# Ecophysiological Perspective on Co-Occurring Invasive and Endangered Species; The Case of *Lantana strigocamara* and *Lantana depressa*

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**Contents.**

## Abstract.

Invasive plant species pose a major threat to ecosystems, landscapes, and species around the world by lowering biodiversity, outcompeting natives for resources, and, in some cases, pushing endangered species out of existence. By comparing co-occurring invasive and native species, we can gain insight into how and why invasive species are able to thrive and persist. Ecophysiology and stress-response is one insightful avenue because it provides an estimate of overall fitness and adaptation capabilities. I compared the invasive, ornamental *Lantana strigocamara*, which has invaded tropical ecosystems across the globe, with its close relatives that co-occur in the pine rocklands the endangered *Lantana depressa* as well as *Lantana involucrata*. *L. strigocamara* and *L. depressa* grow together in Miami-Dade County and may be hybridizing, which could contribute to driving *L. depressa* towards extinction. I measured stress-resistance traits for both species and their possible hybrids to determine whether there are physiological differences between these two species, anticipating the invasive *L. strigocamara* to show higher stress-tolerance. I also used genome size to test for hybridization. I found that *L. depressa* and *L. strigocamara* are remarkably similar physiologically, though they have very different genome sizes. I also found no clear evidence of hybridization between these two species, even though many individuals show intermediate morphologies. This study has implications for further research into the differences between these two species, as well as their hybridization, and provides a morphological key for distinguishing between the species.

## Introduction.

Invasive species are alien or non-native species that encroach on a new environment (di Castri 1990). In plants, invasion typically occurs due to disturbance, low native diversity, resource availability, and/or human influence (Stohlgren et al. 1999; Davis et al. 2000). Invasive species are considered one of the biggest threats to global biodiversity (Walker and Steffen 1997) and pose threats at the ecosystem-, landscape-, and species- level. They have been shown to modify hydrology, disturbance frequency and intensity, soil characteristics, and fire regimes (Vitousek 1990; Ehrenfeld et al. 2001; Bradley et al. 2018). They are also likely to increase in prevalence in more disturbed areas and have a higher propensity to survive in harsh conditions (Bradley et al. 2010; Essl et al. 2020). At the species level, invasive plants pose particular threats to rare or endangered species through hybridization, genetic swamping, or displacement (Levin et al. 1996). When a new exotic moves into an environment, native species that are already threatened by habitat destruction, a native with a small population and/or weak genetic barriers can be pushed further toward extinction through genetic crossing with an invasive, leading to hybrids rather than “pure” natives. Overall, invasive plants pose many threats to native plants and ecosystems, while simultaneously costing economies millions of dollars (Pimentel et al. 2005).

After a plant is introduced to a new location, the chances of that introduced plant becoming an invasive is about one in 10 (Williamson and Fitter 1996), although this chance fluctuates based on the particular introduced species (Caley et al. 2008; Ni et al. 2021). If an introduced species is able to establish and persist, beginning to cause problems for its new habitat, it is very difficult to eradicate. Ornamental plants, introduced to a new area through planting in gardens or along streets, pose one of the largest threats, making up an estimated 75 - 90% of global invasives (Van Kleunen et al. 2010). While it is not always clear how or why this occurs, it may be due to hybridization and selection used during the horticultural development of the plant, i.e., selecting for particular traits that create a lovely, easy-to-care-for garden plant, but which now possesses traits such as year-round flowering, high drought resistance, and fast growth – all traits that support invasion. As such, how and why certain species are invasive has been a looming question in invasion biology to hopefully predict and prevent future invasion through ornamental plantings.

In this study, we focused on one specific invasive species, *Lantana camara*, one of the top 100 most invasive species in the world (Richardson and Rejmánek 2011). Though it is endemic to South America, after its introduction to Europe in the 1700s it became a highly popular ornamental house plant. While *L. camara* began as an ornamental plant it has since jumped from gardens and begun to invade ecosystems around the globe (Sharma et al. 2005). Where it has invaded, there have been noted decreases in species richness, altered fire regimes, lower soil absorption and subsequently possible increased soil erosion, and decreased pasture area and poisoned livestock (Day et al. 2003). The name *Lantana camara* has been applied both to a species inhabiting Central and South America, and also to many hybrid cultivars that have been transported and planted around the world. Because of this complex taxonomic history surrounding *Lantana camara* and its many cultivars, in this paper we refer to the invasive species as *Lantana strigocamara*, as identified by Roger Sanders (Sanders 2006).

To better understand the invasive nature of *L. strigocamara*, we compared the physiology and genome size between three co-occurring species of *Lantana* in the pine rocklands of Miami-Dade County. *L. strigocamara*, the highly invasive species, was most likely introduced to Miami through ornamental plantings. *L. depressa*, a close relative to *L. strigocamara* (Lu-Irving et al. 2021), is native to the pine rocklands and can only be found there. In fact, it is qualified as endangered and critically imperiled by the state of Florida. *Lantana involucrata* is also native to the pine rocklands, but it is not endangered and is also more distantly related to *L. strigocamara* and *L. depressa*. The pine rocklands are a harsh ecosystem with low to no canopy cover, rocky and dry soil, and frequent fires (Florida Natural Areas Inventory 2010). The pine rocklands are also becoming more fragmented due to urbanization throughout Miami-Dade county. As such, this harsh ecosystem requires plants to tolerate very specific conditions which are uncommon to the surrounding ecosystems in Florida.

Ecophysiological traits are often used to compare native species to invasive species within an area to understand performance differences (Cavaleri and Sack 2010; Van Kleunen et al. 2010). By comparing similarities and differences between invasive and native plants in one area, we can begin to uncover what strategies the invasive may be employing. Meta-analyses comparing native and non-native plants have identified certain traits that may contribute to a species becoming invasive (Daehler 2003; Cavaleri and Sack 2010; Davidson et al. 2011). While these studies often present different results and, as of yet, have not yielded many clear patterns in which traits may contribute to invasiveness, they can aid in biocontrol efforts for specific invasive genera or species. That being said, one emerging theory is that the ability to respond to stress and maintain fitness in stressful environments is a common characteristic of invasive species. Though fitness can be tough to measure, physiological properties have been shown to be a great indicator of fitness in some instances (Molina-Montenegro et al. 2013). Overall, the combination of multiple physiological traits can give insight into the stress-resistance or stress-avoidance strategies employed by plants as well as the overall fitness.

In addition to making interesting comparisons between closely related species' adaptations to one rare, harsh habitat, this study aids in the protection of the endangered *L. depressa*. It has been hypothesized for decades that *L. strigocamara* has been hybridizing with its close relative *L. depressa* (Sanders 1987; Day et al. 2003; Maschinski et al. 2010). As such, it is possible that hybridization may be another factor driving *L. depressa* towards extinction due to the loss of "pure" *L. depressa* individuals and the increased prevalence of hybrid individuals. Hybridization has shown to threaten rare and endangered species (Levin et al. 1996), so the same issue may be occurring in the case of *L. strigocamara* and *L. depressa*. However, these two species bear many morphological similarities (Maschinski et al. 2010) but have very different genome sizes – *L. strigocamara* is a tetraploid with a larger genome size than *L. depressa*, a diploid (Sanders 1987). Any "hybrids" may be morphologically ambiguous because of the many similarities between species but are not true hybrids. Hybridization across genome sizes producing viable offspring is also rare and difficult (Ramsey and Schemske 1998). Considering this, I included possible hybrids – named “intermediate morphologies” here – as another point of comparison physiologically and genetically within the co-occurring Lantana community in Miami-Dade County.

The main questions this study aims to address are as follows: 1) Does the invasive *L. strigocamara* show greater drought resistance? 2) Are differences in physiology more associated with habitat or relatedness? 3) Are plants with intermediate morphologies truly hybrids? To answer these questions, I measured a variety of physiological traits (turgor loss point, percent loss of rehydration capacity, stomatal conductance, photosystem II efficiency, electron transport rate) and genome sizes for multiple individuals of each species and intermediates. The comparison of turgor loss points and percent loss of rehydration capacity in leaves can be used as an indicator of drought resistance. Stomatal conductance, photosystem II efficiency, and electron transport rate compare the physiological efficiency of all individuals within their environment and how these photosystem strategies differ among distinct species. Lastly, genome size shows the genetic differences between species and can reveal any hybridization that may be occurring. I hypothesized that the invasive, *L. strigocamara*, would demonstrate traits associated with higher drought resistance and better utilization of resources compared to the other species. I also hypothesized that the so-called hybrids would bear more physiological resemblance to the species they were closest to morphologically. This project intends to understand what makes *L. strigocamara* so invasive, to inform future biocontrol and management protocols for this plant, and to aid in the conservation of the endangered *L. depressa* by better understanding possible hybridization.

## Materials and Methods.

### *Plant ID and Field Collections.*

Greenhouse plants were used for preliminary testing of physiological, morphological, and anatomical similarities and differences. Two *Lantana strigocamara* individuals were collected in Boulder, Colorado, from ornamental plantings and grown in the Ramaley greenhouse at the University of Colorado-Boulder. Three *Lantana depressa* individuals were obtained from the Fairchild Tropical Botanic Garden’s conservation nursery in Miami, Florida. For comparison, two individuals of *Lantana involucrata* (grown from cuttings collected at Selby Botanic Garden) were also grown in the greenhouse. The majority of the data were collected during field work in July 2022 in and around Miami-Dade County or in the laboratory using tissue or plant samples collected during field work. Communication with land and preserve managers in the area and consultation of GBIF and iNaturalist identified sites for collection. Because leaf tissue was removed from each plant, permits were obtained for collection. We sampled a total of 73 individuals from eight sites across Miami-Dade County including Larry and Penny Park, Martinez Luiz Reserve, Navy Wells Preserve, and Rockdale Preserve. Our sites primarily consisted of protected pine rockland or other areas where herbarium records indicated pure

**Figure 1**. Images show typical morphology of intermediates, *L. depressa, L. strigocamara*, and *L. depressa*. The specific features can be seen in Table 1. Note the combination of features from *L. strigocamara* and *L. depressa* on the intermediates.

*L. depressa* has historically grown. For each plant we collected from, we also took physiology measurements on three leaves per individual using a portable porometer (LI-600, LI-COR, Lincoln, Nebraska 68504, USA). These physiology measurements would allow us to test differences in stomatal conductance, photosystem II efficiency, and electron transport rate between species. Stomatal conductance (gsw), photosystem II efficiency (ΦPSII), and electron transport rate (ETR) are easy to measure and provide insight into the stress-response strategies of plants (Turner 1991; but see Toro et al. 2019). Stomatal conductance measures the opening and closing of stomata on the leaves of the plant. During times of water stress, plants tend to close their stomata to limit water loss via transpiration (Chaves et al. 2003). However, closed stomata also reduce photosynthetic rate because the leaves can no longer take up as much carbon dioxide. Plants must balance this trade-off in drought conditions, and thus understanding the physiological strategies of invasive species helps to explain why and how they adapt to novel environments that experience water stress. Chlorophyll fluorescence (photosystem II efficiency) and electron transport rate show us how efficiently the plant harnesses available energy. Measuring these traits can provide quick and accessible measurements of a plant's overall fitness (Molina-Montenegro et al. 2013).

Collections included individuals from the species *L. depressa*, *L. strigocamara*, *L. involucrata*, and possible *L. strigocamara* x *L. depressa* hybrids (intermediates). Identification of each plant was based on known morphological features of each species as described by Roger Sanders (1987; 2006). The factors used to characterize each individual are shown in **Figure 1** and **Table 1**. If a plant showed multiple characteristics from each species, it was classified as an "intermediate," denoting its ambiguous morphology and possible *L. strigocamara* x *L. depressa* hybrid state. The most common indicators of *L. depressa* included narrow leaves with a small basal angle which were typically rolled or curled at the lateral sides. For *L. strigocamara*, the best identifier characteristics were larger leaves with flat bottoms and often thicker stems. Intermediates were easier to identify than pure species because they often showed one identifier from each category (**Table 1**); typically, we found intermediates to have leaves that resembled *L. depressa* but either had orange or red flowers characteristic of *L. strigocamara,* or an upright posture which is also characteristic of *L. strigocamara*. Intermediates often looked different at different sites depending on light and water availability and some plants had small, *L. depressa*-like leaves on one area of the plant and much larger *L. strigocamara*-like leaves on other areas.

### *Percent Loss of Rehydration Capacity.*

Percent loss of rehydration capacity (PLRC) measures the ability of leaves to dehydrate and rehydrate without damaging too much internal structure (John et al. 2018). The lower the relative water content (RWC) in the leaves when a PLRC threshold is met, in theory, the plant can take more water loss before it reaches a point of irreparable internal damage. Most commonly, two thresholds are used to signify important points in the dehydration of leaves: $PLRC_{10}$ as a point of wilting (or permanent turgor loss point; see "*Pressure-Volume Curves*") and $PLRC_{50}$ as a point of irreparable damage (John et al. 2018; Trueba et al. 2019).

| Characteristic | Species | |
|---|---|---|
| | *L. depressa* | *L. strigocamara* |
| Basal angle | Small, acute | Large, approaching 90° |
| Leaf size/shape | Narrow, oval, curled | Wide, rectangular, flat |
| Growth habit | Low, crawling | Tall shrub |
| Flower color | Yellow | Deep orange, white, purple, and/or red |

**Table 1**. Each plant identified in the field was classified based on the traits indicated here. Intermediates showed multiple traits from each column.

Initial measurements for time needed for total dehydration and maximum rehydration were conducted using similar methods to that of John et al. (2018). I determined that 24 hours was needed for both maximum dehydration and maximum rehydration in the leaves. In order to measure percent loss of rehydration capacity, I used a modified version of the PLRC methods (Trueba et al. 2019). We collected a total of 27 leaves from each individual to measure three leaves per individual at each of 9 time points during the dehydration and rehydration cycle. Immediately after removal from the plant, the leaves were placed into humidified plastic bags. Next, the petiole of each leaf was placed into water and allowed to rehydrate for 12 hours to ensure all leaves started at maximum hydration. After 12 hours, each leaf was weighed for saturated mass ($M_s$) then set on a dry paper towel lined baking sheet to begin dehydration. At each time point (0.5 hr, 3 hr, 6 hr, 9 hr, 12 hr, 15 hr, 19 hr, 21 hr, and 24 hr) during dehydration, three (3) leaves per individual were weighed for dehydrated mass ($M_{de}$) and then allowed to rehydrate for 24 hours. After all leaves had rehydrated for 24 hours, each leaf was weighed for rehydrated mass ($M_r$) then oven-dried at 70°C for at least 72 hours, and then finally weighed for dry mass ($M_d$).

I calculated saturated water content (SaC) and rehydrated water content (ReC) as follows:

$$SaC = \frac{M_s - M_d}{M_d}$$

$$ReC = \frac{M_r - M_d}{M_d}$$

Using these values, Percent Loss of Rehydration Capacity (PLRC) was calculated as:

$$PLRC = 100 * \left(1 - \frac{ReC}{SaC}\right)$$

and Relative Water Content (RWC) as:

$$RWC = 100 * \left(\frac{M_{de} - M_d}{M_s - M_d}\right)$$

We plotted the inverted RWC values against PLRC values for each species to find the overall curve of rehydration capacity loss over declining relative water content. We then fit a linear, exponential, and sigmoidal curve to the data from each species to determine the modeled line that best fit. In all three cases, for *L. depressa*, *L. strigocamara*, and *L. involucrata*, the exponential curve was the best fit based on the Akaike information criterion (AIC) value, or maximum log-likelihood, for each model. This allowed the calculation of the RWC values at 10% and 50% loss of rehydration capacity ($RWC_{PLRC10}$ and $RWC_{PLRC50}$, respectively). These two critical points have been identified as important thresholds for damage in leaves (Trueba et al. 2019). Reaching one of these thresholds at lower RWC indicates higher water-loss resistance and thus the plant is likely less sensitive to drought. Thus, I hypothesized that *L. strigocamara* would reach the thresholds at lower RWC values compared to *L. depressa* and *L. involucrata*. Lastly, I estimated the average water loss (1 – RWC) for each species at each time point to compare the time of dehydration required to reach each threshold.

*Pressure-Volume Curves.*

One method of estimating drought-resistance in plants is measuring the pressure-volume curve for multiple individuals of a species and calculating an average turgor loss point. The turgor loss point is the threshold at which a plant has lost too much water and can no longer maintain normal function. These measurements vary widely based on typical environmental conditions – namely water availability and drought frequency – but typically a lower (more negative) turgor loss point indicates a plant can lose more water before it reaches a point of water stress and are thus more drought resistant.

In order to calculate pressure-volume curves for each species/intermediate of interest, I used previously established methods (Roddy et al. 2019). We collected plants in the early morning just after sunrise. We cut shoots about 6 – 8 inches long then immediately recut another inch of the stem underwater. During transport back to the lab, cut ends were kept submerged in water and shoots were covered in a dark bag. In total, measurements were made on seven intermediates, three *L. depressa*, four *L. involucrata*, and four *L. strigocamara*, for a total of 18 individuals across four sites (Larry and Penny Thompson Memorial Park, Crandon Park, Navy Wells Pineland Preserve, and Seminole Wayside Park). One leaf per individual plant was measured.

Pressure-volume curves were constructed from sequential measurements of leaf water potential and leaf mass made periodically as leaves slowly desiccated. Water potential measurements were made using a Scholander-style pressure chamber (PMS Instruments, 0.01 bar resolution). During measurement, the chamber was lined with wet paper towels to prevent desiccation during chamber pressurization. After each water potential measurement, leaf mass was recorded using a digital scale (Sartorius BCE224I, 0.0001 g resolution). A total of about 12-15 measurements of water potential and fresh mass per leaf were made over the course of several hours. Afterwards, leaves were dried at 60°C for five days and weighed for dry mass. From the relationship between water potential and water content, I calculated a variety of hydraulic traits, including the osmotic potential and relative water content at full turgor pressure and the water potential at turgor loss. These measurements are another possible indicator of water-stress resistance. I hypothesized that *L. strigocamara* would have a lower water potential at turgor loss ($\Psi_{tlp}$) because a lower (more negative) turgor loss point typically indicates higher drought resistance.

*Genome Size.*

Genome size data was collected to determine if the *L. depressa* plants found are diploid and *L. strigocamara* plants tetraploid, as previously believed. Additionally, this allowed us to compare the genome size of the intermediates collected and determine whether the patterns are consistent with hybridization. We followed standard protocols for measuring genome size in plants using flow cytometry (Doležel et al. 2007; Pellicer and Leitch 2014). Approximately 50–100 mg of fresh leaves tissue was finely chopped over ice using a fresh razor blade along with fresh standard leaf material ( Zea mays L., 1C = 2.71 pg; Lysak and Doležel 1998) in 1500-2000 µl ice-cold Galbraith's buffer [45 mM MgCl2, 20 mM MOPS, 30 mM sodium citrate, 0.1% (v/v) Triton X-100, pH 7.0] (Galbraith et al. 1983). Seeds of the plant standard were generously provided by the Institute of Experimental Botany, Czech Academy of Sciences. The homogenate was filtered through a 30-µm nylon mesh filter (CellTrics™, Sysmex, Germany) and 50–100 µg/mL propidium iodide was added. Samples were incubated on ice for 15 minutes prior to analysis. Flow cytometry was performed using a BD Accuri C6 Flow Cytometer (BD Biosciences, San Jose). At least 5000 nuclei were counted for unknown samples, with a coefficient of variation < 5% for measured peaks. The 2C-value was determined following Pellicer & Leitch (2014) as:

$$2C\ DNA\ content\ target\ (pg) = \frac{target\ sample\ mean\ G_1\ peak}{standard\ sample\ mean\ G_1\ peak} * \ 2C\ DNA\ content\ standard\ (pg)$$

*Statistical Analysis.*

All statistical tests were run in R (R Core Team 2021) using the package tidyverse (Wickham et al. 2019) to plot data. From the LICOR data set, I was able to collect data on stomatal conductance ($g_{sw}$), photosystem II efficiency (ΦPSII), and electron transport rate (ETR) for each of the 73 individuals collected in the field. The distribution of $g_{sw}$ and ΦPSII were relatively normal and I used a square root transformation for ETR, which was initially not normally distributed. Next, I ran a linear model and linear mixed effects model for each variable – either accounting for a random effect of site, or not, and accounting for time of day, or not – using the packages lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2017). I compared residuals and fitted values for each followed by AIC values to choose the best fit model, then ran a pairwise comparison using the emmeans package (Lenth 2022). For the PLRC data, I fit a linear, exponential, and sigmoidal curve to the data for each species and compared data fit based on the $R^2$ value. I used the model outputs to determine the equation of the curve and used the equation to calculate the RWC values at two thresholds of $PLRC_{10}$ and $PLRC_{50}$. Rather than running a complex model, I ran a loess model to fit a local regression to the RWC values for each species across the time points in order to predict the time point at which each species met the determined PLRC threshold.

## Results.

### *LI-COR Physiology.*

Stomatal conductance (gsw) was significantly higher in *L. involucrata* than *L. depressa* (T-ratio = -4.983, df = 63.2, P-value = <0.0001), *L. strigocamara* (T-ratio = 2.967, df = 62, P-value = 0.0216), and intermediates (T-ratio = -3.563, df = 60.9, P-value = 0.0039) (**Figure 2a**).. In the model including the effect of light intensity (Qamb) on photo system II efficiency (ΦPSII), *L. depressa* shows a significantly lower efficiency than *L. involucrata* (T-ratio = -3.292, df = 66, P-value = 0.0085) (**Figure 2b**). Electron transport rate (ETR) was found to be significantly higher in *L. involucrata* than in *L. depressa* (T-ratio = -2.886, df = 63.5, P-value = 0.0267) (**Figure 2c**)

### *Percent Loss of Rehydration Capacity.*

Using the equations of the exponential curves modeled to each species' set of PLRC data points, where each point represents one leaf, I calculated the predicted RWC value for each PLRC threshold of $PLRC_{10}$ (permanent turgor loss) and $PLRC_{50}$ (irreparable cell damage) (**Figure 3**). I found that *L. strigocamara* reached these thresholds at higher RWC vales compared to *L. depressa* and *L. involucrata*. This indicates that *L. depressa* can lose more water before reaching a cell damage threshold. *L. strigocamara* reached $PLRC_{10}$ at 66% RWC, *L. depressa* at 59% RWC, and *L. involucrata* at 52% (**Table 2**). For $PLRC_{50}$, *L. strigocamara* reached the threshold at 17% RWC, *L. depressa* at 11% RWC, and *L. involucrata* at 19% RWC. Based on the local regression fit to the scatter plot of RWC values across time points, *L. strigocamara* reached the $PLRC_{10}$ threshold after 2 hours of dehydration, whereas *L. depressa* did not reach it until an estimated 4.2 hours and *L. involucrata* after 9 hours of dehydration. *L. strigocamara* reached $PLRC_{50}$ after 11 hours of dehydration. I was unable to estimate the RWC values at $PLRC_{50}$ for *L. depressa* and *L. involucrata*, but the data suggests that they do not reach this threshold until after at least 24 hours of drying.

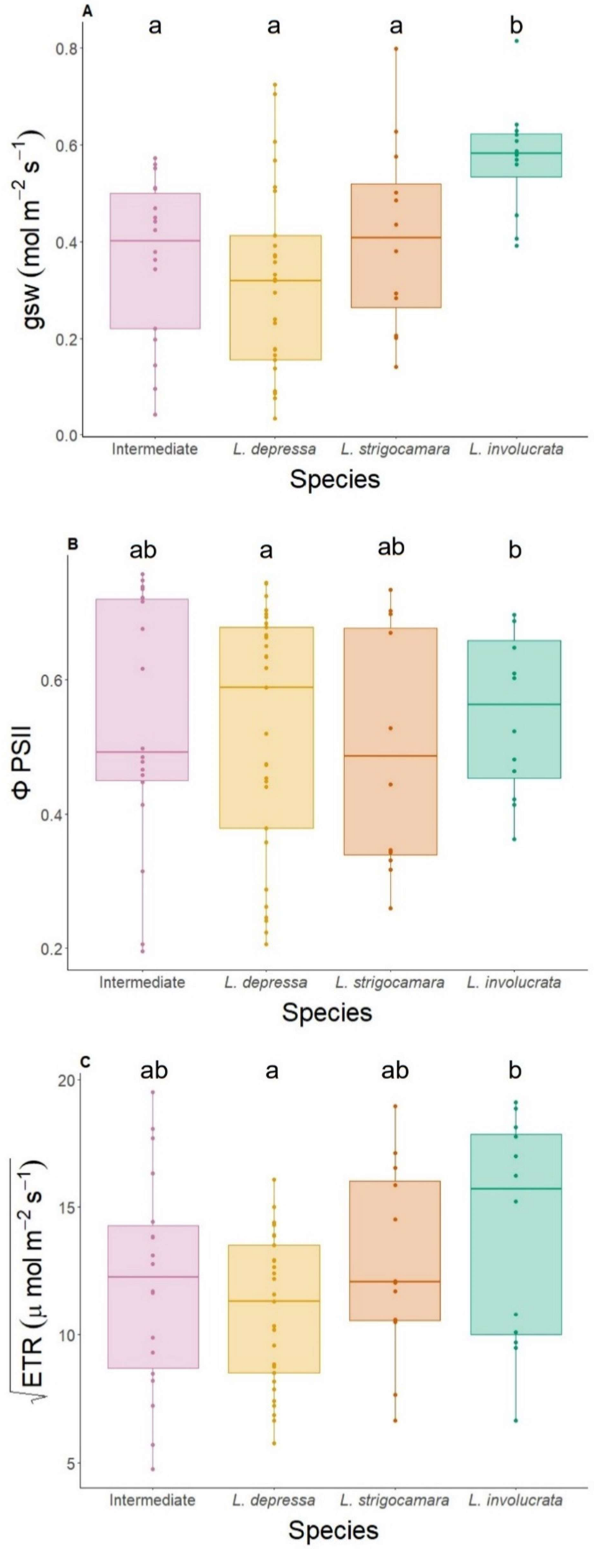

**Figure 2**. Comparison of stomatal conductance (A), quantum yield of photosystem II (B), and electron transport rate (C) among the intermediate and three species. Electron transport rate values are square root transformed. The letters above each boxplot show significant difference between species.

*Pressure-Volume Curves.*

In looking at the change in water potential of each species and the intermediates, there was no significant difference in turgor loss point ($\Psi_{TLP}$) (**Figure 4**). The average $\Psi_{TLP}$ for each species was slightly different, but the model accounting for a fixed effect of species and random effect of site found no significant difference.

*Genome Size.*

We determined that *L. depressa* has a mean genome size of 3.20 (± 0.07), *L. strigocamara* had a mean of 6.05 (± 0.25), and *L. involucrata* had a mean of 5.05 (± 0.07) (**Table 3**). It is important to note that while the intermediates have a mean of 4.25 (± 1.57), the points are either at ~3 C or ~6 C (**Figure 5**). We measured no intermediates of intermediate genome size.

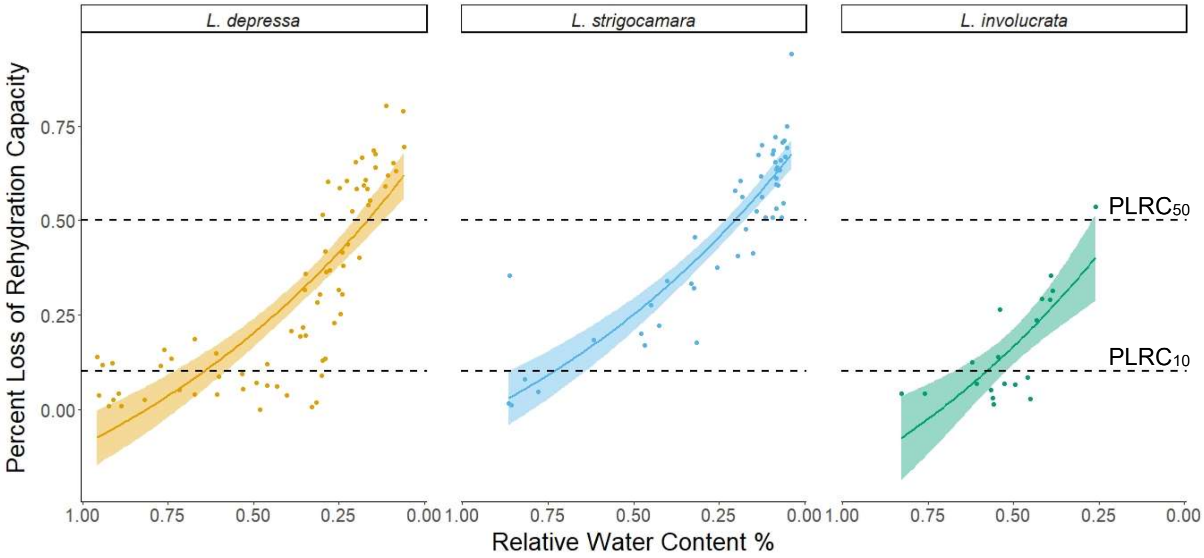

**Figure 3.** Exponential curves show the trend of each species' leaf percent loss of rehydration capacity (PLRC) over decreasing relative water content (note axis is inverted). Points of note are $PLRC_{10}$ and $PLRC_{50}$.

| | *L. depressa* | Time | *L. strigocamara* | Time | *L. involucrata* | Time |
|---|---|---|---|---|---|---|
| $RWC_{PLRC_{10}}$ | 59% | 4.2 hr | 66% | 2 hr | 52% | 9 hr |
| $RWC_{PLRC_{50}}$ | 11% | >24 hr | 17% | 11 hr | 19% | >24 hr |

**Table 2.** Relative water content for each species at thresholds of $PLRC_{10}$ and $PLRC_{50}$ and the estimated dehydration time (Time) to reach said threshold. The RWC values were calculated using the equations pulled from modeled exponential curves for each species. The time spent dehydrating to reach each threshold was predicted using a modeled local regression.

## Discussion.

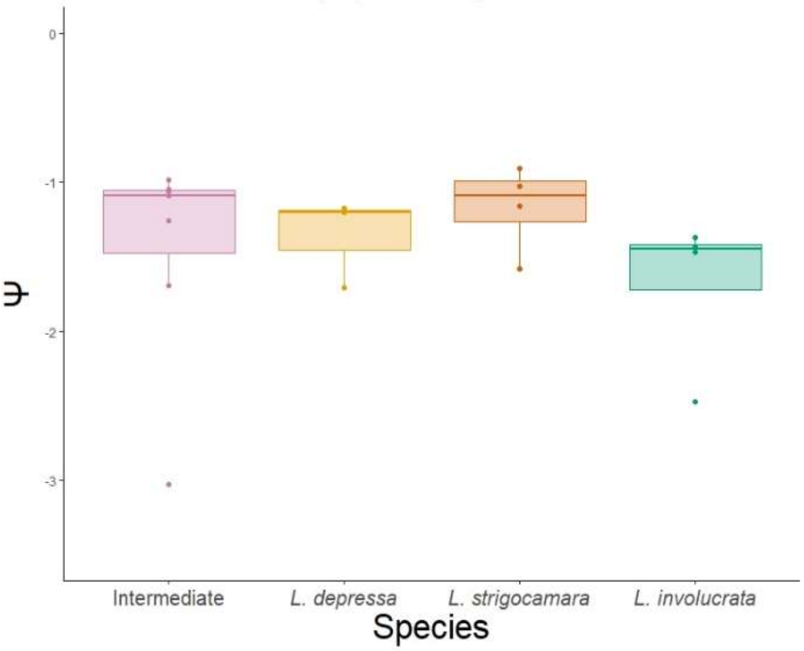

**Figure 4.** Comparison of water potential (Ψ) at turgor loss point for three species and intermediates. No significant difference was found.

Invasive plants often outcompete native plants in the process of establishing in new environments. To do this, invasive species may allocate the majority of their resources to fast growth and reproduction, superior resource acquisition, and/or better stress resistance compared to natives (Ren and Zhang 2009). *Lantana strigocamara* has demonstrated its ability to adapt and encroach in a variety of ecosystems around the world, taking advantage of disturbed lands and quickly spreading. In India, Australia, and South Africa, *L. strigocamara* has taken over as the dominant understory vegetation in many forests, choking out native plants and presenting a challenging restoration effort for conservationists (Richardson and Rejmánek 2011; Bhagwat et al. 2012; Lone et al. 2022).

Here, we used the co-occurrence of three *Lantana* species in Miami-Dade County to test whether the invasive *L. strigocamara* has differing physiological strategies when growing in the pine rocklands compared to *L. depressa* and *L. involucrata,* and whether *L. strigocamara* exhibited more characteristics of drought-resistance. We also tested whether the plants demonstrating intermediate morphologies, showing similar traits to *L. depressa* and *L. strigocamara*, were hybrids and how they compared with the "pure" species. We found that, in some instances, *L. strigocamara* actually showed lower drought resistance compared to *L. depressa* and *L. involucrata*. *L. strigocamara* was similar physiologically to *L. depressa* and the intermediates, while *L. involucrata* had slightly different operating levels (higher gsw, ΦPSII, and ETR), physiologically, though in the same habitat. For the intermediates, we found no genetic evidence of hybridization based on genome size. In the following sections, I will discuss the implications of the results found here considering the invasive nature of *L. strigocamara*, differences between species of *Lantana*, and hybridization considerations for the future conservation of the endangered *L. depressa*.

*Drought-Resistance.*

The pine rocklands of Miami-Dade are harsh, low-canopy cover, rocky ecosystems. It is home to a handful of rare species that, like *L. depressa*, can only be found in the pine rocklands (Florida

| Species | 2C DNA Content | Standard deviation |
|---|---|---|
| Intermediate | 4.247074 | ± *1.57352051* |
| *L. depressa* | 3.197235 | ± *0.06991707* |
| *L. involucrata* | 5.054646 | ± *0.07393313* |
| *L. strigocamara* | 6.048189 | ± *0.25469232* |

**Table 3.** Average genome size in 2C DNA content and standard deviation of each species and intermediates from flowcytometry results on 23 measured individuals.

Natural Areas Inventory 2010). Few plants are accustomed to the ecosystem and new encroaching species must be able to tolerate these same harsh conditions. *L. depressa* is adapted only to this particular environment, while *L. strigocamara* is able to grow in a much wider range of soil types, water and nutrient availabilities, and light conditions. In fact, a team of researchers estimated the climatic niche (typical climate range of areas it inhabits) across the continents it has invaded and found that *L. strigocamara* was able to spread to areas much warmer than that from its home range (Goncalves et al. 2014). This provides strong evidence for the ability of *L. strigocamara* to easily tolerate a range of environmental conditions in order to invade a new area. Considering this, we anticipated that drought resistance, and particularly the phenotypic plasticity required to adjust to different water availabilities, would be highest in *L. strigocamara*.

Measurements of percent loss of rehydration capacity were used as an initial test of possible differences between the two main species of interest. These thresholds of rehydration capacity loss are known to indicate differences in drought tolerance, particularly in species from similar areas (John et al. 2018). We found that *L. strigocamara* showed lower drought resistance during this experiment compared to both *L. involucrata* and *L. depressa*. It is important to note, however, that these experiments were performed

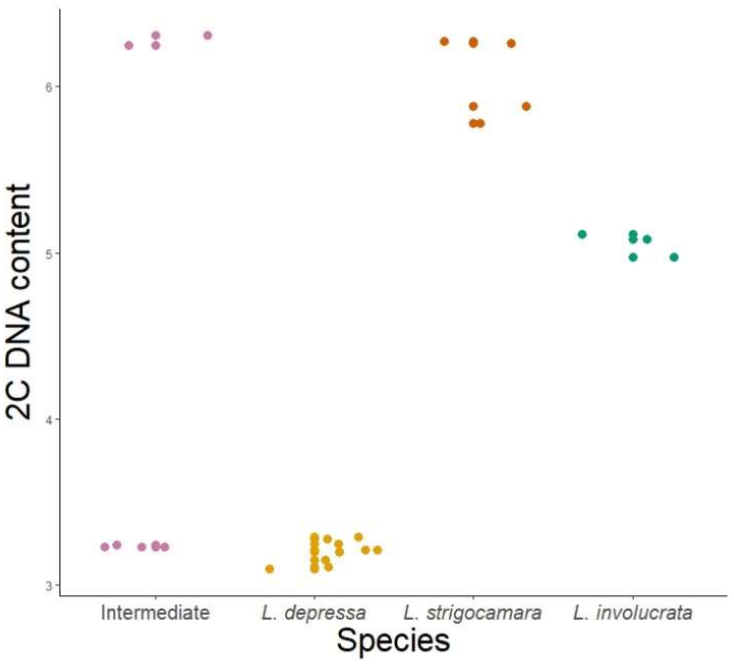

**Figure 5.** Data show genome sizes of intermediates, *L. depressa*, *L. strigocamara*, and *L. involucrata* based on 2C DNA content. Note that points are jittered to better visualize distribution of data for each species.

on individuals of each species that had been growing for many months in a greenhouse in Colorado. So, it is possible that these plants grown in greenhouse conditions are not a good proxy for the physiological processes of plants growing the pine rocklands. In particular, greenhouse conditions generally do not expose plants to low water levels because plants are watered daily, while the pine rocklands likely experience a much greater range of water availability.

Using plants from the pine rocklands, we also quantified pressure-volume curves for each species and the intermediates in order to estimate a turgor loss point. The turgor loss point is the threshold at which the pressure inside the cell, which is typically a negative pressure that pushes out on the plant cell wall and keeps plant cells plump and functioning, is lost and the cells begin to collapse, losing water. The water potential at turgor loss ($\Psi_{tlp}$) is an important indicator of plant water-loss resistance (Zhu et al. 2018). I initially anticipated *L. strigocamara* to reach $\Psi_{tlp}$ at a lower water potential than *L. depressa*. Instead, we found no significant difference between turgor loss points among the species and intermediates. However, *L. strigocamara* had a higher mean water potential at turgor loss, indicating lower drought resistance.

It is possible, considering the trends in our results, that more replicates of each species would reveal more distinct patterns. Invasive species have, in past studies, been shown to reach turgor loss at a higher water potential than native species indicating lower water loss resistance (Petruzzellis et al. 2021). On the other hand, Garcia-Serrano et al. (2009) found a similar lack of difference between invasive and native *Senecio* species, suggesting that a wider combination of traits may contribute to the differing range sizes of these two species. I theorize that this may be the case here, where *L. strigocamara* is relying on a jack-of-all-trades method for invasion rather than focusing on drought resistance in particular (Richards et al. 2006). Again, looking back to the expanding climatic niche of this invasive species, phenotypic plasticity and local adaptation may be a better explanation for the invasive capabilities of *L. strigocamara.*

### *Relatedness versus Habitat.*

The co-occurrence of three *Lantana* species in the pine rocklands presents an interesting opportunity to test whether relatedness or local adaptation underlies the physiology of these species. We can use this shared relatedness and habitat to understand whether the invasive species operates differently compared to species native to the pine rocklands under the same conditions. In particular, we wanted to assess how these species are able to tolerate an ecosystem as harsh as the pine rocklands and any different strategies they may employ to do so.

Typically, stomatal conductance and photosystem II efficiency are paired, each being affected by outside factors and imposing that effect on the other. Stomatal conductance decreases with increasing water stress as the plant attempts to limit water loss, therefore limiting the uptake of $CO_2$ and slowing photosynthesis (Fracheboud and Leipner 2003). Past metanalyses have found invasive species to have, on average, higher stomatal conductance rates than their native counterparts (Cavaleri and Sack 2010). As such, I expected the invasive *L. strigocamara* to display these traits. However, *L. strigocamara* and *L. depressa* showed similar physiological capabilities, showing comparable stomatal conductance ($g_{sw}$), quantum yield of photosystem II

(ΦPSII), and electron transport rate (ETR), indicating comparable ability to utilize the available light and stave off water stress. The intermediate morphologies also showed similar patterns. *L. involucrata*, however, showed higher $g_{sw}$ than the other species and intermediates as well as higher ΦPSII and ETR than *L. depressa.* Our samples were from field plants that grew within ~20 ft of each other and thus were exposed to similar environmental conditions yet exhibited some differences in physiological operations.

Based on the patterns observed, evolutionary relatedness underlies the ability to utilize $CO_2$ and energy, measured through gsw and ETR, respectively, rather than adaptation to the pine rockland habitat. The species that are more closely related – *L. strigocamara*, *L. depressa*, and their intermediates – were more similar physiologically than they were to the more distantly related *Lantana involucrata*. Because our models of ΦPSII included a light intensity variable (Qamb), it is understandable that there are no patterns of relatedness, but habitat and environmental conditions lead the operational levels of photosystem II for this physiological variable. Different species appear to utilize sunlight at different rates and this is partially dependent on the light intensity and availability in the specific environment.

Because there are so many factors that can affect these physiological parameters, not finding significant differences among *L. depressa* and *L. strigocamara* tells us that these plants are even more similar than we previously expected. Furthermore, it suggests that all of the species can tolerate the drier conditions in the pine rocklands, otherwise we would've detected signs of stress. That being said, these parameters can vary greatly for one plant, even, across the day as the temperature changes and the sun moves, but also across multiple days due to variation in weather conditions and water availability (Fracheboud and Leipner 2003; Allred et al. 2010). It is possible that, with long-term measurements of these traits across a time series and under different water or light conditions, we may find a more marked difference between species.

*Hybridization.*

One question that remains is whether *L. strigocamara* and *L. depressa* are truly hybridizing in Miami, Florida. Hybridization has been widely assumed after first being reported by Roger Sanders (1987), and more recent work (Maschinski et al 2010) also inferred hybridization using Amplified Fragment Length Polymorphisms (AFLPs) – a method used to detect polymorphisms (differences) between DNA sequences. Sanders published multiple papers reviewing and attempting to tease apart the complex taxonomy of the Lantaneae family, particularly the many hybrids and cultivars surrounding the invasive *L. camara* (2006). In one study, Sanders identified diploid, triploid, and tetraploid hybrids of *L. strigocamara* and *L. depressa* and claimed that because these two species were able to hybridize, the triploids were back-crossing into *L. depressa* populations leading to genetic swamping and possible future extinction of the beloved, native, diploid *L. depressa* (Sanders 1987). Maschinski et al. (2010) used both genetics and morphology to distinguish between *L. strigocamara*, *L. depressa*, their possible hybrids, and another variety of *L. depressa* previously described, *L. depressa* var. *floridana*. These researchers propose that while *L. depressa* and *L. strigocamara* are genetically and morphologically distinct, there is still evidence of introgression and hybridization. A further confounding variable is the fact that cultivated *L. strigocamara* plants are often sold under the name *L. depressa* when they display yellow flowers (rather than multi-colored flowers, which is more typical of *L. strigocamara*), which exacerbates the confusion over the identity of true, wild, *L. depressa* in the Miami-Dade area (Hammer 2004). This is, therefore, adding more morphologically ambiguity to *Lantana* to the system.

I found no evidence of triploid hybrids in our sample despite sampling many intermediate phenotypes. Of the six plants with intermediate phenotypes that we measured for genome size, four had a comparable size to *L. depressa* and two had a similar size to *L. strigocamara.* The four intermediates with *L. depressa*-sized genomes were found at Crandon Park and were characterized as intermediates because, while their leaves and flowers generally resembled *L. depressa*, they grew in large bushes, up to 6 feet off the ground. The two intermediates with *L. strigocamara*-sized genomes were found at Larry and Penny Thompson Park, and they displayed the rolled leaves and growth pattern characteristic of *L. depressa* but with the orange-red flowers characteristic of *L. strigocamara*.

The formation of hybrids between species with different genome sizes is rare and difficult. Here, we confirm that *L. strigocamara* is tetraploid, meaning that it possesses four sets of chromosomes, and has double the genomic DNA found in *L. depressa*, a known diploid. For hybridization to occur, the species with the smaller genome must produce an unreduced gamete (*double the normal size*) in order to allow for the creation of a triploid hybrid. Then, a triploid can backcross into the diploid population to form a tetraploid. This process, however, is rare and not well understood (Ramsey and Schemske 1998). Another barrier to this formation is known as the "triploid block," in that triploids are often sterile and thus are not able to reproduce and contribute to the gene pool (Petit et al. 1999; Köhler et al. 2010). Although we did not find any triploid hybrids, they have been reported previously by Roger Sanders. This presents a conundrum: if the tetraploid *L. strigocamara* is hybridizing with the diploid *L. depressa* to produce triploid hybrids, are those triploids fertile and able to back-cross with diploid *L. depressa* individuals? This back-crossing is necessary for genetic swamping to occur (where the prevalence of hybrids essentially drowns out the endangered native species). In order to understand the contribution of hybridization to the endangerment of *L.* depressa, it is critical to better understand the extent to which hybridization is occurring, whether hybrids tend to have diploid, triploid, or tetraploid genomes, and whether they are fertile or sterile.

In conclusion, while hybridization may contribute to the extinction of *L. depressa*, a much more evident threat is the destruction of its native habitat. Only 2% of the pine rocklands remain in Miami and surrounding areas (Florida Natural Areas Inventory 2010), and as such only 2% of the native habitat of *L. depressa* remains. *L. strigocamara*, on the other hand, is easily able to move into both the areas of remaining pine rockland, as well as other environments around Miami. We must gather more information on the *Lantana* hybrid complex within Miami-Dade County, but the conservation of the remaining pine rockland is also imperative in preserving this endangered species and the many other rare and endangered species that live there.

### *Future Directions and Conclusions.*

*L. strigocamara* has the ability to invade new ecosystems outside its native climatic range (Goncalves et al. 2014) and as a consequence range of *L. strigocamara* will likely continue to expand with increasing global temperatures and human disturbance (Mungi et al. 2018). As such, it is important to understand the mechanisms and characteristics of this species that allow it to inhabit such a wide variety of habitats. This will aid in the prevention of its future spread as well as assist mitigation efforts where it has already invaded. Additionally, because it is a hybrid cultivar often introduced through garden plantings, this species can be used as a tool for predicting and preventing further invasive species from developing out of the horticultural trade (van Kleunen et al. 2018).

Another large issue caused by invasive species is the extinction of rare species. Invasive plants are known to reduce biodiversity, *L. strigocamara* in particular (Lone et al. 2022), but they can also pose a threat to isolated, remaining populations of rarer species. The intention of this project was to, in part, better understand the hybridization occurring between *L. strigocamara* and *L. depressa* to hopefully aid in the protection of the native species. Whether or not this hybridization is occurring is still unclear and requires a more thorough analysis of the genetic differences between species and populations across the Miami-Dade area. Using herbarium specimens of both species from the area and comparing these with current individuals will reveal more clear hybridization patterns and differences between species.

While conducting field work in Miami-Dade County, we interacted with land managers tasked with preserving the remaining protected pine rockland areas. One of the fundamental issues limiting any work in the eradication of *L. strigocamara* invasion is the morphological similarities between the invasive *L. strigocamara* and the endangered *L. depressa*. Here, we identify at least four identifying features of each species that may aid in alleviating this issue. The changing climate and diminishing native ecosystem may be forcing a change in growth strategy in *L. depressa*. In order to preserve the endangered *L. depressa*, more research is needed distinguishing between these two species and zeroing in on an effective eradication method for *L. strigocamara*.

In this study, we used physiology and genetics to measure differences between three species of *Lantana* along with possible *L. strigocamara* x *L. depressa* hybrids to understand 1) physiological differences between the species 2) the importance of adaptation to the pine rockland habitat vs phylogenetic conservatism in explaining the pattern of trait variation across the species, and 3) possible hybridization between *L. depressa* and *L. strigocamara*. We stress the importance of understanding the mechanisms that enable some species to become invasive for predicting and preventing future invasions. Furthermore, the conservation and protection of the endangered, Miami-native, *L. depressa* is dependent on the removal of *L. strigocamara* from the system and the protection of remaining pine rocklands.

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