Investigating the allelopathic effects of pale swallowwort (*Cynanchum rossicum*) on the growth success of common milkweed (*Asclepias syriaca*) and pale swallowwort

A Senior Honors Thesis

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Abstract

Invasive species seriously threaten both biodiversity and ecosystem functionality. One mechanism that makes invasive plants successful is allelopathy, which is the release of chemical compounds that have negative effects on other plants. A vine that is native to the Ukraine region and is now highly invasive in Western New York is Pale swallowwort (*Cynanchum rossicum*). It has the capability to change the growth situation in favor of itself by releasing allelochemicals into the soil. Thus far, little research has been conducted to examine the direct effects of swallowwort allelopathy on the growth of native plants. To accomplish this, two types of soil from two local sites were collected; the first that contained swallowwort remains, and the second that did not contain any swallowwort. The native and confamilial species *Asclepias syriaca* (common milkweed) and swallowwort were planted in these soil types where growth was compared. It was hypothesized that the growth of milkweed would be limited by the swallowwort soil due to allelopathy and that swallowwort growing in soil containing remains of swallowwort would thrive. Analysis of growth data indicated that there were no significant differences in the success of swallowwort growing in both soil types and that milkweed growing in swallowwort soil was significantly smaller than milkweed planted in the control for one of two sites. This suggested that allelopathy or other changes to the soil induced by swallowwort may affect the growth of milkweed and improve the overall competitive ability of swallowwort.

Introduction

Global ecological changes have produced new combinations of species in communities. One such global change is the occurrence of invasive species, which represents a substantial challenge to those attempting to conserve and maintain native biodiversity and ecosystem processes. They are one of the greatest threats to ecosystems worldwide because they can withstand a variety of ecological niches and environments by surpassing different biological filters in order to successfully invade and outcompete (Lym 2005). They are among one of the greatest threats to ecosystems since this process is responsible for eliminating the presence of native species, facilitating changes in soil properties, contributing to habitat degradation, and, as well as the loss of native wildlife and tree species (Richardson et al. 1989; Gaertner et al. 2009; Zhang et al. 2009; Zahid et al. 2013; Vitousek 1996; Pimentel et al. 2005; Davis 2003).

One mechanism that makes some invasive plants successful is allelopathy, which is, "The stimulatory and inhibitory negative effects of a plant on other plants through the release of chemical compounds into the environment" (Rice 1984). Chemicals released from plants that stimulate allelopathic influences are termed allelochemicals and are produced secondarily to plant's primary metabolic pathways (Koocheki et al. 2013). When vulnerable plants are exposed to allelochemicals, germination, growth, and development may be impacted. The most commonly reported morphological effects on plants are inhibited or hindered seed germination, effects on coleoptile elongation, and on radicle root and shoot development (Kruse et al. 2000). Allelopathy has been suggested as a mechanism for the success of invasive plants, partly because invaders often establish effective monocultures where diverse communities once thrived. To explain remarkable biogeographical patterns of many successful invaders, the establishing of monospecific stands suggests the need for remarkably potent mechanisms that must be more effective in recipient communities than in native communities (Hierro and Callaway 2003).

Pale swallowwort, *Cynanchum rossicum*, is a perennial herb or small vine in the Apocynaceae family that was first introduced into North America about 120 years ago from the Ukraine region of Eastern Europe (Weston et al. 2005). This species is currently expanding its range in northeastern North America at a high rate, threatening predominantly natural and semi-natural forested habitats due to its ability to thrive in a wide range of environmental conditions (Weston et al. 2005; Tewksbury 2002; Magidow et al. 2013). Dense populations of swallowwort tend to cover substantial areas due to its growth habit of twining on other plants. As a result, swallowwort can outcompete native vegetation and decrease faunal and floral biodiversity in New York State, as well as serve as a host for various insect pests (Weston et al. 2005).

It is speculated that swallowwort influences the soil it grows in, which may contribute to the displacement of resident vegetation (Weston et al. 2005). Nonnative invasive species such as swallowwort can influence a range of abiotic components including soil chemistry through allelopathy (Adalsteinsson et al. 2016). The success of swallowwort as an invader could be due in part to the release of allelochemicals into the rhizosphere from either tissue leachates or standing biomass (Douglass et al. 2011). This recognized allelopathic activity has been hypothesized to alter mycorrhizal fungi associations that significantly benefit the competitive dominance of *Cynanchum* spp. (Douglass et al. 2011). The roots of related *Cynanchum* species are known to contain the poison glycoside vincetoxin, which may have an effect on the growth and survival of native species such as *Asclepias syriaca* (common milkweed), allowing swallowwort to have a greater competitive advantage (Bhowmik 1994; Wyatt and Broyles 1994). These allelopathic features can directly impact the growth of nearby plants and indirectly through the alteration of soil microorganism balance, thus changing the growth situation in favor of swallowwort (Golzardi et al. 2014). An example of alteration of microorganism's population was observed in a study conducted by Mogg et al. (2008). They reported crude swallowwort extracts inhibited the growth of gram negative and positive bacteria and contained significant antifungal properties against yeast-like fungi in soil (Mogg et al. 2008).

Although no published studies have directly investigated the allelopathic properties of pale swallowwort given its complex secondary chemistry, this mode of inference could help to explain the formidable success of this species in open areas where no supporting woody vegetation is present (Weston et al. 2005).

The invasion of swallowwort, potentially aided by allelopathy, has been shown to displace common milkweed, a confamilial species that is native to eastern North America (Bhowmik 1994; Wyatt and Broyles 1994). The lifecycle of monarch butterflies (*Danaus plexippus*) is critically dependent on milkweed as a host plant to survive, grow, and reproduce, and has evolved to withstand the toxic cardenolides in which they produce (Malcom 1994). Pale swallowwort also produces a toxin lethal to monarch larvae. Monarchs laying larvae on the now wide-spread swallowwort plants has led to increased monarch offspring morality across much of the Eastern United States (Malcom 1994, Pahlevani et al. 2008, Tewksbury 2002). In order to help conserve these migratory insects, it is important to understand what mechanisms swallowwort uses to out-compete native milkweed. Studies comparing phylogenetically related native and invasive species are useful in understanding how adaptive capabilities differ between species (Bolnick et al. 2011; Daehler 2003; Davidson et al. 2011). Common milkweed and pale swallowwort can be studied in this framework to understand the role of interactions that can determine the outcome of competition.

Many experiments have reported on the phytochemical constituents of swallowwort species, focusing on their cytotoxic and antibiotic properties; however, allelopathic interactions of the chemical compounds with neighboring plant species have been less studied. The purpose of this experiment was to compare the growth of common milkweed and pale swallowwort in soil containing remains of pale swallowwort in order to understand more about how pale swallowwort has a greater competitive advantage over its phylogenetically related native competitor common milkweed. It was hypothesized that the remains of pale swallowwort would limit the growth of common milkweed, but have no effect on its own growth success.

Methods

Common milkweed and pale swallowwort seeds were collected during October 2016 from Oatka Creek Park in Monroe County, New York. The seeds underwent cold stratification from mid-November 2016 until early March of 2017, and were then moved into a greenhouse to begin germination. In early June, at random, two seedlings of identical size and species were placed into 46 pots of non-swallowwort soil (control) and 46 swallowwort soil (treatment), for a total of 92 pots (Figure 1).

Soil was collected from Oatka Creek Park, in the town of Wheatland, Monroe County, New York and North Hampton Park in Brockport, Monroe County, New York in early June of 2017, from areas that contained dense swallowwort and native herbs, and from areas that contained strictly remains of native herbaceous materials such as common milkweed, moss (*Bryophyta* sp.), wild strawberry, (*Fragaria vesca*) and graminoids (Poales). The habitat for soil collected from Oatka Creek was comprised of a swallowwort understory with a forest canopy of oak and hickory, where light emittance was limited. Soil from North Hampton was collected from an open grassy area, where there was no canopy, where swallowwort dominated. The top 10 cm of soil was collected, kept intact using a bulb corer, and placed directly into pots (Figure 1).

The ambient conditions of the greenhouse were set at 26°C and controlled by an evaporative cooling system. The lamps provided 16 hours of light each day, and the soil was kept moist, as both species favor more mesic soil conditions (Bhowmik 1994; Pahlevani et al. 2008; Wyatt and Broyles 1994). Temperatures in the greenhouse tended to exceed 26°C and remained around approximately 80% humidity throughout the summer. Plants were watered regularly and did not experience water stress.

After seedlings were planted in their designated soil types, initial measurements were made on reproductive phenology, reproductive structure height (cm), plant height (cm), stem stretch height (cm), largest leaf length (cm), largest leaf width (cm), and number of leaves. These measurements were conducted weekly for 11 weeks. During the study, some pots had either no plants, 1 plant, or both plants survive (Table 1). In week 12, each individual plant had its two youngest and most fully expanded shade leaves removed to be used for determining specific leaf area (SLA) analysis in the lab, where values were determined using methods described by Cornelissen et al (2003). Leaf areas were calculated by scanning leaf images into the computer and analyzed using ImageJ software (Schneider et al. 2012):

$$SLA\ (cm^2 \cdot g^{-1}) = \frac{\text{One-sided area of fresh leaf}}{\text{Oven-dry mass}}$$

Data analysis was completed in Excel and Minitab (Microsoft Excel ® 2017; Minitab 17 Statistical Software 2010). During week 13, plants were harvested for root to shoot ratio measurements which were then oven dried using the methods described by Cornelissen et al. (2003):

$$Root{:}\,Shoot\ Ratio\ (g) = \frac{\text{Root Mass (g)}}{\text{Shoot Mass (g)}}$$

Statistical Analysis

Data were tested for normality using the Anderson-Darling method. Parametric statistical analysis was performed in Minitab on the maximum leaf number data using ANOVA to compare means. Even after several data transformations, the data for measured plant traits, specific leaf area, and root to shoot ratio did not appear normal. Consequently, non-parametric statistical analyses were performed in Minitab using the Mann-Whitney U test to compare medians across the control and treatment soil types for these parameters (Minitab 17 Statistical Software 2010). Chi-squared analyses were also performed on the total number of survivors between species, sites, and soil type.

Results

Overall, after running several Chi-Squared analyses, there were no statistically significant differences between the number of survivors among species, site, and soil type (Table 2).

North Hampton

Plant height and stem stretch for milkweed grown in swallowwort soil during week 3 was significantly less than the control ($P<0.05$, Figure 2). There were no statistical differences in largest leaf length, largest leaf width, or number of leaves for milkweed between both soil types. In week 7, common milkweed grown in swallowwort soil had a significantly smaller stem stretch than the control plants. Plant height for milkweed growing in swallowwort soil during week 11 was significantly less than the control, with median heights of 7.25 cm and 10.85 cm (Figure 2). Correspondingly, stem stretch for milkweed growing in swallowwort soil was significantly less than milkweed grown in the paired control. The median length for milkweed grown in swallowwort soil was 7.6 cm, while the median for milkweed grown in the control was 11.05 cm (Figure 2). There were no significant differences in the largest leaf lengths and widths for common milkweed during this week.

Swallowwort soil type had an impact on largest leaf length for pale swallowwort ($P<0.006$), as it was larger than the controls during week 3. However, during week 7, swallowwort's largest leaf length and width were significantly larger than swallowwort grown in control soil (Figure 2). Conversely there were no statistically significant differences for any of the measured traits for pale swallowwort growing in either soil type in week 11.

There was no difference in the average specific leaf area for milkweed, but there was a large, significant difference in the specific leaf area for pale swallowwort grown swallowwort soil, with medians of 035 and 0.27 $m^2 \cdot kg^{-1}$ (Figure 3).

Root to shoot ratios for common milkweed grown in soil containing remains of swallowwort was significantly less than milkweed growing in the control soil, with corresponding medians of 1.5 and 1.1 g. There was also a significant difference in pale swallowwort growing in swallowwort soil remains. These plants contained higher biomasses than the controls with medians of 2.4 g compared to 1.6 g (Figure 4).

Oatka Creek

There were no statistically significant differences in measured traits for common milkweed grown between soil types.

For pale swallowwort grown in swallowwort soil during week three, stem stretch was significantly greater than the control soil. However, largest leaf length for swallowwort grown in swallowwort soil was significantly less than the control (Figure 5). In week 7, there were no statistically significant differences in measured traits among both soil types, and pale swallowwort's largest leaf width grown in swallowwort soil was significantly greater than pale swallowwort grown in the control, with medians of 2.05 and 1.5 cm, respectively in week 11. There were no differences in average plant height, stem stretch, and largest leaf length.

There was a significantly greater difference in the specific leaf are for common milkweed, as plants grown in the control soil had larger SLA measurements than plants grown in soil containing remains of swallowwort, with medians of 0.27 and 0.13 $m^2 \cdot kg^{-1}$, respectively. There was no difference observed between SLA for pale swallowwort grown in both soil types (Figure 6).

Root to shoot ratios for common milkweed and pale swallowwort soil types did not differ between soil types (Figure 7).

Discussion

It was hypothesized that the remains of pale swallowwort would limit the growth of common milkweed, but have no effect on its own growth success, suggesting its ability to thrive throughout different soil conditions through allelopathic interactions. Analysis of growth data indicated that there were no significant differences in the success of swallowwort growing in either soil type for both sites. This suggested that soil type had a limited impact on swallowwort growth, supporting observations that swallowwort is currently found growing in a variety of soil types. At one site, milkweed growing in swallowwort soil was significantly smaller than milkweed planted in control soil for one of two sites, which partially supported our hypothesis, suggesting that allelopathy in some soil types may affect the growth of milkweed and improve the overall competitive ability of swallowwort. For all weeks analyzed, stem stretch and plant height for North Hampton common milkweed planted in swallowwort soil were less than the control. Similarly, in a study conducted by Douglass et al. (2011), pale swallowwort exudates reduced common milkweed shoot growth. Black and pale swallowwort that were used in this particular experiment demonstrated the ability to interfere with the establishment and growth of neighboring plant species' common milkweed and butterflyweed (*Asclepia tuberosa*) in controlled settings. Both common milkweed and butterflyweed were significantly inhibited by swallowwort root exudates or tissue leachates. The growth inhibition of milkweed that was observed in our experiment perhaps was caused by the remains of swallowwort roots, leaves, and stems that leached allelochemicals within the soil, as the root to shoot ratio for common milkweed was less than those planted in the control soil.

Contrary to North Hampton milkweed, there were no significant differences in growth characteristics for Oatka Creek milkweed that were analyzed in addition to root to shoot ratios. This may have resulted from the type of soil that was collected for the experiment. Oatka Creek Park's forest is mainly dominated by *Quercus* species including red (*Quercus rubra*) and white oak (*Quercus alba*). Consequently, the majority of collected soil was beneath a layer of decomposing red and white oak leaves. Oak leaf litter can produce extracts that contain highly localized lignin and tannin residues which affect microbiota density and diversity (Anderson 2005). These residues are also hypothesized to influence the soil nitrogen cycle through their ability to bind protein, forming polyphenol-protein complexes that are resistant to decomposition (Talbot and Finzi 2008). A combination between these two mechanisms could have inhibited the ability of swallowwort remains to release leachates comprised of allelochemicals, un-affecting the growth of common milkweed and pale swallowwort plants growing in the Oatka Creek soil.

Additionally, soil from North Hampton Park was predominately collected from an open, grassy area, where swallowwort was dominant. The growth and fecundity of swallowwort have been shown to be substantially greater in open, sunny sites or forest gaps than in the understory of dense forests, which may explain why there were more differences observed in milkweed and swallowwort grown in the swallowwort soil (DiTommasso et al. 2005). The differential impact of potentially allelopathic leachates on milkweed's reduced performance strongly supported the hypothesis that invasive plants can differentially inhibit growth of native confamilial milkweed through allelopathic mechanisms, and as a result, it is likely that invasive plants like swallowwort can strongly influence plant community dynamics by altering the relative abundance of native plant species and improve the overall competitive ability of itself.

Several pots also had more than 1 individual survive, perhaps causing intraspecific competition that limited the growth of both plants within a given pot. In North Hampton common milkweed planted in swallowwort and control soil, there were a total of 8 and 10 pots that contained more than 1 plant, respectively. North Hampton swallowwort with 2 individuals planted in swallowwort soil had 9 pots and the swallowwort control had 5 pots. For Oatka Creek milkweed planted in swallowwort soil and control soil, there were 3 pots with 2 individuals. Oatka Creek swallowwort grown in swallowwort soil contained 4 pots with 2 individuals, while the control had 6 pots total with 2 individuals. Classical competition theory predicts intraspecific competition should be greater than interspecific competition because individuals of the same species share similar resource requirements (Mangla et al. 2011). Competition during early stages of growth can critically influence individual plant growth, and intraspecific competition is known to be dominant for invasive species (Mangla et al. 2011), which may help to explain the nonexistent differences in swallowwort SLA for both Oatka Creek and North Hampton plants grown in swallowwort soil in addition to the lack of significant differences for the total number of survivors among species, site, and soil type (Table 2).

Allelopathic interference might provide a partial explanation for the invasive capabilities of swallowwort species, as they might possess a greater competitive advantage over neighbors from the interaction of released biologically-active compounds with soil microbes or fungal species (Douglass et al. 2011). However, since much of the prior research on allelopathy originates from agricultural or laboratory settings, limiting the ability to infer the ecological relevance of the results for native plant and soil communities is challenging. Ultimately, our experimental design cannot determine if these results are directly affected by of allelopathy, or if they occurred indirectly from differences in the microbial associations of soil, leaf, or litter, or from differential nutrient levels among pots. An important next step in this experiment would be to test for allelopathic effects of swallowwort on these factors such as microbial associations and nutrient content.

The reliance on arbuscular mycorrhizal fungi (AMF) by invasive plants is another factor to consider since plants can benefit from these associations because fungi can enhance nutrient uptake such as phosphorus for host plants (Smith et al. 2008). Pale swallowwort plants can form associations with AMF in its introduced range and it has been suggested that its colonization of new habitats is associated with a change in the composition of AMF communities in the affected soils. The survival of pale swallowwort can also be significantly greater in soil collected from areas of dense monocultures of swallowwort than in sterilized soil, which is perhaps why there were more total swallowwort survivors that were grown in swallowwort soil in our experiment, as AMF was in favor for the growth of swallowwort instead of milkweed, suggesting that AMF occurring in swallowwort (invaded) habitats could have advantageous effects on pale swallowwort survival and growth, limiting the competitive ability of milkweed.

The inclusion of soil testing would have also been extremely beneficial to the study since soil properties like pH and nutrient type could have further supported our hypothesis and perhaps explain effects of allelopathy in greater detail. Although in other common garden field experiments such as one conducted by Madigow et al. (2013) that assessed if the performance of swallowwort was affected by soil type and/or soil pH, plants grown in an alfisol produced a smaller root mass than inceptisol soil type. Plants grown in low pH soils also contained smaller root dry mass compared to plants grown in higher pH soils. Contrary to their expectations, interactions between swallowwort and their associated soil type or with their "preferred" soil pH were weak and contradictory, suggesting that swallowwort can colonize and thrive in a relatively wide range of soil pH conditions. For our experiment, more research is certainly required to determine if and how much of a role soil type, pH, or some other related soil characteristics like nutrient content had on the growth of swallowwort and milkweed. Therefore, we could detect how allelopathy might influence the continued growth of swallowwort and inhibit the growth of native milkweed, and also enhance the general applicability of our findings.

Tables and Figures

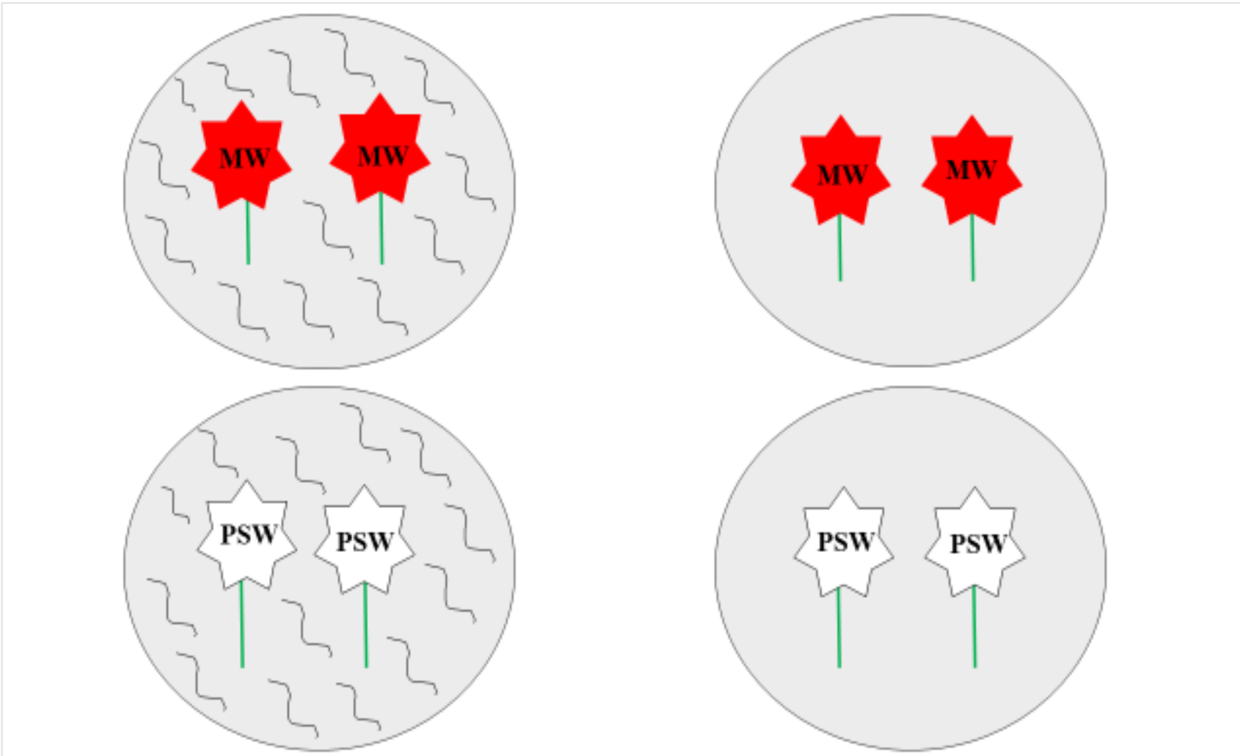


Figure 1. Design of experimental layout. There were two soil treatment types; control soil (bare) and swallowwort soil (squiggles). Two individuals of identical size and species for common milkweed (MW) and pale swallowwort (PSW) were planted in the same pot, where growth traits were measured weekly. There was a total of 60 pots for North Hampton and 32 pots for Oatka Creek.

Table 1. Total number of common milkweed and pale swallowwort survivors (0, 1, or 2) per pot upon completion of the experiment for each soil type and site (North Hampton and Oatka Creek).

	NH				OC			
	0	1	2	Total	0	1	2	Total
SW MW	3	4	8	15	3	2	3	8
SW SW	4	3	8	15	2	2	4	8
C MW	0	5	10	15	3	2	3	8
C SW	3	7	5	15	3	1	4	8

Table 2. Results for Chi-squared analyses for number of survivors among species, site, and soil type.

	χ^2	df	P value
Species	0.63	2	0.73
Site	3.40	2	0.15
Soil Type	1.07	2	0.59

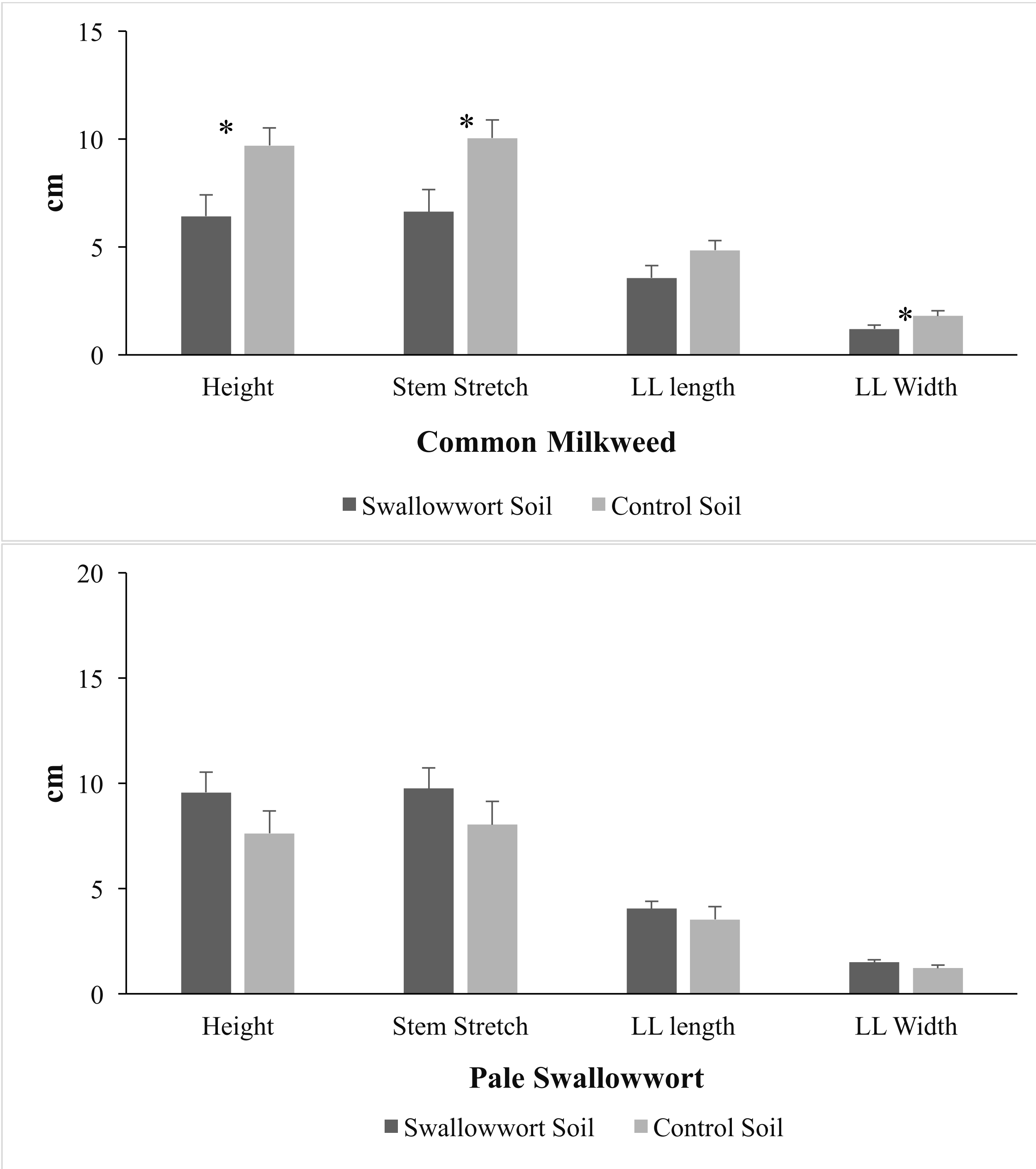


Figure 2. Week 11 average height, stem stretch, largest leaf length, and largest leaf width (cm) for common milkweed and pale swallowwort grown in both soil types. Asterisks represent significance via the Mann-Whitney U test (P<0.05).

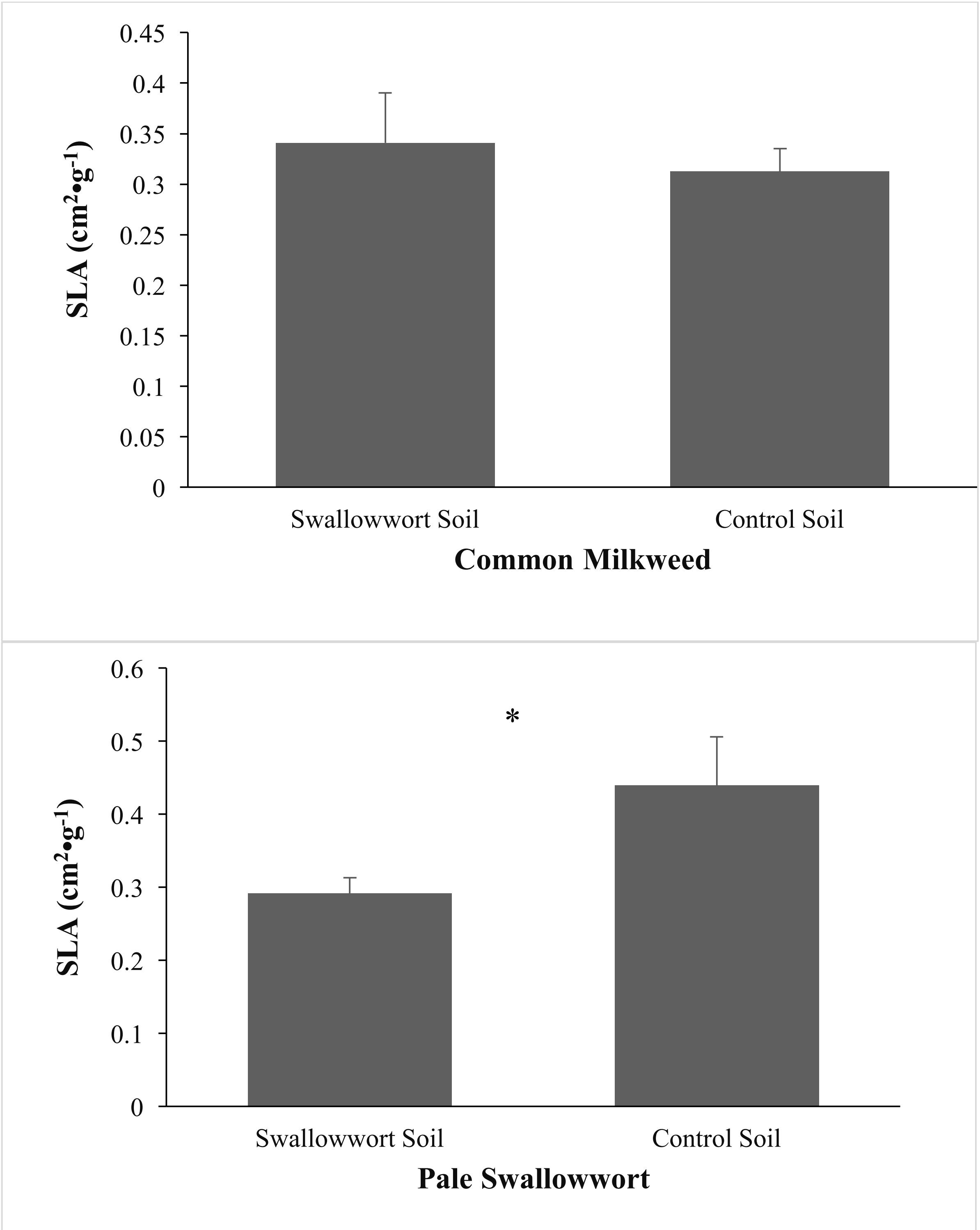
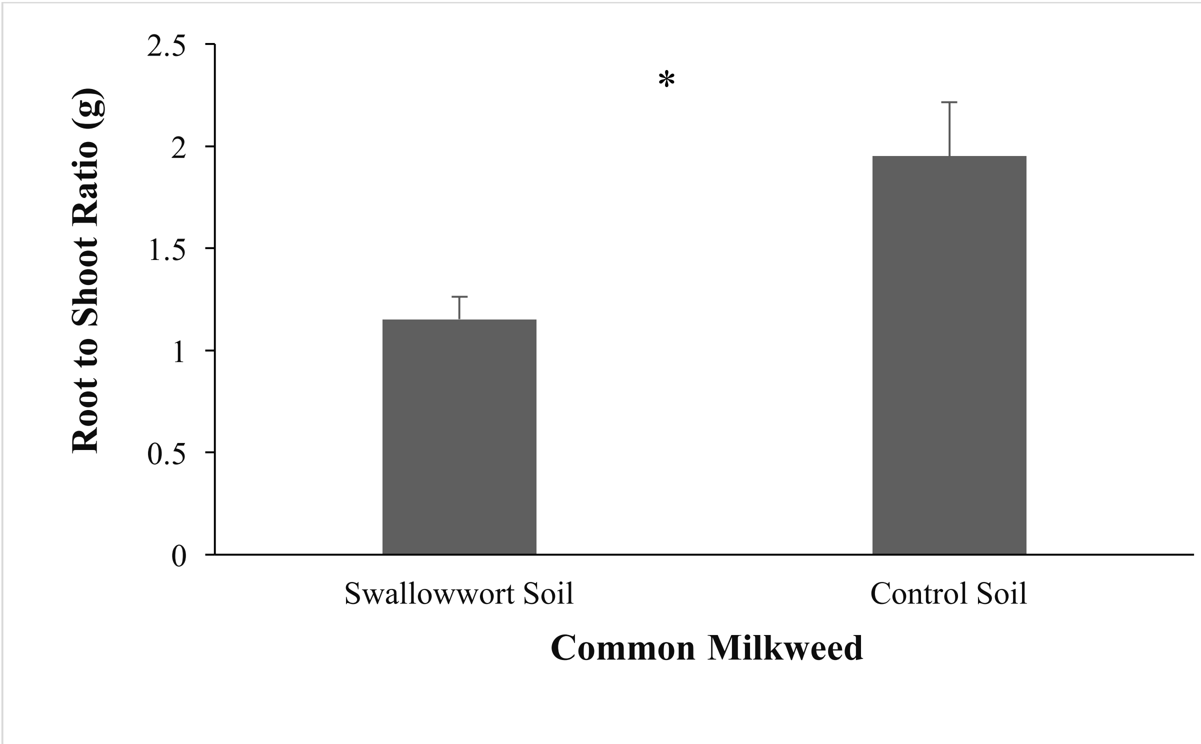


Figure 3. Average specific leaf area ($cm^2 \cdot g^{-1}$) for milkweed and swallowwort grown in the different soil types. Asterisks represent significance via Mann-Whitney U test ($P<0.05$).



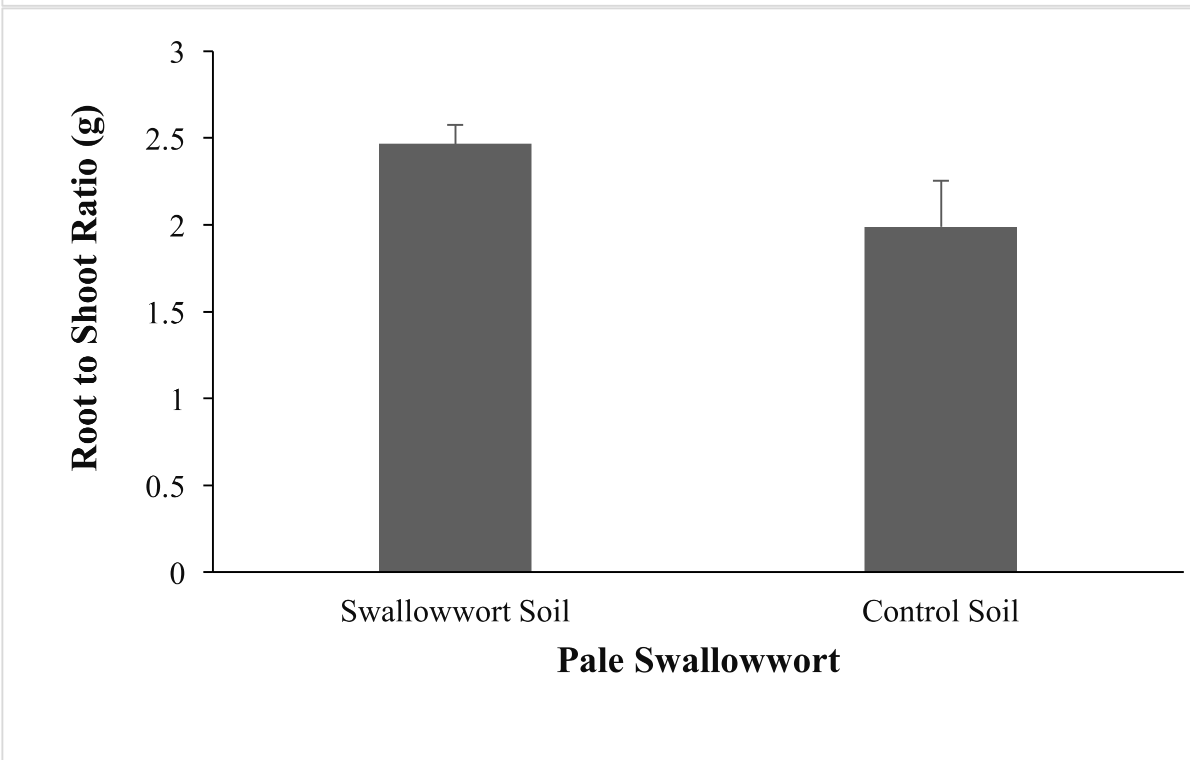
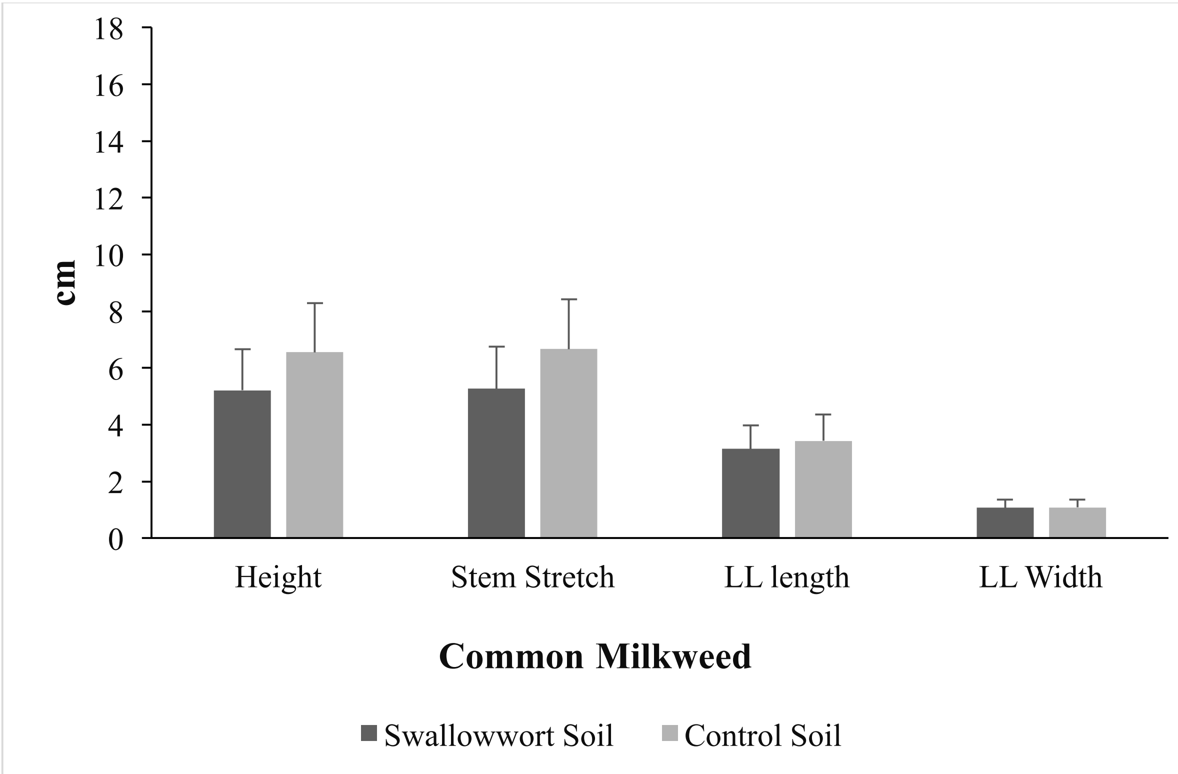


Figure 4. Average root to shoot ratio (g) among the different treatment types for milkweed and swallowwort. Asterisks represent significance via the Mann-Whitney U test ($P<0.05$).



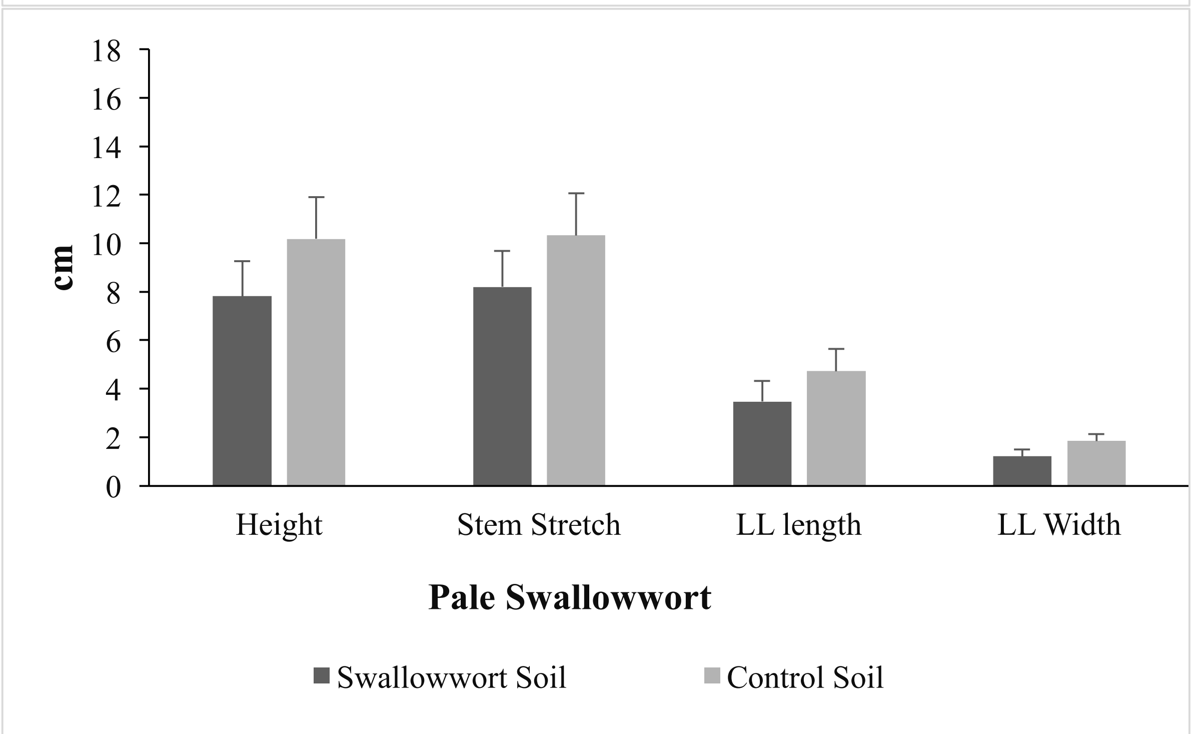
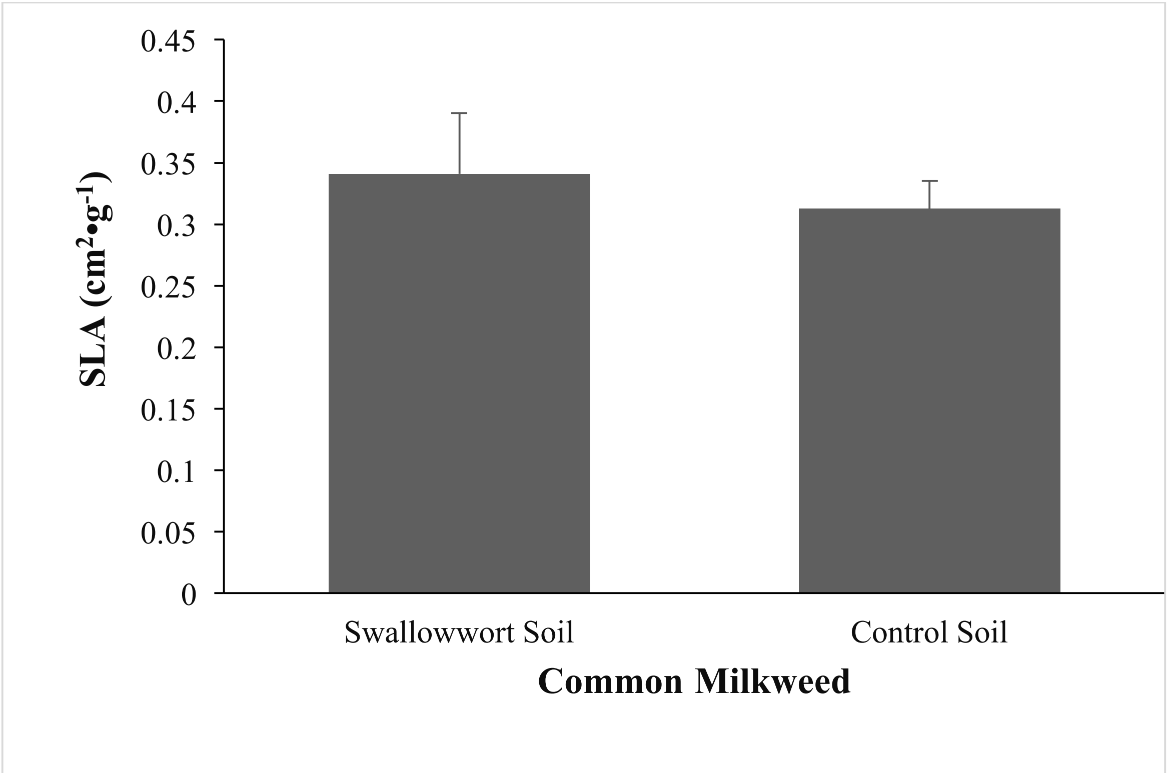


Figure 5. Week 11 average height, stem stretch, largest leaf length, and largest leaf width (cm) for common milkweed and pale swallowwort grown in both soil types.



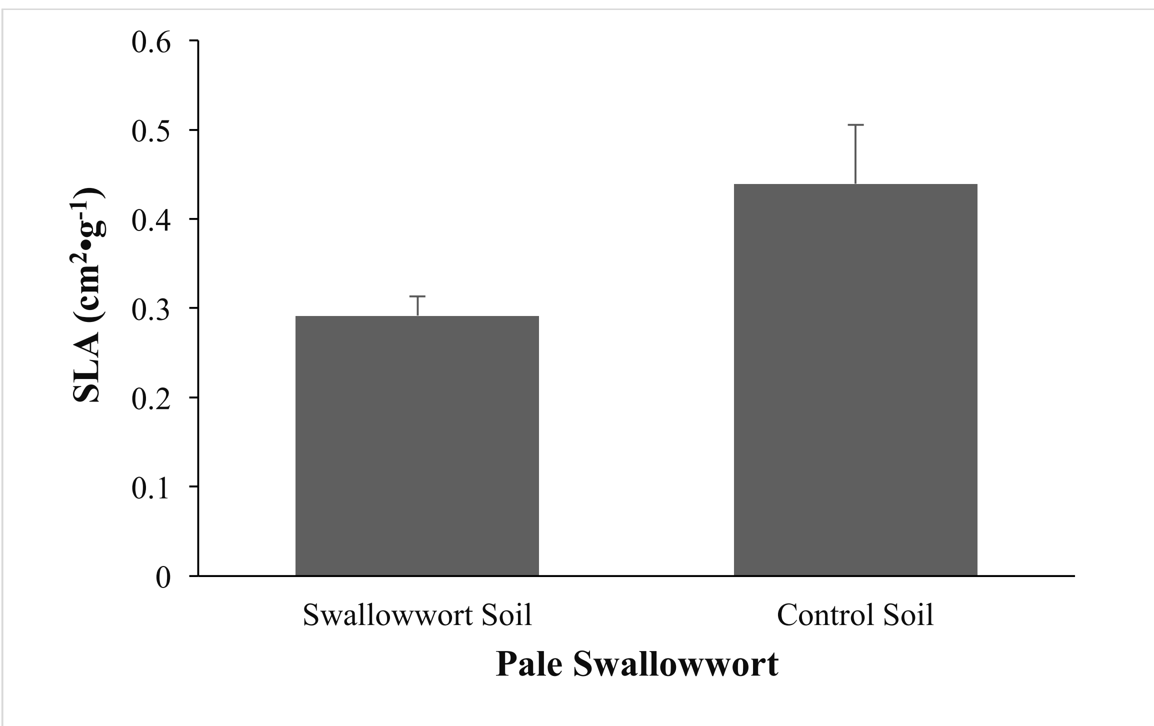
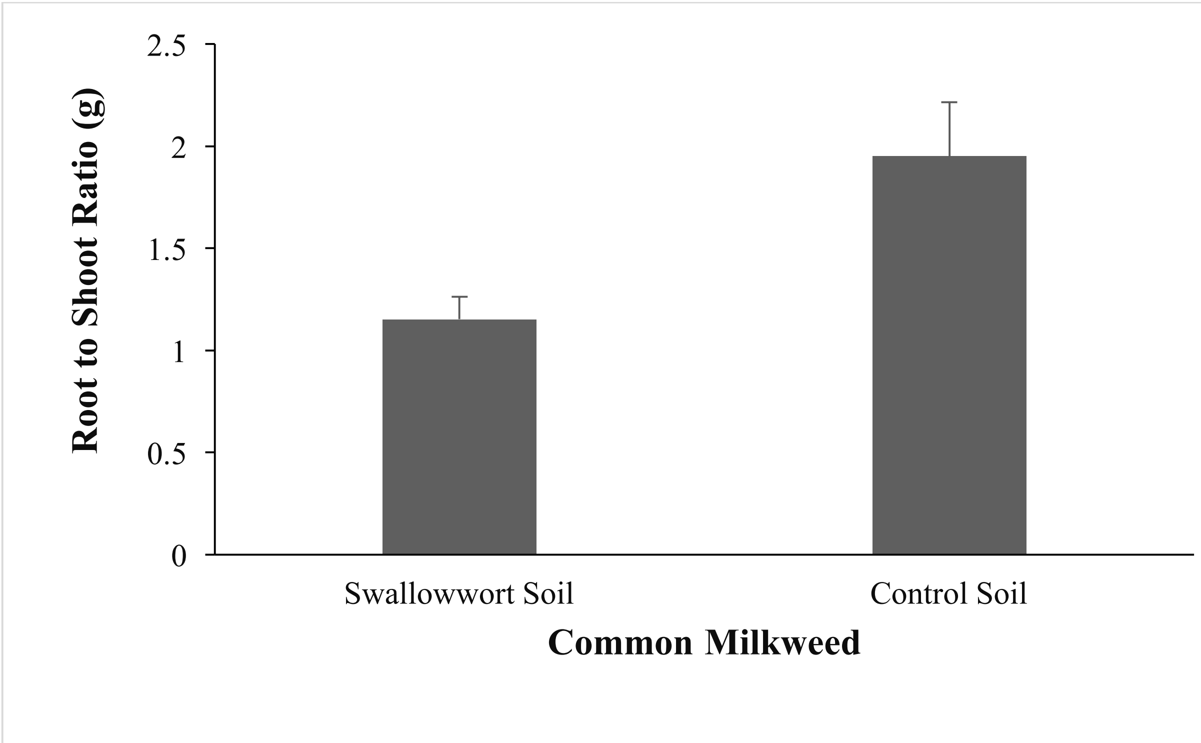


Figure 6. Average specific leaf area ($cm^2 \cdot g^{-1}$) for milkweed and swallowwort grown in the different soil types.



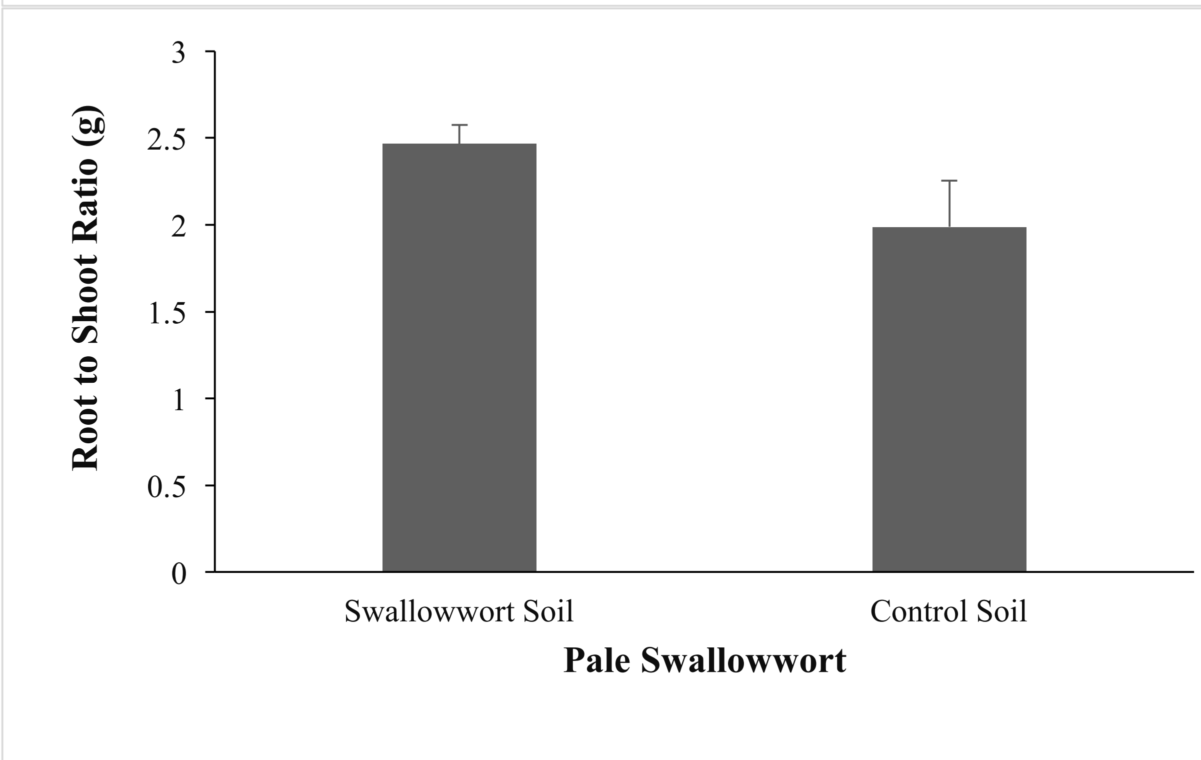


Figure 7. Average root to shoot ratio (g) among the different treatment types for milkweed and swallowwort.

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