

Quantifying the effects of Japanese knotweed (*Fallopia japonica*) allelopathy on native plant
growth

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By

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Abstract

Biological invasions are one of the leading causes of decreasing biodiversity around the world. Japanese knotweed (*Fallopia japonica*) is an invasive perennial herb native to east Asia that has become increasingly abundant in New York state. One way that invasive plants such as Japanese knotweed interfere with native communities is through the release of allelochemicals, which are secondary compounds that inhibit the growth of competitors. While knotweed is known to produce several potentially allelopathic compounds, their isolated effects on native plant growth are understudied. I performed a manipulative growth experiment treating three native species with knotweed leachate or tap water and potting them in control soil or knotweed soil collected from beneath a local knotweed population. Native species were grown for eight weeks, and their growth among treatments compared. I hypothesized that the growth of natives receiving knotweed leachate and natives potted in knotweed soil would be limited by allelochemicals. Contrary to my hypothesis, there were no significant differences between leachate and control plants. When significant results were found, plants potted in knotweed soil grew better than control plants. This may be due to increased nutrient availability or changes in soil biota caused by knotweed. My results suggest that Japanese knotweed allelopathy plays a minimal role in the species' success and its allelochemicals are not a major concern for restoring native communities.

Introduction

The prevalence of biological invasions is a global consequence of increasing connectedness and human activity (Pyšek et al. 2020). Invasive species are now one of the largest drivers of global biodiversity loss and pose significant economic costs -- from 1960 to 2020 the reported costs of biological invasions in the United States alone surpassed \$1.22 trillion (Fantle-Lepczyk et al. 2022). Invasive species degrade ecosystem structure and function through predation, competition, disease transmission, and facilitation of other invasive species (Doherty et al. 2016, Valtonen et al. 2017, Siddiqui et al. 2021). One of the largest groups of invasive species, terrestrial plants, can also disrupt native ecosystems through belowground resource exploitation and altering the rhizosphere (Torres et al. 2021).

Invasive plants alter rhizosphere dynamics through the release of secondary metabolites called allelochemicals (Meiners et al. 2017, Koza et al. 2022). As per the novel weapons hypothesis, plants outside of their native range produce allelochemicals that interfere with native plants and soil biota (Izhaki 2002). Allelochemicals enter ecosystems primarily in the rhizosphere, exuded by roots or leached from plant litter. Allelochemicals inhibit the establishment and growth of competing natives through a process called allelopathy (Izhaki 2002, Callaway and Ridenour 2004, Cheng and Cheng 2015). In the soil, these compounds suppress the germination, growth, and nutrient uptake of susceptible plants, providing a competitive advantage to the donor species (Grove et al. 2012, Dommanget et al. 2014). While chemically diverse, allelochemicals largely consist of phenolic compounds such as tannins, flavonoids, and quinones (Kong et al. 2019). Allelochemicals have been documented in several common invasive plants including European buckthorn (*Rhamnus cathartica* L.), amur honeysuckle (*Lonicera maackii* Rupr.), garlic mustard (*Alliaria petiolata* M. Bieb.), and Japanese

knotweed (*Fallopia japonica* Houtt.) (Chu et al. 2005, Cipollini et al. 2008b, Lankau 2010, Warren et al. 2017).

If allelopathy contributes to plant invasions, then efforts to restore invaded communities must consider the lasting effects of allelochemicals on the rhizosphere. Research has shown that allelochemicals can be impactful in soils even after the removal of the donor species (Grove et al. 2012). At low concentrations, soil microorganisms can naturally degrade allelochemicals in the soil, however, dense monocultures of allelopathic species often pollute soils beyond the mediating capacity of soil biota (Scavo et al. 2019). In these cases, the removal of topsoil or the addition of activated carbon, a material used to neutralize organic compounds may be needed to promote the re-establishment of native communities (Lau et al. 2008). Understanding the allelopathic effects of specific invasive species on native plants and their allelochemicals' residence in soils is key for informing successful restoration practices.

Japanese knotweed is an invasive perennial herb in the family Polygonaceae that was introduced to North America in the late 1800s via ornamental trade from its native range in east Asia (Gillies et al. 2016). In New York, knotweed is an aggressive invader of agricultural, roadside, and riparian habitats due to its high tolerance for abiotic stress (Weston et al. 2005). Knotweed's spread is facilitated by an extensive system of hardy rhizomes that can generate new shoots with as little as 0.5 g of material (Lawson et al. 2021). The species produces tall stems with high shoot ramification, forming dense monotypic stands that displace native vegetation and contribute to its dominance. Efforts for controlling Japanese knotweed and other invasive knotweeds in Europe and North America have proved labor and cost intensive. The annual cost of knotweed management has been estimated at € 32 million in Germany and £ 1.6 billion in the United Kingdom (Murrell et al. 2011). Part of this failure to successfully manage Japanese

knotweed invasions is due to our limited ecological understanding of what makes this species such a successful invader.

One thing that may make Japanese knotweed a successful invader is allelochemical production. Tannins, flavonoids, and quinones have all been documented in knotweed's roots, stems, and leaves -- most notably the anthraquinone emodin (Chu et al. 2005, Beňová et al. 2010, Chen et al. 2013, Dommanget et al. 2014, Békési-Kallenberger et al. 2016, Kato-Noguchi 2021). Emodin has been shown to exhibit allelopathic effects in 17 different plant families and is therefore suggested as an agent for knotweed's success (Izhaki 2002, Murrell et al. 2011, Kato-Noguchi 2021). It is hypothesized that emodin is released from the rhizomes as glycoside or as aglycone, where it is decomposed in the soil to form emodin aglycones, the active form that disadvantages competitors. Previous research in France found that leachates derived from Japanese knotweed contain high concentrations of phenolic compounds and limit the growth of native plants (Dommanget et al. 2014). However, the concentration of various allelochemicals have been shown to vary among geographically distant populations of Japanese knotweed, suggesting that their allelopathic effects may vary as well (Chen et al. 2013).

Research analyzing the allelopathic effects of Japanese knotweed populations in western New York is lacking. Previous research has demonstrated that local populations contain measurable emodin, but its impacts on native plants have not been investigated (A. Graziano, unpublished data). The purpose of this study is to quantify the isolated effects of Japanese knotweed allelopathy on native plant growth with two experimental treatments: knotweed leachate and knotweed soil. I hypothesized that these treatments would limit the growth of native plants relative to control conditions.

Methods

The effects of Japanese knotweed allelopathy on native plant growth were evaluated with a manipulative experiment. Three native knotweed associates were selected as test plants based on habit, abiotic tolerance, and phylogenetic proximity to Japanese knotweed. Swamp milkweed (*Asclepias incarnata* L.) is a perennial herb that occupies open, wet environments along stream banks. Gray dogwood (*Cornus racemosa* Lam.) is a hardy perennial shrub. The confamilial species, jumpseed (*Persicaria virginiana* L.) is a shade-tolerant perennial herb belonging to the same family as knotweed, Polygonaceae. Thirty plants of each species were purchased from the Missouri Wildflower Nursery.

Knotweed and control soil was collected from Northampton Park in Brockport, Monroe County, NY from 28 May to 2 June 2023. Knotweed soil was collected from beneath a roadside monoculture of Japanese knotweed under a canopy of knotweed at 43°11'18"N 77°53'03"W. Control soil was collected from an area uninvaded by Japanese knotweed at 43°11'18"N 77°53'04"W, within the same soil zone as the knotweed stand, verified using the USDA web soil survey (Figure 1). Species composition in this area included poison ivy (*Toxicodendron radicans*), grape (*Vitis sp.*), goldenrod (*Solidago sp.*), buttercup (*Ranunculus sp.*), and Virginia creeper (*Parthenocissus quinquefolia*). The top 10 cm of soil was collected and lightly sifted to remove extraneous organisms and seeds.

Eight knotweed donors were harvested from Northampton Park one month prior to soil collection. Young shoots were collected using a soil corer, rooted in tap water for two weeks, then potted in 0.7-liter pots with knotweed soil, following the same potting procedure above. All eight donors were placed in a single plastic container and watered with tap water until exactly 1.5 L of leachate had accumulated.

Test plants were placed into three experimental groups: “control,” “knotweed soil,” and “leachate,” each with ten replicates. Plants were repotted into one-liter pots with a small circle of shade cloth fitted at the bottom of pots to reduce the amount of soil loss through drainage holes. Before being transferred to their new pots, excess soil was removed from the roots of test plants by hand. Pots were filled a third of the way with the respective soil treatment, packed down, then the test plant added and filled with soil. A small circle of shade cloth was fitted over the surface of the soil to prevent excessive drying. Plants in the control group were potted in control soil and received tap water only. Plants in the knotweed soil group were potted in knotweed-grown soil to simulate knotweed’s unique belowground microclimate and received tap water only. Plants in the leachate group were potted in control soil and watered with 50 mL of fresh leachate twice a week, supplemented with tap water to combat heat stress. All test plants were watered twice a week based on percent soil moisture measured with a LabQuest soil moisture probe. The soil moisture of two randomly selected pots from each treatment group per species was monitored each week.

Plants were grown outside at SUNY Brockport’s ponds facility, an early successional grassland enclosed by fences to keep out large mammalian herbivores. Test plants were grouped by species, then raised on wooden pallets to eliminate interspecific plant competition and placed under netting for extra protection (Figures 2, 3, and 4). The location of pallets was selected based on light availability, measured with a LabQuest light meter. Milkweed and dogwood plants were grown in an open field with minimal shade, receiving an average of $96.81 \pm 5.74\%$ and $81.28 \pm 28.31\%$ photosynthetically active radiation (PAR) during daylight hours respectively. Jumpseed plants were grown under a canopy of box elder (*Acer negundo*), receiving an average of $18.93 \pm 8.11\%$ PAR during daylight hours.

Plant measurements

Initial measurements were made on plant height (cm), largest leaf length (cm), largest leaf width (cm), number of stems, and number of leaves. Leaf herbivory (Table 1) and overall condition were recorded using ordinal scales (Table 2). Height and condition were measured weekly while leaf and stem measurements were taken bi-weekly for 8 weeks from 3 June 2023 to 2 August. Hereinafter, a day-of-year numbering system will be used to indicate time during the treatment period (3 June = day 154, 2 August = day 214). On day 214, each individual plant had its two youngest fully expanded shade leaves collected for specific leaf area (SLA) analysis, following the procedure described by (Cornelissen et al. 2003). Leaf areas were calculated by scanning leaves with a CanoScan LiDE 110 and scans were processed using ImageJ software (Schneider et al. 2012):

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

On day 214, each individual plant was harvested for root and shoot biomass measurements and oven dried using methods described by Cornelissen et al. (2003):

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

Statistical analysis

Data analysis was completed in Excel and Minitab (Microsoft Excel ® 2019, Minitab 20 Statistical Software 2020). Data were tested for normality using the Ryan-Joiner method. Even after several attempted data transformations, growth and leaf number data did not appear normal for any species. Thus, non-parametric statistical analyses were performed in Minitab using the Kruskal-Wallis method to compare median soil moisture, growth, number of leaves, shoot

biomass, root biomass, root:shoot ratio, and specific leaf area among treatments. When the null hypothesis was rejected, Wilcoxon signed rank tests were used to determine pairwise differences.

Results

All *A. incarnata* and *P. virginiana* plants survived and remained healthy through the duration of the experiment. Thirteen of thirty *P. virginiana* plants achieved reproductive status and it was the only species to do so (Table 3). On day 170, all *C. racemosa* plants were presumed dead from heat stress. However, by day 214, all but five individuals recovered and exhibited new growth (Figure 5). Percent soil moisture did not differ between treatments for *A. incarnata*, *C. racemosa*, or *P. virginiana* plants and fluctuations throughout the treatment period were minimal (Table 4, Table 5, Table 6, Figure 6).

A. incarnata and *P. virginiana* plants exhibited linear growth, gaining an average of 27.94 and 28.09 cm in height respectively (Figure 7). *C. racemosa* growth plateaued very early in the experiment and plants gained an average of 0.42 cm in height. *A. incarnata* potted in knotweed soil grew significantly taller than other treatments (Table 4), confirmed with a pairwise test between the control and knotweed soil groups ($T=9$, $p=0.067$). In contrast, neither *C. racemosa* nor *P. virginiana*'s growth was significantly affected by treatment (Table 5, Table 6).

A. incarnata and *C. racemosa* had similar numbers of leaves in all treatments and treatments did not statistically affect leaf number (Figure 8, Table 4, Table 5). *P. virginiana* in knotweed soil had on average 34% more leaves than control plants, but this difference was not statistically significant (Figure 9, Table 6). *P. virginiana* was the only species to experience leaf herbivory by insects. Only 13% of all leaves on day 214 had more than 1% of their total area

eaten (Figure 9). SLA of all three native plants was not affected by treatments (Tables 4-6, Table 8).

A. incarnata root and shoot biomass were both significantly greater in knotweed soil, each confirmed with a pairwise test between the control and knotweed soil groups (T=2, p=0.011 and T=3, p=0.014, respectively; Table 4, Table 5, Figure 10). *A. incarnata* root:shoot ratio did not differ among treatments (Table 4, Figure 10). *C. racemosa* showed no difference in root biomass, shoot biomass, nor root:shoot ratio among treatments (Table 5, Figure 10). *P. virginiana* shoot biomass was significantly greater in knotweed soil, confirmed with a pairwise test between the control and knotweed soil groups (T=8, p=0.053, Table 6, Figure 10). However, there was no difference in root biomass nor root:shoot ratio.

Discussion

Three native species were treated with knotweed soil and knotweed leachate to determine the allelopathic effects of Japanese knotweed. Contrary to my hypothesis, the three native plants used in this experiment did not experience any negative effects of knotweed leachate or soil. In contrast, when significant differences among treatments occurred, native plants appeared to perform better in soil collected from under a local population of Japanese knotweed.

When significant results were found, plants grown in knotweed soil were taller and grew more biomass than control plants. Given that both soil types were collected within the same soil zone, I hypothesize that these differences are the result of knotweed-specific impacts. After the growing season, all of knotweed's dead stems litter the ground beneath it, adding massive amounts of organic material to the soil. Organic material increases the soil's capacity to store and supply nutrients, which may explain why natives grew better in knotweed soil than control soil. Alternatively, soil differences may be due to knotweed's unique belowground microclimate, created through knotweed's interactions with soil biota. The ability of soil biota to enhance the performance of invasive plants is well documented (Bray et al. 2003, Smith et al. 2008, Parepa et al. 2013). Soil biota enhance plant growth by increasing the availability of nutrients such as phosphorus, but tend to benefit invasive plants more than natives that co-exist in the same soil (Bray et al. 2003). Parepa et al. (2013) explored the role of soil biota in the invasion success of Japanese knotweed and found that the overall presence of soil biota increased knotweed growth and regeneration compared to sterilized soils. They discovered that adding activated carbon to knotweed soil erased the benefits provided by soil biota, suggesting that the advantages were chemically mediated. Perhaps in its early establishment, knotweed's allelochemicals serve to attack its plant competitors, but once established, they foster microbial communities that enhance

soil conditions -- but ultimately favor knotweed. This idea is supported by the fact that the knotweed population from which this soil was collected is well established and experiences little to no interspecific competition with native plants. These findings suggest that allelopathy may play out on two-time scales: through immediate, direct impacts on native plants as well as through long-term, indirect impacts caused by the alteration of rhizosphere dynamics (Grove et al. 2012).

Contrary to expectations, the addition of leachate from living knotweed plants did not affect the growth or survival of native species differently than tap water. One possibility for this may be that local knotweed and the soil and leachate derived from it lack the allelochemical concentration necessary to exhibit negative effects on test plants. An unpublished independent study using high performance liquid chromatography verified the presence of allelochemical emodin in local samples of knotweed root, but the concentrations have not been quantified (A. Graziano, unpublished data). Chen et al. (2013) quantified the concentration of emodin in Japanese knotweed from both China and Canada and found that Chinese samples contained 1.3 times more emodin than Canadian samples. This finding advances the idea that there is significant global variation in allelochemical concentration even within a single species. Perhaps the local knotweed population selected for this study produces relatively low concentrations of emodin.

In a similar study, Dommanget et al. (2014) administered a near identical knotweed leachate to Salicaceae cuttings and analyzed this leachate for phenolic compounds. They reported high concentrations of phenols in the leachate and significant inhibition of plants that received it. However, in this experiment test plants that received leachate grew no differently than control plants. According to the National Center for Biotechnology Information (2024), the one

allelochemical verified in this population, emodin is practically insoluble in water – meaning that it was potentially only transferred from the donor pots at low concentrations. Several studies have found success in isolating allelochemicals by preparing extracts directly from plant leaves or rhizomes (Cipollini et al. 2008b, Abgrall et al. 2018). Future studies should optimize the preparation of an allelochemical leachate or extract from this population.

Another mechanism that may have interfered in the transfer of allelochemicals is soil biota. A 2010 study by Lankau explored the allelopathic effects of garlic mustard and found allelopathic inhibition only in sterilized soils – suggesting that soil biota contained in the other soils may have degraded the allelochemicals. If soil microbes were present in the soil from Northampton Park, they could have neutralized the influx of allelochemicals from both the leachate and the soil itself. It is not uncommon for allelopathic treatments on the same target species to yield varying results, suggesting that the strength of allelopathic inhibition may depend on the specific microclimate of the soil (Lankau 2010). Determining the soil compositions that reduce or eliminate Japanese knotweed allelopathy could lead to a greater understanding of its invasion success. Further research should analyze the nutrient content, microbial communities, and soil composition at this site.

It is widely accepted that native plants are particularly sensitive to allelochemicals because they have not evolved to tolerate them (Dommanget et al. 2014, Cipollini and Greenawalt Bohrer 2016). A growing hypothesis in allelopathy research is that natives may be more resistant to allelochemicals produced by members from the same plant family, but evidence for this is two-sided. In 2008, Cipollini et al. found no effect of garlic mustard's allelochemicals on thale cress (*Arabidopsis thaliana* L.), another member of Brassicaceae, which was attributed to their evolutionary relationship and shared physiology. But a later study investigating invasive

lesser celandine (*Ficaria verna* Huds.) allelopathy found no such family-specific resistance for other Ranunculaceae species (Cipollini and Flint 2013).

Considering that knotweed soil and leachate lacked negative effects on all test species equally, it makes sense that either all or none of these species possess an allelochemical tolerance. *P. virginiana* belongs to the same family as Japanese knotweed, Polygonaceae, *A. incarnata* is closely related to *Asclepias syriaca* L., which is known to produce allelochemicals in its invaded range, and *C. racemosa* belongs to the same genus and family as the allelopathic *Cornus controversa* Hemsl. (Popov et al. 2021, Chen et al. 2024). While *P. virginiana* is the only test species that could potentially possess a family-specific tolerance to knotweed allelopathy, it is possible that *A. incarnata* and *C. racemosa* have had separate phylogenetic exposure to some of the same compounds.

This study contradicts previous work on Japanese knotweed's allelopathic potential. My results suggest that allelopathy may not be directly contributing to local knotweed invasions. Since native plants potted in knotweed soil experienced no negative effects, I conclude that allelochemicals from this population present little to no threat to restoration efforts. Considering the global variation of allelochemical concentrations in Japanese knotweed, I propose that knotweed does not utilize allelopathy as an invasion mechanism equally across the species' range. Understanding regional differences in knotweed's invasion strategy can help inform land managers on the best practices for restoring areas invaded by knotweed and re-establishing native communities.

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Tables and Figures

Table 1. Ordinal scale used to describe the percentage of herbivory on test plant leaves.

Score	Herbivory (%)
0	0
1	<1
2	1-10
3	11-25
4	26-50
5	>50

Table 2. Ordinal scale used to describe the overall, health, growth, and reproductive phenology of test plants.

Score	Condition
0	Dead
1	Dying
2	Healthy- no growth
3	Healthy- new growth
4	Healthy- reproductive

Table 3. The number of individuals alive on day 154, the number of individuals alive on day 214, and the number of reproductive individuals on day 214 respectively.

Treatment	<i>Asclepias incarnata</i>	<i>Cornus racemosa</i>	<i>Persicaria virginiana</i>
Control	10, 10, 0	10, 9, 0	10, 10, 3
Leachate	10, 10, 0	10, 9, 0	10, 10, 5
Knotweed soil	10, 10, 0	10, 7, 0	10, 10, 5

Table 4. Results of Kruskal Wallis analyses ran on *Asclepias incarnata* data with treatment as the factor. All methods adjusted for ties. Significance differences indicated with (*).

Response	DF	H value	P value
Soil moisture (%)	2	2.42	0.298
Growth (cm)	2	6.26	0.044*
Number of leaves	2	0.15	0.926
Shoot biomass (g)	2	12.16	0.002*
Root biomass (g)	2	13.13	0.001*
Root:shoot ratio	2	4.67	0.097
Specific leaf area (mm ² /mg)	2	0.05	0.976

Table 5. Results of Kruskal Wallis analyses ran on *Cornus racemosa* data with treatment as the factor. All methods adjusted for ties.

Response	DF	H value	P value
Soil moisture (%)	2	0.26	0.878
Growth (cm)	2	2.54	0.281
Number of leaves	2	3.89	0.143
Shoot biomass (g)	2	1.99	0.370
Root biomass (g)	2	3.07	0.215
Root:shoot ratio	2	0.26	0.877
Specific leaf area (mm ² /mg)	2	1.45	0.484

Table 6. Results of Kruskal Wallis analyses ran on *Persicaria virginiana* data with treatment as the factor. All methods adjusted for ties. Significant differences indicated with (*).

Response	DF	H value	P value
Soil moisture (%)	2	0.88	0.644
Growth (cm)	2	1.41	0.495
Number of leaves	2	1.13	0.569
Shoot biomass (g)	2	6.20	0.045*
Root biomass (g)	2	2.79	0.247
Root:shoot ratio	2	3.22	0.200
Specific leaf area (mm ² /mg)	2	0.33	0.849

Table 7. Mean largest leaf length and width (cm) of test plants on day 214. Standard error given in parentheses.

Treatment	Largest leaf (cm)	<i>Asclepias syriaca</i>	<i>Cornus racemosa</i>	<i>Persicaria virginiana</i>
Control	Length	7.48 (0.29)	4.60 (0.39)	14.73 (0.52)
	Width	1.35 (0.11)	2.19 (0.22)	7.32 (0.30)
Leachate	Length	7.98 (0.20)	5.46 (0.28)	14.72 (0.51)
	Width	1.40 (0.09)	2.53 (0.19)	6.85 (0.29)
Knotweed soil	Length	8.49 (0.39)	4.93 (0.72)	13.63 (0.50)
	Width	1.55 (0.08)	2.37 (0.32)	7.01 (0.29)

Table 8. Mean specific leaf area ($\text{mm}^2 \text{mg}^{-1}$) of test plants on day 214. Standard error given in parentheses.

Treatment	<i>Asclepias syriaca</i>	<i>Cornus racemosa</i>	<i>Persicaria virginiana</i>
Control	2.572 (0.048)	4.161 (0.097)	12.499 (0.454)
Leachate	2.580 (0.065)	3.986 (0.169)	13.080 (0.448)
Knotweed soil	2.693 (0.073)	4.326 (0.156)	12.482 (0.364)



Figure 1. USDA web soil survey map showing the sites of knotweed (grey) and control (white) soil collection at Northampton Park, Monroe Co., New York.



Figure 2. *Asclepias incarnata* test plants on day 187.



Figure 3. *Cornus racemosa* test plants on day 154.



Figure 4. *Persicaria virginiana* test plants on day 177.

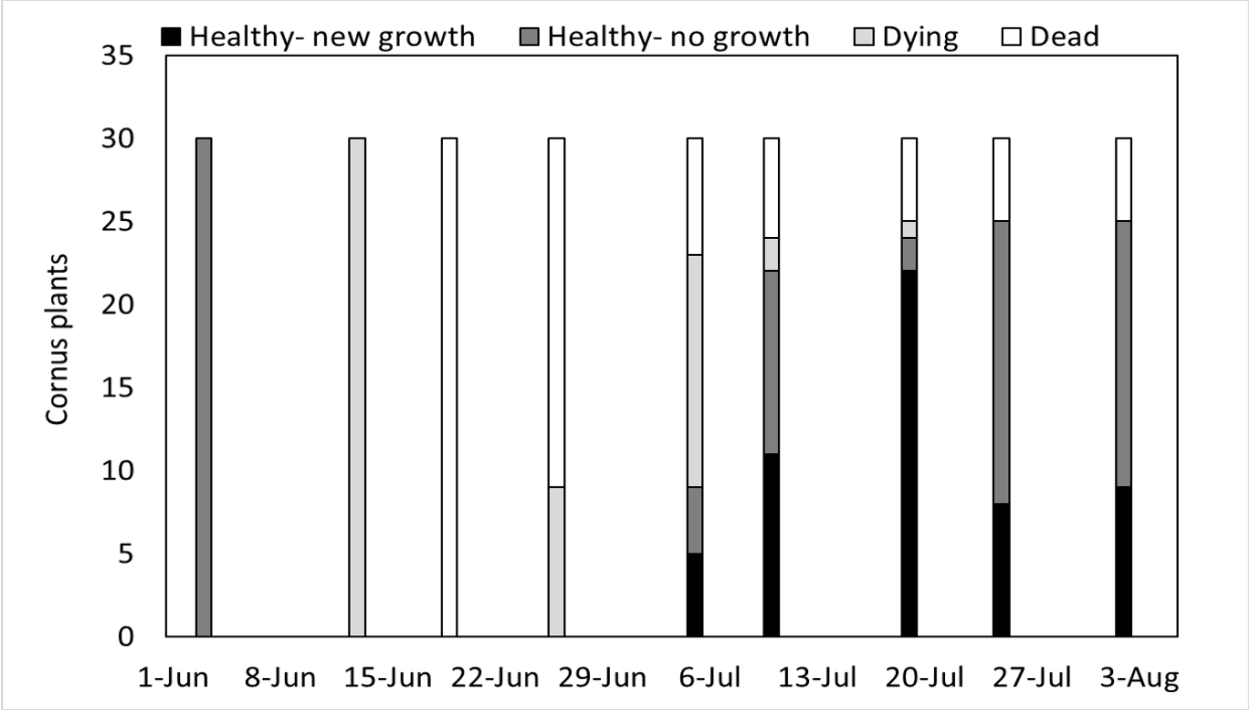


Figure 5. The overall condition of *C. racemosa* plants throughout the treatment period.

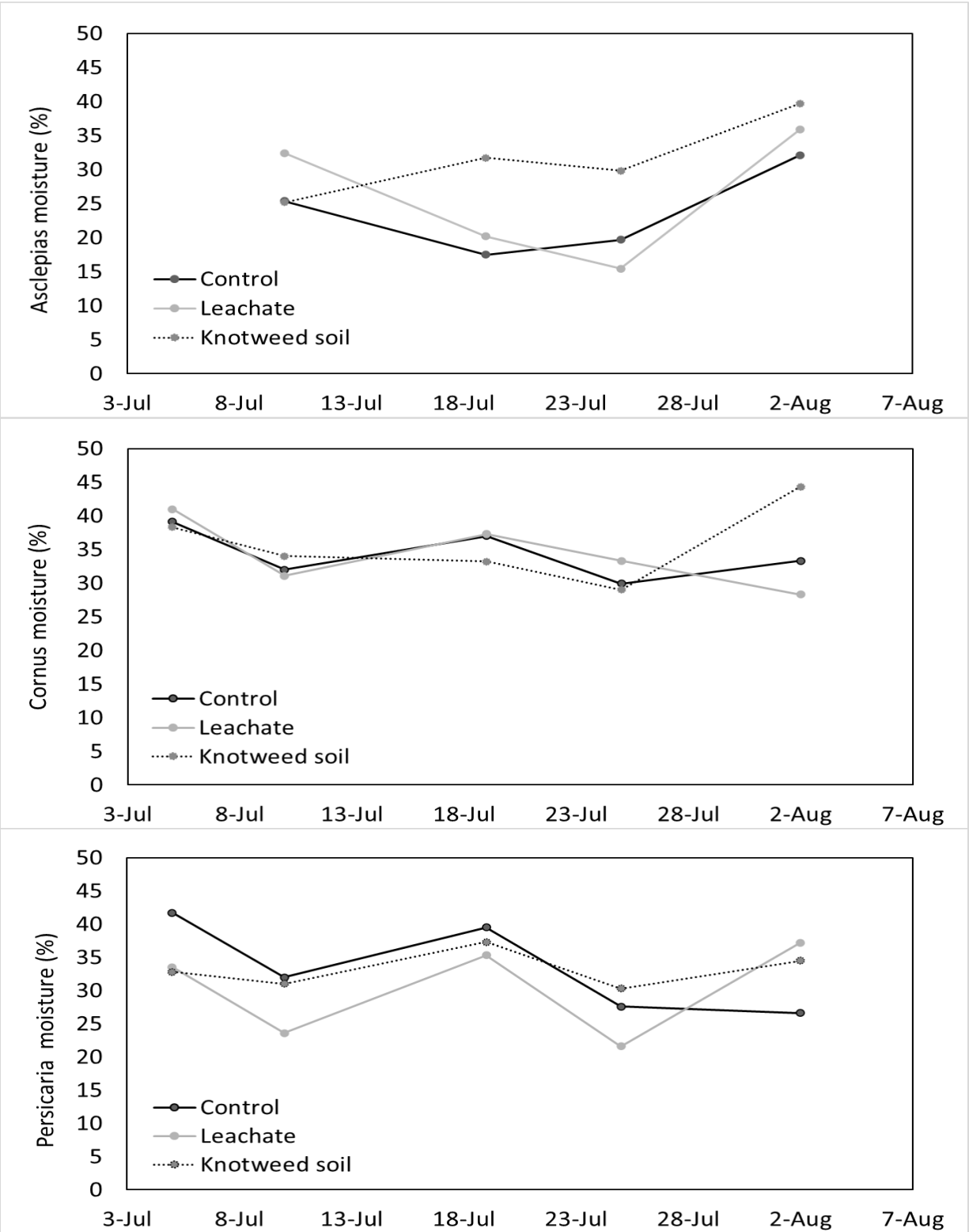


Figure 6. Mean percent soil moisture of *A. incarnata* (A), *C. racemosa* (B), and *P. virginiana* (C) pots throughout the treatment period.

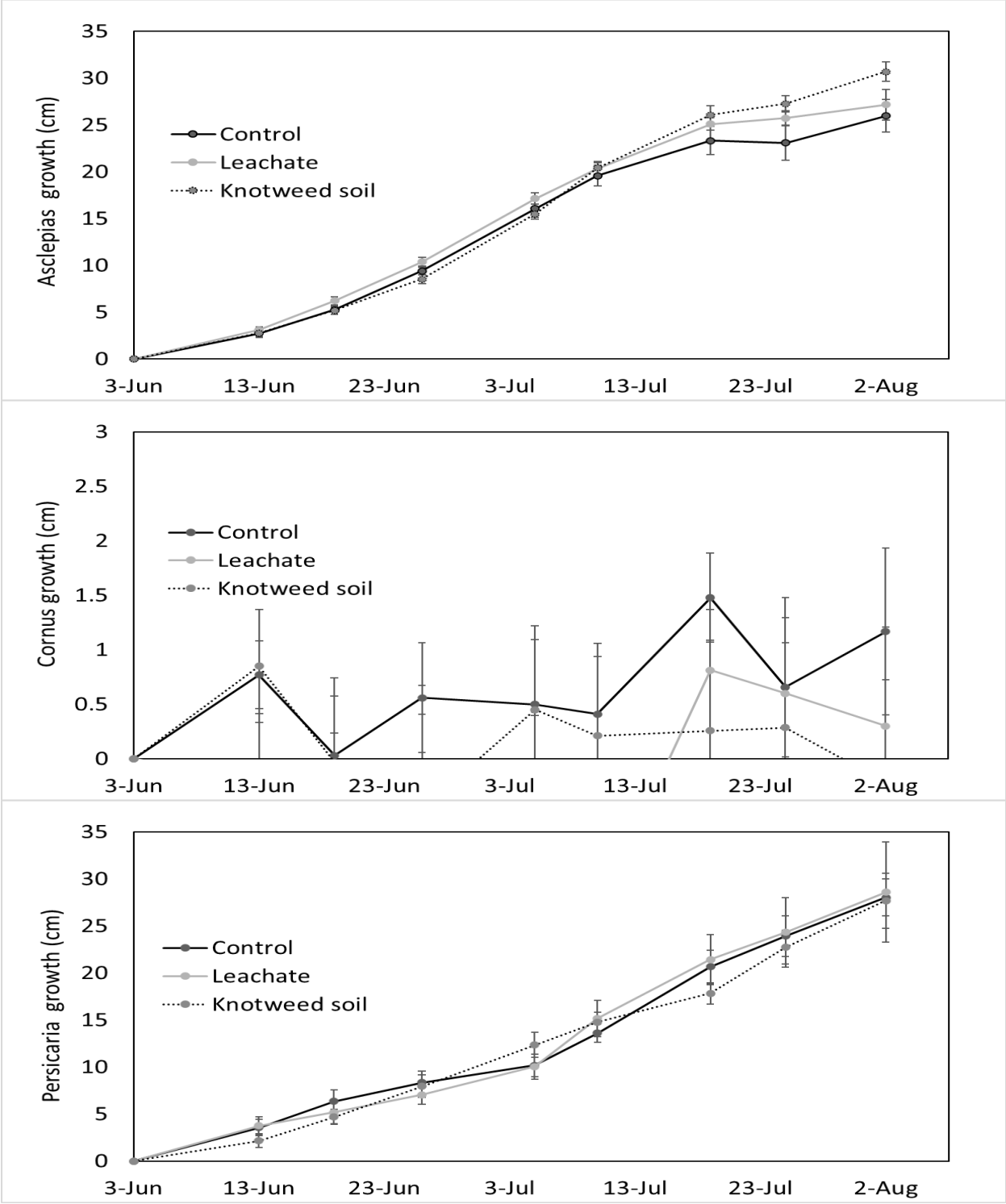


Figure 7. Mean growth of *A. incarnata* (A), *C. racemose* (B), and *P. virginiana* (C) plants based on sequential measures of plant height (cm). Error bars indicate standard error. Note differences in y-axis scaling.

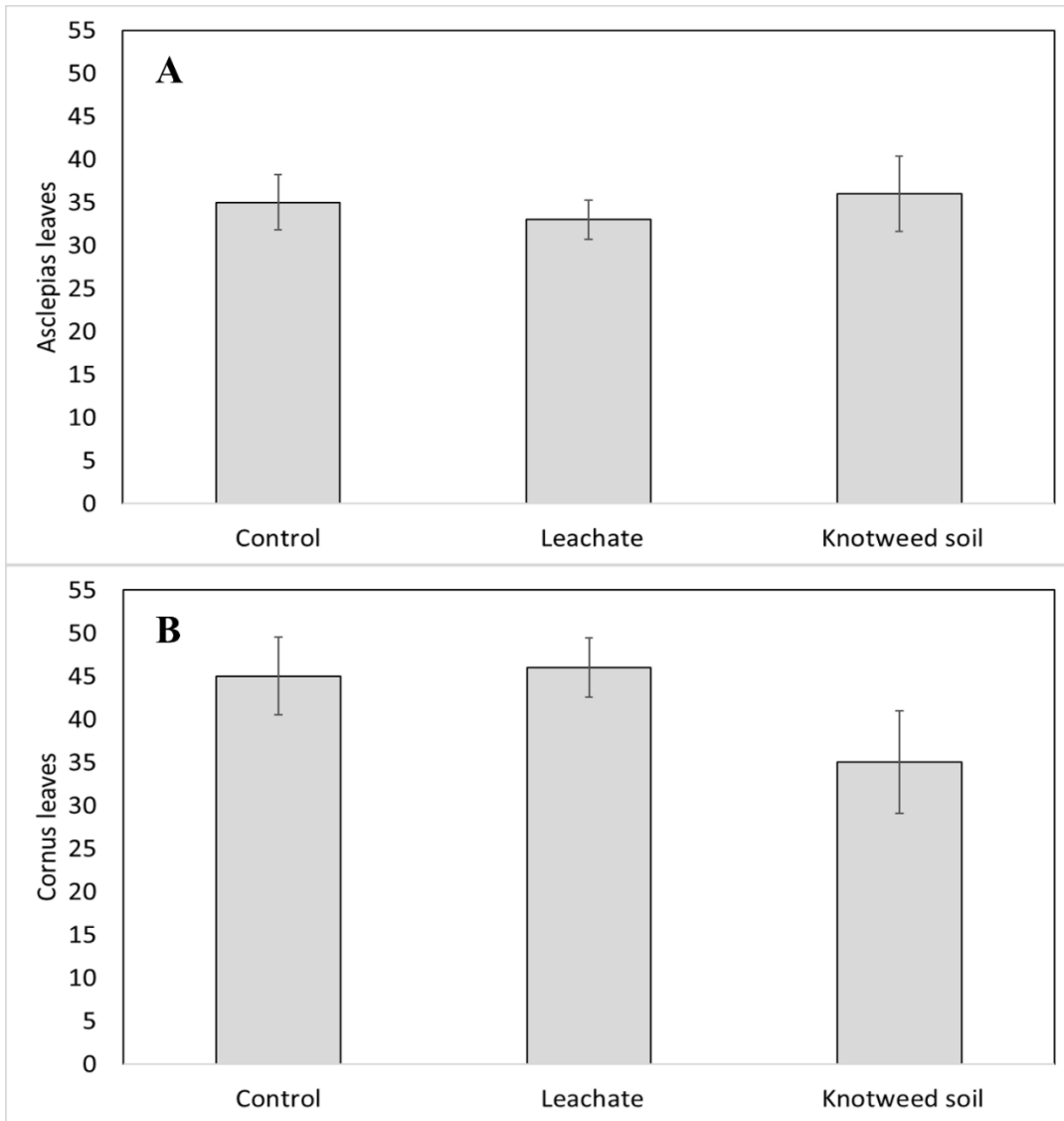


Figure 8. Mean number of leaves for *A. incarnata* (A) and *C. racemosa* (B) plants on day 214.

Error bars indicate standard error.

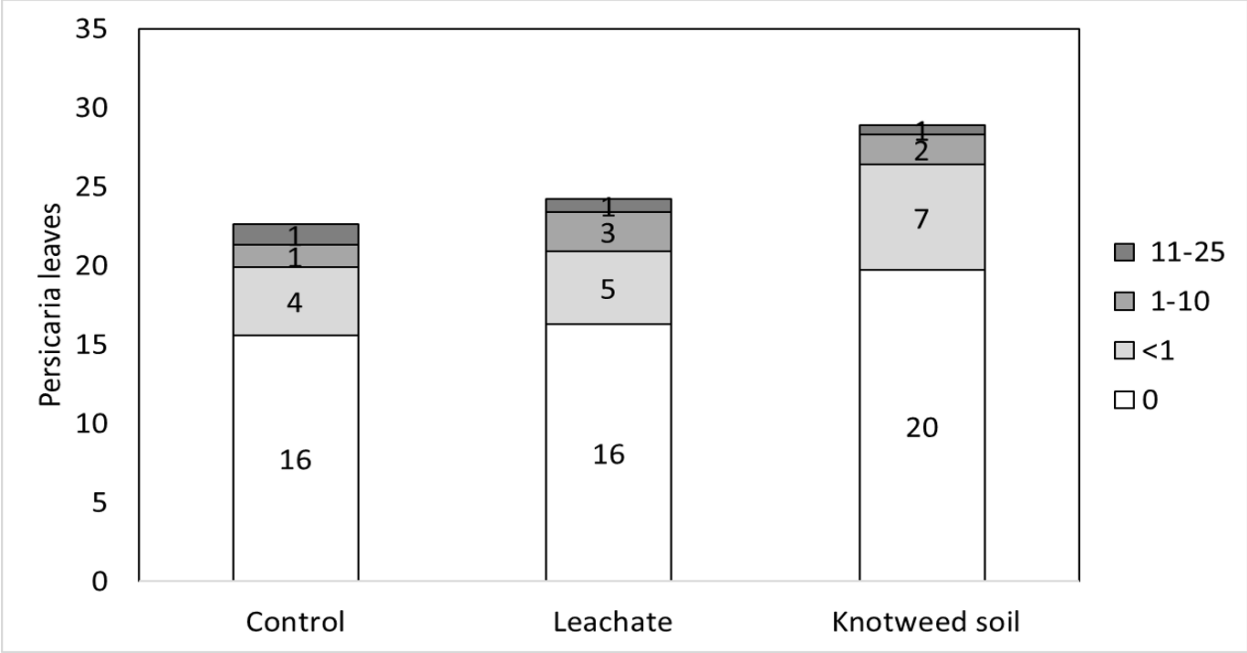


Figure 9. Mean percent herbivory and number of leaves of *P. virginiana* plants on day 214.

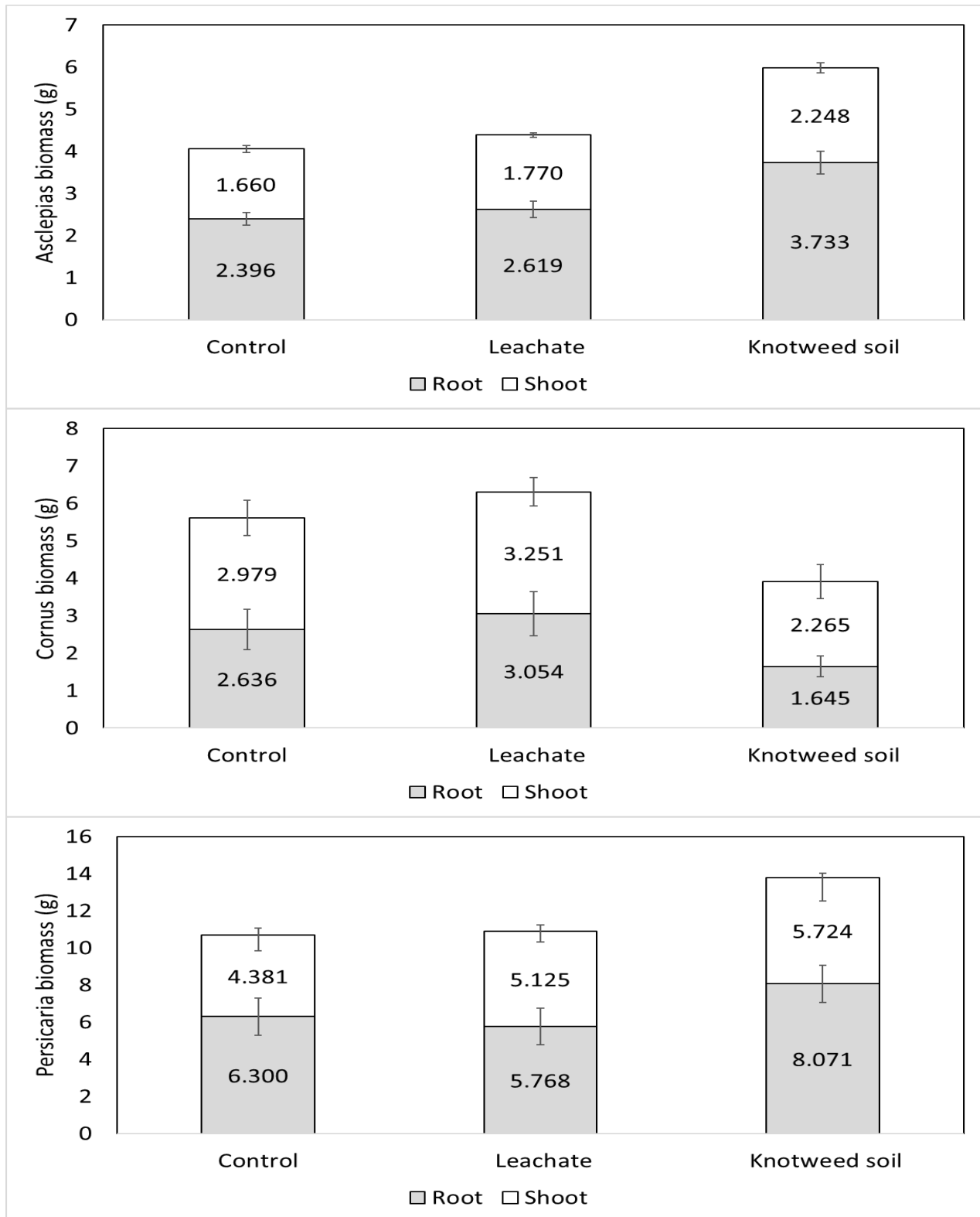


Figure 10. Mean biomass (g) of *A. incarnata* (A), *C. racemosa* (B), and *P. virginiana* (C) plants on day 214. Error bars indicate standard error.