, and Mar. 4. The leaf samples were dried at 70°C for 4 days and were ground into fine particles using a mortar and pestle. Two replicates of the dried leaves were sent to the JR Peters Laboratory (Allentown, PA, USA).



Fig. 1 - Various degrees of leaf tip burn (LTB) symptoms in *Ornithogalum dubium* leaves. Leaf without evident LTB symptom (A) and different degree of LTB symptoms (B, C, and D).

Bulbs purchased on Sept. 15, 1999 were used in this experiment. Before potting, *O. dubium* bulbs were stored at constant temperatures of 10, 16, and 22°C for 40 days from Sept. 21 (ST-A). Bulbs were planted 2 cm deep of the nose from the surface of the growing medium, and leaf emergence through the growing medium was recorded from potting when temperature treatment of ST-A started. Bulbs were potted (one bulb per 6.3 cm pot) filled with the ProMix medium, and the pots were placed in a greenhouse maintained at 15/12, 18/15, and 21/18°C for 35 days from Nov. 2 (ST-B). On Nov. 2, a slow-release fertilizer was applied to the surface of the growing medium, and plants received 200 ppm N water-soluble fertilizer once a month. The leaves emerged (about 0.5 cm or less above the nose of bulbs) before potting on Sept. 21. On Dec. 7, plants were divided into two groups and grown in a greenhouse maintained at 15/12 and 21/18°C for 30 days (ST-C) (Table 1). There were 16 plants per treatment. Starting from Jan. 6, all plants were grown in a greenhouse maintained at 19/16°C until flowering, and data were collected at anthesis.

Criteria establishment for quality evaluation

When 3-4 flowers reached anthesis, the date of flowering, the number of total leaves showing LTB (Fig. 1) excluding the incipient LTB (Fig. 2) and the scape length, number of flowers, leaf spread, scape growth (Fig. 2, 3) were recorded. The leaf spread was based on the angle of the leaf measured from the base to the tip using the following scores: 1: greater than 60° (pointing upward, erect); 2: between 30 and 60°; 3: old leaves prostrate and drooping downward at less than 15°. In addition, the scape growth was used as a quality criterion using the following scores: 1: no bending of the scape; 2: leaning sideways without bending at the base of scape; 3: scape at the base starting to grow at about 40° and continued sideways growth.

Data analysis

Data were analyzed using SAS® System Version 9 for Microsoft® Windows® (SAS Institute Inc., 2002). The number of total leaves, number of leaves with LTB, and the scores of the leaf spread and scape orientation were analyzed following a square root transformation $(x + 0.5)^{1/2}$. Means were compared using Duncan's Multiple Range test (DMRT) at the significant level indicated in the tables.

Table 1 - The number of total leaves and of leaves showing leaf tip burn by leaf position, and the total number of leaves showing leaf tip burn symptoms in *Ornithogalum dubium*

Individual plant (number)	10°C ST-A, 15/12°C ST-B, 21/18°C ST-C ^y			22°C ST-A, 18/15°C ST-B, 15/12°C ST-C ^x		
	No. of total leaves per plant	Tip burn symptom		No. of total leaves per plant	Tip burn symptom	
		Leaf position ^z	Total number of leaves		Leaf position number	Total number of leaves
1	5	2.5	2	5	1,3,4,5	4
2	4	1,2,3,4	4	6	2,3,4,5,6	5
3	4	-	0	6	3,4,5,6	4
4	5	5	1	6	5,6	2
5	4	-	0	6	1,3,4,5,6	5
6	5	3	1	5	3,4,5	3
7	6	4.5	0	5	1,2,3,4,5	5
8	6	4	1	6	3,4,5,6	4
9	5	-	0	6	2,3,4,5,6	5
10	6	-	0	5	1,2,3,4,5	5
11	5	4,5,6	3	5	2,3,4,5	4
12	5	-	0	8	1,2,3,4,5,6,7,8	8
13	6	-	0	4	1,3,4	3
14	5	-	0	6	3,4,5,6	4
15	5	1,2,3,4,5	5	5	3,4,5,6	4
16	6	5	1	6	2,3,4,5	4
Average	5.13		1.13 (22%) ^w	5.62		4.31 (76.7%)

^z Leaf position was counted from the crown and upward, the first leaf (leaf number 1) is the most matured leaf.

^y Bulbs received 10°C from Sept. 21 (ST-A), 15/12°C from Nov. 2 (ST-B) and 21/18°C from Dec. 7 (ST-C).

^x Bulbs received 22°C from Sept. 21 (ST-A), 18/15°C from Nov. 2 (ST-B) and 15/12°C from Dec. 7 (ST-C).

^w Percentage of leaves showing LTB symptoms.

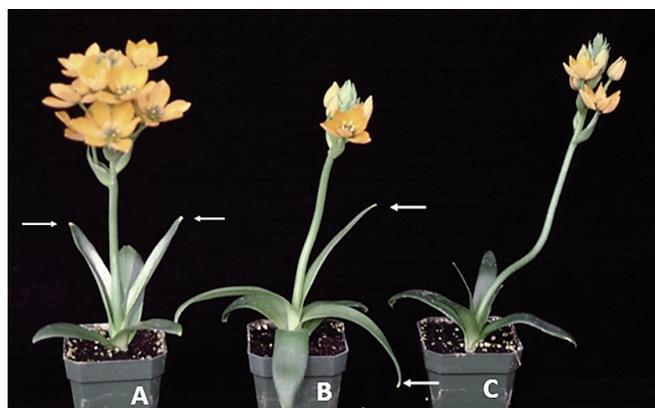


Fig. 2 - Patterns of scape growth of *O. dubium*. A) straight growth and no bending of the scape (score 1). B) leaning to sideways without bending at the base of scape (score 2). C) scape at the base started to grow at about 30-degree angle, and continue to grow leaning to sideways (score 3). Incipient leaf tip burn (LTB) symptoms are indicated with arrow sign. Plant A is considered the most ideal and high-quality plant.

3. Results

Leaf tip burn symptoms and analysis of macro- and micro-elements in different leaf parts

The number of total leaves ranging from 4.8 to 5.6

was not affected significantly by the temperature treatments (data not presented). When bulbs were stored at 10°C/ST-A, followed by forcing at 15/12°C/ST-B and 21/18°C/ST-C, the number of leaves showing LTB was 1.1 out of 5.1 leaves. When the bulbs were stored and forced at 22°C/ST-A, 18/15°C/ST-B, and 15/12°C/ST-C, 4.3 out of 5.6 leaves showed LTB (Table 1).



Fig. 3 - Leaf spread of *Ornithogalum dubium*. A) all leaves growing upward (erect) (score 1), B) Old leaves showing prostrate growth (spreading), while young leaves growing upward (score 2), and C) Old leaves drooping and curved inward toward the pot (prostrate) (score 3). Evident leaf tip burn (LTB) symptoms are indicated with arrow sign.

The macro- and micro-element analysis of the leaves without apparent LTB revealed that the B concentration at the tip (distal end) of the leaves was 119 ppm, which is within the suggested acceptable range (30-150 ppm) for general ornamentals (Table 2). The B concentrations in the middle and base (proximal end) of the leaves were 34 and 21 ppm, respectively, which was close to the lower end of the range.

When the samples were collected in the early growth stages when no or incipient symptoms were observed on Jan. 14, Jan. 26, and Mar. 4, the B concentrations were 109, 96, and 108 ppm, respectively, which fell within the suggested acceptable range (Table 3). Macro- and micro-element analysis of the leaves without apparent LTB revealed that the B concentration in the tip of the leaves when collected on Jan. 14 was lower than 109 ppm (Table 3). However, B concentrations of leaves showing incipient symp-

toms and severe LTP when the leaves were collected on Feb. 3 and Feb. 8 were 218 and 230 ppm, respectively, and these concentrations exceed the suggested acceptable range.

The Ca concentration was high in the leaf tips (0.76%), and the concentrations of the other macro- and micro-elements fell within the acceptable ranges for each element, even when LTB was observed. The exception was zinc (Zn), whose concentration was slightly above the upper level (155 and 159 ppm) of the suggested range (150 ppm) (Table 3). The nitrogen (N) concentration was above the lower level of the suggested range in all samples, regardless of leaf position, LTB symptoms, and leaf sampling time. The concentrations of Ca and Zn were lowest and highest, respectively, in the tip (distal end) and lowest in the middle and bottom (proximal end) of the leaves when LTB was not observed. At the tip of the leaves show-

Table 2 - Macro- and micro-elements of *Ornithogalum dubium* from the distal-, mid- and basal portion of third leaves from the base of the scape that do not show leaf burn symptom

Leaf position	Macro- and micro- elements										
	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Mo (ppm)
Tip (distal end)	3.9 c ^z	0.97 a	3.1 a	0.76 c	0.29 c	119 c	87.2 a	40 a	18 a	56 c	3.1 a
Mid	3.5 b	1.11 a	3.8 a	0.69 b	0.27 b	34 b	76.2 a	34 a	17.1 a	46 b	2.3 a
Base (proximal end)	2.8 a	0.88 a	4.3 a	0.55 a	0.19 a	21 a	60.4 a	29 a	15.3 a	37 a	1.4 a
Level of significance ^y	***	NS	NS	***	***	***	NS	NS	NS	***	NS
Suggested acceptable range ^x	3.5-5.5	0.35-1.0	2.0-8.8	0.8-3.0	0.2-1.5	30-150	60-200	50-200	mag-25	30-150	0.5-5

^z Comparisons of means by Duncan's multiple range test, $P < 0.01$, F -test.

^y ***, NS= significant at $P < 0.001$ and not significant.

^x JR Peter's Laboratory (Allentown, PA. USA).

Table 3 - Macro- and micro-elements of leaf analysis of *Ornithogalum dubium* from the tip and base of third leaves from the crown showing different degree of leaf burn symptom

Leaf position	Leaf tip burn	Sampling date	Macro- and micro- elements										
			N (%)	P (%)	K (%)	Ca (%)	Mg (%)	B (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Mo (ppm)
Base	No	Jan. 14	5.30 c ^z	1.31 a	6.3 b	1.55 b	0.33 bc	25 a	67.5 a	40 e	10.9 d	62 a	1.4 d
Tip	No	Jan. 14	4.78 b	1.59 c	7.3 c	1.36 a	0.27 b	109 c	103.5 e	31b	13.1 e	114 bc	1.3 c
Base	No	Jan. 26	5.21 c	1.32 a	5.5 a	1.96 de	0.37 c	22 a	59.1 a	35 d	6.9 a	51 a	1.2 b
Tip	Incipient	Jan. 26	4.62 b	1.60 d	6.1 b	1.76 c	0.30 b	96 b	84.3 d	30 b	8.8 b	106 b	1.1 a
Tip	Evident	Feb. 3	3.92 a	1.44 b	6.0 a	1.94 de	0.30 b	218 d	91.6 bc	27 c	8.3 b	155 e	1.2 ab
Tip	Severe	Feb. 10	3.83 a	1.49 c	5.7 a	2.08 e	0.37 c	230 d	91. bc	25 bc	10.2 d	159 e	1.6 de
Tip	No	Mar. 4 (flowering)	3.90 a	1.31 a	5.4 a	1.50 b	0.19 a	108 c	93d	22 a	8.2 b	118 d	1.71 e
Level of significance ^y			***	***	***	***	***	***	***	***	***	***	**
Suggested and acceptable range ^x			3.5-5.5	0.350-1.0	2.0-8.8	0.8-3.0	0.2-1.5	30-150	60-200	50-200	mag-25	30-150	0.5-5

^z Comparisons of means by Duncan's multiple range test, $P < 0.01$, F -test.

^y ***, ** = significant at $P < 0.001$, and $P < 0.01$, F -test.

^x JR Peter's Laboratory (Allentown, PA. USA).

ing evident and severe LPB Ca concentration was higher than in leaves showing incipient LTB symptom. The Mn concentration ranged from 25 to 40 ppm, which was lower than the lower level of the range (50 ppm). The concentrations of phosphorus (P), potassium (K), iron (Fe), Mn, Cu, and molybdenum (Mo) did not differ between the tips, middle, and bottom of the leaves.

The effect of the pre-planting and post-planting forcing temperatures on growth, flowering, and LTB expression

Leaf emergence was affected significantly by temperature treatments during ST-A and ST-B and the interaction of ST-A and ST-B (Table 4). Leaves emerged late when bulbs were stored at 10°C before

potting during ST-A (10°C/ST-A) compared to when bulbs were stored at 16 and 22°C/ST-A, and the forcing temperatures were 15/12°C or 18/15°C/ST-B and 15/12°C or 18/15°C/ST-B (Table 4). The number of days to flower was affected significantly by temperature treatment during ST-A, ST-B, and ST-C and the interaction of ST-A and ST-B. The earliest flowering (87 days) occurred when bulbs were treated at 10°C/ST-A and 21/18°C/ST-B and ST-C. Bulbs stored at 22°C/ST-A took longer than 112 days to flower, regardless of the temperature during ST-B and ST-C. The average days to flowering was 116 days, whereas the days to flowering was <100-105 days at 21/18°C/ST-C.

The number of flowers (<36 flowers) was only affected by bulb storage temperature during ST-A

Table 4 - The effect of temperature treatment during three different growth and development periods on the growth and flowering of *Ornithogalum dubium*

Treatment stage (ST-)/ temperature (°C) ^z			Number of days ^v to		No. of flowers	Scape length (cm)	No. of leaves showing leaf tip burns	Score of angle u		Leaf	
A ^y	B ^x	C ^w	Leaf emergence	Flowering				Scape	Leaf	Length	Width ½ length
10	15-dic	15-dic	47.9 a	113 f	33 c	20.5 ij	2.00 b	1.0 a	1.0 a	8.4 a	2.2 ab
10	15-dic	21/18	47.9 a	98 c	33 d	20.7 hij	1.13 a	1.5 b	1.2 ab	8.5 a	2.1 a
10	18/15	15-dic	47.6 a	105 ef	36 d	22.7 j	3.31 fgh	1.1 ab	2.5 ab	9.6 abc	2.3 ab
10	18/15	21/18	47.5 ab	90 b	27 a	20.4 hij	2.50 bcde	1.1 ab	2.8 c	10.4 cdef	2.2 ab
10	21/18	15-dic	46.4 cd	100 c	25 a	22.6 ij	2.44 bcd	1.4 b	2.9 ab	9.5 abc	2.2 ab
10	21/18	21/18	46.4 cd	87 a	29 ab	19.4 h	0.50 a	1.5 b	2.5 b	9.9 bcd	2.2 ab
16	15-dic	15-dic	46.0 d	113 f	55 ef	15.3 defg	2.19 bc	2.3 c	1.8 c	9.1 ab	2.5 abc
16	15-dic	21/18	46.2 d	105 ef	44 d	15.8 fg	2.44 bcd	2.3 c	1.9 c	10.1 bcde	2.4 abc
16	18/15	15-dic	46.1 d	123 g	52 ef	15.4 defg	3.13 efg	2.9 e	2.7 de	11.5 fgh	2.4 abc
16	18/15	21/18	46.0 d	107 ef	51 e	20.3 hi	4.00 j	3.0 e	2.7 de	11.4 fg	2.5 abc
16	21/18	15-dic	46.0 d	116 fg	52 ef	12.4 ab	3.31 fgh	2.5 cd	2.8 e	11.3 efg	2.6 bcd
16	21/18	21/18	46.1 d	102 cd	48 e	13.6 bcdefg	2.94 defg	2.3 c	2.5 d	11.3 efg	2.4 abc
22	15-dic	15-dic	47.1 abc	129 h	52 ef	13.3 bcde	2.75 cdef	2.4 cd	2.8 e	12.1 h	2.8 cd
22	15-dic	21/18	47.0 bc	116 fg	58 fg	13.5 bcdef	3.50 ghi	2.6 de	2.9 e	12.2 hu	3.1 d
22	18/15	15-dic	46.8 bcd	125 g	59 fg	14.7 bcdefg	4.31 j	2.9 e	2.7 de	13.9 hi	2.8 cd
22	18/15	21/18	46.8 bcd	114 f	63 gh	12.8 bc	3.50 ghi	2.4 cd	2.8 e	12.8 hi	2.9 cd
22	21/18	15-dic	46.4 cd	123 g	61 gh	10.4 a	3.50 ghi	2.8 de	2.9 e	12.0 gh	2.6 bcd
22	21/18	21/18	46.4 cd	112 f	57 fg	13.2 bcd	3.88 hij	2.4 cd	2.7 de	13.6 i	2.6 bcd
Level of significance ^t											
ST-A			***	***	***	***	***	***	***	***	***
ST-B			***	***	NS	**	***	NS	***	***	**
ST-C			NS	***	NS	NS	NS	NS	**	NS	NS
ST-A × ST-B			***	***	NS	NS	***	NS	***	NS	***
ST-A × ST-C			NS	NS	NS	NS	**	NS	***	NS	NS
ST-B × ST-C			NS	NS	NS	NS	NS	NS	NS	NS	NS
ST-A × ST-B × ST-C			NS	NS	NS	NS	NS	NS	NS	NS	NS

^z Comparisons of means by Duncan’s multiple range test, P<0.01, F-test.

^y ***, **, ns = significant at P< 0.001, P<0.01, and not significant.

^x JR Peter’s Laboratory (Allentown, PA. USA).

when treated at 10°C/ST-A, 18/15°C/ST-B, and 15/12°C/ST-C (Table 4). More than 44 flowers were produced when bulbs were treated at 16 and 22°C/ST-A, regardless of the temperature during ST-B and ST-C. The scape length was >20.4 cm when bulbs were stored at 10°C/ST-A, regardless of the temperature during ST-B and ST-C. When the temperature during ST-A was increased from 10 to 22°C, a significantly higher number of flowers was produced on short scapes when bulbs were stored at 22°C.

For plants that required an average of 115 days to flower, the scape length was 12.4 cm, and 52 flowers were produced in the treatment of 16°C/ST-A, 21/18°C/ST-B, and 15/12°C/ST-C (Table 4). However, a significantly higher number of leaves (3.31) showing LTB was produced compared to 0.50 leaves in treatment (10°C/ST-A, 21/18°C/ST-A, and 21/28°C/ST-C) and 1.13 leaves in treatment (10°C/ST-A, 15/12°C/ST-A, and 21/28°C/ST-C). As the bulb storage temperature was increased to 16 or 22°C, the number of leaves showing LTB increased to 2.19 leaves (16°C/ST-A, 15/12°C/ST-A, and 15/12°C/ST-C) to 4.31 leaves (22°C/ST-A, 18/15°C/ST-A, and 15/12°C/ST-C).

The scape growth was primarily influenced by the temperature during ST-A, resulting in scores of <1.5 when bulbs were stored at 10°C/ST-A, regardless of the temperature during ST-B and ST-C (Table 4). The scape growth when bulbs were stored at 16 and 22°C/ST-A had scores of >2.3, regardless of the temperature during ST-B and ST-C. The leaf spread showed a complex response; ST-A, ST-B, and ST-C had a significant effect, as did the interaction of ST-A and ST-B and ST-B × ST-C. However, the leaf spread had scores <1.9 when the plants were forced at 15/12°C/ST-C, followed by storing the bulbs at 10 or 16°C/ST-A. In contrast, the scores were >1.9 regardless of the temperature treatments during ST-A, ST-B, and ST-C. The length of the leaves was <10.4 cm when bulbs were stored at 10°C/ST-A, regardless of the forcing temperature during ST-B and ST-C, and was >12 cm when bulbs were stored at 22 °C/ST-A. Bulbs stored at 16°C/ST-A had leaf lengths between those of the 10°C and 22°C/ST-A treatments. The leaf width was <2.3 cm when bulbs were stored at 10°C/ST-A.

4. Discussion and Conclusions

Quality criteria of the finished potted plants

Criteria for the quality of finished potted *O. dubium*

plants have not been established even in 2019. The quality can be evaluated in reducing the incidence leaf tip burn (LTB) at the tip of the leaves (Fig. 1) and by the rate of flowering without sacrificing the number of flower buds. Areas of LTB are indicated by the arrows in *O. dubium* seedlings in figures 2 and 3. However, the criteria used to assess the visual quality were not specified (Lee and Miller, 2015).

Therefore, the following three quantitative criteria to assess the plant quality were established in this study. Criterion I: LTB occurs on <1.5 leaves per plant. The lower the score of the scape growth and leaf spread, the higher the plant quality is. Criterion II: the number of days to flowering is <115 days, the length <11.5 cm, the width of the third leaf from the crown is <2.5 cm, the scape length is <15 cm with >45 flowers. Criterion III: the leaf spread and scape growth have a score <1.5.

Analysis of the macro- and micro-elements in the third and/or fourth leaves showing various symptoms and suggested concentration for quality plant production

The cause of LTB was investigated based on the macro- and micro-element concentrations in the tissue. Macro- and micro-elements analysis of *O. dubium* leaves without apparent LTB revealed that the B concentration in the leaf tips (distal end) was 119 ppm, which was within the suggested acceptable range for general ornamentals (30-150 ppm) (Table 2 and 3). The concentrations in the middle and bottom (proximal end) of the leaves were slightly higher or lower, respectively, than the lower level of the range of B concentration. When the samples were collected in the early growth stages when no or incipient LTB symptoms were observed on Jan. 14, Jan. 26, and Mar. 4, the B concentrations were 109, 96, and 108 ppm, respectively, which fell within the suggested acceptable range.

Leaf tip burn in *O. dubium* in young and old leaves is considered to be different from marginal leaf burn observed in old leaves of *Curcuma* 'CMU Price'. The latter is related to high concentrations of B (119 ppm), Fe (189 ppm), and Mn (273 ppm) and low N concentration (1.7%) (Roh et al., 2006). Boron may accumulate at the tip or margin of leaves (Jones, 1970) and may cause toxic effects (Oertli, 1962). Therefore, LTB in *Ornithogalum* is a unique physiological expression resulting from high B concentration at the leaf tips and developing regardless of the leaf age; these symptoms were observed in *O. thyr-*

soides and *O. arabicum* (Roh, personal observation). The incidence of LTB in *Watsonia laccata* (Jacquin) Ker Aawler is considered a similar disorder as in *O. dubium* and adversely affects potted plant production in *W. laccata* (Suh *et al.*, 2011). Leaf tip scorch observed in *O. dubium* (De Hertogh and Gallitano, 1997), upper leaf necrosis observed in Oriental hybrid lilies (Chang *et al.*, 2008), and burn appearing at certain developmental stages before the visible bud stages in *Lilium* 'Pirate' (Berghoef, 1986) may be different from LTB in *O. dubium*.

The LTB is, therefore, caused by a high B concentration (218-230 ppm) and possibly a high Zn concentration (155-159 ppm), which was higher in this study than the high level of the suggested range, even when grown in medium low in B and Zn. Boron concentration in the growing medium was <6 ppm (Tech Data PRO-MIX_HP_Mycorrhizae_4253; accessed on Nov. 1, 2020). The source of B accumulated at the tip of leaves showing LTB in *Ornithogalum* is considered resulting from B accumulation at the margin of old leaves after translocation from rhizome and leaves as discussed in *Curcuma* (Roh *et al.*, 2006; Roh and Lawson, 2009). Since Zn has intermediate mobility and occurs in the middle leaves (Shelp *et al.*, 1995; McCauley *et al.*, 2009), it may not be associated with LTB observed in the young and old leaves of *O. dubium*.

The effect of the temperatures in the pre-planting bulb (ST-A) and post-planting forcing periods (ST-B and ST-C) on growth, flowering, and plant quality

Regardless of the temperature treatments during the pre-planting bulb (ST-A) and post-planting forcing (ST-B and ST-C) periods, the LTB symptoms were not entirely eliminated in *O. dubium*, which was in agreement with the results for *W. laccata* (Suh *et al.*, 2011). In our experiment, the number of leaves showing LTB was 1.1 out of 5.1 leaves which was the fewest when received 10°C/ST-A, followed by forcing at 15/12°C/ST-B and 21/18°C/ST-C (Table 1). Therefore, the flowering responses and leaf and scape morphologies, as evaluated in criteria II and III, respectively, were further considered. The overall average number of days to flower was 116 days (Table 4), which was close to the 115 days established for criterion I. Therefore, it is suggested to store bulbs at lower than 22°C, which is lower than the 25°C suggested by De Hertogh and Gallitano (1997). Our data suggested that storing bulbs at 10°C

ensures that plants will flower in <115 days.

Low pre-planting temperature treatments in the range of 9 to 27°C for 3 weeks were shown to accelerate flowering (Lee and Miller, 2015). However, this depends on the cultivar and bulb-handling methods since leaves did not emerge and plants failed to flower when bulbs were treated at 10°C. The initiation of the inflorescence can be inhibited by more than 6 weeks because breaking dormancy may require temperatures higher than 10°C (Roh and Joung, 2004). The optimum pre-planting temperature was reported as 22 to 28°C immediately after harvesting the bulbs to promote the early development of the first inflorescence and initiation of the second inflorescence (Roh and Joung, 2004). In *O. arabicum*, flower initiation and development were accelerated by storing initially at 30°C for 12 weeks, followed by 20°C for 4 weeks, and finally at 13°C for 8 weeks before planting (Shoub and Halevy, 1971; Shoub *et al.*, 1971).

Forcing at 21/18°C/ST-B and ST-C after potting the bulbs caused the plants to flower in 100 to 105 days, and the response was faster when bulbs were treated at 16°C/ST-A. Therefore, the recommended temperatures for storing bulbs to ensure flowering in <115 days are 10 or 16°C/ST-A followed by forcing at 21/18°C/ST-B or preferably 21/18°C/ST-C (10°C/ST-A or at 16°C/ST-A, 21/18°C/ST-B or 21/18°C/ST-C). De Hertogh and Gallitano (1997) suggested to force bulbs in a greenhouse at a minimum of 22/18°C to flower in 96 days (this is similar to the period in ST-B and ST-C in this study). Temperatures below 15°C during an unspecified growth period did not increase the growth rate (Littlejohn and Blomerus, 2000), although a constant temperature of 17 to 19°C was most suitable for forcing, considering the forcing time, plant height, and visual quality of *Ornithogalum* cultivars. However, the criterion for visual quality was not specified (Lee and Miller, 2015).

The number of flowers was significantly influenced by bulb storage temperature during ST-A, and plants produced <36 flowers (10°C /ST-A, 18/15°C /ST-B, and 15/12°C/ST-C). However, more than 44 flowers were produced when bulbs were treated at 16 and 22°C during ST-A, regardless of the temperature during ST-B and ST-C. The number of flowers was reduced when forcing occurred at 32/27°C; however, the flowering rate was not mentioned (Luria *et al.*, 2002). A significantly higher number of flowers (52 flowers) produced on the short scapes (12.4 cm)

when bulbs were stored at 22°C/ST-A and forced at 21/18°C/ST-B) and 15/12°C/ST-C. Depending on the bulb size, 20 flowers each were produced on two flower stalks, and the bulbs stored at 9°C produced floral stalks (31.3 cm) (De Hertogh and Gallitano, 1997).

Three criteria were established to determine the quality of *O. dubium* plants. Criterion I focus on the occurrence of LTB that are observed in less than 1.5 leaves per plant. Leaf tip burn cannot be avoided, but can be controlled by maintaining the forcing temperature at a minimum of 10°C or potentially 16°C during ST-A. This treatment does not delay flowering. Leaf tip burn was observed in *O. dubium* in both young and old leaves and was caused by a high B concentration (218-230 ppm) and possibly by a high Zn concentration (155-159 ppm). Calcium was not the primary cause for LTB. Criterion II focuses on growth and flowering, leaf and scape morphologies; flowering occurs less than 115 days, the length and width of the third leaf is shorter than 11.5 cm and narrower than 2.5 cm, respectively, the scape length is shorter than 15 cm, and there are more than 45 flowers. Criterion III focuses on the leaf morphology and the scape growth have a score of less than 1.5. The recommended treatment to meet the three criteria and to produce high quality plants at flowering (plant A, Fig. 2) is to treat bulbs at 10 or 16°C before planting (ST-A), and at 15/12°C in ST-B and at 21/18°C during ST-C during forcing. However, it was not possible to produce finished plants without severe LTB symptoms. Although LTB can not be prevented in *O. dubium*, it can be minimized by temperature manipulation.

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