



# Nighttime stomatal conductance and transpiration in *Castilleja* spp., a genus of root hemiparasites



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## BACKGROUND

### Nighttime stomatal conductance and transpiration

We assume plants do not transpire at night because stomata close in the dark. Darkness halts crucial aspects of photosynthesis and consequently stomatal closure limits water loss when there is no carbon to be gained<sup>1</sup>. However, nighttime stomatal opening and consequent nighttime transpiration has been observed across many taxa and ecosystems<sup>2–4</sup>. Various explanations have been proposed for why nighttime transpiration occurs, both adaptive and non-adaptive, including nutrient acquisition, leaky stomata, and delivery of O<sub>2</sub> to parenchyma<sup>4–8</sup>, however, nighttime transpiration remains poorly understood and often ignored<sup>9</sup>.

### Why would parasitic plants open their stomata at night?

#### 1. A unique carbon–water tradeoff:

Parasitic plants are partially released from two of the major drivers of stomatal closure at night:

- Inability to gain carbon in darkness: parasites also derive C from their host
- Water conservation: water can be relatively “cheap” for parasites because they can steal directly from a host rather than build their own extensive root systems.

#### 2. Nutrient acquisition:

This is a possible explanation for nighttime transpiration in non-parasites, which could also drive nighttime transpiration in hemiparasites<sup>8–12</sup>.

Hemiparasites typically only have access to host xylem from which they acquire water as well as dilute C and nutrients<sup>13,14</sup>. The N parasitism hypothesis: hemiparasites are N limited and N acquisition via the host xylem stream drives observed high transpiration rates and resulting low water-use-efficiency (WUE)<sup>15</sup>.

N limitation would lead to nighttime transpiration because N supply via the host xylem stream is unaffected by daylight (as is N supply in a non-parasitic plant).

### How are stomatal conductance and transpiration related?

Stomatal conductance ( $g_s$ ) is evidence of transpiration but is not directly equal to transpiration (E). E is a function of  $g_s$  and the vapor pressure deficit (VPD) between the leaf and air, canopy structure and atmospheric mixing. VPD is controlled by temperature and relative humidity and is typically lower at night. Given the same  $g_s$ , actual transpirational water loss will typically also be lower at night than during the day<sup>5</sup>.



## METHODS

### Stomatal Conductance Measurements

Using a decagon porometer I measured the stomatal conductance of each individual during daytime and nighttime. At each time, I measured 3 leaves/individual, recording the stomatal conductance, air temperature and time. Daytime and nighttime measurements for each site were made within 48 hours of each other. Nighttime was defined as past astronomical twilight. In cases where the leaf did not cover the porometer aperture (diameter: 6.35 mm), I made a typical measurement and subsequently scaled the measurement up, proportionate to the leaf area within the aperture. To do this, I collected and scanned the leaves then digitally analyzed the leaf area using the LeafArea package in R<sup>16</sup>.

### Statistics

I analyzed the data from all the *Castilleja* spp sites together, except *C. aplegatei* ssp. *pinetorum* because the differing collection protocols resulted in a different data structure. For both sets of data I used a type III ANOVA on a linear mixed effects model fit by restricted maximum likelihood estimation on log-transformed data<sup>17</sup>. I used single term deletions (F-tests) and compared AIC and BIC scores to determine the best fitting model<sup>18</sup>. The final model for *C. aplegatei* ssp. *pinetorum* had PlantType, Temperature, DayVsNight, and the interaction between DayNight and PlantType as fixed effects and Location (i.e. transect), plot, and individual plant as nested random effects to account for spatial autocorrelation as well as repeated measures. PlantType here is a factor combining species and presence of a parasite (+P), resulting in seven levels. I then conducted pairwise comparisons on estimated marginal means using a Turkey adjustment for multiple comparisons of means<sup>19</sup>. For the other seven sites, the final model had DayVsNight, PlantType, Site and all interactions as fixed effects and Plot and Individual Plant as nested random effects to deal with spatial autocorrelation and repeated measures. PlantType here is a factor with three levels: 1) Parasite, 2) Non-Parasite w/o parasite neighbor and 3) Non-Parasite with parasite neighbor. Each Site represents a different location and combination of species, identified in the results by the species of *Castilleja* at the site. I then conducted planned contrasts on estimated marginal means to compare the effects of DayVsNight and PlantType within each site, again using a Tukey adjustment.

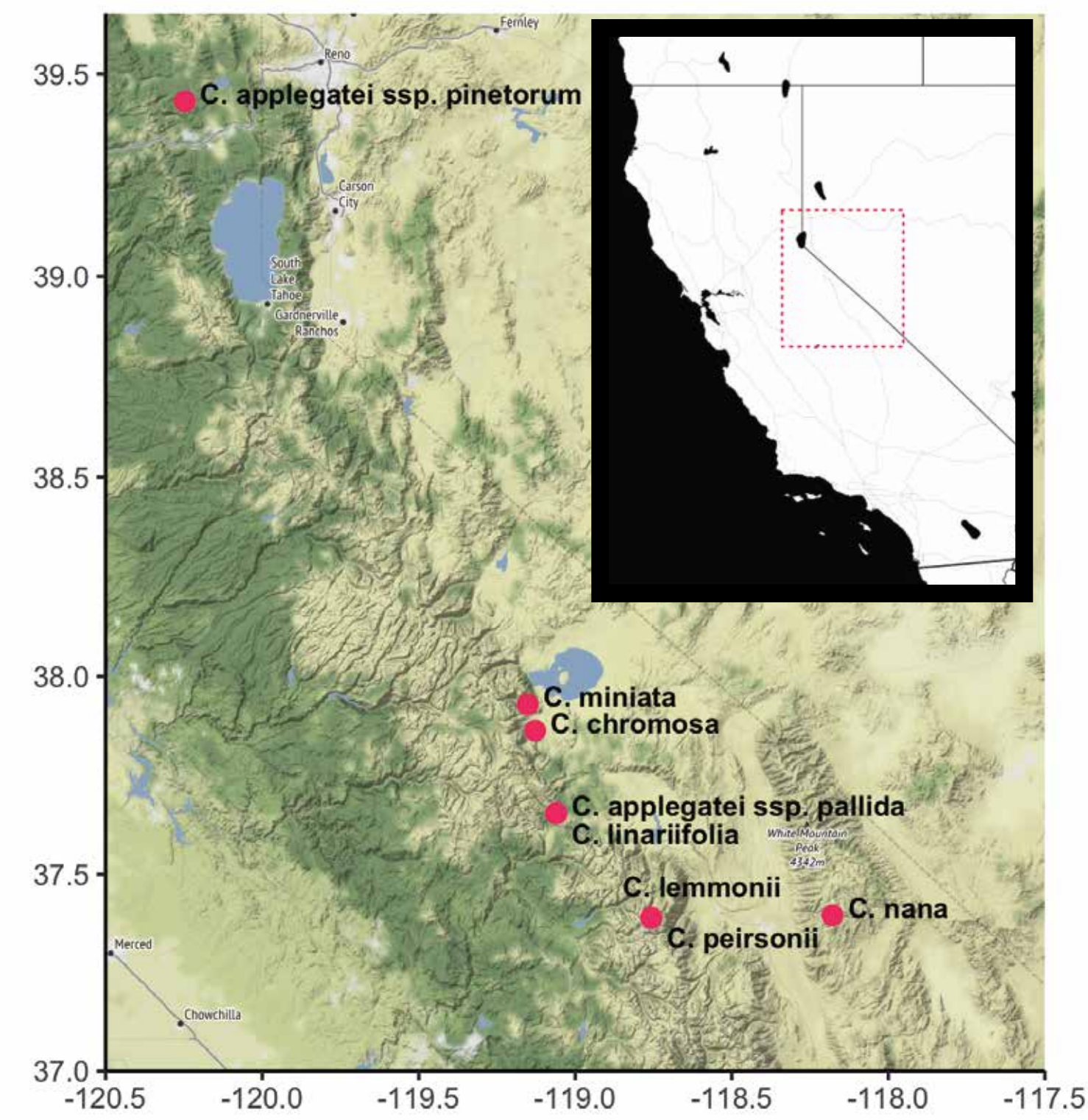


Figure 1: Map of field sites

## SUMMARY

Here I present evidence of nighttime transpiration in eight *Castilleja* species/subspecies (family: Orobanchaceae), a widespread genus of root hemiparasites. On average, nighttime stomatal conductance in *Castilleja* spp was 235% higher than in non-parasites, adding *Castilleja* spp to the growing group of plants known to open stomata at night.

## METHODS

### Species and field sites

I measured daytime and nighttime stomatal conductance ( $g_s$ ) in eight species or subspecies of *Castilleja* (Paintbrush or Owl's Clover), a common widespread genus in the Orobanchaceae family of root hemiparasites, each at their own site. I also compared each species to a common non-parasitic plant at each site.

I selected 18 individuals per site:

- 6 *Castilleja* sp individuals,
- 6 neighboring individuals of a common non-parasitic species
- 6 non-neighboring individuals of the same non-parasitic species

This resulted in 12 plots/site (6 *Castilleja* sp + neighbor plots, and 6 non-neighbor plots).

Neighbor was defined as the nearest neighbor for that individual *Castilleja* sp (in nearly all cases the canopies overlapped with the *Castilleja* sp individual).

Non-neighboring individuals were at least a designated distance (>1-5m depending on the size of the *Castilleja* species) from any *Castilleja* sp individuals.

Table 1: Species list and site descriptions

<i>Castilleja</i> species	Neighbor species	Habitat type
<i>C. aplegatei</i> ssp <i>pallida</i>	<i>Symphoricarpos rotundifolius</i> (Mountain snowberry)	High elevation sagebrush scrub
<i>C. aplegatei</i> ssp <i>pinetorum</i>	<i>Ceanothus prostratus</i> (Mahala mats) <i>Wyethia mollis</i> (Wooly mule's ears) <i>Artemisia tridentata</i> (Common sagebrush)	Mixed-conifer and shrubland
<i>C. chromosa</i>	<i>Artemisia tridentata</i> ssp <i>tridentata</i> (Big sagebrush)	Mid-montane sagebrush scrub
<i>C. lemmonii</i>	<i>Carex spectabilis</i> (Showy sedge)	Wet sub-alpine meadow
<i>C. linariifolia</i>	<i>Artemisia tridentata</i> ssp <i>vaseyana</i> (Mountain sagebrush)	Sub-alpine rocky slope
<i>C. miniata</i>	<i>Phleum pratense</i> (Timothy grass)	Riparian forest
<i>C. nana</i>	<i>Stenotus acaulis</i> (Stemless mock goldenweed)	Alpine plateau
<i>C. peirsonii</i>	<i>Vaccinium cespitosum</i> (Dwarf bilberry)	Wet sub-alpine meadow

*C. aplegatei* ssp *pinetorum*, was measured in July of 2015 as part of a different project with different sampling methods. Thus it has three non-parasitic neighbor species (instead of just one) and more individuals measured (60 day and 20 night *C. aplegatei*; 85 day non-parasites and 28 night non-parasites, split between the three species).

All other measurements were taken in July and August of 2019.

## RESULTS

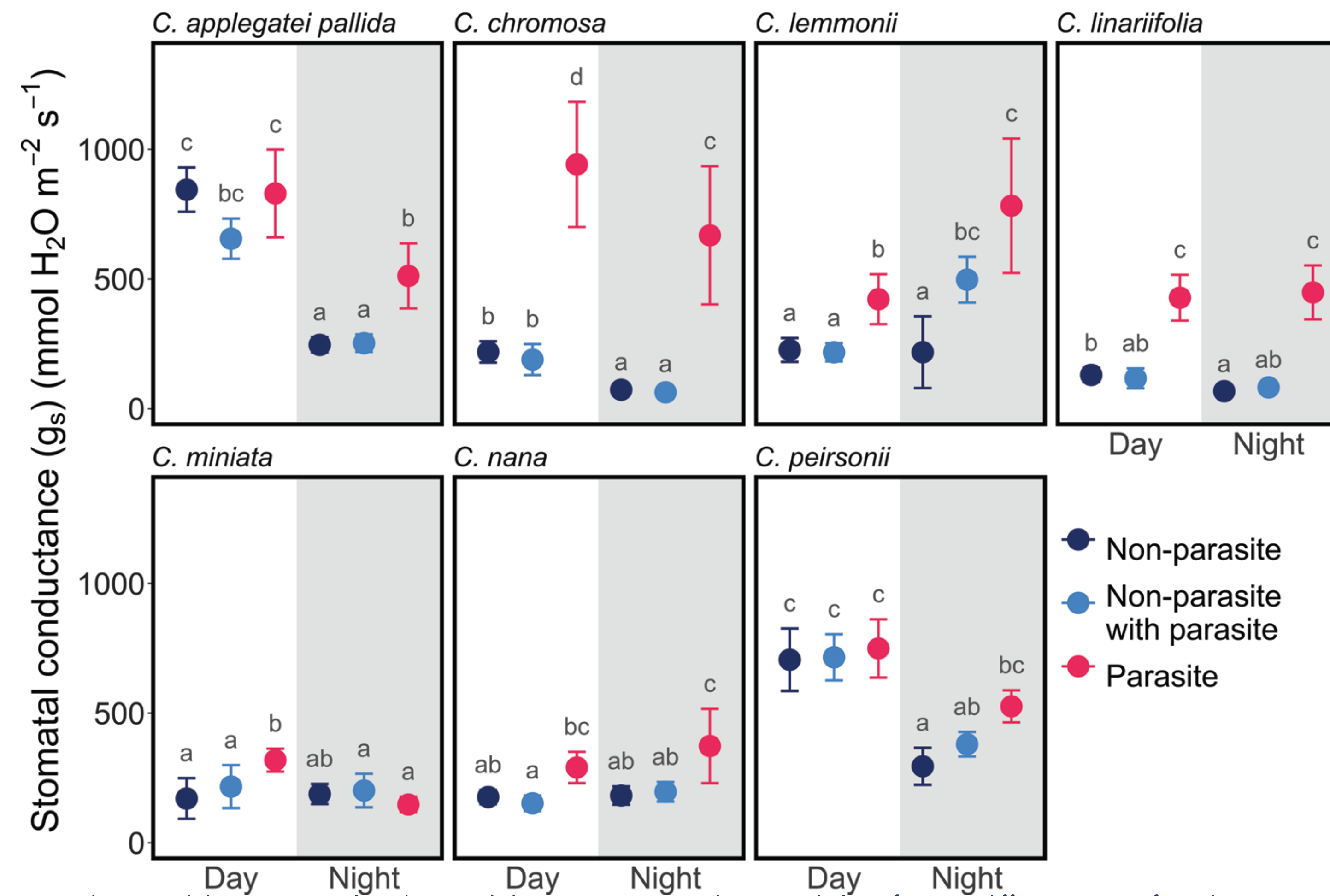


Figure 2 Nighttime and daytime stomatal conductance ( $g_s$ ) among parasites and associated plants for seven different species of root hemiparasites in the genus *Castilleja*. Each panel represents a different site with a unique parasite, non-parasite pair (see Table 1 for species list). The *Castilleja* species is shown at the top of each panel. Error bars are 95% confidence intervals. Letters represent significant differences (alpha = .05) from post-hoc Tukey adjusted pairwise comparisons within each site. Pairwise comparisons were run on a mixed effects model including all the sites.

## RESULTS

- All the plants had non-zero nighttime stomatal conductance ( $g_{\text{night}}$ ).
- $g_{\text{night}}$  in parasitic plants was similar to or exceeded daytime stomatal conductance ( $g_{\text{day}}$ ).
- $g_{\text{night}}$  in non-parasitic plants was generally lower than or similar to  $g_{\text{day}}$ .
- Parasites'  $g_{\text{night}}$  was 235% higher than the non-parasites' on average and ranged from 24% lower in *C. miniata* to 877% higher in *C. chromosa*.
- Parasitic  $g_{\text{day}}$  was mixed: On average parasites'  $g_{\text{day}}$  was 104% higher than the non-parasites'  $g_{\text{day}}$ . But the parasites'  $g_{\text{day}}$  significantly exceeded both group's of neighbors'  $g_{\text{day}}$  in only half of the species (*C. chromosa*, *C. lemmonii*, *C. linariifolia*, and *C. miniata*).
- There was little difference between the two groups of non-parasites: those neighboring a parasite and those without a parasite neighbor.
- Temperature and soil moisture did not play a large role in the  $g_s$ .

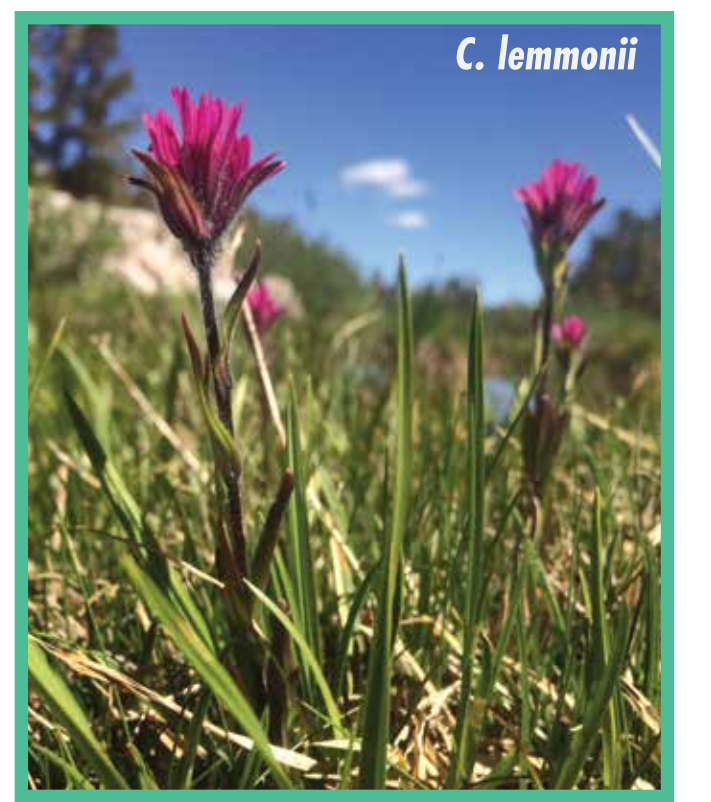


Figure 3 Nighttime and daytime stomatal conductance among the root hemiparasite, *C. aplegatei* *pinetorum* and three associated plants. The top panel represents non-parasitic plants with no parasitic neighbors, while the bottom represents parasitic individuals and neighboring non-parasitic individuals. Error bars are 95% confidence intervals. Letters represent significant differences (alpha = .05) from post-hoc Tukey adjusted pairwise comparisons run on a mixed effects model.

## DISCUSSION

- The parasites, *Castilleja* spp, represented a significant departure from typical patterns of stomatal conductance with both high  $g_{\text{night}}$  relative to  $g_{\text{day}}$  and high  $g_{\text{night}}$  overall.
- In parasitic plants the ability to gain carbon, regardless of sunlight, fundamentally alters their water–carbon tradeoffs. The elevated  $g_{\text{night}}$  observed in *Castilleja* spp supports the theory that this altered tradeoff drives nighttime transpiration because it facilitates the acquisition of water and dilute carbon from the host xylem stream. Similarly, and not mutually exclusive, acquisition of N or other nutrients may also drive nighttime transpiration.
- Nighttime transpiration could be the result of a release of evolutionary pressure on physiology traits due to a water supply buffered by the host association. However, observations of nighttime transpiration in *Rhinanthus*, root hemiparasites also in the Orobanchaceae family, showed that individuals display typical stomatal regulation when unattached to a host and then keep their stomata open continuously when attached to a host<sup>20</sup>. Given that, elevated  $g_{\text{night}}$  is more likely regulated within individual plants and a direct result of their attachment to hosts.
- Parasitic plants are thought to generally be profligate water users. The high  $g_s$  in both the day and night supports this while adding the additional wrinkle that high water use occurs at all hours. If stomata always remain open, overall transpirational water loss via a parasite is of course higher, possibly exacerbating host water stress.
- Although nighttime stomatal opening is more common than previously thought, in most plants  $g_{\text{night}}$  is typically relatively low, falling within 0-150 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, especially compared to  $g_{\text{day}}$  for the same species<sup>4,5</sup>. Here the non-parasitic species were somewhat elevated compared to previous measurements, with means ranging from 63 to 497 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, but not out of the range of previous observations.

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