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Royal Catchfly (Silene regia) Growth and Floral Development in Response to Fertilizer and Photo Period

Amanda Wildenberg

Royal Catchfly (*Silene regia*) growth and floral development in response to fertilizer and photoperiod

by

Amanda Wildenberg

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ABSTRACT

Royal Catchfly, *Silene regia*, is a prairie forb that is endangered in multiple states. More information on the plant's development and propagation is essential to successful recovery programs. Little is known about *S. regia's* response to fertilizer or how floral initiation is triggered. Within Caryophyllaceae floral initiation often is linked to photoperiod. The objectives of my study were to investigate how fertilizer affects growth of *S. regia* to transplant stage and to determine if flowering of *S. regia* is initiated by photoperiod or developmental age. For fertilizer, treatments consisted of a control, a 20-20-20 liquid fertilizer solution at 1.25 g/L applied weekly, and an Osmocote 14-14-14 four-month slow release solid fertilizer at 14.6 g/L. Various shoot and root parameters were measured. For flowering, treatments of either long day photoperiod (16 hours light/8 hours dark) or short day photoperiod (8 hours light/16 hours dark). Basal rosette leaf number, node number on elongated stems, axillary stem number, and flower bud number were recorded weekly. Overall, fertilizer increased growth relative to the control for shoot and root parameters. Solid fertilizer was better than weekly liquid at producing healthy shoots, but had no difference in root parameters. For long day photoperiod plants, 38.5% had elongated stems, but for short day photoperiod plants, 0% had elongated stems. Short day plants were smaller than long day plants but by week 16 were equal or greater in size, yet still no plants flowered. Plants grew axillary stems and 19.4 ± 8.8 flower buds. Solid fertilizer mixed into the soil was the best for growth. A long day photoperiod triggered floral initiation in *S. regia*. Future studies should use this information to increase survival in transplants planted into natural areas.

INTRODUCTION

Silene regia (Royal Catchfly) is a perennial prairie forb in Caryophyllaceae. The plant's early growth habit is a leafy rosette that produces an elongated stalk between 60-66 cm tall when flowering (Gerlica and Parsons 2006). Its red flowers are pollinated by ruby-throated hummingbirds, some butterflies, and can also self-pollinate. *Silene regia* prefers well-drained soil in open woods, woodland degrade, or prairies. The plant is distributed throughout the southeastern United States and is threatened or rare in Arkansas, Georgia, and Indiana, and endangered in Tennessee, Kentucky, and Illinois (Herkert and Ebinger 2002, Kartesz 2011).

One reason for *Silene regia's* endangered status in Illinois is the destruction of its habitat. The prairie ecosystem has been exploited for agriculture and prairies are now rare habitats in the United States (Noss *et al.* 1995). The fragmentation of prairie habitat is associated with a decrease in pollinator populations which leads to decreased genetic variation for *S. regia*, along with lower germination and seedling establishment rates (Dolan 1994, Menges 1991). The lack of habitat has lead *S. regia* to colonize opportunistically in Illinois, attempting to establish in less than ideal environments (Ketzner *et al.*1989). Recovery programs, which seek to establish a species in a natural area through the use of transplants grown in greenhouses, are needed to maintain this species.

Seedlings used in recovery programs often are grown from seed that might be dormant. The use of fire improved *S. regia*'s reproductive success and cold stratification increased seed germination rates (Flocca *et al.* 2004, Menges 1995, Menges and Dolan 1998). Recovery programs can have a low success rate due to lack of knowledge regarding reintroduction conditions for each species and lack of monitoring after transplanting (Godefroid *et al.* 2011). A focus on how cultural or environmental aspects affect growth and development of *S. regia* might provide insight into protocols to improve germination, flowering, or successful transition from greenhouse to natural areas.

When cultured in greenhouses plants often require certain minerals or nutrients to grow. The most commonly applied nutrients in fertilizers are nitrogen, phosphorus, and potassium which may be formulated to apply as a liquid or a solid (Boodley 1998, Dole and Wilkins 2005). Deficiencies in these minerals can lead to stunting of growth, necrosis, and leaf discoloration (Dole and Wilkins 2005). Fertilizing plants allows them to grow faster and healthier; however, different species grow best with different concentrations of fertilizer (Hartman *et al.* 1998). Liquid fertilizer quickly releases all nutrients as soon as it is applied. Solid fertilizer may be controlled or slow release which allows for less nutrient loss and leaching as nutrients become available more slowly (Dole and Wilkins 2005, Oertli 1980). Controlled release fertilizers also may be beneficial for transplants in a recovery program as the fertilizer will remain in the soilless mix of the transplant and continue to be available slowly in the field. This continued supply of fertilizer might provide the plant more time to adjust to the new nutrient levels in the prairie soil. Most studies reporting how fertilizer affects plant growth focus on horticultural species which often continue to be fertilized after they are transplanted into garden situations. Studies concerning the success of fertilization of native species in the greenhouse and subsequent transplants to natural areas where no additional fertilizer is provided are absent in the literature. *Silene nurtans* show differing nutrient uptake depending on soil composition and nutrient level (De Bilde 1978). This species will most likely need to adapt to the changes in the soil and nutrients when from greenhouses transplanted to natural areas.

Another aspect of *Silene regia* that would provide useful information for recovery programs is a better understanding of its floral development. Floral initiation is an essential aspect of plant reproduction, as more flowers will be capable of producing more seeds. Initiation commonly is triggered by three possible factors, including vernalization, photoperiod, and developmental age, although internal factors are not fully understood (Dole and Wilkins 2005, Taiz and Zeiger 2006). Vernalization occurs when floral initiation is induced by cool

temperatures (Dole and Wilkins 2005). Photoperiod response occurs when exposure to a specific photoperiod triggers floral initiation, although the critical factor is the exposure to certain hours of darkness. If a short day plant is exposed to even a flash of light during its darkness hours, flowers will not develop (Boodley 1998, Taiz and Zeiger 2006). Temperature affects the number and gender of flowers in *Silene noctiflora*, but not the actual initiation of the process (Folke and Delph 1997). Multiple species within Caryophyllaceae show floral initiation as a response to photoperiod, including carnations and *Cerastium regelii* (Harris 1968, Heide and Pedersen 1990). A field study determined that light was the limiting factor in reproductive growth in *S. regia* (Ketzner *et al.* 1989). Floral initiation might be either a response to reaching a certain developmental stage, or a photoperiod response under constant temperature in greenhouse situations (pers. obs.) How floral initiation is triggered in *Silene regia* is unknown.

Additional research concerning healthy transplants of native species for recovery programs in natural areas is needed, including fertilizer and floral initiation to increase population stability. The objectives of my study were to understand how growth and development of *S. regia* were affected by liquid and solid fertilizers for production of transplants to use in recovery programs for natural areas, and how floral initiation was affected by photoperiod (long day, short day or day neutral). This information will be useful for developing the best strategies for successful recovery programs.

METHODS

Cultural Conditions

All *Silene regia* seeds were cold moist stratified (Baskin and Baskin 1998, Black *et al.* 2006) by placing about 300 seeds into filter paper folded into packets which then were wrapped

in cheesecloth and soaked in a Bonide® Captan (50% wettable powder) fungicide solution (4.93 g/L) for one minute to prevent fungal growth. Packets were placed in a plastic container (Rubbermaid® 25 x 15 x 35 cm) and were covered with a moistened sand/sphagnum mix (70:30 % by mass). Seeds were kept at 4-6 °C in a laboratory refrigerator (Fisher Scientific Isotemp, Pittsburgh, Pennsylvania) for 2 weeks.

After stratification, 2-3 seeds were planted into individual Cone-tainers™ (Stuewe & Sons, Inc., Corvallis, Oregon) that were 4 cm wide x 20 cm deep with removable sleeves. The planting consisted of High Porosity SB 500 soilless mix (Sun Gro Horticultural Distribution Inc., Bellevue, Washington) composed of composted pine bark, Canadian sphagnum peat moss, vermiculite, perlite, dolomitic limestone, gypsum, and wetting agent. All Cone-tainers™ were kept in a Conviron® growth chamber (Conviron CMP 4030, Winnipeg, Manitoba, Canada) at 25 ± 0 °C and watered as needed.

Parameters Measured in Fertilizer Study

Rosette diameter and leaf number of each plant were measured weekly for 8 weeks starting when fertilizer treatments began, 2 weeks after planting. Crown diameter was measured using a Digimatic IP65 micrometer (Mitutoyo Corporation, Japan). Leaf area was measured by removing the leaves and using a leaf area meter (LI 3100 leaf area meter, Li-Cor, Inc., Lincoln, Nebraska). The number of dead leaves and number of side rosettes were recorded. The fresh mass of each shoot was recorded using an analytical balance. Each plant was given a root score of 1-3, where 1 had very little to no visible roots, 2 had visible roots, and 3 had numerous roots, often with a large central root and/or root growth around the Cone-tainer™ insert (Figure 1). Roots then were soaked in water and physically manipulated to remove as much excess soilless mix as possible. Root length and fresh mass were taken.

Then shoots and roots were dried in an oven (Despatch, Minneapolis, Minnesota) at 100 °C for 48 hours. Dry masses of shoots and roots were measured.

Fertilizer Study

Silene regia seeds were collected in fall 2010 from Minonk in Woodford County Illinois by Janice Coons. Plants were exposed to a photoperiod of 16 hours light/8 hours dark at an intensity of 295 μmol/m^2/sec measured by an Apogee® quantum meter (Logan, Utah). Plants were sorted after 2 weeks into 30 replicates for each of the three treatments: a control with no fertilizer, a liquid fertilizer (20-20-20 Peter's solution at 250 ppm) applied weekly, and a solid fertilizer, 14.6 g Osmocote® 14-14-14 four-month slow release solid fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) that was evenly mixed into each liter of soilless mix at planting. Each replicate was a single plant in a Cone-tainer™. The liquid fertilizer started 2 weeks after seeds were planted. Plants were harvested after 9 weeks.

Parameters Measured in Photoperiod Study

The crown diameter of each plant was measured using the Digimatic IP65 micrometer (Mitutoyo Corporation, Japan) at 9 weeks after planting and using a ruler at 24 weeks. Basal rosette leaf number was recorded weekly, and when elongated flower stems appeared, number of nodes, number of axillary stems, and number of flower buds also were recorded. If a plant grew more than one elongated stem they were measured under the same parameters.

Photoperiod Study

Seeds were obtained from Prairie Moon Nursery (Winona, Minnesota) in August 2010. For this study, seeds were stratified for 2 weeks and planted using Cone-tainers™ containing a soilless mix with Osmocote® 14-14-14 four-month slow release solid fertilizer mixed at 14.6 g/L. Cone-tainers™ were placed in separate but identical Conviron growth chambers. One chamber

had a long day photoperiod with 16 hours light/8 hours dark at an intensity of 185 μmol/m^2/sec, and the other chamber had a short day photoperiod with 8 hours light/16 hours dark at an intensity of 368 μmol/m^2/sec. The light intensity was higher in the short day chamber to attempt to provide similar total amounts of radiation.

At 9 weeks after planting, plants were sorted visually by size, choosing the largest 36 plants from each photoperiod. Each set of 36 plants was divided into three replicates of twelve plants each to allow for replication in comparisons for percentage of plants that develop elongated stems. These plants were transplanted into 2.83 L Treepots™ (TPOT1 Stuewe & Sons, Inc., Corvallis, Oregon) with high porosity soilless mix and Osmocote® 14-14-14 four-month slow release solid fertilizer at 3.65 g/L soilless mix. Twenty-three weeks after planting, the photoperiod for short day plants was changed to a long day photoperiod.

Statistics

Means and standard errors of all data were calculated and analyzed using a univariate one way analysis of variance ANOVA. A Duncan's multiple range test was used for the fertilizer studies. Arcsine transformation was used to stabilize the percentages and then analyzed using a Student's t-test.

All statistics were performed with SPSS software (Version 17), and an x-value set at 0.05.

RESULTS

Fertilizer Study

In the solid and liquid fertilizer study, differences occurred between all treatments for number of leaves and rosette diameter (Table 1). Plants receiving the solid fertilizer treatment were greater than both the weekly liquid fertilizer and the control for all shoot measurements (Table 1). Also, plants receiving weekly fertilizer were greater than the control for all shoot measurements, except dead leaves (Table 1). For all root measurements, except root length, the control plants were lower than the two fertilizers, but the two fertilizers were not similar (Table 2). For root length, plants in the control and weekly liquid fertilizer treatment were different from each other, but the solid fertilizer was not different from either treatment (Table 2). Both number of leaves and rosette diameter increased almost linearly, and the plants in the solid fertilizer treatment started at a greater number when counts began (Figure 2, Figure 3).

Photoperiod Study

The percentage (38.5 ± 1.7) of elongated stems for plants in the long day photoperiod was greater than the percentage (0.0 ± 0.0) of elongated stems for plants in the short day photoperiod after 23 weeks ($t = 23.1$, $df = 4$, $p < 0.0001$). Plants in the long day treatment had more basal leaves than those in short days at 9 weeks from planting ($f = 23.7$, $df = 1$, $p < 0.0001$), but after 21 weeks, the two groups had no difference in number of basal leaves ($f = 0.98$, $df = 1$, $p = 0.33$; Figure 4). Plants in the long day photoperiod had a larger crown diameter than short day at 9 weeks after planting, but short day plants had a larger crown diameter than those in the long day photoperiod at 23 weeks (Table 3). Ten of the fourteen plants that grew elongated stems started within the first 9 weeks after planting, with the last 4 starting over the next 5 weeks (Figure 5). When photoperiod of short day plants was changed to long day they began to grow elongated stems within the first week with 36.1% of plants growing elongated stems after 11 weeks from change to long day (Figure 6). Once a stem began to elongate,

nodes developed in a fairly linear pattern with one every week, and leveling around 16 nodes at week 11 (Figure 7).

Some zero values occur in the number of axillary stems and flower buds since not all plants had flowered by the end of the study. Continued observation of the plants revealed that almost all the plants with elongated stems did eventually flower (Table 4). A negative correlation occurred between the weeks until the first flower and the total number of flowers and the weeks from stem initiation to the first flower and total number of flowers ($p = 0.006$; Table 5). A positive correlation occurred between total number of axillary stems and total number of flower buds ($p = 0.0002$; Table 5).

DISCUSSION

Fertilizer Study

Silene regia grew consistently better with fertilizer than without fertilizer. This response agrees with previous information showing that fertilizer aids plant growth (Boodley 1998, Dole and Wilkins 2005, Hartman *et al.* 1998). Among the fertilizer treatments, the solid slow release fertilizer was the best for shoot growth, but the same as weekly liquid for root growth. The increased growth is consistent with findings stating that solid fertilizer retains nutrients in the soil over time with less leaching (Dole and Wilkins 2005). The shoot and root discrepancy may be caused by the limitations of the Cone-tainers® in which the plants grew. The Cone-tainers® are 164 mL, and the plants became root bound as they grew larger. Many of the solid and some liquid fertilizer plants had roots wrapped around the Cone-tainer® insert, supporting the observation that their growth became limited by container size. The solid treatment had a greater number of leaves and rosette diameter at the start of counts because it had been

receiving fertilizer since planting, while the other treatments had not. The high number of dead leaves in the solid fertilizer treatment is likely due to over-shading, so as the leaf number increased, the older leaves were not able to survive or just abscised naturally.

Future studies will plant *S. regia* into natural areas using transplants grown with each of these three fertilizer treatments and a control to see how fertilizer affects the success rate of transplants. I suggest that transplants grown with solid fertilizer may establish better than those grown with liquid or no fertilizer. This response is hypothesized for various reasons, including that the solid fertilizer allows the plants to grow faster than liquid during transplant growth. Because solid fertilizer is in the soil from the start but liquid was not added until the plants were about two weeks old, the plants in the solid fertilizer treatment had a two week head start, and more growth by the end of the study. This increased growth means that plants can be transplanted quicker so requiring less time in greenhouses. The plants may also be sturdier and healthier when transplanted. The solid fertilizer in the soil also will be transplanted with *S. regia*, allowing the plant to continue to wean itself slowly from the fertilizer rather than stop abruptly as with no additional liquid fertilizer after transplanting.

Photoperiod Study

Floral initiation in *Silene regia* was triggered by a long day photoperiod as demonstrated by the long day photoperiod grown plants and the change from short day to long day photoperiod. The percentage of elongated shoots being less than 100% for the plants suggests that other factors are involved between the plant receiving the stimulus and actually starting to elongate. *S. regia* had a long flowering period, lasting the entire summer, indicating that the results of this study are not uncommon (Ketzner *et al.* 1989). Long day photoperiod also is the trigger for carnations, another species in Caryophyllaceae (Harris 1968).

The size difference between the long day and short day plants at the beginning of the study could be due to total light exposure. While I tried to equalize the radiation, the growth chambers experienced a problem that lowered the light intensity of both groups. This reduction was corrected as quickly as possible, and the short day plants began to catch up with the long day, but this problem could have caused the initial discrepancy. Despite these problems, the short day plants eventually reached and exceeded the developmental age (based on basal leaf number and crown diameter) of the long day plants, with no stem elongation. Hence, developmental age was not the trigger for stem elongation and flower production.

Plants quickly developed stems after exposure to the long day photoperiod, with most of the stems starting between 8-9 weeks after planting. This response indicates that early perception of the stimulus when the plant is still very young is possible. The plateau in node growth corresponded with a start of flowering approximately 2 weeks later. Most likely resources were diverted to flower production. The negative correlations between total number of flowers and weeks till the first flower shows that the faster the plant begins to produce flowers, the more it is capable of generating. Positive correlations between the number of axillary stems and the number of flower buds is because the flowers mainly grow from the axillary stems.

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Table 1. Shoot growth parameters (mean ± standard error) for *Silene regia* after 9 weeks with various fertilizer treatments. Means within an experiment and a column followed by different letters are significantly different based on Duncan's multiple range test at P=0.05 level.

Treatment[a]	Crown Diameter (mm)	Side Rosettes	Dead Leaves	Leaf Area (cm^2)	Fresh Shoot Mass (g)	Dry Shoot Mass (g)	Rosette Diameter (cm)	Number of Leaves
Control	1.02 ± 0.07[c]	0.0 ± 0.0[c]	0.2 ± 0.1[b]	3.00 ± 0.49[c]	0.11 ± 0.02[c]	0.014 ± 0.004[c]	2.5 ± 0.1[c]	8.2 ± 0.5[c]
Wk Lqd	2.75 ± 0.18[b]	1.5 ± 0.3[b]	0.6 ± 0.1[b]	47.63 ± 3.74[b]	1.95 ± 0.16[b]	0.349 ± 0.032[b]	7.3 ± 0.3[b]	16.4 ± 1.0[b]
Solid	3.60 ± 0.24[a]	3.3 ± 0.4[a]	1.6 ± 0.3[a]	71.05 ± 7.28[a]	3.17 ± 0.32[a]	0.543 ± 0.058[a]	9.2 ± 0.4[a]	25.2 ± 1.8[a]

[a] Fertilizer Treatment: Control- no fertilizer; Bi-Wk Lqd- 20-20-20 Peter's solution applied bi-weekly; Wk Lqd- 20-20-20 Peter's solution applied weekly; Solid- Osmocote® 14-14-14 four-month slow release solid fertilizer applied in soil

Table 2. Root growth parameters (means ± standard errors) for *Silene regia* after 9 weeks with various fertilizer treatments. Means within an experiment and a column followed by different letters are different based on Duncan's multiple range test at P=0.05 level.

Treatment[a]	Root Score[b]	Root Length (cm)	Fresh Root Mass (g)	Dry Root Mass (g)
Control	1.2 ± 0.1[b]	16.92 ± 0.73[b]	0.84 ± 0.19[b]	0.097 ± 0.021[b]
Wk Lqd	2.6 ± 0.1[a]	18.94 ± 0.34[a]	5.25 ± 0.70[a]	0.737 ± 0.105[a]
Solid	2.7 ± 0.1[a]	17.69 ± 0.51[ab]	6.67 ± 0.96[a]	0.900 ± 0.118[a]

[a]Fertilizer treatments: Control- no fertilizer; Bi-Wk Lqd- 20-20-20 Peter's solution applied bi-weekly; Wk Lqd- 20-20-20 Peter's solution applied weekly; Solid- Osmocote® 14-14-14 four-month slow release solid fertilizer applied in soil

[b]Scale of 1-3: 1 had very little to no visible roots, 2 had visible roots, and 3 had extreme roots, often with a large central root and/or root growth around the Cone-tainer™ insert

Table 3. Crown diameter (mean ± SE) for *Silene regia* grown in either long day or short day photoperiod. Means that within the same week different letters are different based on Duncan's multiple range test at P=0.05 level

Treatment	Weeks after Planting	Mean ± Standard Error
Long Day	9	4.6 ± 0.2[a]
Short Day	9	2.6 ± 0.1[b]
Long Day	23	8.1 ± 0.3[b]
Short Day	23	9.3 ± 0.3[a]

Table 4. Flowering characteristics for *Silene regia* grown in long day photoperiod at 23 weeks after planting.

Characteristic	Mean ± SE	Range
Number Nodes on Elongated Stems	26.9 ± 4.2	16-78
Number Flower Buds	19.4 ± 8.8	0-114
Number Axillary Stems	7.1 ± 2.1	0-28
Number Basal Leaves	28.0 ± 3.9	6-56
1st Node with Axillary Stem	8.9 ± 1.2	4-17
% Nodes with Axillary Stems	31.2 ± 9.1	0-122
Weeks after Planting with 1st Elongated Stem	9.9 ± 0.5	9-15
Weeks after Planting with 1st Flower Bud	22.4 ± 1.1	17-25
Number of Elongated Stems	1.7 ± 0.3	1-4
Weeks from 1st Elongated Stem to 1st Bud	13.0 ± 1.1	8-16

Table 5. Correlations (r-value) between flowering characteristics in *Silene regia* grown in a long day photoperiod at 23 weeks after planting.

	Total Nodes	Total Flower Buds	Total Basal Leaves	Total Axillary Stems	1st Node with Axillary Stem	% Nodes with Axillary Stems	Week with 1st Elongated Stem	Week with 1st Flower Bud	Total Elongated Stems
Total Nodes									
Total Flower Buds	-0.19								
Total Basal Leaves	0.02	-0.09							
Total Axillary Stems	-0.19	0.69	-0.02						
1st Node with Axillary Stem	-0.13	0.14	-0.07	0.30					
% Nodes with Axillary Stems	-0.25	0.74	-0.03	0.99	0.24				
Week with 1st Elongated Stem	-0.20	-0.21	0.18	-0.25	-0.28	-0.21			
Week with 1st Flower Bud	0.20	-0.92	0.40	-0.78	-0.28	-0.80	0.10		
Total Elongated Stems	-0.15	0.10	0.23	0.47	-0.06	0.46	-0.14	-0.19	
Weeks from 1st Elongated Stem to 1st Flower Bud	0.25	-0.88	0.41	-0.75	-0.39	-0.79	-0.18	0.96	-0.07

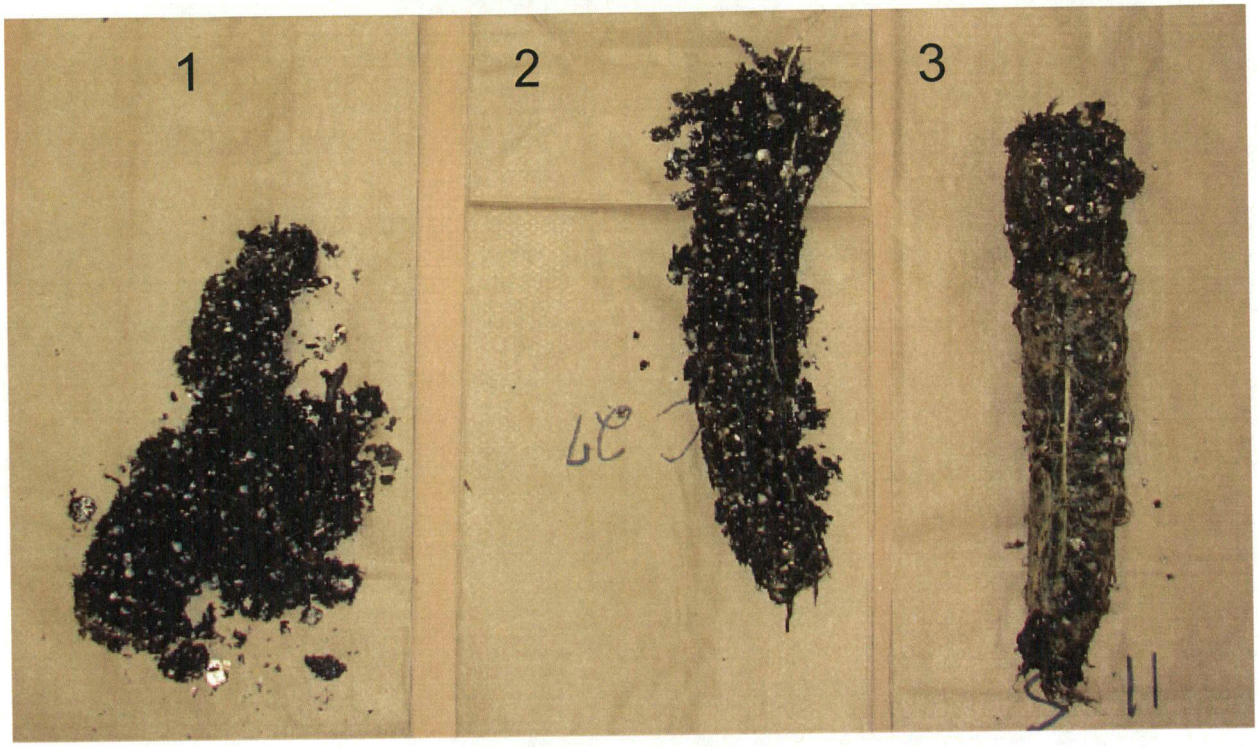

Figure 1. Representations of root scores for *Silene regia* after 9 weeks of fertilizer treatments: 1- very little to no visible roots, 2- visible roots, 3- numerous roots, often with a large central root and/or root growth around the Cone-tainer™ insert.

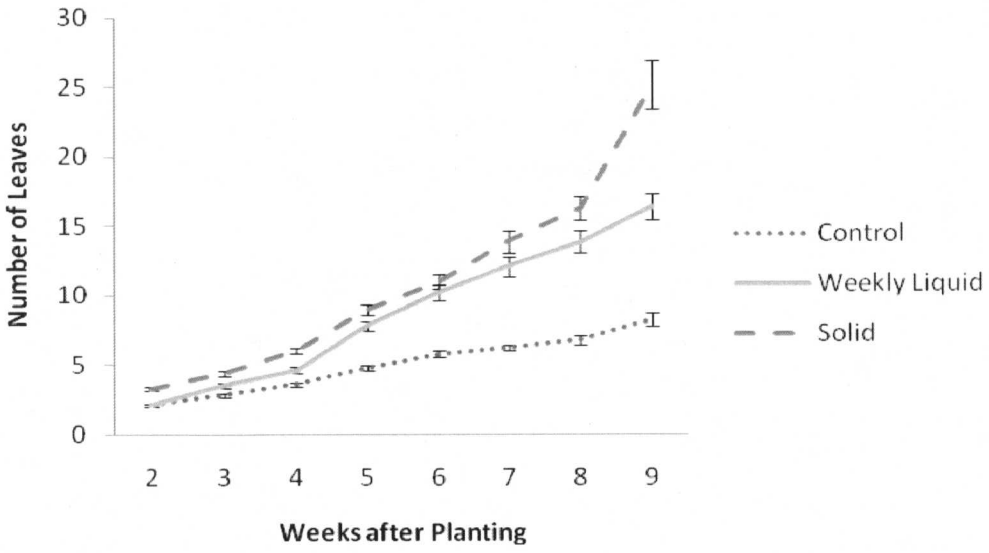

Figure 2. Number of leaves (mean ± SE) as a function of time in *Silene regia* given either Peter's 20-20-20 liquid, Osmocote™ 14-14-14 solid, or no fertilizer. Weekly counts began with the first liquid fertilizer treatment when plants were 2 weeks old.

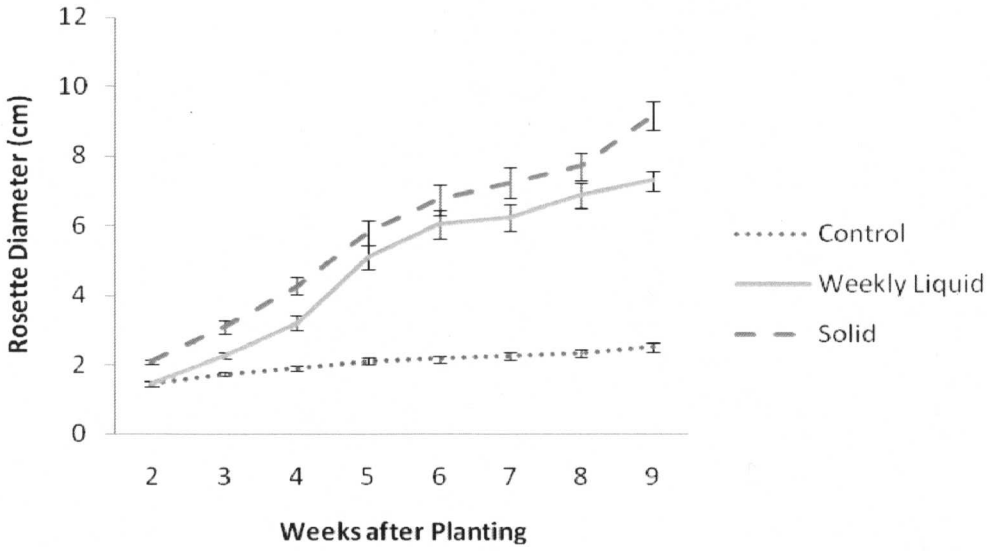

Figure 3. Rosette diameter (mean ± SE) as a function of time in *Silene regia* given either Peter's 20-20-20 liquid, Osmocote™ 14-14-14 solid, or no fertilizer. Weekly counts began with the first liquid fertilizer treatment when plants were 2 weeks old.

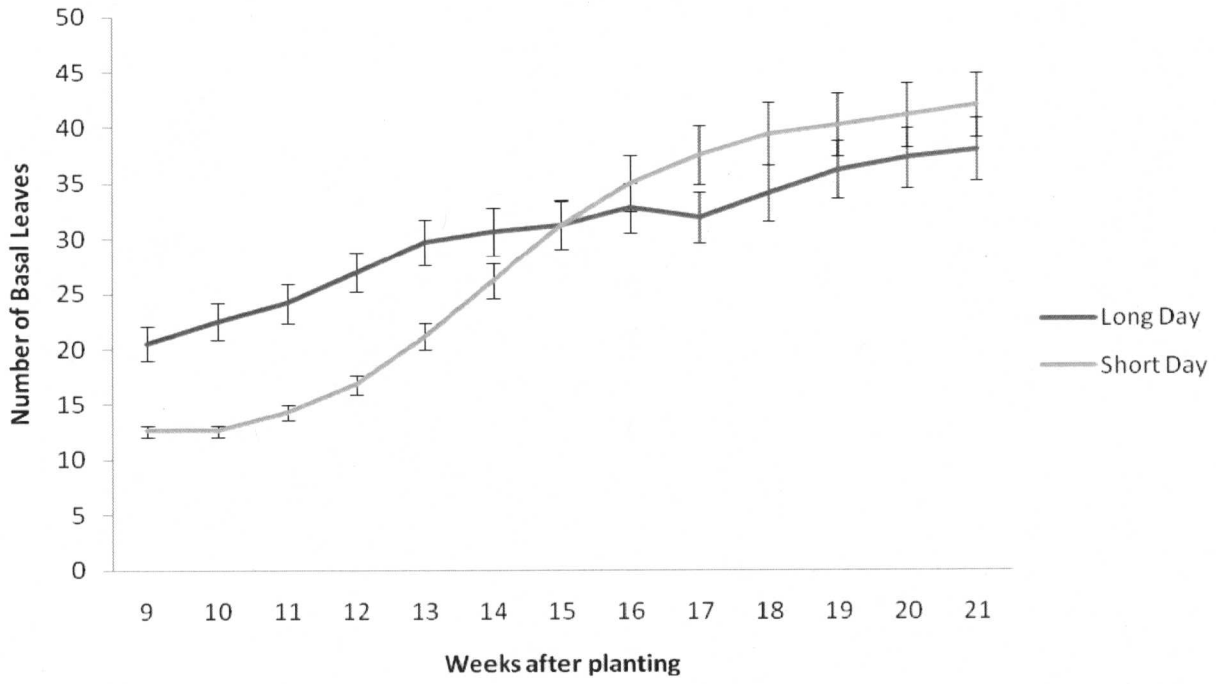

Figure 4. Number of basal leaves (mean ± SE) as a function of time in *Silene regia* plants grown with either a short day or long day photoperiod. Weekly counts began 9 weeks after planting seeds.

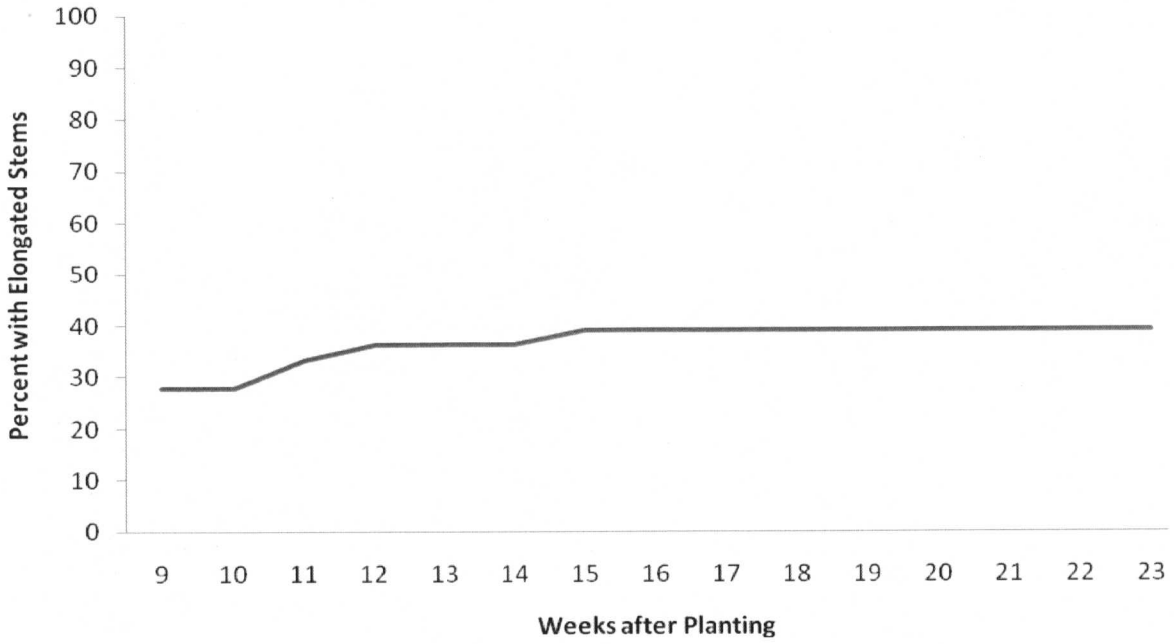

Figure 5. Percentage of plants with elongated stems as a function of time in long day photoperiod. Weekly counts began 9 weeks after planting seeds.

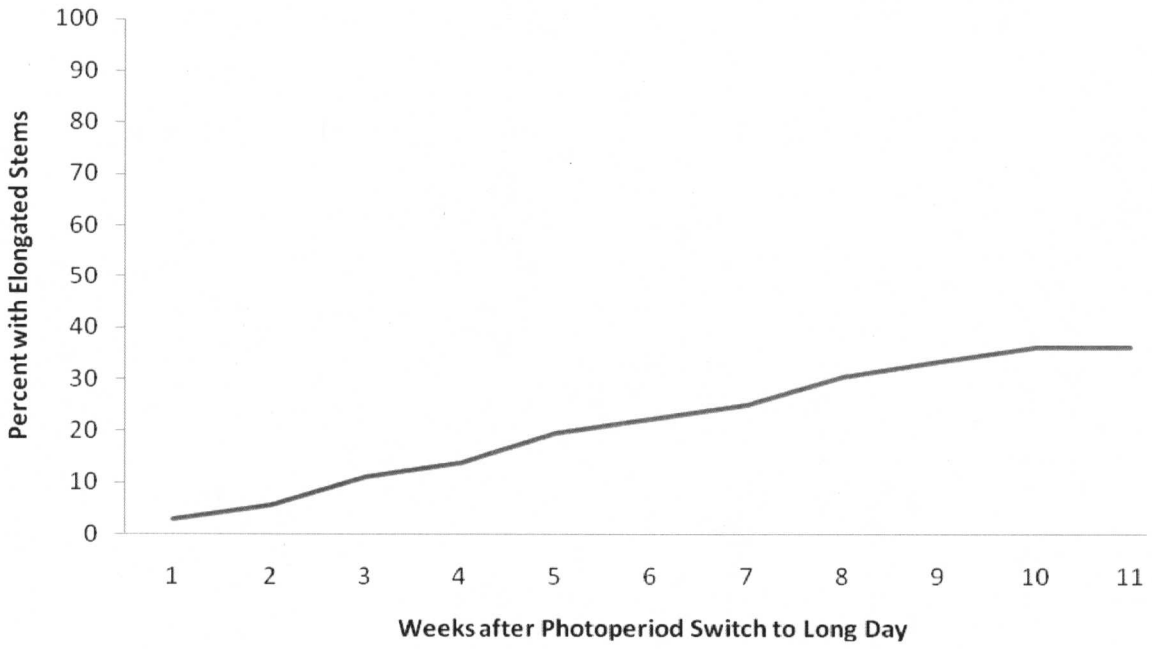

Figure 6. Percentage of plants with elongated stems when switched from a short day to a long day photoperiod. Weekly counts began with the start of the long day photoperiod when plants were 32 weeks old.

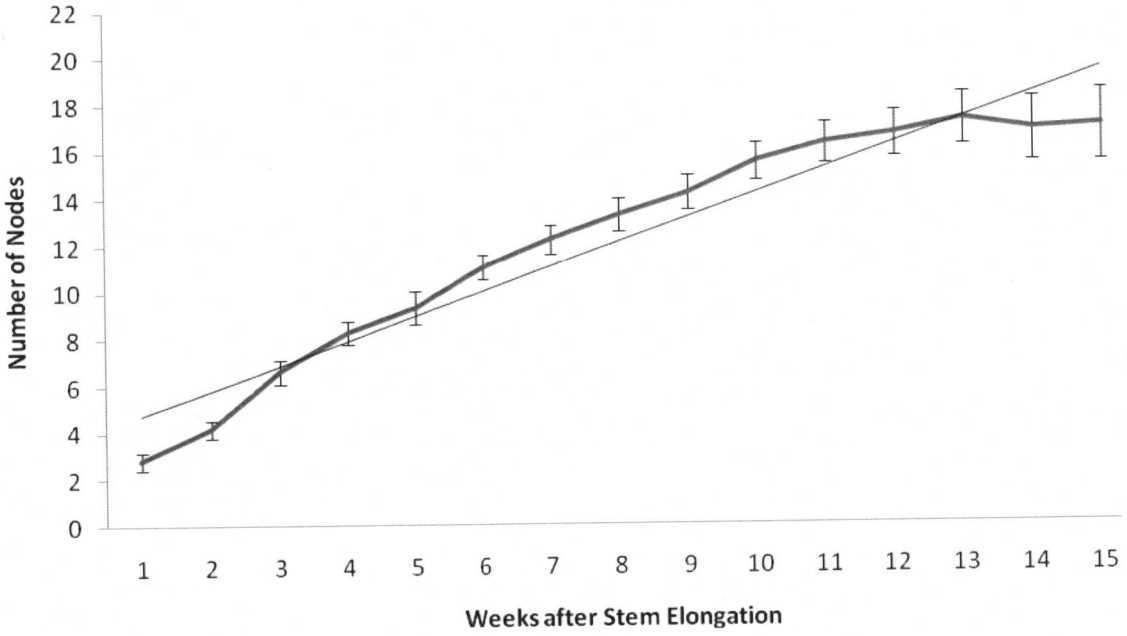

Figure 7. Number of nodes (mean ± SE) on elongated stems for plants in long day photoperiod. Weekly counts started with the first week of elongated stem growth in each individual plant.