CORN LILY (*VERATRUM CALIFORNICUM*) RESPONSE TO CALCIUM AND DAY LENGTH

Cheryl A. Parris, Myrtle P. Shock, and Clinton C. Shock, Malheur Experiment Station, Oregon State University, Ontario, OR, 2011

Introduction

Corn lily (*Veratrum californicum*) produces a steroidal alkaloid, cyclopamine, that has shown promise as a precursor for pharmaceuticals to treat cancer. The possible increase in the demand of cyclopamine for cancer research and therapy necessitates that commercial production methods be developed, since field cultivation practices are presently unknown.

During 2010, corn lily plants in growth chamber and irrigation field trials exhibited uncommon leaf symptoms that are not usually seen in their natural habitats. Visual symptoms included yellowing leaf tips that turned brown with subsequent tissue necrosis. In some cases leaf symptoms were followed by plant mortality. New leaf growth failed to expand, causing the leaves to stick together and reducing new growth formation. The plants appeared to exhibit symptoms of nutrient deficiency, specifically calcium deficiency. Another possible interpretation of the symptoms was that the corn lily plants exhibited signs of transplant shock, provoked from the root mass being cut and then replanted. The visual symptoms observed on plants in the trials may have occurred due to the transplanting alone or due to the change in environmental conditions, such as calcium deficiency.

In July 2010, undisturbed native corn lily plants growing in the Manti-La Sal National Forest, Utah, were compared to plants that had been inadvertently dug and replanted in the same proximity during a 2009 fall root harvest. The leaves of the replanted corn lily plants exhibited the same visual leaf yellowing symptoms as seen in the 2010 irrigation and growth chamber trials. Leaf nutrient analysis of plants from disturbed areas showed a reduction in calcium content by 46 percent, decreases in plant top total calcium uptake of 78 percent, and increases in plant top nitrate, copper, manganese, and iron content compared with undisturbed plants (Shock et al. 2011).

Consequently, in 2011, a calcium nutrition response trial was integrated into a growth chamber day-length response trial. Dormant corn lily rhizomes with partial root systems were planted in a lower calcium control or received substantial calcium in the form of gypsum. Three pots in each of three day-length chambers were planted with and without gypsum. The following report focuses on calcium supplementation using gypsum under varying day-length treatments and evaluating the subsequent growth responses of corn lily. Corn lily plant height and stem diameter were evaluated over time. Calcium content of leaves and final root weight were determined.

Materials and Methods

Dormant corn lily root systems were dug from Boulger Canyon, Manti-la Sal National Forest, Utah (N 39° 37.475', W 111° 17.113', 9,401 ft elevation) on September 11, 2010. Roots were dug using a modified nursery root digger, transported on ice, and held in cold storage growth chambers at 0°C (32°F) until healed in new potting mix in 3.7 L (1-gal) plastic bags in November, 2010. The roots in the bags were buried in the field to over-winter and in the spring they were returned to the growth chamber and held at 0°C. Dormant corn lily plants consisted of a bud, rhizome, and roots. The bud is encased in the previous year's shoot tissue, attached at the proximal end of a rhizome. The rhizomes varied in diameter and length. In some instances, most or part of the distal end of the rhizome appeared to be alive, but was soft to touch, without living tissue and was considered dead vegetative material. The root system projects out from the rhizome in various configurations. When the rhizome is growing in the soil, roots radiate out and downwards in all directions from around the rhizome, which after digging is rarely the case for transplanted rhizomes. In most instances, the rhizome has only part of its roots remaining and is positioned in a horizontal manner and the roots form mid-way down from the rhizome's uppermost surface, much like the legs of a centipede. When the rhizome is growing naturally in the soil, roots radiate out 50 to 60 cm (20 to 24 inches) in all directions, but transplanted rhizomes rarely have roots longer than 10 to 12 cm (4 to 5 inches). For this trial, "roots" are considered to be a dormant corn lily plant containing the bud, rhizome and root system.

The roots were washed, weighed, sorted, and selected April 22, 2011 for inclusion in the trial. The roots were washed to remove bulk soil in running tap water. The roots were then rinsed in rain water to remove the salts deposited from the tap water. Roots were drained on newspaper and weighed. Rhizome length was measured and in many cases the length included a dead decomposing end.

Planting occurred on the same day. Potting mix Sunshine LC1complete (Sun Gro Horticulture Canada Ltd.) from the previous year's day-length chamber trial was reused. Each of three chambers contained fifteen 56.6 L (2-ft^3) pots. The potting mix from the 2010 chamber trial from each chamber was dumped on a tarp and mixed to eliminate nutrient composition differences among pots. The calcium response trial reused the pots from 2010. Pot bottoms were lined with one sheet of newspaper to avoid the loss of potting mix and a single newly harvested corn lily stem with roots was planted with the base of the bud's growing point 10 cm (4 inches) below the potting mix surface. Each pot had its own corresponding tray to confine nutrient runoff losses and each pot and tray pair was numbered similarly.

After planting, gypsum was mixed into the top 10 cm of all non-control pots at the rate of 250 g/pot (0.55 lb). Two liters (0.53 gal) of water with a mixture of root growth stimulator and Captan were poured on the top of each potted root. For 20 L (5.25 gal) of water, 25 g (0.88 oz) of Captan (50%) and 258 ml (17.5 tablespoons) of rooting hormone solution (0.0004% indole-3-butyric acid, Root Stimulator, Ferti-Lome, Bonham, TX) were added.

The calcium nutrient trial was arranged with two calcium treatments (with or without calcium), three repetitions (individual plant pots) per day length, and three day-length treatments. In total there were 18 plant pots. Half of the plant pots, those with added calcium, were also part of a larger day-length trial. The growth chamber settings were as follows:

- Treatment 1, chamber 1: 12-hour day (8 am to 8 pm), 14 hours of heat (8 am to 10 pm)

- Treatment 2, chamber 2: 14-hour day (8 am to 10 pm), 14 hours of heat (8 am to 10 pm)
- Treatment 3, chamber 3: 16-hour day (8 am to midnight), 14 hours of heat (8 am to 10 pm)

Day length was controlled with grow lights on timers in chambers 1 and 3. In chamber 2 the 14-hour day-length lights were controlled on the same timing system as the heating.

Temperatures in each chamber were set to 25°C (77°F) for 14 hours, beginning at 8 am, and a temperature of 10°C (50°F) was set for the remaining 10 hours. Heating and cooling were controlled with three timers and three independent systems. During the day there were two electronic temperature controls, one set to cool and the other set to heat. During the night there was one electronic temperature control set to cool to 9°C (48°F).

Each chamber had a datalogger installed to record hourly air and soil temperatures. The datalogger in chamber 1 was installed April 26. The dataloggers in chambers 2 and 3 had been used in previous chamber trials, were evaluated for accuracy and battery strength, and reused starting April 22 for this trial.

Single low tension tensiometers (Irrometer Model LT, Irrometer Co., Riverside, CA) were installed in every plant pot on April 27. Low tension tensiometers have blue porcelain tips that are more sensitive in the wet soil water tension (SWT) range (0 – 30 kPa). The tensiometers were primed and checked for accuracy. Tensiometers were serviced weekly to ensure accuracy.

Plants were watered two times per week in accordance with tensiometer SWT readings. At the outset of the trial irrigation criteria were 7 kPa, receiving 1 l (about 1 quart) of rain water, and 12 kPa, receiving 2 l of rain water. After 60 days the irrigation criteria were changed to 10 kPa and 15 kPa, receiving 1 or 2 l of rain water respectively.

All plants were fertilized at the rate of 55 kg/ha (50 lb/ac) nitrogen in 2 l of rain water on day 15 of the trial. On day twenty five of the trial, 8 l of rain water were leached through all pots because there was some concern as to the accumulation of salts in the soil.

Plant pot locations were randomized within each chamber once per week to reduce the effects of variability of light intensity favoring any particular plant.

Leaf Analysis

All pots were planted with two corn lily plants. One plant was selected and marked to be sacrificed at a height when the leaves were considered fully expanded to analyze for leaf nutrient content. These plants were designated with the pot number and the letter "t" for tissue. The tops of these plants were cut (day 60) and then washed in de-ionized water, dried, and sent to Western Laboratories (Parma, Idaho) for analysis.

After the end of the trial in November 2011, the remaining senesced plant tops from the calcium treatment trial were washed in de-ionized water, dried at 60°C (140°F) for 2 days, and weighed in brown paper bags. Two 10-bag bundles were also dried and weighed to determine average empty bag weight. This weight was used to determine the adjusted plant top weight. The plant tops were sent to Western Labs, Parma, Idaho, for analysis.

Calcium Treatment

This trial was composed of two treatments (with and without added calcium), three day lengths (12, 14, and 16 hours), and three repetitions (individual plant pots). In total there were 18 pots.

- Treatment 1, plus-calcium pots were marked with green flags. Pots in treatment 1 were also part of a larger day-length trial.
- Treatment 2, no-calcium pots excluded the planting step of adding gypsum to the soil in the plant pot. Pots were marked with yellow flags. Pots in treatment 2 were not part of the larger day-length trial.

Each pot had two plants, one that was the "official" plant for growth and one that was harvested for tissue analysis upon reaching full leaf development. The tissue analysis plant side of the pot was labeled with a stick in the pot and "tissue" was written on the exterior below the pot rim. Tissue sample plants were deliberately chosen to be those with smaller root systems that would probably grow into smaller plants.

Potting Media

The calcium content of the potting mix at the start of the 2010 trial contained 100-150 ppm available calcium from manufacturer's addition of limestone. Due to symptoms of tip burn during the 2010 day-length trial, three subsequent applications of 1g $Ca(NO_3)_2$ (calcium nitrate), for a total of 3g $Ca(NO_3)_2$ had been applied to each pot in 2010. The resulting calcium concentration at the start of the 2011 trial was 123 ppm as determined by growth medium analyses (Table 1).

Limestone had been added by the potting medium manufacturer as a buffering agent to counter the low pH found in potting medium that contains a high proportion of peat moss. Limestone was also added by manufacturers as part of a complete nutrient supplement required for plant growth. Finding a pre-mix containing all nutrients except calcium for the 2010-2011 trial was problematic. Due to time constraints when working with easily desiccated plant material and the readily visible deficiency symptoms in 2010, the decision was made to compare the plant results based on the knowledge that there was a base calcium level involved in the check treatment.

The trial began on April 22, 2011.

Table 1. Potting medium analysis indicating the calcium concentration change prior to and during the 2011 calcium response trial. Oregon State University Malheur Experiment Station, Ontario, OR.

Veratrum Native Soil & Chamber Media Comparisons in Trials 2010 & 2011					
Nutrients (ppm)	Average native corn lily soil analysis 2011	Sunshine LC1 start 2010 trial May 2010	End 2010 trial Dec 2010 - start 2011 trial	End trial Dec 2011 no added - calcium pots	End trial Dec 2011 +-calcium pots
pH	6.3	5.5	6.8	6.6	6.6
	ppm				
nitrate	35.9	17	16	15	32
ammonium		9	1	0	0
phosphorus	24.8	22	10	18	15
potassium	304.8	76	80	65	113
calcium	2100	114	123	86	681
sulfur	16.2	107	87	48	891
sodium	32.1	39	55	33	43
iron	135	23.4	2.3	12.5	7.5

Results and Discussion

Plant Growth

In all chamber and calcium treatments, emergence of the corn lily bud began near day five of the experiment (Figure 1). The no-calcium, 16-hour day-length treatment (NC16) plants had a delayed emergence response by 3 days. By day 18, the NC16 treatment plant grew to the same average height of 26.5 cm (2.54 cm = 1 in), as all other treatments except the no-calcium, 14-hour day-length treatment (NC14), which only reached a height of 22 cm. By day 60 the NC16 treatment had the greatest plant height at 56 cm, while the 16-hour calcium (C16) and 12-hour calcium (C12) treatments reached 46 cm and 44.3 cm respectively. The 12-hour no-calcium treatment (NC12) and the 14-hour calcium (C14) treatment plants were both shorter, with heights of approximately 37 cm. Average plant heights are complicated by three plants that bloomed, starting around day 30 of the trial. Plants in bloom grow to a greater height. The plants that bloomed were one each in NC16, C12, and C16.

The stem diameters of the corn lily were not measured until day 11(Fig. 2). As the corn lily bud begins to emerge from the potting media, the swollen base is extremely delicate and vulnerable to tissue damage. It is difficult to distinguish between leaves and stem tissue of emerging corn lily plants. To reduce damage to leaf tissue, the early measurements were withheld. As the stem matures, the outer tissue hardens and a more accurate measurement can be taken without harming the leaf tissue. Through day 15, the NC16 treatment showed a markedly thicker stem diameter, to a maximum 2.7 cm. As the trial progressed, the average stem diameter of all treatments decreased to between 1.3 and 1.7 cm, with NC16 having among the thickest stems and C12 having among the thinnest.

The effect of day-length alone on the height of corn lily plants (Fig. 3) mirrored the average effects seen in the day-length by calcium treatments. The calcium treatments alone (Fig. 4) had little effect on plant height with averages of 41.3 cm for no-calcium and 42.6 cm for calcium supplementation after 60 days.

Observations

The shoot growth was observed and recorded for indications of plant response to calcium or no calcium treatments. The initial change in growth patterns began as a discoloration of the young green leaf to light green, while the older leaves remained green. The second change in leaf growth pattern found was an upward cupping of the light green leaves, where the tip (1 cm) of the leaf curled inward toward the center of the corn lily leaf. The third observed change associated with the same leaves that had exhibited light green color and cupping was the development of burnt-like tissue that was dry and necrotic at the leaf tip, and that over time progressed to more of the leaf.

The expression of the undesirable growth patterns was not specific to treatment, day-length, root weight, or leaf calcium. The symptoms were found on some corn lily leaves at an early stage of development (day 12), and progressed rapidly to necrosis by day 18. Other corn lily plants had early development of leaves with light green upper portions and then did not show the other symptoms until later in the trial (day 25).

Plant-top Calcium Uptake

The greatest total plant-top calcium content was found in NC16, the treatment without supplemental calcium and with the longest day length (Fig. 5). The second highest total plant-top calcium content was in the C16 treatment. The average leaf weight of C16 was less overall.

Root Development

The effect of the NC16 treatment on root weight change from trial start to trial end showed the only positive response (Fig. 6). Root fresh weight desiccation during plant storage following the trial in the plant pots in the growth chamber may be a partial reason for root weight losses.

Sources of Uncertainty

Root fresh weight deviations could have been due to an inconsistent degree of desiccation between measurement dates.

Harvesting corn lily by hand or mechanical means from rocky mountainous soils can reduce the amount of root mass up to 75 percent compared to unharvested native plants. The important root tips and root hairs are eliminated when harvested. The root tip area is where mass flow of water and calcium takes place. The corn lily plant must first replace lost root tips before translocation of calcium will occur. It remains unknown whether considerable transplant shock may be occurring and manifesting itself as nutrient deficiencies.

The examination of light intensity in native corn lily locations was not evaluated, nor was it compared with the light within the growth chambers. If the light intensity in the growth chambers was less than the required amount for sustained corn lily growth, there may be implications that complicate the interpretation of the day-length response data.

Summary

Although there is evidence that the no-added-calcium, 16-hour day-length treatment was most effective, the concern arises that the data may be biased due to over-application of gypsum in the calcium treatments, uneven plant flowering, or other unintended changes in the nutrient balance. Although soil calcium in native corn lily fields is found to be approximately 2100 ppm and the supplemented potting mix contained only 681 ppm, other nutrients also departed substantially from typical conditions: the sulfate concentration was significantly higher in the potting media, 891 ppm, compared to about 16 ppm in native soil. The average available iron in native corn lily soil was 135 ppm, whereas the iron content in the amended potting medium was only 7.5 ppm. The heavy gypsum application may have reduced the growth response by limiting absorption of other nutrients, such as iron, or made available excessive supplies of other nutrients.

Future Trials

1. Study the allocation and distribution of calcium in native plants that have maintained an abundant root system.
2. Study the effect of different calcium treatments and root stimulating hormones to reduce transplant shock.
3. Evaluate alternative light sources, such as LED lighting to maximize light intensity and reduce chamber overheating issues.

References

Shock, C.C., C.A. Parris, S. Monsen, and D. Mann. 2011. Possible key role of calcium for the future cultivation of corn lily (*Veratrum californicum*). Oregon State University Agricultural Experiment Station, Malheur Experiment Station Annual Report 2010, Ext/CrS 132:208-215.

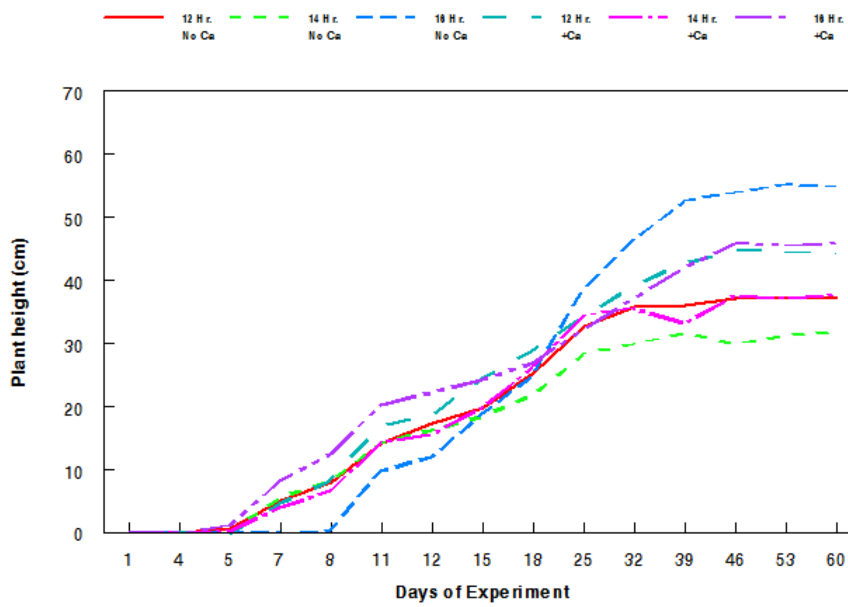

Figure 1. Plant height over time of corn lily (Veratrum californicum) *in response to three day-length and two calcium treatments (2.54 cm = 1 in). The corn lily showed a delayed emergence response in the no-calcium 16-hour day-length treatment compared to all other treatments. After recovery, the same no-calcium 16-hour day-length treatment plant height surpassed all others, Oregon State University Malheur Experiment Station, Ontario, OR.*

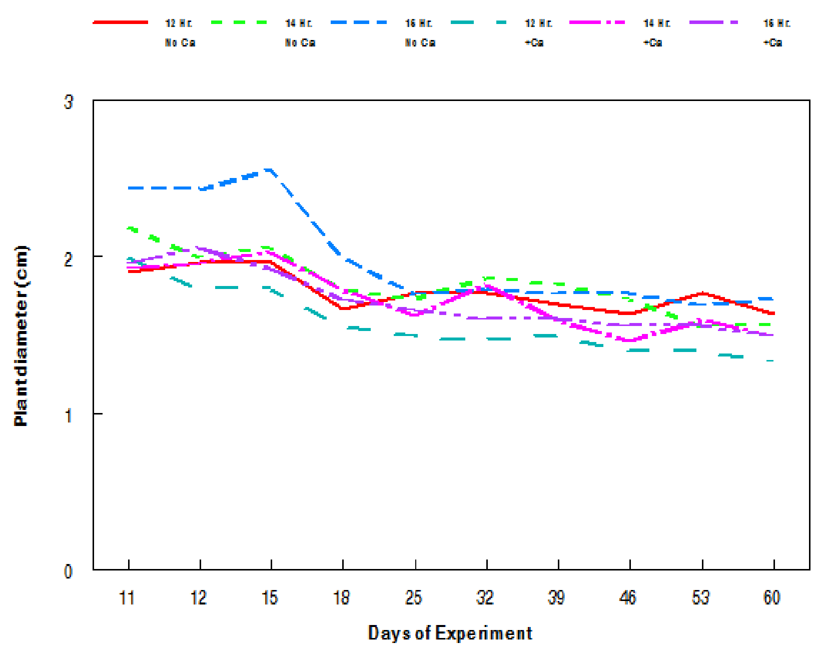

Figure 2. Plant stem diameter over time of corn lily (Veratrum californicum) *in response to three day-length and two calcium treatments (2.54 cm = 1 in) Oregon State University Malheur Experiment Station, Ontario, OR.*

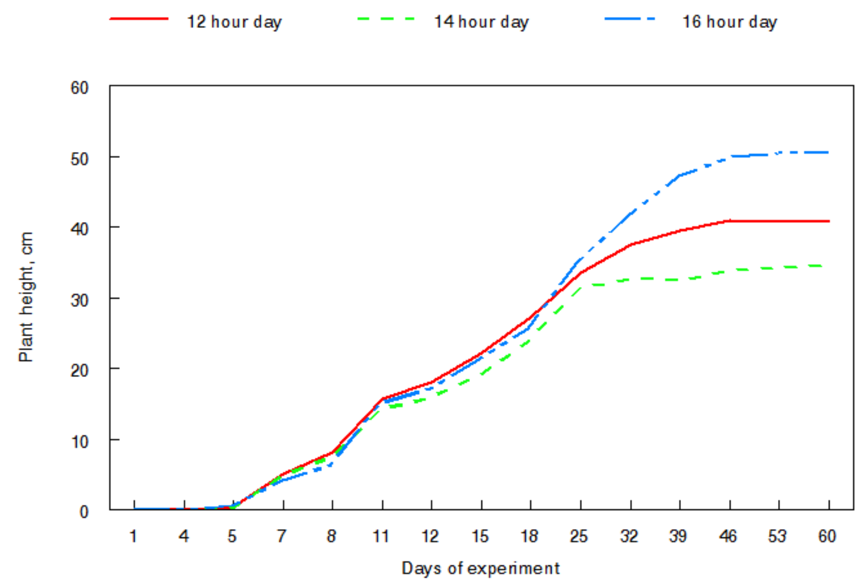

Figure 3. Plant height of corn lily (Veratrum californicum) *over time in response to three day-length treatments (2.54 cm = 1 in). The plants in all day-length trials grew rapidly through the 25th day, after which time the increases in plant height slowed. Plants grown with 16-hour day length were taller, followed by the 12-hour and 14-hour day-length treatments, Oregon State University Malheur Experiment Station, Ontario, OR.*

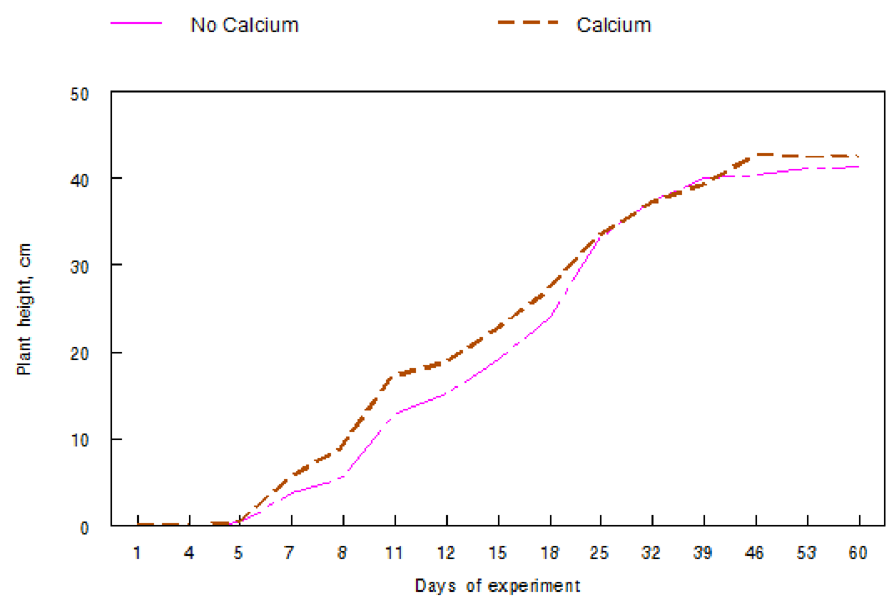

Figure 4. Average plant height growth response of corn lily (Veratrum californicum) *to supplemental calcium, over time. Reasons for the minimal effect of supplemental calcium may be due to the over-application of gypsum or nutrient imbalances or deficiencies inadvertently created, Oregon State University Malheur Experiment Station, Ontario, OR.*

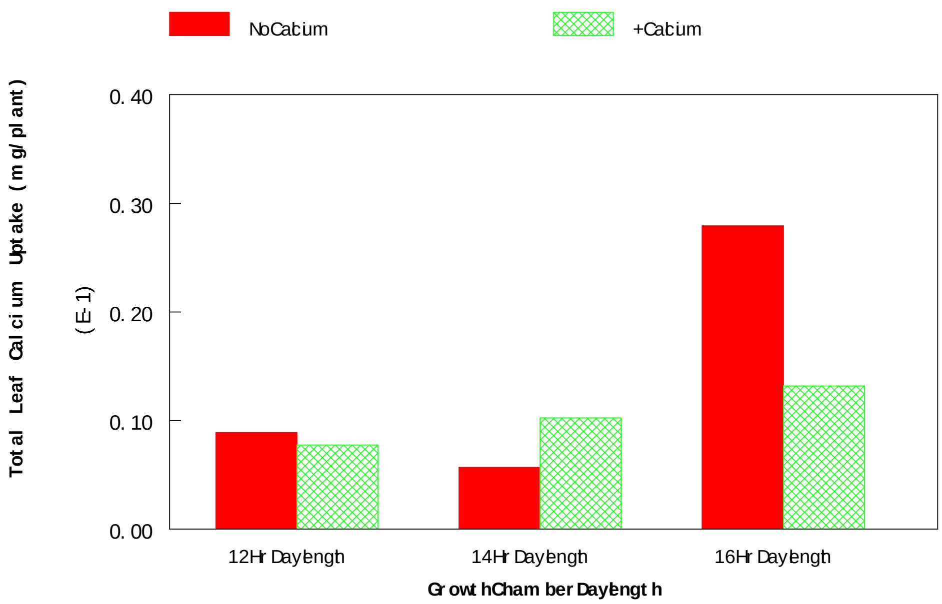

Figure 5.Total plant-top calcium content of corn lily (Veratrum californicum) *in response to added gypsum and three day-length treatments. Plants grown with no supplemental gypsum in 16-hour day lengths had the greatest plant-top calcium content of all treatments, Oregon State University Malheur Experiment Station, Ontario, OR.*

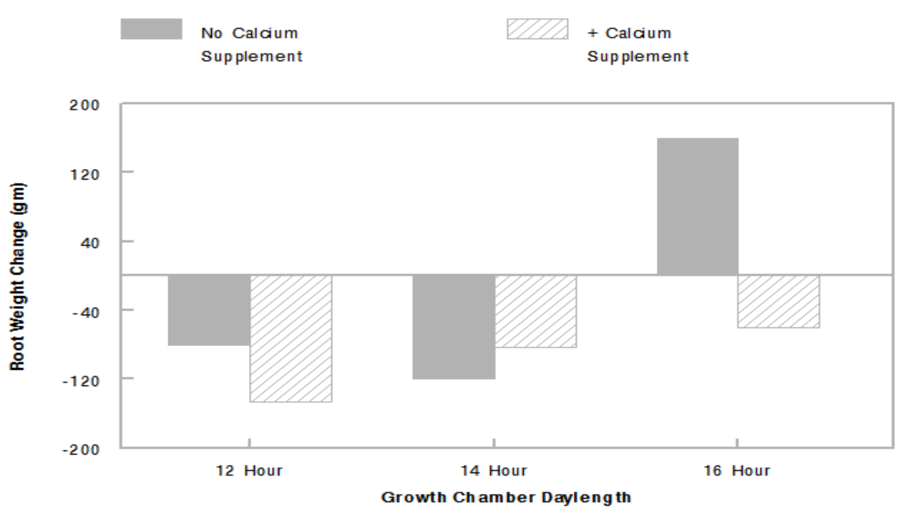

Figure 6. Root fresh weight of corn lily (Veratrum californicum) *response to supplemental calcium and three day-length treatments. The roots were weighed at trial start in April 2011 and again at trial end, November 2011 with the change noted above. Positive fresh weight development occurred in the plants grown at 16-hour day length with no gypsum supplement. The calcium treatment may have exhibited over-supplementation of calcium and nutrient imbalances. Root desiccation during plant storage in the growth chamber may have been a contributing factor in root weight losses, Oregon State University Malheur Experiment Station, Ontario, OR.*