Host tree circumference, litter depth, and slope affect snow plant (Sarcodes sanguinea) size at James San Jacinto Mountain Reserve

Sean Hinson¹, Mina Sadek²

¹University of California, Santa Barbara; ²University of California, Riverside

Plant-ectomycorrhizal relationships dominate temperate forest ecosystems, and for many Pinaceae tree species, this mutualistic relationship is an essential aspect of their ability to gather nutrients. The snow plant (Sarcodes sanguinea) is an ectomycorrhizal parasite that steals nutrients from host trees by accessing their root systems via the mycorrhizae. We examine microhabitat variables that affect snow plant size and phenology by measuring 51 snow plants within the James San Jacinto Mountain Reserve. We investigate the effects of distance to the largest host tree, its circumference, the depth of litter surrounding the snow plants, and the slope compared to the snow plants’ sizes and the number of reproductive parts. We find an association between larger snow plants to larger host trees. A relationship between deeper litter and steeper slopes with larger snow plants is also found and discussed. None of the microhabitat variables we tested are found to influence the number of reproductive structures. The interconnected system of the snow plant, ectomycorrhizae, and tree involves organismal specificity that can have subsequent evolutionary influences in their ecosystems.

Keywords: snow plant (Sarcodes sanguinea), Monotropes (Monotropoideae), Rhizopogon ellenae, Pinaceae, ectomycorrhizae.

INTRODUCTION

The over four thousand species of various parasitic plants have been shown to have profound impacts on the ecosystems in which they are found (Press & Phoenix 2004). A parasitic plant obtains all or some of its nutritional requirements from a host plant in a relationship that provides no apparent benefit to the host. Many of these parasitic plants become specialized and exhibit a high degree of host-specificity (Press & Phoenix 2004).

Species within the Monotropoideae subfamily display host-specificity to ectomycorrhiza fungi (ECM) (Kretzer et al. 200; Press & Phoenix 2004; Bidartondo & Bruns 2002). They indirectly parasitize trees by exploiting a mutualistic relationship between the tree and the ECM (Bidartondo & Bruns 2002). In this way, the mycorrhizae act as a fungal intermediate between the host and the parasite.

The largest Monotropoideae species, Sarcodes sanguinea, commonly known as the snow plant (Bidartondo et al. 2000; Kretzer et al. 2000), obtains its nutrients through association with a specific ectomycorrhiza, Rhizopogon ellenae. In addition, R. ellenae grows in close
association with the roots of the pine family (Family: *Pinaceae*) (Cullings et al. 1996). Modification to components of this three-unit relationship between *S. sanguinea*, *Rhizopogon*, and *Pinaceae* results in consequent effects to other trophic relations involving the *Pinaceae* host and the *S. sanguinea* parasite (Press & Phoenix 2004).

Changes in the microhabitat of *S. sanguinea* constitute a modification to the three-unit relationship. A comparable parasitic species, *Nematoda*, shows an extreme specificity to the microhabitat conditions it prefers to reside in (Adamson & Caira 1994). This leads us to believe that, likewise, the parasitic *S. sanguinea* has preferential microhabitat conditions.

By measuring microhabitat factors surrounding snow plants, we aim to determine conditions affecting their growth and phenology. Phenological events include the sprouting and number of reproductive parts (Dubé et al. 1984). The microhabitat of a snow plant includes the host tree, surrounding leaf litter, and the slope of the ground. A deeper amount of leaf litter will favor larger snow plants that are better suited to “breaking through” it. Trees on steeper slopes will have fewer resources to grow due to water runoff and soil erosion (Goenadi 1987), thus providing the parasitic snow plant with fewer resources to grow. As in Vreeland et al. 1980, we assume that larger trees will have more extensive root networks resulting in a more established mass of ECM capable of supporting larger snow plants with a higher number of reproductive parts. This assumption also leads us to predict that bigger snow plants with a greater number of reproductive parts will be found closer to larger trees. We also predict that a greater depth of leaf litter will surround larger snow plants. Additionally, we expect to find smaller snow plants on steeper slopes.

**METHODS**

2.1 Site

This study was conducted at the James San Jacinto Mountain Reserve (James Reserve), located in Idyllwild-Pine Cove, San Bernardino County, California (33.81, -116.78). The James Reserve is located on a steep slope and consists of mixed conifer and hardwood forests, montane riparian forests, and montane chaparral habitats. These communities host various flora, including Sierra mixed conifer riparian forest, oak woodlands, and alder-willow-cedar riparian forest. Species of pines found on the reserve are *Pinus lambertiana*, *Pinus jeffreyi*, *Pinus coulteri*, and *Pinus ponderosa*. The study was conducted from May 6 to 9, 2021.

2.2 Observational Study.

We walked around the reserve trails and systematically surveyed every snow plant we found, not including those surrounding the same tree. In total, 51 snow plants were measured. We used a ruler to measure snow plant height and calipers to measure the width. Only snow plants over five centimeters above the soil were included in this study because snow plants under that limit were considered not mature enough in terms of their phenology. We also counted the number of reproductive parts (buds and flowers) appearing from the snow plants as a measure of their phenological maturity. When multiple plant stems were found, we standardized them by measuring only the tallest stem.
A transect tape was used to measure the circumference of the largest tree within a five-meter radius and the distance to it. This tree was assumed to function as the host for *S. sanguinea* (Vreeland et al. 1980). We also identified the species of the tree.

Additionally, the litter depth surrounding the snow plants was measured by sticking a ruler into the litter at four sides around the snow plant, five centimeters away at each side, until the soil underneath was reached. The average of the four values was used in our analysis. Finally, we used a compass to measure the incline of the slope that the snow plants were on.

### 2.3 Statistical Analysis.

We ran two multivariate multiple regressions. The first multivariate multiple regression observed the effects of slope and litter surrounding the snow plants on their size and phenology. The second multivariate multiple regression observed the effect of distance to the largest tree and its circumference on the size and phenology of snow plants. All statistical analyses were performed in JMP® Pro Version 16 (JMP®, 2021).

**RESULTS**

Several of the microhabitat variables we measured affected the size of snow plants. Larger trees were proximal to larger snow plants (Whole model: N = 51; LogWorth = 2.178; p = 0.00663; Fig. 1). Larger snow plants were surrounded by deeper litter (N = 51; LogWorth = 1.640; p = 0.02291; Fig. 2) and found at steeper slopes (N = 51; LogWorth = 3.372; p = 0.00043; Fig. 3). No microhabitat variable we measured impacted the number of a snow plant’s reproductive parts. Distance to host trees did not impact snow plants’ size or phenology. A detailed description of the results can be found in Table 1.

Most of the snow plants we surveyed (63%) were found near Jeffrey pine trees (*Pinus Jeffreyi*), but no measurement of the abundance of Jeffrey pines was made to determine if a relationship exists between the two species. 84.3% of the snow plants surveyed were on south-facing slopes.

**DISCUSSION**

Our prediction that larger snow plants with a greater number of reproductive parts would be found closer to larger trees was partially supported. Although larger trees were found to have larger associated snow plants, no relationship was found between the distance to the tree and snow plant size. We also expected to find more reproductive parts on snow plants located proximally to larger trees. However, the number of reproductive parts was not affected by the host tree’s proximity or size.

While our results do not indicate a relationship between the number of reproductive parts observed on the plant with any of the microhabitat variables we measured, further research is needed to determine microhabitat factors that can impact the phenology of snow plants. Interspecies competition between mycorrhiza has been shown to reduce the abundance of the mycorrhizal fungi involved (Engelmoer et al. 2013), which could affect snow plant phenology. There may be variables affecting the associated ECM’s fitness and the host plant’s resources that could affect the snow plant.
Figure 1. The effect of host tree circumference on snow plant width. We measured the circumference of the largest host tree in proximity to the snow plant and compared it to the width of the associated snow plant at James Reserve. The width was determined to be an indicator of the size of the snow plants. Larger snow plants tended to be associated with larger trees (N = 51; $R^2$=0.12; p<0.005).

Figure 2. The effect of litter depth surrounding the snow plant on its width. We measured the depth of litter surrounding the snow plants using a ruler and compared it to the width of the associated snow plants at James Reserve. The width was determined to be an indicator of the size of the snow plants. Larger snow plants tended to be found at sites with deeper litter (N = 51; $R^2$=0.33; p<0.05).
**Figure 3. The effect of slope on snow plant width.** We measured the incline of the slopes that the snow plants were on and compared them to the width of their associated snow plants at the James Reserve. The slope was determined to be an indicator of the size of the snow plants. Larger snow plants tended to be found at steeper slopes ($N = 51; R^2=0.30; p<0.005$).

**Table 1. Multivariate multiple regressions outputs.** We surveyed 51 snow plants at the James Reserve. We compared the microhabitat variables to the snow plant variables through two multivariate multiple regressions. The first compares distance to largest tree and its circumference to snow plant variables (height, width, number of reproductive parts). The second compares litter depth and slope to snow plant variables (height, width, number of reproductive parts). A combination of all the results is shown in the table. Statistically significant results are marked with an asterisk.

<table>
<thead>
<tr>
<th></th>
<th>Circumference of largest tree (m)</th>
<th>Distance to largest tree (m)</th>
<th>Litter Depth (cm)</th>
<th>Slope (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. sanguniea</strong></td>
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<tr>
<td>height (cm)</td>
<td>$N = 51$</td>
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<td>$R^2 = 0.23$</td>
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<td></td>
<td>$p &lt;0.01^*$</td>
<td>$p = 0.69$</td>
<td>$p &lt;0.05^*$</td>
<td>$p &lt;0.05^*$</td>
</tr>
<tr>
<td><strong>S. sanguniea</strong></td>
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</tr>
<tr>
<td>width (cm)</td>
<td>$N = 51$</td>
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<td></td>
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<td>$R^2=0.12$</td>
<td>$R^2=0.33$</td>
<td>$R^2=0.30$</td>
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<td></td>
<td>$p &lt;0.005^*$</td>
<td>$p = 0.46$</td>
<td>$p &lt;0.05^*$</td>
<td>$p &lt;0.005^*$</td>
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<tr>
<td><strong>Number of reproductive parts on S. sanguniea</strong></td>
<td>$N = 51$</td>
<td>$N = 51$</td>
<td>$N = 51$</td>
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<td></td>
<td>$R^2 = 0.16$</td>
<td>$R^2 = 0.16$</td>
<td>$R^2=0.0039$</td>
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<tr>
<td></td>
<td>$p = 0.76$</td>
<td>$p = 0.93$</td>
<td>$p = 0.67$</td>
<td>$p = 0.98$</td>
</tr>
</tbody>
</table>
We based our initial prediction involving tree distance and size with the perception that the root system of a larger tree would feature more ECM, thus facilitating a greater degree of nutrient acquisition for the snow plant. However, we did not account for the niche specializations of *Rhizopogon* fungi involving multiple mutualistic root systems from neighboring trees (Massicotte 1994). Thus, optimal conditions for increased snow plant size may occur between several host trees where these particular *Rhizopogon* niches reside instead of nearby any specific host tree.

Confirmed by our prediction that a deeper amount of leaf litter will favor larger snow plants, we can also propose that deeper leaf litter allows for increased moisture and nutrient retention (Luo & Sims 1999), thus providing a more advantageous environment for the mutualistic relationship between the tree and ECM for the snow plant to parasitize.

One possible explanation for finding larger snow plants on steeper slopes is that plants located on steeper, south-facing slopes in the northern hemisphere receive more sunlight (Barbour et al. 1999). Increased exposure to sunlight and a higher rate of photosynthesis could be beneficial to the snow plants’ acquisition of nutrients from the host plant. For example, in a study conducted by Koike et al. 2009, pine needles from trees on south-facing slopes contained more nitrogen and chlorophyll than trees on north-facing slopes. Future studies may seek to explore the relationship between a host tree’s photosynthetic rate with the snow plant’s size and phenology. A second explanation could be the presence of more ECM on steeper slopes to help the mutualistic tree with the acquisition of water and nutrients to mitigate the effects of water runoff and soil erosion on steeper slopes.

Microhabitat effects on *S. sanguinea* may affect *Rhizopogon* and *Pinus* due to the interconnection between them. Conditions that are favorable for *S. sanguinea* will allow for more resource acquisition from the host tree, providing favorable conditions for the pollinators of *S. sanguinea*. On the other hand, due to their interconnection, herbivory on the host tree could reduce the fitness of the tree, leaving fewer resources for the snow plant to acquire. In addition, this three-unit system is complex due to the host selectivity of two very different organisms. Specialization involving parasites could result in evolutionary pressures on hosts in the ecosystems they reside in. Future studies involving other multi-unit relationships may benefit from using the snow plant as a model system for microhabitat factors affecting associated organisms.

**ACKNOWLEDGMENTS**

This study was performed at the University of California, Riverside James San Jacinto Mountains Reserve, doi:10.21973/N3KQ0T We want to extend our gratitude to reserve manager Jennifer Gee, for her help with and accommodation of this research. We thank our instructors, Tim Miller and Sarah Kingston, for their guidance throughout this research. Additional thanks to Luis Orozco Sanchez and Benjamin Early for their aid in pine tree identification.

**REFERENCES**


