Oriental Hybrid Lily Cultivars Vary in Susceptibility to Upper Leaf Necrosis

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Abstract
Upper leaf necrosis (ULN) in Oriental hybrid lilies (Lilium L.), commonly observed as leaf tip “burn”, is a calcium (Ca) deficiency disorder. In this study, we compared the susceptibility of four cultivars, ‘Star Gazer’, ‘Acapulco’, ‘Sissi’, and ‘Alliance’, and investigated some calcium nutrition characteristics that may contribute to ULN susceptibility. We found that ‘Star Gazer’ and ‘Acapulco’ are susceptible cultivars, ‘Sissi’ and ‘Alliance’ are not, and ‘Star Gazer’ suffers from more severe ULN than ‘Acapulco’. At planting, the Ca concentration in the bulb scales was similar in the four cultivars, approximately 0.03% DW. However, the susceptible cultivars had lower initial Ca concentration in leaf primordia than the non-susceptible ones, and the most susceptible ‘Star Gazer’ had the lowest. Thirty days after planting, leaf Ca concentration was highest (1.01%) in ‘Acapulco’, with no difference among the other three cultivars (in the range of 0.54 - 0.65%). During this 30-day period, the net Ca gain from the media in ‘Acapulco’ was 43 mg/plant, about 60% more than the other cultivars. The results agreed with the observation that ‘Acapulco’ had the highest transpiration rate. Shoot growth rate in the most susceptible period for ULN (30 - 40 days after planting) was approximately 0.18 g·day$^{-1}$ for ‘Star Gazer’, ‘Acapulco’, and ‘Sissi’, but only 0.09 g·day$^{-1}$ for ‘Alliance’. Although the low initial Ca concentration in leaf primordia may play a role, it is difficult to isolate a single characteristic to explain the observed cultivar variation to ULN in Oriental hybrid lilies.

INTRODUCTION
Calcium (Ca) deficiency disorders have been found in many economically important crops (Shear, 1975), upper leaf necrosis (ULN) in Oriental hybrid lilies (Lilium L.) has been recently identified to be another case (Chang and Miller, 2005). In general, calcium deficiency is not due to an insufficient Ca content in the soil or medium (Bangerth, 1979; Chang and Miller, 2005; Kirkby, 1979) instead, an imbalanced Ca distribution within the plant is the major cause. Many factors contribute to insufficient Ca translocation to the desired organs, such as low transpiration of the organs (Bangerth, 1979; Chang and Miller, 2004; Collier and Tibbitts, 1982), high humidity during the day (Adams and Ho, 1995b; Collier and Tibbitts, 1982), rapid growth rate (Bangerth, 1979), high salinity in the soil or medium (Adams and Ho, 1995a; Ehret and Ho, 1986), and low root pressure (Bradfield and Guttridge, 1979; Palzkill and Tibbitts, 1977; Palzkill et al., 1976). In addition to the factors mentioned above, low calcium concentrations in the bulb is also involved in the onset of Ca deficiency on upper leaves in Oriental lilies (Chang and Miller, 2003).

Attempts to control Ca deficiency disorders are not consistent (Bangerth, 1979), mainly because the influencing factors are very complicated. In general, methods that relieve the Ca-deficiency causal factors reduce the disorder to some extent. For example, increasing transpiration of young leaves in ‘Star Gazer’ lilies by means of increased airflow or artificial leaf unfolding greatly reduced ULN symptoms (Chang and Miller, 2004). Foliar Ca sprays have also been attempted for reducing Ca deficiency disorders in various crops, however, with conflicting results (Chang et al., 2004; Collier and Tibbitts,
Our previous studies indicated that directing Ca sprays onto the desired young organs is crucial (Chang et al., 2004). As for the insufficient amount of Ca content in ‘Star Gazer’ bulb scales, it seems that it can only be increased by future genetic manipulation, and not by changing cultivation methods. The Ca concentration in scales of commercially-available bulbs is low, approximately 0.04% dry weight (Chang and Miller, 2003). We have cultivated bulbs with modified Johnson’s solution in which Ca concentration was high (4 mM), but the Ca concentration in harvested bulbs was just 0.03% DW (Chang and Miller, 2003), the same as for commercially grown bulbs.

Susceptibility to Ca deficiency disorders seems to be cultivar dependent, for example, in blossom-end rot (BER) in tomato (Lycopersicon esculentum Mill.) (Ho et al., 1995), bract distortion in poinsettia (Euphorbia pulcherrima Willd.) (Jacques et al., 1990) and ULN in Oriental hybrid lilies. To gain better insight into Ca deficiency in Oriental hybrid lilies, we compared the susceptibility of four lily cultivars to ULN. Our objective was to determine if any of the following factors contribute to the different susceptibilities to ULN between cultivars: shoot growth rate, leaf transpiration, initial Ca concentration in various bulb organs, and net Ca gain from the growing medium.

**MATERIALS AND METHODS**

Bulbs (16- to 18-cm circumference) of four lily cultivars, ‘Star Gazer’, ‘Acapulco’, ‘Sissi’, and ‘Alliance’, were grown in 15-cm pots with 1 soil : 2 peatmoss : 1 perlite mix (by volume) supplemented with lime amendments. Ninety-eight bulbs of each cultivar were planted, and pots arranged using a completely randomized design. The average fresh weight of bulbs was 64.1 ± 0.4 g, 64.5 ± 0.5 g, 63.9 ± 0.4 g and 64.1 ± 0.5 g for ‘Star Gazer’, ‘Acapulco’, ‘Sissi’, and ‘Alliance’, respectively. Plants were grown in a greenhouse with day/night temperature set at 17/17°C. Plants were fertigated with 15N-2.2P-12.5K fertilizer (Excel 15-5-15, Scotts-Sierra Hort. Prod. Co., Marysville, Ohio) at the concentration of 200 mg·L⁻¹ N. Calcium concentration in the fertilizer solution was 67 mg·L⁻¹.

On 0 and 30 days after planting (DAP), eight plants of each cultivar were harvested and dissected into scales, basal roots, stem roots, stem, and leaves for dry matter measurement and Ca determination. At planting, the bulb bore a shoot that was 5-6 cm long with several young scale-like leaves. We refer to those young leaves on 0 DAP as ‘leaf primordia’. On Day 40, another eight plants were harvested and dry matter was measured to calculate shoot growth rate between 30-40 DAP. Stomatal conductance (an index of leaf transpiration) of the most susceptible leaf U1 (the first leaf beneath the flower buds) was determined on 38 and 44 DAP by a Li-1600 steady state porometer (LI-COR Inc., Lincoln, Nebr.). Ten replications were randomly selected for this measurement. On 104 DAP, those leaves used for stomatal conductance measurement were harvested for Ca determination.

As for calcium analysis, harvested tissues were washed briefly in reverse osmosis water, rinsed in distilled water twice, then dried at 70°C to constant weight in a forced-air oven. Plant tissues were milled to pass a 20-mesh screen, then microwave-assisted digestion of plant tissue was performed according to U.S. Environmental Protection Agency (EPA) Method 3052 modified to deliver a reaction temperature and acid matrix of 170°C and 100% nitric acid, respectively. Tissue (500 mg) was digested in 10 mL of concentrated HNO₃ (trace metal grade) at 300 psi and 170°C for 10 min. in a microwave (model Mars 5, CEM Corp., Mathews, NC). Tissue digests were brought to volume in 100 ml volumetric flasks and filtered (no. 41; Whatman Paper, Maidstone, Kent, UK). Calcium level was determined by an Inductively Coupled Plasma Spectrometry [ICP (IRIS 1000 HR Duo, ThermoElemental, Franklin, MA.)] according to U.S. Environmental Protection Agency (EPA) Method 6010B.

The incidence of ULN occurrence was defined as the percentage of plants that had any level of symptom expression. ULN severity of a single leaf was determined by an index from 0 (healthy) to 5 (severely necrosed), based on symptom progression and
necrosed leaf area (Chang and Miller, 2005). When the severity level was between two index categories, the average was used. Single leaf severity was then summed for all leaves to yield a whole-plant severity. When the whole-plant severity is lower than 5, the symptoms were very light and would not be noticed by consumers.

RESULTS AND DISCUSSION

Within 74 plants examined, no symptoms were found in ‘Sissi’ and ‘Alliance’, indicating they were not susceptible to ULN. Both ‘Star Gazer’ and ‘Acapulco’ were susceptible cultivars, with 95% and 58% of plants showing ULN, respectively (Table 1; Fig. 1). The whole-plant severity of ‘Star Gazer’ was 13, much higher than that of ‘Acapulco’, which was 1.7. These data suggest that ‘Star Gazer’ is more susceptible to ULN than ‘Acapulco’. According to The International Lily Register (Leslie, 1982), ‘Acapulco’ and ‘Sissi’ were bred from the same seed parent - ‘Star Gazer’. However, they do not harbor the same characteristics of ULN susceptibility from ‘Star Gazer’.

Rapid growth rate increases Ca demand, and often makes plants more prone to Ca deficiency (Bangerth, 1979; Collier and Tibbitts, 1984). This relationship has been reported in many crops, such as lettuce (*Lactuca sativa* L.) (Collier and Tibbitts, 1984) and Asiatic hybrid lily (Berghoef, 1986). For ‘Star Gazer’ grown from 16-18 cm bulbs, the susceptible period was 25-50 DAP, when the shoot was in a rapidly-growth phase (Chang and Miller, 2003). In the current study, shoot growth rates in the first month of the four cultivars were between 0.08-0.13 g·day⁻¹ on a dry weight basis. During the most susceptible period to ULN (30-40 DAP), shoot growth rates significantly increased to 0.18-0.19 g·day⁻¹ for ‘Star Gazer’, ‘Acapulco’, and ‘Sissi’; but were unchanged for ‘Alliance’ (Table 1). Since ‘Sissi’ is a non-susceptible cultivar but had a relative high shoot growth rate when compared with the non-susceptible ‘Alliance’, a clear correlation between shoot growth rate and ULN susceptibility between cultivars was not observed.

Low Ca concentration in bulb scales was an internal cause of ULN (Chang and Miller, 2003). Our previous studies in ‘Star Gazer’ lilies demonstrated that bulb scales could supply Ca to the shoots during the early growth stage. However, Ca concentration in scales was low, thus was not able to meet the high demand of rapidly-grown shoot. As a result, the plants became susceptible 25 DAP (Chang and Miller, 2003). Therefore, the Ca budget in the bulbs at planting might affect ULN susceptibility when plants enter the rapid growth phase. In this study, we thus examined the initial Ca status in different organs of both susceptible and resistant cultivars, when bulbs were planted and again on 30 DAP. Since bulb size could affect ULN susceptibilities (Chang, 2002), we used very uniform bulbs, as described in the Material and Methods.

The initial Ca concentrations (on 0 DAP) in scales of four cultivars were all 0.03% DW (Table 2), indicating that this should not be a significant factor influencing cultivar susceptibility to ULN. Since the scale dry matter of ‘Acapulco’ was higher than that of other cultivars, its Ca content in scales (6.3 mg) was higher than others (in the range of 4.8-5.4 mg; Table 2); though it is a susceptible cultivar. The total Ca contents in the bulbs were 7.0 mg for ‘Star Gazer’ and 7.7 mg for ‘Acapulco’, which were higher than that of the resistant cultivars (Table 2). These data suggest that Ca content in the bulbs was not the cause of different susceptibilities within the four cultivars investigated.

Since the susceptible young leaves started to unfold 30 DAP (Chang and Miller, 2005), they were not able to acquire much Ca via transpiration flow before leaf unfolding occurred. The initial Ca concentration in the leaf primordia might be important in the early stage of leaf expansion. The initial leaf Ca concentrations of the susceptible cultivars were lower than that of non-susceptible ones; among them, the most susceptible ‘Star Gazer’ had the lowest, 0.12% DW. The concentration was only about half of that in the non-susceptible ‘Sissi’ and ‘Alliance’ (Table 2). As regards the Ca content in the leaf primordia, the susceptible cultivars had lower content (0.34 mg and 0.38 mg) than that of resistant ones (0.64 mg and 0.45 mg; Table 2). The low initial leaf Ca concentration may play a role in the different ULN susceptibilities between cultivars, though further experimentation is needed to confirm this hypothesis.
Thirty days after planting, when plants became susceptible to ULN, the susceptible ‘Acapulco’ had the highest Ca concentrations in scales, stem roots, and leaves. There were essentially no differences between the other three cultivars (Table 2). This result indicated ULN susceptibility was not directly correlated to absolute leaf Ca concentration. For future investigations, the Ca concentrations in upper leaves and lower leaves should be separately determined in order to illustrate the potential cause of ULN susceptibility between cultivars. ‘Acapulco’ seems to have a better Ca-acquiring mechanism from soil mix by roots and scales. By subtracting the total Ca content on 0 DAP from the Ca content on 30 DAP, net Ca gain per plant from the media in the first month was obtained: 26.7 mg for ‘Star Gazer’, 43.2 mg for ‘Acapulco’, 26.5 mg for ‘Sissi’, and 24.5 mg for ‘Alliance’ (calculated from Table 2). The net Ca gain of ‘Acapulco’ was about 60% more than other three cultivars. This agreed with the observation that ‘Acapulco’ had the highest transpiration rate (measured on 38 and 44 DAP) and highest Ca concentration on leaf U1 on 104 DAP (Tables 1 and 2).

Although high growth rate, low leaf transpiration, low initial Ca concentration in scales, and poor stem root development all have been demonstrated to be factors promoting ULN in ‘Star Gazer’ lilies (Chang, 2002; Chang and Miller, 2003, 2004, 2005), no single characteristic was able to explain differential susceptibilities between the four cultivars investigated. For example, ‘Star Gazer’ had a lower (0-30 DAP) or a similar (30-40 DAP) growth rate at compared with others, yet it is the most susceptible cultivar (Table 1). ‘Acapulco’ had the highest leaf transpiration and the best stem root development on 30 DAP, resulting in the highest net Ca gain per plant for the first month and the highest leaf Ca concentration on 30 DAP (Tables 1 and 2). When planted, ‘Acapulco’ also had the highest Ca reserved in the whole bulb (Table 2), yet it is a susceptible cultivar. The susceptible cultivars may have higher Ca demand, or a less efficient Ca translocating mechanism.

The genetic variation in susceptibility to Ca deficiency disorders seemed to have complicated causes. In poinsettia, attempts were made to compare Ca uptake or translocation between susceptible and non-susceptible cultivars. However, no clear differences were found. In a few instances, Ca uptake was even greater in the susceptible cultivars (Jacques et al., 1990). In the study of susceptibility to BER between tomato cultivars, Ho et al. (1995) concluded BER susceptibility was not correlated to Ca-acquiring ability. Although the Ca-efficient cultivars had higher Ca uptakes than the Ca-inefficient cultivars, some of them were still susceptible to BER. The higher Ca uptake in Ca-efficient plants might be due mainly to greater canopy transpiration and possibly better root absorption of water. However, a more crucial factor was the ability to divert sufficient Ca to the tomato fruits. In this study, ‘Acapulco’ seemed to be more Ca-efficient than other cultivars (Tables 1 and 2), but might be inefficient in translocating Ca to young upper leaves.

In this investigation, the initial Ca concentrations in leaf primordia were low in the susceptible cultivars, ‘Star Gazer’ and ‘Acapulco’. Whether the initial leaf Ca concentration plays a role on the different susceptibilities between cultivars needs further studies.

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Literature Cited


Table 1. Comparisons of susceptibility to upper leaf necrosis, initial scale calcium concentration, shoot growth rate, stomatal conductance, and leaf calcium concentration between four Oriental hybrid lily cultivars.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Necrosis</th>
<th>Initial scale Ca concn (% DW)</th>
<th>Shoot growth rate (g day⁻¹)</th>
<th>Stomatal conductance (mmol m⁻² s⁻¹)</th>
<th>Leaf U₁ Ca concn (% DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incidence (%)</td>
<td>Severity</td>
<td>0 – 30 DAP⁺</td>
<td>30 – 40 DAP⁺</td>
<td>38 DAP</td>
</tr>
<tr>
<td>Star Gazer</td>
<td>94.6***</td>
<td>13.0 a</td>
<td>0.03 a</td>
<td>0.079 c</td>
<td>0.19 a</td>
</tr>
<tr>
<td>Acapulco</td>
<td>58.1***</td>
<td>1.7 b</td>
<td>0.03 a</td>
<td>0.130 a</td>
<td>0.18 a</td>
</tr>
<tr>
<td>Sissi</td>
<td>0 ns</td>
<td>0.0 b</td>
<td>0.03 a</td>
<td>0.101 b</td>
<td>0.18 a</td>
</tr>
<tr>
<td>Alliance</td>
<td>0</td>
<td>0.0 b</td>
<td>0.03 a</td>
<td>0.096 bc</td>
<td>0.09 b</td>
</tr>
</tbody>
</table>

*Means in the column followed by a different letter are significantly different at P ≤ 0.05 by Duncan’s multiple range test.
⁺DAP: days after planting.
⁺⁺⁺⁺ U₁: first leaf beneath flower buds.
ns, *** non significant or significant at P ≤ 0.0001 compared with ‘Alliance’ by chi-square test.
Table 2. Calcium concentration and content in different organs of four Oriental hybrid lilies, 0 and 30 days after planting (DAP).

<table>
<thead>
<tr>
<th></th>
<th>0 DAP</th>
<th>30 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal roots</td>
<td>Scales</td>
</tr>
<tr>
<td>Dry matter (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Star Gazer</td>
<td>0.53 a</td>
<td>15.8 b</td>
</tr>
<tr>
<td>Acapulco</td>
<td>0.33 b</td>
<td>19.0 a</td>
</tr>
<tr>
<td>Sissi</td>
<td>0.38 ab</td>
<td>15.9 b</td>
</tr>
<tr>
<td>Alliance</td>
<td>0.35 ab</td>
<td>16.1 b</td>
</tr>
<tr>
<td>Calcium concn (% DW)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Star Gazer</td>
<td>0.17 b</td>
<td>0.03 a</td>
</tr>
<tr>
<td>Acapulco</td>
<td>0.19 a</td>
<td>0.03 a</td>
</tr>
<tr>
<td>Sissi</td>
<td>0.17 b</td>
<td>0.03 a</td>
</tr>
<tr>
<td>Alliance</td>
<td>0.13 c</td>
<td>0.03 a</td>
</tr>
<tr>
<td>Calcium content (mg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Star Gazer</td>
<td>0.89 a</td>
<td>5.4 b</td>
</tr>
<tr>
<td>Acapulco</td>
<td>0.65 ab</td>
<td>6.3 a</td>
</tr>
<tr>
<td>Sissi</td>
<td>0.63 ab</td>
<td>5.1 b</td>
</tr>
<tr>
<td>Alliance</td>
<td>0.48 b</td>
<td>4.8 b</td>
</tr>
</tbody>
</table>

\(^2\) Means in the column followed by a different letter are significantly different at \(P \leq 0.05\) by Duncan’s multiple range test.
Fig. 1. Different susceptibility to upper leaf necrosis in four Oriental hybrid lily cultivars: (A) ‘Star Gazer’, (B) ‘Acapulco’, (C) ‘Sissi’, and (D) ‘Alliance’. Arrows indicate leaves with upper leaf necrosis.