Bridging the physiology and ecology of root hemiparasitic plants

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Abstract

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Metabolism based on photosynthesis is a defining feature of plants. However, some flowering plant lineages have adopted a heterotrophic lifestyle based on parasitism, either reducing or completely abandoning their ability to photosynthesize. Approximately 1-2% of angiosperms directly parasitize other plants, attaching to the vascular tissue of one or more host plants using specialized structures called haustoria. All lineages that have evolved away from autotrophy display profound changes in morphological, biochemical, and molecular traits. In addition, the adoption of a parasitic lifestyle necessitates physiological adaptations that allow the parasite to locate, attach to, and effectively siphon resources away from the host. As a consequence of the expression of these physiological traits, parasitic plants can occupy unique ecological roles in their natural communities. Hemiparasites, parasitic plants that can photosynthesize, both compete with and parasitize host plants, simultaneously acting as producers and consumers. Hemiparasites often disproportionately parasitize certain species in a community, changing the competitive dynamics between host and non-host species and affecting

vegetation structure and diversity within a plant community. However, while research into the role of hemiparasites in their communities is growing, it is still largely based on only a few genera. Furthermore, while some hemiparasitic species are considered to be keystone species, profoundly impacting community structure, others exert little to no effect. However, due to a relative lack of representation of diverse genera in hemiparasitic plant literature, we cannot adequately address the mechanisms of such variation.

I examined the physiology and ecology of the Castillejinae tribe in the Orobanchaceae, a species rich yet largely understudied group of annual and perennial genera. In several greenhouse experiments I assessed resource flow between host and hemiparasite and investigated whether the pattern of resource gain varied based on hemiparasite life form (annuals or perennials). Like other hemiparasitic plants, these genera had higher concentrations of mineral nutrients than their hosts. However, by experimentally allowing or preventing parasitism, I was able to show that phosphorous limits the growth of unattached hemiparasites. Furthermore, unattached hemiparasites invested more in their roots than attached hemiparasites. Thus, hemiparasites in the Castillejinae conform to physiological patterns observed with other hemiparasites for several key traits.

I expanded my study group to North American hemiparasites as a whole to study the interactions of these hemiparasites with their natural communities. Through analysis of vegetation plots across national parks in the United States, I found that hemiparasites are associated with higher evenness in plant communities across the United States, but that their relationship with richness is pronounced. Almost all the species in this dataset are absent from hemiparasitic plant literature, suggesting that the current research is only capturing some of the mechanisms by which a hemiparasite can affect its community. Building on my research and the existing literature, I propose that life history may be an important mediator influencing the physiology of hemiparasites and, therefore, their relationships with their hosts and communities.

This body of physiological, ecological, and theoretical work enhances our understanding of hemiparasitic plant biology. Given that hemiparasites are a common component of the world flora, this research represents an important step towards incorporating hemiparasites into community ecological theory.

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Acknowledgements

The final stage of a PhD—communicating the answers to my research questions—was, not, in my opinion, the hard part. The hard part was the beginning: trying to find interesting and different questions to make my doctoral work matter. I struggled with the niche nature of a PhD, fearful of the idea that it would ultimately only serve to make me an expert in a very narrow subject field. I have come to realize that the opposite is true. Over the past four and a half years, I have been tasked with learning what the right questions to ask are and discovering how to best seek out their answers. This is a skill which I will never fully master—one that takes as much creativity as it does knowledge, and which will only serve to enrich and enhance my life.

This document compiles and presents the culminating results of several scientific experiments I have carried out. It captures the facts and findings of my experiments, but it says nothing of the convoluted journey I have undergone. It does not describe the vulnerability I felt while carrying out my work, since my experiments often depended upon the survival of hundreds of delicate plants under my care. It does not describe my awe upon observing them grow and respond to my treatments, nor the intense frustration and concern when, for seemingly no reason, some plants did not make it. It fails to relay the electrifying buzz that crackled through me when I suddenly connected disparate ideas with an elegant theory. What's more, it does not recount the moments when things went wrong. The reader cannot feel the stunning force of the weight that pressed upon me, for example, when an experiment I conducted for over a year failed ultimately because of a risk that is inherent in all novel research: an unpredictable and unknowable aspect of a study system revealed itself. Certainly, it makes no mention of the people I met along the way. It cannot capture the particularly diaphanous evening light of a Seattle spring, nor the feeling of levity that coursed through me as I zipped down the Burke-Gilman trail on my way to Ravenna Brewery to meet my friends. It in no way touches upon the simple act of being in my mid-20s in a new city embarking on a journey that, at first, seemed to be wholly my own. Now I know that, though my name appears on the first page of this document, I have leaned heavily on many others throughout this process.

I am most indebted to the mentorship of my primary advisor, Dr. Jon Bakker, who made his belief in me clear from the moment I began what was initially a master's project. I moved to Washington in 2017, at the beginning of what would be one of Seattle's wettest and greyest winters. I was new to the area and wholly in over my head, spending many a long evening in the campus greenhouse, scheming up project after project, most of which turned into dead ends. Jon never faltered in his conviction that these experiences were as important as the research that is presented here, a lesson that has reverberated through many arenas in my life. Equally important was the guidance he never hesitated to provide as I struggled to align my research goals with my personal life; without his consideration and abundance of patience, I am certain this work would never have been completed.

I am also grateful to the members of my committee, Dr. Soo-Hyung Kim, Dr. Dick Olmstead, and Dr. Ian Pearse for their mentorship and invaluable expertise in plant physiology, systematics, and ecology.

The first lecture I attended at the University of Washington was for Soo-Hyung Kim's plant ecophysiology course. I was full of energy and attentiveness, though his clear command of the subject and articulate mannerisms would be obvious to anyone. What really stuck, though, was the often-subtle wit and humor he would inject into his lectures and meetings. Soo maintains an air of seriousness and consideration, while also keeping things light— a balance I have not known many to successfully strike. I also am grateful that he served as the chair of my qualifying exam committee, particularly for the Belgian wafers he brought.

As for Dick, I will never forget—though this was probably just another morning of teaching for him—when I was approached at the end of one of his botany lectures by his PhD student and my TA, who, with clear mild confusion, gave me a Ziploc bag with a dried-up specimen of *Triphysaria pusilla,* a common yet small and underappreciated hemiparasitic plant that Dick had dug up for me to examine. Clearly, Dick was excited to have another parasitic plant geek on board. Turns out we shared another common passion in rock-climbing, which made for many an engaging conversation, at least for me.

I first called Ian after hearing of his proposal for an NSF internship. I ended up walking my bike three miles while talking to him to delay my arrival home, just to keep our conversation going. Ian's engaging questions and instant curiosity into my small world lit me up. His positivity and his knowledge of statistics and ecology, together with his connections to federal agencies, bolstered and improved my work. Finally, he displayed endless patience as we batted around ideas for how best to answer a tough research question with limited materials: once we had a plan, the coronavirus pandemic began. If Ian was exasperated as we returned to the drawing board, he never showed it.

I wish to express my most sincere gratitude to the following individuals who helped grow and harvest plants, prepared samples, collected data, and inspired project ideas: Will Braks, Helen Ganahl, Courtney Matzke, Erin Weisman, Victoria Fox, Bridgette Jones, Dongsen Xue, Andrew Sher, and Pierre Joubert.

Dr. Nate Haan, Dr. Claire Wainwright, and Loretta Pedersen welcomed me warmly into the Bakker lab and served as mentors and friends, helping me navigate both the technical and emotional aspects of graduate school.

My friends and family have built the foundational support upon which I stand. Maggie Keating, Marian Hsieh, Kate Kistler, and Hew Carrot were on the receiving end of many a long conversation and always found ways to make me laugh when I needed it most. Joanna Marquez kept her door open for me every time I returned to Seattle; she always let me bounce against the walls with pent up energy and, I would like to think, took mild amusement in it. Sage Stowell provided beautiful illustrations that have certainly enhanced this dissertation, and, more importantly, stayed up with me as we talked about pretty much everything in the slightly rickety yet charismatic house we shared for a year and a half in Wallingford. Some of my fondest memories of graduate school are from that period in which we both worked on our graduate research while trying to tame a wild and obstinate Basenji. Derek Allen and I kept those Wallingford house conversations (as well as taco night) going after Sage departed to bigger and better things.

Nic Thune was my first consistent climbing partner in Seattle and is now one of my best friends. A PhD can be a lonely endeavor, both during the long hours of writing and analysis, and simply mentally, as one dives into a topic more deeply than anyone else has ever before. Nic, an arborist and fellow plant lover, alleviated this by making a consistent and genuine effort to truly understand my research. We had plenty of time to talk about it during our ill-advised weekend trips to Oregon's Smith Rock State Park, the only two people in Seattle crazy enough to do it nearly every weekend for an entire winter and spring.

I received many gifts from Margareta and Hajrudin Hodžić; the list would be longer than the pages of this dissertation. The largest, though, may be the understanding of genuine gratitude, the type one gains when one learns that nothing in life is guaranteed, no situation immutable. When I was young and made any kind of claim, my older brother, Zlatan, would always retort "citation needed"— I blame him for my career choices. For as long as I can remember, I always knew he was much more intelligent and talented than me (you may have seen him on *Jeopardy!*), something that made me proud and, of course, jealous. These three are the most intelligent people I know, and I can only hope to make them proud.

Lastly, Tom Moulin, as I careened through the last three years, you were always there to steady me. In that simple act, you made me feel loved, trusted, and safe. Despite years of trying, I still cannot find the words to describe just how lucky I was to have met you that February afternoon in the Lime Kiln Canyon of Arizona. At the time, I was a rock-climbing nomad, living in my white 1999 Toyota Corolla with the bent muffler, enjoying life on the road before graduate school. A lot has changed since then, but, more importantly, a lot has stayed the same.

Dissertation overview

This dissertation threads together the physiological adaptations of a plant with its ecological role in natural communities, using hemiparasitic plants as a study group. While some chapters describe a physiological trait of a specific species of hemiparasite, others have been written with the intent of advancing ecological theory and inspiring future lines of research. I hope to have accomplished both. I also wish to inspire interest and curiosity in hemiparasites, as well as describe their largely underappreciated diversity.

I studied species in the subtribe Castillejinae (tribe Pedicularidae, Orobanchaceae), with a focus on the genus *Castilleja. Castilleja* consists of ~200 species of plants, distributed across various ecosystems. Commonly known as "paintbrushes," species in the genus *Castilleja* are some of the most beloved wildflowers in North America. Furthermore, *Castilleja* are mostly perennial hemiparasites, which have been understudied compared to their annual counterparts. The role that life history plays in mediating a hemiparasite's physiological and ecological adaptations to a heterotrophic lifestyle appears as a common conceptual theme throughout this dissertation.

Chapter 1 and Chapter 2 summarize the findings of several manipulative greenhouse experiments in which I examined what types of resources a hemiparasite gains from its host. In Chapter 1, I analyze the leaf mineral nutrition of *Castilleja* and its host, *Achillea millefolium*. By experimentally preventing and allowing parasitism, I show that the parasitism status of the hemiparasite influenced both the content and concentration of most mineral nutrients. Additionally, both unattached and attached *Castilleja* vary in their mineral nutrient profile from the host, indicating that there are fundamental physiological differences in nutrient uptake between hemiparasites and this host. Finally, I propose that phosphorous (P), which limited the growth of unattached hemiparasites, may be a particularly important nutrient for hemiparasites. The availability of P, thus, may influence the host preference of *Castilleja*. In Chapter 2, I show for the first time that both perennial and annual hemiparasites in Castillejinae can uptake and utilize host carbon. This study builds upon research showing that access to host carbon, once thought to be unimportant for hemiparasites, should be re-examined as a potential driver for hemiparasitism in plants.

In Chapter 3 and Chapter 4 I propose and examine ecological questions surrounding root hemiparasites. Using a combination of empirical and theoretical approaches, I propose potential mechanisms underlying why some hemiparasites impact their communities while others do not. In Chapter 3, I analyze a national vegetation dataset and show that North American hemiparasites are associated with increased evenness in plant communities. However, in contrast to other studies, I found no patterns between hemiparasite abundance and richness, suggesting the mechanisms for community impact differ between hemiparasites. In Chapter 4, I identify and examine trends found in the literature and in my own work to propose a novel framework in which life history is integrated with physiology to predict the role of a hemiparasite in its community.

As an appendix to this document, I include pilot studies that, for one reason or another, were not written as full chapters, yet I feel are important and worthwhile to document.

Note to the reader: All chapters are written as separate manuscripts for publication. Therefore, some of the content may be repetitive and formatting differs between chapters.

Background on parasitic plants

Hemiparasites are the focus of this dissertation, yet they only represent one sub-group of parasitic plants. To provide context for my research, I summarize core concepts of parasitic plant biology and discuss current research trends in the field.

Classification and diversity:

There are two main types of parasitic lifestyles in plants; haustorial parasites attach to and siphon resources away from another plant using a specialized organ called a haustorium whereas mycoheterotrophs parasitize the mycorrhizal networks of other plants (Nickrent 2020). Hereafter "parasitism" will be used to discuss haustorial parasites, which are only found in angiosperm clades. A widely adopted strategy amongst plants, parasitism is thought to have evolved independently around 12 times in angiosperms (Westwood et al. 2010). Accordingly, parasitic angiosperms are ubiquitous members of the world flora, representing ca. 4,750 species across 292 genera in a variety of ecosystems (Nickrent 2020). These plants can occur as vines, herbaceous annuals, trees, and long-lived perennials. Furthermore, they occur in all major biomes of the world, from xeric grasslands and tropical rainforests to the boreal forest (Nickrent 2002, Těšitel 2016). Some facultative hemiparasites like *Castilleja* appear "normal" aboveground, with green tissues. On the extreme end, endoparasitic plants live primarily underground or internally within a host, emerging only to flower. In the case of *Rafflesia,* this flower happens to be the largest in the world (Davis et al. 2018). Such a diversity in evolution, morphology and distribution has allowed for the classification of parasitic plants into sub-groups, varying in their degree of host dependence.

Parasitic plants are first classified based on their ability to photosynthesize. Endoparasitism has been suggested as being the "peak" of host-dependency. However, in the most comprehensive and recent review on the functional biology of parasitic plants, Těšitel (2016) proposed separating endophytic parasites from the two most common groups of parasitic plants, hemi- and holoparasites. Holoparasites (from Greek "holo-," whole, entire, complete) are non-photosynthetic and are completely dependent on their host to survive. Hemiparasites, (from Greek "hemi-", half) are "half" parasitic, in that they attach to hosts yet also photosynthesize. Hemiparasites are further distinguished based on their level of host dependency. Obligate hemiparasites cannot survive without a host, while facultative hemiparasites can complete their life cycle autotrophically. A further classification separates parasitic plants based on where they attach to the host, via the root or the stem.

While such classifications are useful, it is important to recognize that most parasitic plants do not fit rigidly into such groupings and instead exist on a spectrum of host dependence, photosynthetic ability, and even whether they exclusively rely on phloem or xylem host materials. For example, root hemiparasites can obtain some reduced carbon from their hosts in the form of xylem-mobile organic elements (Těšitel et al. 2010) and there are xylem-only feeding holoparasites like *Lathraea* (Nickrent 2002, Samigullin et al. 2016). Functionally, the phloem-feeding genus *Cuscuta* is holoparasitic, yet most species retain very limited photosynthetic capacity and evolutionarily are considered to be "transitioning" from hemiparasitism to holoparasitism (Krause 2008, Těšitel et al. 2015, Sun et al. 2018). Furthermore, given this variation in photosynthetic ability and host dependence, the distinction between "facultative" and "obligate" parasitism is difficult to demonstrate.

Bearing in mind the variability of the degree of host dependence observed, most hemiparasites are considered facultative root hemiparasites. This group of plants is the focus of this dissertation.

Adaptations to parasitism

Although parasitic plants are diverse in growth-form and physiology, they are unified by the presence of the "haustoria", from the Latin "haustor-," meaning to drain or draw (Lewis and Short 1879). However, there are interspecific differences in haustoria structure, development and function described in most detail by the comprehensive review of Yoshida et al. (2016). For example, obligate hemiparasites all have primary haustoria, which form at the end of the growing radicle while the plant is a germinant. In contrast, facultative hemiparasites only possess secondary haustoria which grow out of lateral roots (Dörr 1997, Yoshida et al. 2016). In addition to the evolution of a haustoria, parasitic plants have converged on certain physiological strategies and morphological adaptations to successfully locate and parasitize hosts.

Parasitic reduction syndrome

Almost all plant lineages that have evolved away from autotrophy and towards heterotrophy display profound changes in morphology, biochemical, and molecular traits. In general, the evolutionary release from a fully autotrophic lifestyle has caused a reduction in the investment of morphological traits and certain genes. This phenomenon is collectively referred to as "parasitic reduction syndrome." The degree of overall reduction is correlated with host dependence as the parasite is released from particular physiological constraints (Heide-Jørgensen 2013). For example, holoparasites like *Cuscuta* experience very little selective pressure to photosynthesize or forage below-ground; they contain very low levels of chlorophyll, their leaves are minute, and their roots have been reduced to haustorial stubs (Dawson et al. 1994). In contrast, facultative hemiparasites dramatically reduce their root biomass upon attachment to a host (Atsatt and Strong 1970) but retain chlorophyll and green, obvious leaves. In hemiparasites, the degree of reduction seems to be correlated to life history, with annual hemiparasites having a more reduced root mass than perennials, who must retain some root mass during dormant parts of the year (Matthies 2017, *cf.* Table 6).

Parasitic reduction syndrome also affects the genome of parasitic plants. As a plant becomes increasingly heterotrophic, the plastome, which mainly contains genes for photosynthesis, is released from functional constraints and accumulates gene losses and pseudogenes (Krause 2008, Sun et al. 2018). In addition, the parasitic lifestyle seems to cause an increase in the genome-wide rate of molecular evolution with faster substitution rates having been reported for numerous genes in several parasitic plant lineages (Wicke and Naumann 2018). Though these genetic markers mostly occur in holoparasites, a study of hemiparasitic genomes suggests that they also have gene deletions in the plastome (Li et al. 2017).

Physiological adaptations

All parasitic plants have adapted physiologically to ensure access to host resources once attached. This is particularly well understood with respect to the unique “water-wasting” strategy common to all parasitic plants. By transpiring at a rate consistently higher than that of the host, parasites create a “sink” effect and siphon the xylem or phloem sap toward the haustorium. To do this, parasites maintain a water potential less than that of the host by keeping their stomata open except under the most severe water stress (Phoenix and Press 2005, Irving and Cameron 2009, Světlíková et al. 2018). In addition, parasitic plants tend to have higher mineral nutrient concentrations in their dry tissue than their hosts. Although some of this may be due to the fact that xylem is uni-directional and therefore materials are not translocated back to the host, some mineral nutrients accumulate in such high amounts that suggest a parasitic plant may have differential selectivity in nutrient uptake than the host. Most notably, parasitic plants have been shown to accumulate up to 20 times more potassium in their tissue than the host (Press and

Graves 1995, Lamont 1983, Glatzel 1983, Jiang et al. 2010). Given the role of potassium in maintaining guard cell turgor, it is thought that its high concentration in parasites may serve to maintain high stomatal conductance (Smith and Stewart 1990). Finally, most parasitic plants that have been studied have extremely elevated levels of abscisic acid (ABA), the function of which is unclear given that ABA is usually involved in stomatal closure, which is counter to a hemiparasite's water-wasting strategy (Jiang et al. 2018). One hypothesis is that that the high levels of ABA may be an adaptation to increase root hydraulic conductivity and further siphon host xylem resources towards the plants (Jiang et al. 2004).

Ecological impacts of parasitic plants

In addition to distinctive physiological, morphological, and genomic adaptations, parasites also disproportionately affect their communities relative to their abundance. Though I focus on hemiparasites here, the influence of stem holoparasites on forested ecosystems has been reviewed (Barbosa et al. 2016, Selosse et al. 2017, Melnyk 2017).

Root hemiparasites are generalists and can parasitize a wide variety of host plant species. However, they disproportionately parasitize certain hosts with which they tend to perform better (Marquardt and Pennings 2010, Mudrák et al. 2016, Sandner and Matthies 2018), a trait termed "host preference." In addition to host preference, hemiparasites tend to parasitize the dominant plant of an ecosystem more, simply by virtue of the abundance of said host (Těšitel et al. 2017). This disproportionate parasitism can change the competitive dynamics between host and non-host species and affect the vegetation structure and diversity of a plant community (Press 1989, Pennings and Callaway 2002, Watson et al. 2011, Li et al. 2012, Těšitel et al. 2017). Hemiparasites can also alter the availability of certain mineral nutrients (Fisher et al. 2013) and have been demonstrated to affect higher trophic levels beyond plants (Watson et al. 2011, Hartley et al. 2015, Haan et al. 2018). As a result, many are considered to be keystone species (Press and Phoenix 2005).

Current trends in parasitic plant research

Given their bizarre and unique lifestyle, parasitic plants have long attracted the attention of a variety of researchers. In the past, attention was focused on developing methods to control invasive parasites like *Striga* which can cause severe economic harm by parasitizing and devastating crop plants like corn and sorghum (Spallek et al. 2013). Over the last several decades, however, research focus has broadened and shifted to answering questions pertaining to the molecular biology, systematics, and ecology of parasitic plants. For example, there has been a renewed effort to undercover "parasitic plant genes" unique to parasites and related to haustorial development. Transcriptome analysis of three parasitic plants in the Orobanchaceae that span the spectrum of host dependence—*Triphysaria versicolor* (facultative hemiparasite), *Striga hermonthica* (obligate hemiparasite) and *Phelipanche aegyptiaca* (obligate hemiparasite) — is currently underway and may very well change our view of the evolution of parasitism in plants (Wickett et al. 2011, Westwood et al. 2012; see ppgp.huck.psu.edu for updates). New molecular methods have helped clarify the closest non-parasitic relatives of holoparasites, as genomic deletions and horizontal gene transfer have made phylogenetic classifications difficult (Nickrent 2020). Additionally, there has been considerable interest in investigating how hemiparasites interact with their communities and whether they could be used effectively in ecological restoration to promote diversity and reduce the abundance of invasive plants (Těšitel et al. 2020).

The unique phylogeny, interesting physiological and genomic adaptations, and compelling ecological roles of root hemiparasites make this functional group of plants a fascinating system in which to explore both theoretical and applied questions connecting plant physiology and ecology.

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Chapter 1 : **Leaf mineral nutrition of hemiparasites and their hosts**

Abstract

Given their partially autotrophic nature, access to mineral nutrients is of particular importance to root hemiparasites. Although it is well known that hemiparasites have higher concentrations of most mineral nutrients than their hosts, this has never been examined for species in the hemiparasitic genera *Castilleja* and *Cordylanthus.* Furthermore, intraspecific comparative studies examining nutrient content and concentration between attached and unattached species are mostly absent from the literature, despite their obvious value in determining which, if any, mineral nutrient limits hemiparasite growth.

I examined the leaf mineral nutrition profile of hemiparasites in the genera *Castilleja* and *Cordylanthus* (Orobanchaceae) to determine if these genera conformed to mineral nutrient patterns seen in other hemiparasites. I also addressed the hypothesis that P limited the growth of unattached hemiparasites. In two experiments, hemiparasites were grown with the host *Achillea millefolium* and parasitism was experimentally allowed or prevented. Levels of potassium (K), sodium (Na), sulfur (S), magnesium (Mg) and phosphorous (P) were analyzed, both as a multivariate suite of responses and as individual nutrients. The multivariate suite of nutrients was subject to Principal Component Analysis. I related nutrient levels to parasitism status (attached and unattached) and type of plant (host or hemiparasite). Like other hemiparasites, nutrient composition of hemiparasites in our study differs considerably from that of hosts. However, we found that P limited growth rates, suggesting that, among the diversity of hemiparasites, it is a general strategy to accumulate nutrients, but that the nutrients limiting growth may be system specific.

Introduction

Since plants are immobile, they are constrained to acquire resources from their immediate environment. The foraging ability of a plant is vitally important for access and uptake of mineral nutrients critical to survival and growth (Weiser et al. 2016). However, to facilitate acquisition of certain nutrients, many plants have developed symbiotic relationships with other organisms such as mycorrhizal fungi (van der Heijden et al. 2015) or bacteria (Mahmud et al. 2020). Other plants, however, rely on a parasitic lifestyle to ensure access to adequate amounts of mineral nutrients. Root hemiparasites are photosynthetic yet also tap into the xylem sap of other plants by attaching to hosts using specialized root structures called haustoria (Těšitel 2016). Given their partially autotrophic nature, access to host mineral nutrients has long been thought of as a primary evolutionary driver for hemiparasitism in plants (Press and Graves 1995). Additionally, most hemiparasites have vestigial and shallow root systems, suggesting their ability to forage for nutrients is limited (Mudrák et al. 2016, Matthies 2017). However, whether root hemiparasitism has evolved as a strategy to ensure access to indiscriminate host resources – i.e., all materials in the host xylem sap– or as a mechanism for garnering a particular type of resource remains an unanswered question in hemiparasitic plant biology.

Considering the importance of mineral nutrition to hemiparasites, the amount of research on the subject is limited. Most of the literature compares the mineral nutrition of hosts to that of phloem feeding hemiparasitic plants in the Loranthaceae and Viscaceae, though studies have assessed the xylem-feeding hemiparasites *Thesium* (Santalaceae) (Fer et al. 1994, Luo and Guo 2010), *Rhinanthus* (Orobanchaceae) (Jiang et al. 2010), *Striga* (Orobanchaceae) (Smith and

Stewart 1990) and *Olax* (Santalaceae) (Pate et al. 1990). From this work, we know that all root hemiparasites tested have higher mineral nutrient concentrations than their hosts.

Mechanistically, the mineral nutrition of a hemiparasite is linked to its water relations. Parasitic plants transpire much more than their hosts, maintaining a water potential less than that of the host and consequently creating a sink effect to siphon xylem sap towards the haustorium (Phoenix and Press 2005). Due to the uni-directional nature of xylem flow and the lack of phloem in haustoria, root hemiparasites harbor large amounts of host xylem mobile solutes. This is particularly well appreciated for potassium (K) which is thought to be used by the hemiparasite not for metabolic reasons but rather as a means of retaining guard cell turgor to allow stomata to remain open even under high drought stress to extract host resources (Smith and Stewart 1990, Press and Graves 1995).

Because hemiparasites tend to accumulate many mineral nutrients from their hosts, it is not always clear which of these limit their growth. To determine this, comparisons of mineral nutrition can be made between attached and unattached hemiparasites, though this type of study is rare. This type of analysis gives us valuable ecological insight into which mineral nutrients may be limiting the hemiparasite growth. For example, Luo and Guo (2010) compared dry leaf material from parasitic and autotrophic individuals of *Thesium chinense* (Santalaceae), and reported that parasitic *Thesium* had higher concentrations of K and phosphorus (P) and nonparasitic *Thesium* had higher concentrations of sodium (Na) and calcium (Ca). Additionally, Jiang (2004) found that parasitic *Rhinanthus* N, P and K were dramatically higher (up to 20-fold for K) than unattached *Rhinanthus.* Similar trends were also observed in *Melampyrum,* with the attached hemiparasite having higher K and P and lower Ca and Mg compared to unattached individuals (Lechowski 1995).

Studies also differ with respect to whether hemiparasites are limited by a particular nutrient; however, some trends do emerge. Seel et. al (1993) found that *Rhinanthus* was limited by P (but not N or K) and Li et al (2013) found that two different species of *Pedicularis* had different nutritional needs, though P limitation suppressed the growth of both species. Additionally, in a xylem sap assessment of unattached hemiparasites, Seel and Jeschke (1999) suggest that low phosphate concentrations in unattached plants may be indicative of growth limitation by P and that uptake of host xylem phosphate may be the most important factor in enhancing *Rhinanthus* growth. It has even been shown that host plants are less damaged by hemiparasites who are fertilized with P than hemiparasites who receive no fertilizer (Davies and Graves 2000). Thus, although it has been proposed that root hemiparasites are primarily parasitic for N, due to their high performance with leguminous hosts, these studies highlight the potential importance of P (Irving and Cameron 2009).

It can be difficult to tease apart the signal of elevated nutrients in attached hemiparasites, i.e., whether nutrients are elevated due to the inherent physiology of a hemiparasite or to host attachment? By comparing the mineral nutrient profiles of unattached hemiparasites with that of their hosts and attached parasitic counterparts, we can pinpoint which nutrients a hemiparasite gains from the host. Those nutrients which are elevated in both unattached hemiparasites and attached hemiparasites are likely more related to the foraging physiology of a hemiparasite. In contrast, nutrients that are similar or lower than those of host concentrations in unattached hemiparasites, yet higher in attached hemiparasites, may be those that the parasite relies on host connections to access.

Finally, comparing the mineral nutrition between attached and unattached hemiparasites is confounded by the fact that, in general, hemiparasites gain biomass as they attach to a host, potentially diluting certain nutrients. Thus, only assessing nutrient concentration (grams of nutrient/gram of dry tissue) can be misleading, giving artificially lowered values due to hemiparasite growth. However, to date, no study has considered changes in both nutrient content (mg per plant) and concentration when studying hemiparasitic plant nutrition.

I analyzed the nutrient content and concentration of leaf tissue of hemiparasites in the genera *Castilleja* and *Cordylanthus* and their host, *Achillea millefolium.* While the exact definitions of concentration and content are contentious, I conform to literature standards in parasitic plant biology, with concentration being µg nutrient/g leaf tissue (or converted to percent) and content being concentration multiplied by the leaf mass of the plant (Tolhurst et al. 2005). By using a novel system to control for parasitism, I addressed the following objectives:

i.) Compare the mineral nutrition of attached and unattached hemiparasites with that of their host, with the expectation that hemiparasites accumulate more of most mineral nutrients compared to hosts.

ii.) Compare nutrient concentrations and contents of attached and unattached hemiparasites to explore which mineral nutrients are accumulated due to parasitism and which may limit the growth of unattached hemiparasites.

I provide the first results that show that species in these genera conform to generalizations of hemiparasitic plant nutrition, having higher nutrient concentrations than their host. By comparing unattached hemiparasites with their attached counterparts and hosts, I conclude that hemiparasites are limited in their growth by P.

Methods

Data source

The data for this experiment comes from two studies. Both experiments were conducted in the Douglas Research Conservatory Greenhouse in the Center for Urban Horticulture at the University of Washington, Seattle, USA. The average temperature was 20.8°C and the average relative humidity was 45.5%. Supplemental lighting was on between the hours of 6 AM t 8 PM, but only illuminated if the outdoor light meter read a value of 500μmol. Watering was performed as needed. In both studies, parasitism was controlled for by planting half of the hemiparasites in root exclusion bags, which allow water and nutrients to pass through but prevent the parasite from making haustorial connections with the host (Figure 1.1). The bags are made of nylon woven mesh of 35- or 45-micron diameter openings (Industrial Netting, MN). The fabric was cut to create 5x5 inch bags and were sealed with waterproof glue. Both mesh sizes successfully prevented hemiparasites from penetrating host roots. In both experiments, SunShine #2 (Sungro Horticulture) was used as a media, which contains mychorrize and a slight nutrient charge.

In both cases, parasitism was not confirmed by checking for haustoria. This was not performed because it is labor intensive and can lead to skewed conclusions. For example, sometimes the haustoria break off at the host root junction and are difficult to find, causing a potentially misleading assumption that parasitism did not occur. Instead, I relied on the observation that attached hemiparasites tend to enter a distinct rapid growth phase (Matthies, 1997, Hodžić , personal observation).

Experiment A:

This experiment was undertaken from fall 2016 -winter 2017. The host plant *Achillea millefolium* (hereafter referred to by its genus) was planted with several *Castilleja levisecta* individuals to test the effects of factorial drought and parasitism treatments on *Achillea.* Half of the pots were watered normally and the other half experienced drought, receiving half as much water as the control pots. Within each drought treatment the state of parasitism was manipulated; in half of the pots *Achillea* was grown with hemiparasites that were allowed to parasitize and in the other half parasitism was controlled for by planting hemiparasites in root exclusion bags. Plants were harvested when they began to flower, or nine weeks. Pots received one liquid dose of Fish & Poop fertilizer (Monterey Lawn & Garden) upon planting

Experiment B:

This experiment was conducted in fall 2020. The host *Achillea* was planted with ten different hemiparasite species to assess interspecific differences in a hemiparasite's reliance on the host. Ten pots were planted per hemiparasite species. Within each pot, half of the hemiparasites were planted normally and half were planted in a root exclusion bag to prevent parasitism. No fertilizer was applied. The harvest was standardized by flowering time; when an individual hemiparasite in a host-hemiparasite combination began to bud, all pots of that hemiparasite species were harvested. Hemiparasite plants were harvested and weighed individually. Samples from five of the ten species planted –*Castilleja levisecta, Castilleja foliolosa, Castilleja wightii*, *Castilleja minor* and *Cordylanthus pilosus*–met the weight threshold for mineral nutrition analysis (see Methods below).

In both experiments, the biomass of attached hemiparasites was significantly higher than that of unattached hemiparasites.

Leaf digestion

Leaf tissue was dried at 70°Celsius for two days. The leaf tissue was ground with a mortar and pestle, weighed, and placed in envelopes for delivery to the School of Environmental and Forest Sciences Analytical Service Center. In almost all cases, the leaves of individual hemiparasite plants did not meet the minimum weight requirement (0.20 mg) for digestion. Consequently, leaves of multiple hemiparasite individuals in the same pot and treatment were combined. Nutrient analysis was performed via a wet acid digestion procedure using nitric acid and hydrogen peroxide (Twyman 2005). I focused on five macronutrients—K, P magnesium (Mg), calcium (Ca), sulfur (S)–and the micronutrient Na. Na is included because it is a xylem-mobile cation that plausibly may be used by parasitic plants to maintain higher osmotic potential than their hosts.

Statistical analysis

All analyses were conducted in R 4.0.2 with a significance level of 0.05 (R Studio Team, 2019). Nutrient levels were analyzed, both as a multivariate suite of responses and individually. The multivariate suite of nutrients was subject to Principal Component Analysis (PCA) (Pearson 1901) using the function *princomp* to reduce the dimensionality of the variables and to find combinations of the variables to produce PC axes that are uncorrelated, as it is well-known that nutrients interact with one another (Chapin 1980). For the PCA, all nutrients were relativized by their maximum to give each nutrient equal weight in analysis.

Hemiparasite nutrient concentrations were expressed as percentages. Additionally, nutrient contents were calculated by multiplying the total aboveground dry biomass per individual plant (or total mass of individual plants if combined for analysis) by the tissue nutrient concentration. When comparing hosts and hemiparasites, only nutrient concentration was used due to inherent size differences between hosts and hemiparasites.

Attached hemiparasite vs. host

To test for differences between hosts and hemiparasites, I subset the dataset to include only parasitic hemiparasites (n=29) and host individuals that were parasitized (n=29). I interpreted each PC axis based on the loadings and ran linear mixed models for the first two PC axes, with experiment identity (A or B) as the first fixed effect term in the model, drought status eedsas the second fixed effect and parasitism status (hemiparasite or host) as the third fixed effect; hemiparasite species identity and pot identity were modeled as random effects. Hemiparasite species identity was assigned as a random effect because we are interested in the overall response of the functional group, not in interspecific differences. If warranted by global PCA, I assessed each individual nutrient with the same model structure.

Effects of parasitism on hemiparasite and host

To test how parasitism affects leaf nutrition of both the hemiparasite and the host, I subset the dataset to include only parasitized hosts (n=10), unparasitized hosts (n=11), attached hemiparasites (n=10), and unattached hemiparasites (n=11). I ran a linear mixed model with PC1 as the response variable and drought as the first fixed effect (to account for drought in the model, though not to test its effects on nutrient load), parasitism status (attached hemiparasite, unattached hemiparasite, parasitized host, unparasitized host) as the second fixed effect and pot identity as a random effect. The same model was run for PC2 as the response variable. If parasitism status was significant, all pairwise comparisons were assessed with the package *emmeans* (Lenth, 2020) with a particular interest in the comparison between unattached hemiparasites and unparasitized hosts. These data were only from Experiment A, since Experiment B had no unparasitized hosts, so models did not need to account for experiment identity or hemiparasite species identity.

Attached hemiparasites vs. unattached hemiparasites:

In assessing attached (n=29) and unattached (n=29) hemiparasites, I ran the same models as for comparing attached hemiparasites to the host, however four models were run: they two for the first two axes of a PCA based on nutrient concentration and two for the first two axes of a PCA based on nutrient content. If warranted by global PCA, univariate analysis was done for both the content and concentration matrices.

Results

Attached hemiparasite vs. host

The nutrient concentration response matrix was partitioned into two PC axes that accounted for 82% of the total variation (Table 1.1, Figure 1.2) PC1 was positively correlated with Ca, Mg and S and was significant for plant type (hemiparasite vs. host, t= -13.2, p <0.001). PC2 was interpreted as K, Na and P and was not related to plant type (t = -0. 77, p =0.450) (Table S1.1)

Univariate analysis revealed that parasitic hemiparasites had significantly higher concentrations of all nutrients except for Na, for which there was no difference between hemiparasite and host individuals (Figure 1.3, Table S1.1).

Effects of parasitism on hemiparasites and hosts

The nutrient concentration response matrix was partitioned via PCA into two axes that accounted for 78% of the total variation (Table 1.2, Figure 1.4). PC1 was interpreted as being highly correlated with Ca, Mg and S and PC2 was interpreted as K, Na and P. PC1 was significant for all comparisons, except between the parasitized host and unparasitized host. PC2 was only significant for differences between parasitism group of hemiparasites, and parasitized host and parasitized hemiparasites (Table S1.2).

Univariate analysis revealed that autotrophic hemiparasites had significantly higher nutrient concentrations for K, Ca, Mg and S than their autotrophic hosts, with similar levels of Na and P. The levels of all nutrients for the hosts did not change significantly with parasitism (Figure 1.5, Table S1.2).

Attached vs. unattached hemiparasites

Concentration

The nutrient concentration response matrix was partitioned via PCA into two axes that accounted for 77% of the total variation (Table 1.3, Figure 1.6A) PC1 was interpreted as being positively correlated with Ca, Mg and S and was significant for parasitism status ($t = -7.50$, $p<0.001$). PC2 was interpreted as K, Na and P was significant for parasitism status ($t = -3.93$, $p <0.001$). (Table S1.3)

Content

The nutrient content response matrix was partitioned via PCA into two axes that accounted for 91% of the total variation (Table 1.3, Figure 1.6B) PC1 was interpreted as total mineral nutrient load and was significantly different between attached and unattached hemiparasites (t = 3.20, p = 0.004) PC2 was interpreted as K, Na and P. PC1 and was significant for plant type (t= -6.13, p <0.001) (Table S1.4).

Univariate

Attachment to a host increased both the content and concentration of K and P in hemiparasites. Na was the only nutrient which did not differ significantly in either concentration or content between treatments (Table S1.3). Unattached hemiparasites had significantly higher concentrations of Ca, Mg and S but the content of these nutrients did not differ between unattached and attached hemiparasites suggesting that this is a dilution effect in which unattached plants, which are smaller, have greater nutrient concentrations (Table 1.4, Figure 1.7).

Tissue nutrient concentrations were plotted as a function of nutrient contents following the approach of Timmer and Stone (1978, cf. Fig. 4) and Friedrich et al. (2012, cf. Fig 4) to evaluate the nutrient limitation of hemiparasite plant growth (Figure 1.7). This approach accounts for the fact that in both experiments, attached hemiparasites had significantly higher biomass than unattached hemiparasites. Trajectories of shifts in nutrient concentration and nutrient content because of parasitism status were plotted in a nutrient content- nutrient concentration space consisting of two lines. The upward line is determined by the respective data point obtained from the average of the control treatment and by the origin of the coordinate system. The horizontal line is determined by the respective data point obtained from the average of the control treatment and runs parallel to the x-axis. For the purposes of this study, both experiments were combined such that the control treatment was the mean of the no parasitism group across both experiments. The trajectories indicate whether an increase in nutrient contents occurred because a nutrient was limiting (a shift into the sector between the upward and the horizontal line, i.e., increases in both nutrient concentrations and biomass), was diluted due to increase in growth (shift below horizontal line, i.e., decreased concentration with gain in biomass) or due to luxury consumption (shifts of values along the upward line, i.e., increased nutrient concentrations without any gain in biomass).

This visualization suggested that K and P were limiting the growth of the hemiparasites while Ca, Mg and S were diluted due to growth. Na did not differ between attached and unattached hemiparasites (Figure 1.7).

Discussion

I have demonstrated that the mineral nutrition of parasitic hemiparasites in the genera *Castilleja* and *Cordylanthus* is like that of other hemiparasites. I show that species in these genera have higher concentrations for most nutrients than their autotrophic hosts and that, for some nutrients, this trend remains even for unattached hemiparasites. Finally, I provide additional support to the theory P limits the growth of hemiparasites.

Hemiparasites have high leaf mineral nutrient concentrations

Nutrient composition of hemiparasites differed considerably from that of hosts (Figure 1.2). This was further substantiated by univariate analysis which showed that hemiparasites had significantly higher nutrient concentrations for all nutrients except for Na. The fact that PC2, correlated with K, P and Na, was not significantly different between attached hemiparasites and parasitized hosts may suggest that these are the nutrients which hemiparasites are gaining from their hosts (Figure 1.2). However, when assessing unattached hemiparasites and unparasitized hosts (whose PC axes were interpreted in the same way), attached *Castilleja levisecta* did differ from parasitized hosts in both PC1 and PC2 (Figure 1.4). Thus, this variation in PC2 may be due to interspecific differences between hemiparasites, as well as variation due to experiment identity (Table S1.1).

Like all other parasitic plants tested, these hemiparasites had significantly more of all nutrients (except for Na), particularly of K and P (Figure 1.3). This provides further support to the idea that attached hemiparasites harbor more mineral nutrients than their hosts. When comparing unattached *Castilleja levisecta* to *Achillea*, the hemiparasite surprisingly still had higher leaf mineral nutrient concentrations of Ca, K, Mg and S. Thus, unattached hemiparasites maintain nutrient profiles distinct from that of their hosts, implying that hemiparasites have different foraging styles than their hosts. Given that a hemiparasite, even when unattached, has a shallow root system, we would expect them to be poor foragers (Matthies 2017). Since, Mg, S and Ca are relatively mobile within the soil, the relative abundance of these nutrients combined with low amounts of the immobile nutrient P may reflect a hemiparasite's lack of selectivity in mineral absorption (Fer et al. 1994, Lechowski 1995). The media used did contain mychorrizae, and so this may reflect that hemiparasites do not form mycorrhizal associations, or did not in this experiment. The high levels of K may suggest that a hemiparasite harbors it even in the absence of parasitism, implying a differential mechanism for K uptake than that of the host.

Hemiparasites are limited in their growth by P

PCA suggested highly significant differences between parasitism status in hemiparasites, with both axes being significant for concentration and content, warranting a univariate analysis. By plotting concentration and content I determined how mineral nutrients fluctuated while accounting for changes in biomass (Figure 1.7). This analysis suggested that P and K, which were the only two nutrients to increase in both content and concentration for the parasitism group, are growth limiting for the hemiparasites. Other trends can be explained by dilution due to growth of the hemiparasite (S, Mg, Ca).

Given that hemiparasites use K to maintain a high stomatal conductance (Press et al. 1988, Talbott and Zeiger 1996, Světlíková et al. 2018) and that my results showed that even autotrophic hemiparasites had high concentrations of K when compared to a host (Figure 1.4), K may simply be increasing as a physiological response to parasitism–i.e., higher demand to maintain high transpiration rates. K is also very mobile within the plant, thus, an increase in content and concentration may simply be the result of a hemiparasite allocating more K to its leaves for use in regulating guard cell turgor to exploit the host rather than an initial growth-limiting deficiency of K.

The importance of P was also highlighted in the analysis of unattached *Castilleja* and unparasitized *Achillea*. P is the only nutrient in which unattached *Castilleja* has an average concentration lower than that of the host (though not significantly so), though the attached *Castilleja* has a significantly higher concentration than the host, suggesting that *Castilleja* was primarily parasitic for P. Thus, our results are in line with previous studies which highlight the potential importance of P for the growth of hemiparasites. P is a vital nutrient, playing a major part in protein synthesis, growth of new tissues, and division of cells (Chapin, 1980). Since most plants access P via mycorrhizal associations, simply assessing the mycorrhizal status of hemiparasites would be important in informing the theory that hemiparasites are limited by P (Li et al. 2013). To my knowledge, a survey of the mycorrhizal status of parasitic plants has not been done.

Ecological implications

We know that most hemiparasites grow larger with certain hosts (Matthies 2017, Sandner & Matthies, 2018) and that, in natural communities and mixed diet greenhouse experiments, they disproportionately parasitize certain hosts relative to their abundance (Press & Phoenix, 2005). Understanding and predicting host preference is a critical research field in parasitic plant biology as it will allow us to not only control hemiparasitic invaders better but predict the proven outsized impacts that native hemiparasites can have in their natural communities (Chapters 3,4).

This study has implications for our understanding of the host-hemiparasite relationships of *Castilleja* and *Cordylanthus*. It suggests that hemiparasites may grow more and perform better if attached to a host with high levels of P. This may have consequences for hemiparasite performance and effectiveness in certain environments. For example, it is well known that hemiparasites prefer open, nutrient poor habitats in which they will not be outcompeted by hosts for light (Těšitel et al. 2015). Determining whether hemiparasites are more associated with P-limited areas would be a promising avenue of research and allow us to connect the mineral needs of hemiparasites with their distribution and ecology.

Furthermore, host quality is also largely determined by host resistance and tolerance. Anecdotally we know that *Achillea* is an excellent host for *Castilleja* and likely *Cordylanthus*; when grown with *Achillea* these hemiparasites tend to grow larger and flower sooner (Haan *et al.*, 2018, Hodžić , personal observation). Here, *Achillea's* mineral nutrition was not affected by parasitism, suggesting high tolerance to the hemiparasite rather than high defense (Figure 1.4). Thus, using mineral nutrition profiles may be a useful proxy for host quality. However, this idea needs further development, as *Achillea* may have manifested damage to parasitism in ways that we did not measure; for example, though its concentrations did not increase, its allometry and water relations may have changed (Shen et al. 2006).

Thus, given that mineral nutrition is highly related to the water relations of the hemiparasite, and the water relations of the hemiparasite is correlated with host damage and community level impact, future research should investigate whether we can use mineral nutrition as a proxy for the degree of parasitism. Given the complications with traditional means of water relations measurements on some hemiparasites, this would be a useful and relatively simple way to get a broad understanding of host-hemiparasite relationships. However, it makes several assumptions regarding any selective uptake of ions at the haustorial interface as well as the lack of unidirectional transfer within the hemiparasite itself. These assumptions could be analyzed via a solute flux model using xylem sap, as done by Jiang (2004) for *Rhinanthus.*

Study limitations

Any analysis into resource uptake by hemiparasites is complicated by the fact that they have two sources of nutrients –host uptake via the haustoria and their own below-ground foraging. Thus, I cannot distinguish how many nutrients came from the host and how many from increased or more efficient foraging. Finally, I compared parasitic hemiparasites to hosts that were being actively parasitized. It is plausible that parasitism changed the mineral nutrition of the host itself, complicating a direct comparison. However, I showed that at least when being parasitized by *Castilleja levisecta*, *Achillea* is not affected by parasitism (Figure 1.4). When comparing parasitic to nonparasitic hemiparasites, I can at least determine whether the nutrient was simply diluted due to increased growth, however we still cannot pinpoint the source of the increased nutrient. Future research using isotopic tracers that follow an individual hemiparasite through autotrophy and heterotrophy would provide valuable insight into the flux of solutes.

Additionally, I only assessed the mineral status of leaf tissue, which is one of several destinations for nutrients gained by the host. All nutrients are not mobile within the plant; amongst this suite, S and Ca are typically not re-translocated once incorporated into the plant. Thus, for other mobile nutrients, any changes could also be interpreted as differential allocation of the hemiparasite as a physiological response to parasitism. However, I would expect that upon attachment to a host, investment into the roots is minimal. Additional xylem sap studies with these genera are needed to see how nutrients in leaf tissue mirror what is found in the hemiparasite xylem tissue.

Furthermore, there is very little information on the haustorial structure of these genera. I assumed that these hemiparasites have direct xylem continuity with the host, that their haustoria have no phloem and that there is no back -transfer to the host. To date, only cursory anatomical studies have been done with *Castilleja*, though none with *Cordylanthus* (Montes-Hernández et al. 2015) Thus, while these assumptions are reasonable, anatomical research into these genera would further refine our conclusions and predictions and allow us to link a hemiparasite's anatomy with its physiology and, ultimately, the ecological role it may play.

Conclusion

Mineral nutrition analysis provides an intersecting and relatively easy way to examine resource flow between hosts and hemiparasites. Here I show that hemiparasitic *Castilleja* and *Cordylanthus* behave like other hemiparasites in that they have relatively high concentrations of mineral nutrients. This analysis also suggests that unattached hemiparasites forage differently than their hosts and that they are limited in their growth by P. Although future physiological and anatomical research are needed for these genera, this study clearly shows that P is a vital nutrient for their growth and likely plays a large role in their host preference.

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Table 1.1. The loadings (correlations) of each variable for the first two components of a PCA based on nutrient concentrations of attached hemiparasites (n=29) and parasitized hosts (n=29). See Figure 1.2 for a biplot.

	Component 1	**Component 2**
Ca	-0.528	0.214
K	0.184	-0.568
Mg	0.542	0.200
Na	-0.293	0.453
P	0.138	-0.593
S	0.537	0.184
Variance explained	50%	32%

Table 1.2. The loadings (correlations) of each variable for the first two components of the nutrient concentration of attached and unattached *Castilleja levisecta* and the host, parasitized and unparasitized *Achillea millefolium*. See Figure 1.4 for a biplot.

	Component 1	**Component 2**
Ca	0.544	0.150
K	0.363	-0.514
Mg	0.548	0.046
Na	0.072	-0.522
P	0.015	-0.615
S	0.516	-0.033
Variance explained	52%	26%

Table 1.3. The loadings (correlations) of each variable for the first two components of the nutrient contents and concentrations of attached and unattached hemiparasites. See Figure 1.6 for a biplot.

	Concentration		*Content*	
	Component 1	**Component 2**	**Component 1**	**Component 2**
Ca	0.530	0.074	0.433	0.334
K	0.356	-0.276	0.425	-0.348
Mg	0.523	-0.168	0.443	0.212
Na	-0.133	0.623	0.366	-0.417
P	-0.129	-0.700	0.388	-0.439
S	0.533	-0.107	0.389	0.597
Variance explained	52%	25%	80%	11%

Table 1.4. Mean concentration (% dry matter) and content (mg), with standard deviation in parentheses, of each nutrient for attached and unattached hemiparasites. Different letters across rows indicate significant differences.

		Parasitism Status	
		Unattached hemiparasite (n=29)	**Attached hemiparasite** (n=29)
Ca	Concentration	1.62 (0.47) [a]	1.21 (0.35) [b]
	Content	0.55 (0.35) [b]	0.57 (0.42) [b]
K	Concentration	2.99 (0.66) [a]	4.11 (0.92) [b]
	Content	1.01 (0.67) [a]	1.94 (1.35) [b]
Mg	Concentration	0.51 (0.17) [a]	0.42 (0.11) [b]
	Content	0.16 (0.09) [b]	0.19 (0.13) [b]
Na	Concentration	0.02 (0.02) [a]	0.02 (0.02) [a]
	Content	0.01 (0.01) [a]	0.01 (0.02) [a]
P	Concentration	0.49 (0.24) [a]	0.72 (0.23) [b]
	Content	0.14 (0.06) [a]	0.33 (0.22) [b]
S	Concentration	0.98 (0.36) [a]	0.67 (0.26) [b]
	Content	0.33 (0.22) [a]	0.30 (0.19) [b]

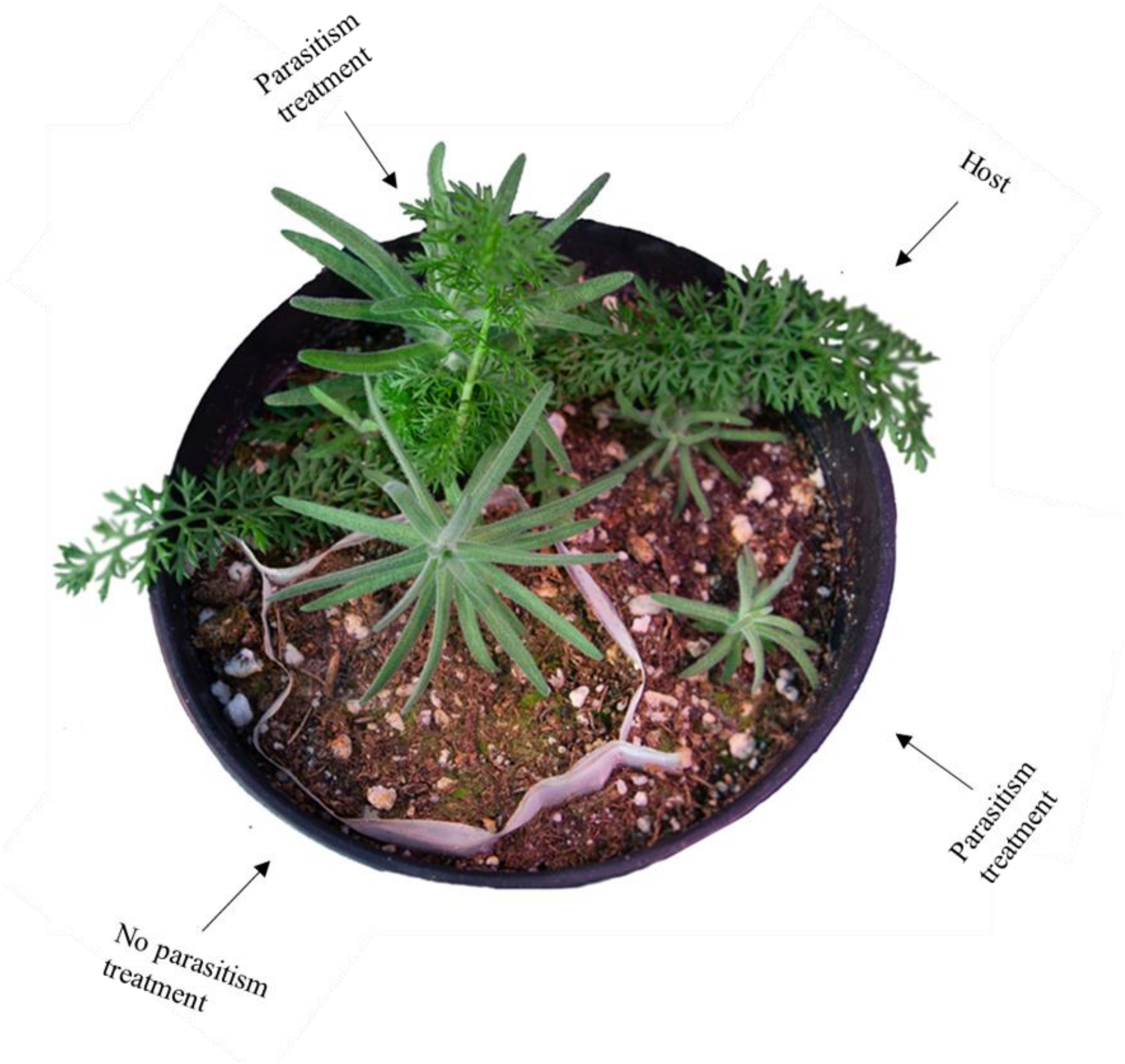

Figure 1.1. Photograph from a pot in experiment B showing the host plant and hemiparasites. Half of the hemiparasite individuals are growing in a root exclusion bag (no parasitism treatment); the others are outside of the root exclusion bag and are able to parasitize the host (parasitism treatment). In experiment A, parasitism treatments did not occur in the same pot. In this image the hemiparasite species is *Castilleja foliolosa* and the host is *Achillea millefolium.*

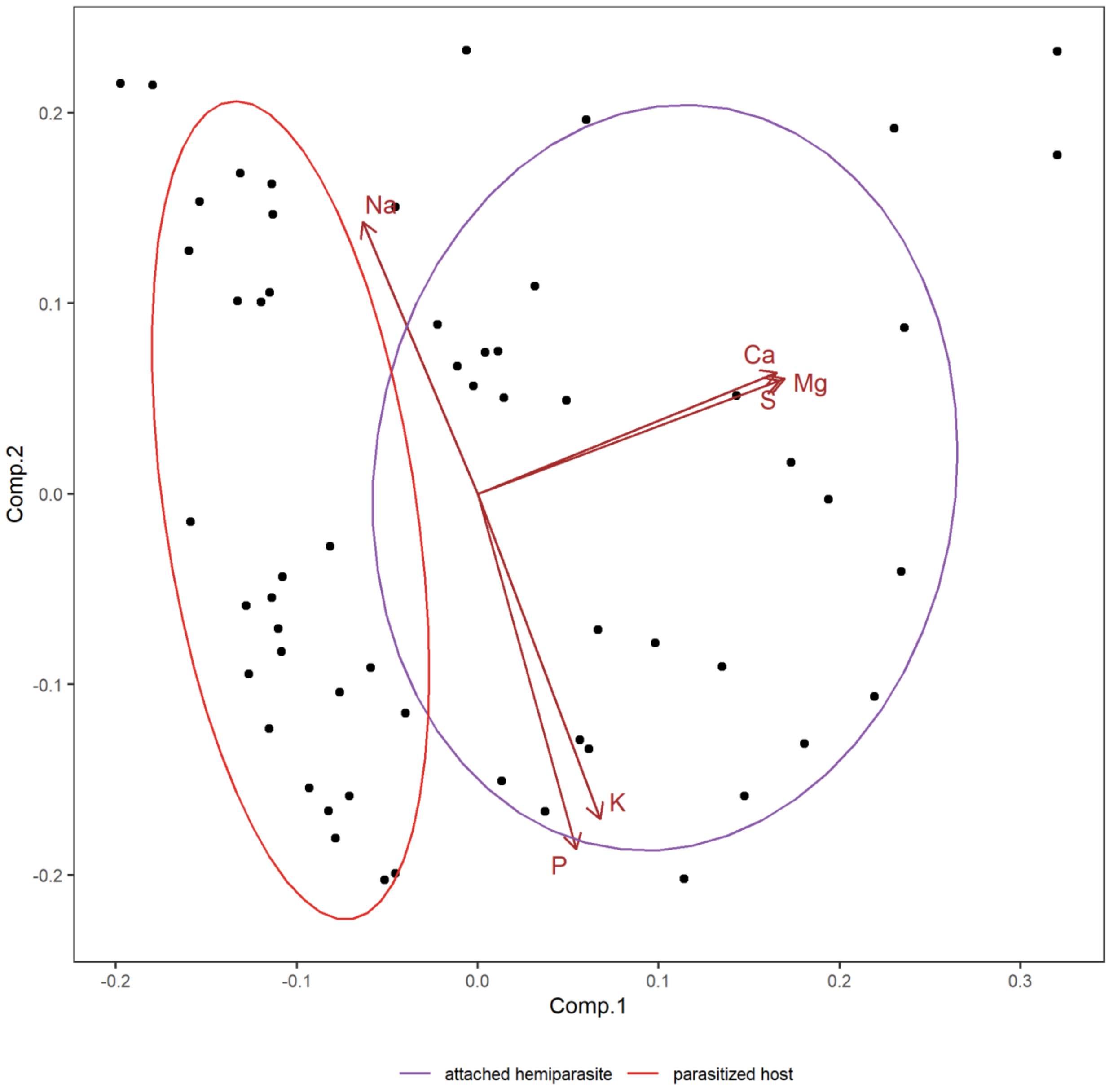

Figure 1.2. PCA ordination plot of the parasitized host and attached hemiparasite nutrient concentration matrix. Groupings are based on plant type (hemiparasite or host). Vectors for all six nutrients have been included. See Table S1.1 for model results for PC1 and PC2.

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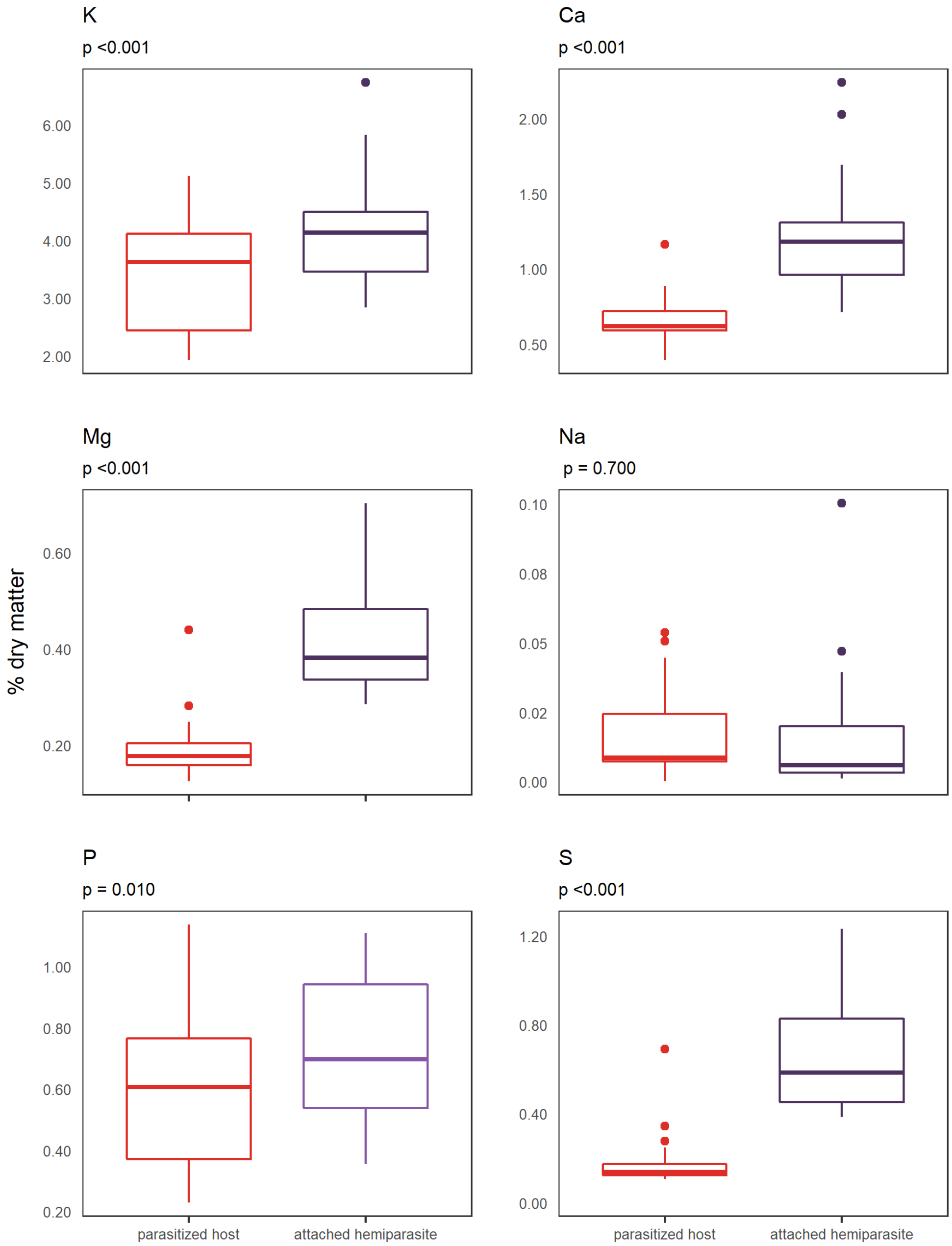

Figure 1.3. Nutrient concentrations of the parasitized host *Achillea* and attached hemiparasites. See Table S1.1 for model results.

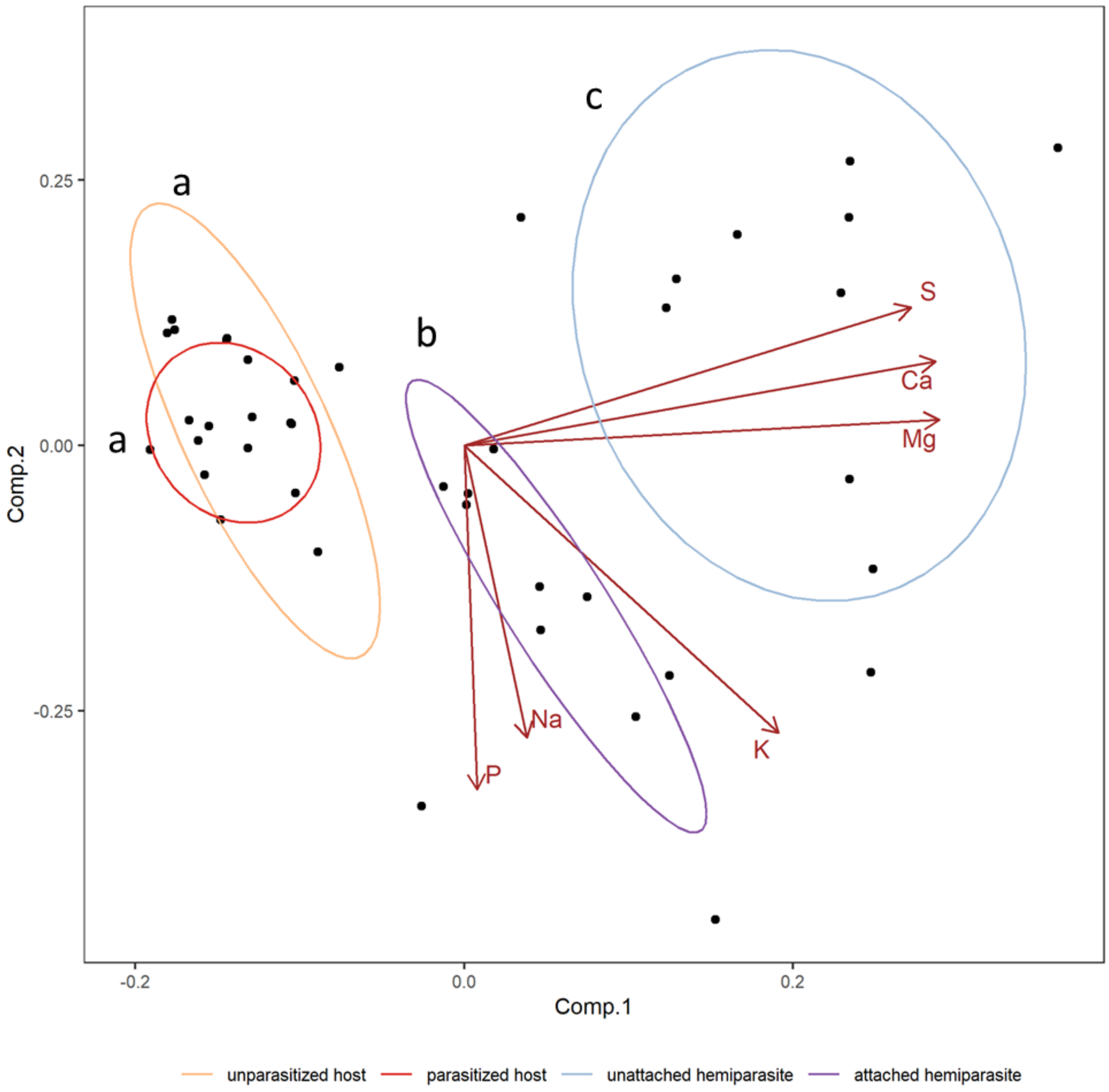

Figure 1.4. PCA ordination plot of the nutrient concentration matrix for the hemiparasite *Castilleja levisecta* and the host *Achillea millefolium.* Groupings are based on plant type (hemiparasite or host) and parasitism status. Vectors for all six nutrients are included. See Table S1.2 for model results. Different letters indicate significant differences between groups for PC1.

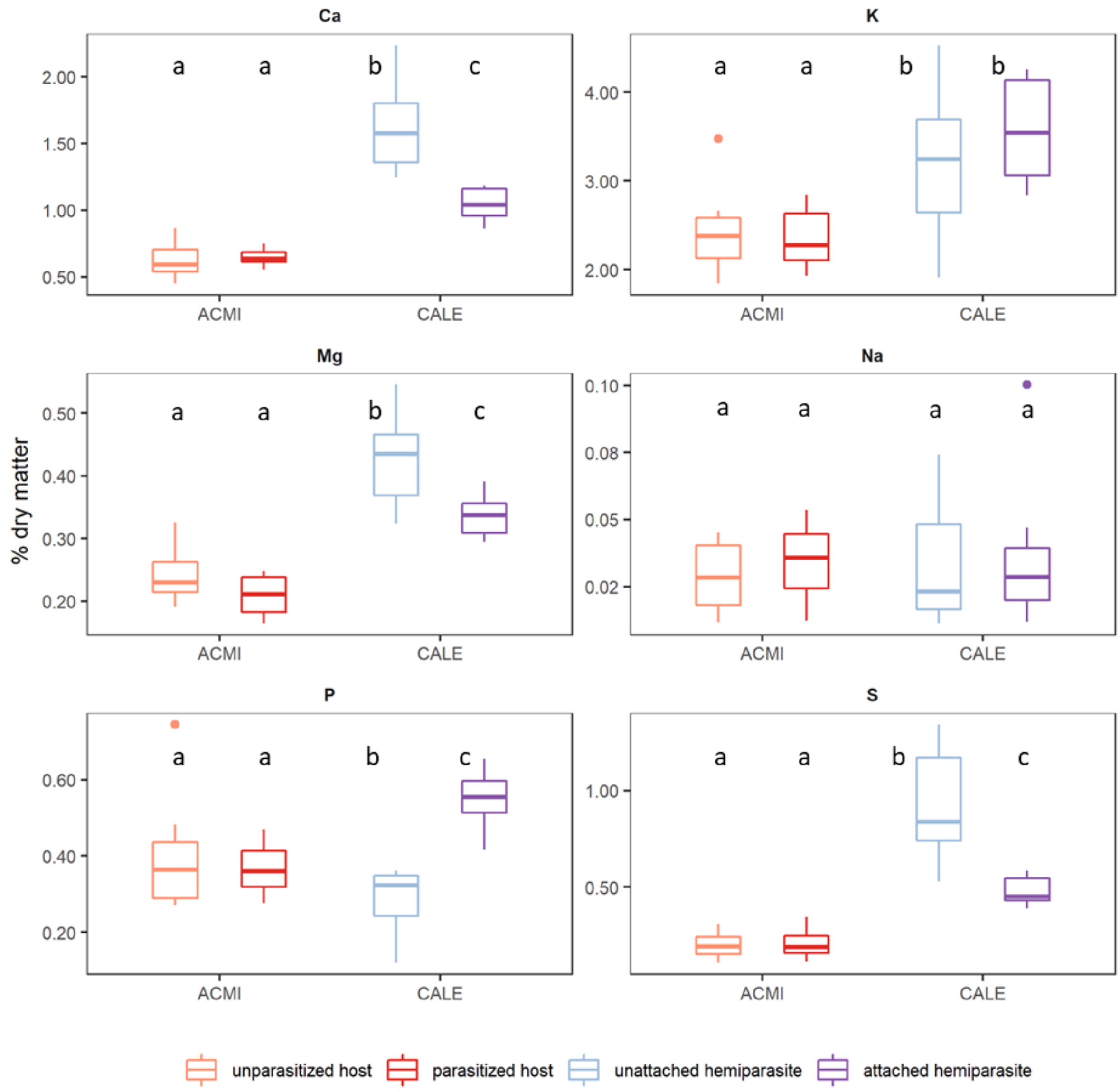

Figure 1.5. Nutrient concentration for *Castilleja levisecta* (CALE) and *Achillea millefolium* (ACMI) grouped by parasitism status. Different letters within each graph indicate significant differences between groups. See Table S1.2 for model results.

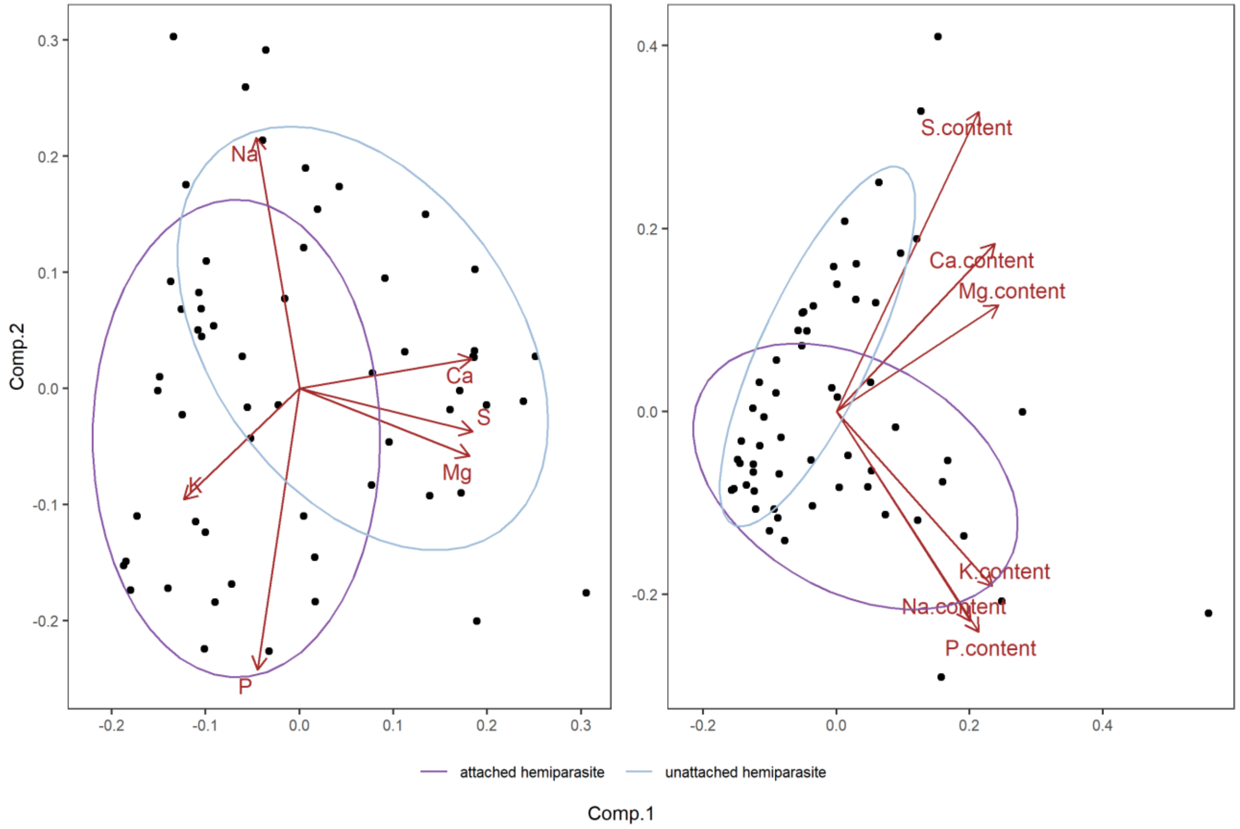

Figure 1.6. PCA ordination plots of the unattached and attached hemiparasitic (A) concentration matrix and (B) content matrix. Vectors for all six nutrients have been included. See Tables S1.3 and S1.4 for model results.

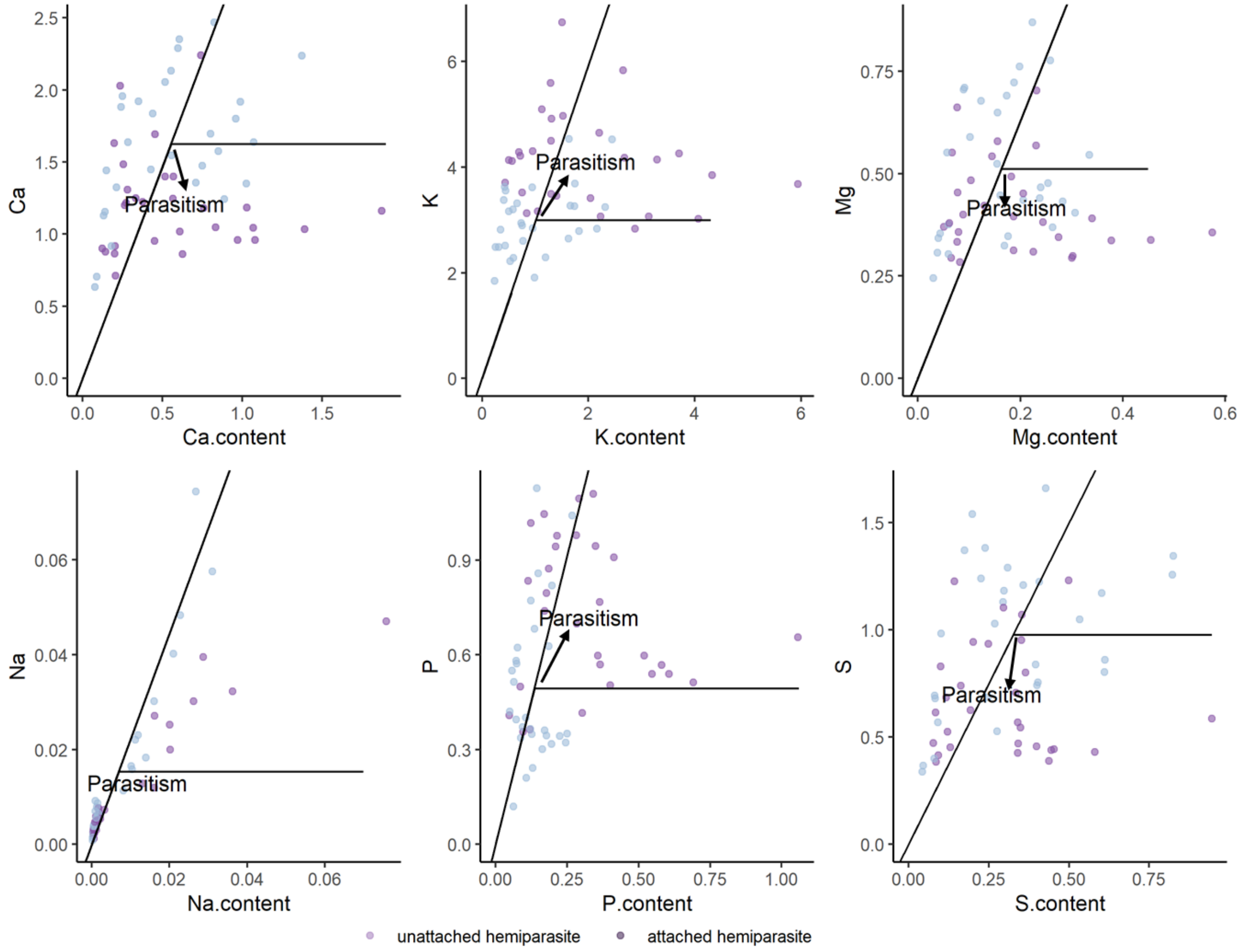

Figure 1.7. Nutrient concentration (% dry weight; vertical axis) vs. nutrient content (mg; horizontal axis) of total aboveground biomass per plant for Ca, K, Mg, Na, P and S. Arrows denote shifts from the mean of the no parasitism group (intersection) to mean of the parasitism group. Shifts of values along the upward line indicate increased nutrient accumulation without gains in biomass (luxury consumption), while shifts of values along the horizontal line indicate increased nutrient content due to biomass growth without a change in concentration. A shift into the upper sector between both lines denotes increases in both nutrient concentrations and biomass, indicating that the initial nutrient level was growth limiting. A shift into the sector below the horizontal line indicates that the nutrient concentration has been diluted due to additional growth.

Chapter 1 **Supplement**

Table S1.1. Model results evaluating the effect of plant type (parasitized host or attached hemiparasite) on PC axes and the individual nutrient concentrations (% dry weight) of six individual mineral nutrients. DenDF = denominator degrees of freedom, F= F-statistic. Bolded p-values indicate statistical significance within a row.

Response	*Predictor*	*DenDf*	*F*	*p-value*
PC 1	Experiment	30.7	10.21	**0.003**
	Drought	50.1	0.62	0.441
	Type	50.1	173.12	**<0.001**
PC 2	Experiment	24.5	18.71	**<0.001**
	Drought	32.6	0.33	0.584
	Type	26.7	0.62	0.44
Ca	Experiment	47.2	0.23	0.64
	Drought	50.2	0.33	0.57
	Type	50.2	88.53	**<0.001**
K	Experiment	12.0	22.00	**0.001**
	Drought	38.4	0.22	0.233
	Type	29.2	20.21	**0.001**
Mg	Experiment	31.2	0.93	0.341
	Drought	50.2	0.40	0.53
	Type	50.2	107.3	**<0.001**
Na	Experiment	54.0	31.2	**<0.001**
	Drought	54.0	8.34	**0.005**
	Type	54.0	0.17	0.700
P	Experiment	25.7	11.81	**0.002**
	Drought	36.2	0.04	0.835
	Type	25.8	8.00	**0.009**
S	Experiment	26.0	1.42	0.244
	Drought	49.9	1.44	0.236
	Type	49.8	108.52	**<0.001**

Note: Pot and hemiparasite identity were included as random effects. *p*-values estimated via t-tests using the Satterthwaite approximations to degrees of freedom.

Table S1.2. Model results evaluating the effect of plant type (parasitized host, unparasitized host, attached hemiparasite, attached hemiparasite) on nutrient concentration (% dry mass) for *Castilleja levisecta* and *Achillea millefolium* PC axes and the concentration of six mineral nutrients. Contrasts shown in Figure 1.4 and 1.5. DenDF = denominator degrees of freedom, F= F-statistic. Bolded p-values indicate statistical significance within a row.

Response	*Predictor*	*DenDf*	*F*	*p-value*
PC1	Drought	35.1	1.67	0.205
	Type	33.2	84.13	**<0.001**
PC2	Drought	35.6	11.22	**<0.001**
	Type	50.2	30.28	**<0.001**
Ca	Drought	37.0	0.05	0.821
	Type	37.0	79.22	**<0.001**
K	Drought	35.9	11.83	**0.001**
	Type	32.6	18.0	**<0.001**
Mg	Drought	34.6	0.07	0.788
	Type	32.5	52.51	**<0.001**
P	Drought	37.0	0.06	0.81
	Type	37	13.73	**<0.001**
Na	Drought	37.0	10.53	**0.002**
	Type	37.0	0.16	0.921
S	Drought	35.1	1.74	0.195
	Type	30.1	70.8	**<0.001**

Note: Pot and hemiparasite identity were included as random effects. *p*-values estimated via t-tests using the Satterthwaite approximations to degrees of freedom.

Table S1.3. Model results evaluating the effect of hemiparasite parasitism status (unattached hemiparasite and attached hemiparasite) on nutrient concentration (% dry mass) PC axes and the concentration of six mineral nutrients. DenDF = denominator degrees of freedom, F= F-statistic. Bolded p-values indicate statistical significance within a row.

Response	*Predictor*	*DenDf*	*F*	*p-value*
PC 1	Experiment	30.1	2.42	0.130
	Drought	47.1	2.00	0.175
	Type	31.2	56.03	**<0.001**
PC 2	Experiment	51.2	25.90	**<0.001**
	Drought	50.2	15.51	**<0.001**
	Type	50.2	30.28	**<0.001**
Ca	Experiment	36.5	0.38	0.540
	Drought	37.1	0.01	0.976
	Type	28.5	39.40	**<0.001**
K	Experiment	44.4	4.00	0.052
	Drought	51.1	2.81	0.100
	Type	34.4	30.1	**<0.001**
Mg	Experiment	40.0	7.60	**0.010**
	Drought	42.8	0.092	0.763
	Type	30.1	16.01	**<0.001**
Na	Experiment	49.6	16.51	**<0.001**
	Drought	52.7	51.26	**<0.001**
	Type	43.6	0.00	0.987
P	Experiment	39.3	15.10	**<0.001**
	Drought	41.5	0.050	0.825
	Type	30.3	36.1	**<0.001**
S	Experiment	39.5	1.06	0.310
	Drought	42.6	2.32	0.136
	Type	30.0	26.6	**<0.001**

Note: Pot and hemiparasite identity were included as random effects. *p*-values estimated via t-tests using the Satterthwaite approximations to degrees of freedom.

Table S1.4. Model results evaluating the effect of hemiparasite parasitism status (unattached hemiparasite and attached hemiparasite) on nutrient content (mg) PC axes and the content of six mineral nutrients. DenDF = denominator degrees of freedom, F= F-statistic. Bolded p- values indicate statistical significance within a row.

Response	*Predictor*	*DenDf*	*F*	*p-value*
PC 1	Experiment	41.5	59.20	**<0.001**
	Drought	49.2	0.22	0.644
	Type	20.7	9.96	**0.004**
PC 2	Experiment	17.4	8.10	**0.01**
	Drought	39.1	23.00	**<0.001**
	Type	18.9	37.62	**<0.001**
Ca	Experiment	41.2	66.34	**<0.001**
	Drought	46.6	1.50	0.230
	Type	30.1	0.85	0.363
K	Experiment	54.0	59.01	**<0.001**
	Drought	54.0	0.07	0.787
	Type	54.0	32.31	**<0.001**
Mg	Experiment	54.0.0	59.0	**<0.001**
	Drought	54.0	0.07	0.787
	Type	54.0	32.25	**<0.001**
Na	Experiment	44.8	23.46	**<0.001**
	Drought	51.1	22.41	**<0.001**
	Type	34.9	5.64	**0.023**
P	Experiment	54.0	21.21	**<0.001**
	Drought	54.0	0.72	0.400
	Type	54.0	31.57	**<0.001**
S	Experiment	38.4	43.85	**<0.001**
	Drought	41.2	7.62	**0.009**
	Type	29.0	0.05	0.826

Note: Pot and hemiparasite identity were included as random effects. *p*-values estimated via t-tests using the Satterthwaite approximations to degrees of freedom.

Chapter 2: **Heterotrophic carbon gain of annual and perennial root hemiparasites**

Abstract

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Root hemiparasites were once considered to be completely autotrophic with respect to carbon gain due to their ability to photosynthesize and lack of host phloem connections. However, it is now well understood that hemiparasites can acquire a substantial amount of host carbon in the form of amino acids and sugars via the host xylem stream. Though heterotrophic gain has been verified for many species, the extent of interspecific reliance on host carbon varies. This variation is not surprising, given the diversity of hemiparasitic growth form and life history. Since the fitness of annuals is based on a single reproductive event, these plants should prioritize resource gain over conservation. For hemiparasites, this is manifested in greater host resource gain and host dependence. Thus, I hypothesize that annual hemiparasites should acquire more host carbon than perennial hemiparasites. In this study, I tested how several morphological and chemical traits of hemiparasites vary with their life history and parasitism status (attached vs. unattached hemiparasites), with the expectation that annuals would show traits consistent with higher host dependency.

I assessed the heterotrophic carbon gain, nitrogen concentration, carbon to nitrogen ratio, and the proportion of biomass allocated to roots and of four annual and three perennial hemiparasitic species in the Castillejinae subtribe (Orobanchaceae). Although attached hemiparasites in general lowered their RMF when compared to unattached hemiparasites, there were no significant differences between attached annual and attached perennial hemiparasites. On average, 31% of hemiparasitic biomass was derived from host carbon, though heterotrophic carbon gain was not affected by hemiparasite life history. This is the first experiment which

illustrates that hemiparasites in the genera *Castilleja* and *Cordylanthus* effectively take up and utilize host carbon and that they reduce their root investment upon attachment.

Introduction

Most terrestrial plants are dependent upon carbon dioxide as their source of carbon and experience a fundamental dilemma – to gain carbon, water loss is inevitable. Stomatal optimization theories describe how a plant copes with this trade-off by minimizing water loss while attempting to maximize carbon gain (Farquhar & Sharkey, 1982). However, some angiosperm lineages have adopted a heterotrophic lifestyle, either partially or completely relying on host plants for carbon, water, and mineral nutrients (Nickrent, 2002). By constantly transpiring at a rate higher than the host, parasitic plants create a sink effect and thereby gain host materials through specialized organs called haustoria (Phoenix & Press, 2005; Yoshida *et al.*, 2016). Thus, hemiparasites sometimes adopt a strategy that is the reverse of the norm– water loss is maximized to maximize resource gain (Stewart & Press, 1990). Among parasitic plants, root hemiparasites (hereafter "hemiparasites") are unique in that, while they attach and parasitize host plants via root connections, they also photosynthesize and some can survive and reproduce without a host (Těšitel, 2016).

Given their autotrophic nature and lack of phloem connections with the host, it was thought that hemiparasites could not access host carbon. However, it is now well substantiated that hemiparasites can uptake significant amounts of carbon via organic molecules dissolved in the xylem sap (Press & Graves, 1995). Furthermore, carbon budget analyses for these hemiparasites show insufficient net carbon gains to maintain growth (attributed mostly to high respiration rates), implying a partial reliance on host carbon (Press *et al.*, 1988). However, quantitative assessments of heterotrophic carbon gain have only been done for few hemiparasitic genera and suggest wide variation in a hemiparasite’s reliance on host carbon (Těšitel *et al.*, 2010).

Since access to host resources, including carbon, is directly related to the strength of the sink the hemiparasite creates, all hemiparasites must balance the evolutionary advantage of parasitism with a water-wasting strategy that is clearly disadvantageous in a dynamic host environment or under drought conditions (Press, 1989). Although further research is needed to substantiate this trend, it has a solid empirical foundation in life history theory. In general, annual species tend to maximize resource acquisition and fast reproduction, whereas perennials should maximize resource conservation and survival (Friedman & Rubin, 2015). Accordingly, it should be advantageous for annual hemiparasites to connect to a host rapidly and then to exploit that host as much as they can by fully adopting the water wasting strategy which may have a strong deleterious effect on the host. Perennial hemiparasites, on the other hand, should benefit more from long-lasting relationships with the same host and would face negative consequences if they killed or severely damage their hosts (Chapter 4). Consistent with these expectations, traits related to resource uptake and conservation, such as specific root length, specific leaf area, relative growth rate and root nitrogen concentration, have been correlated with life history (Roumet *et al.*, 2006). For example, the water relations of a hemiparasite and its relative damage to the host correlate with its life history, with annuals transpiring at higher rates than perennials (Stewart & Press, 1990; Press & Graves, 1995). Thus, we may also expect to see variation in other physiological and morphological traits adapted for host resource uptake.

Besides increased transpiration rates, a reduced investment into root biomass upon attachment to the host is a consistent trait among hemiparasites that may vary based on life history. Root investment is typically expressed as the root mass fraction (RMF) of a hemiparasite, i.e., the percentage of total biomass made up of root mass (Matthies, 2017).

Additionally, a high total carbon: total nitrogen ratio (hereafter C:N) is correlated with higher carbon allocation to the roots (Zhang *et. al*., 2019). However, regardless of host attachment, perennials, particularly in grasslands which house a high diversity of hemiparasites, need to retain a robust below-ground root system for dormancy. Thus, as described above, they likely do not fully embrace the water-wasting strategy, but rather exist in a more conservative middle ground, exhibiting physiological characteristics somewhere between an autotrophic plant and an annual hemiparasite (Chapter 4). Matthies (2017) compiled RMF values for perennials and annuals showing that, in general, the RMF value of annuals is lower for attached hemiparasitic annuals than attached perennials, though these data were not analyzed statistically. Thus, the "degree of parasitism" as measured by physiological and ecological traits associated with parasitic plants, like RMF, the total carbon to total nitrogen ratio (C:N), and transpiration rate, may be correlated with life history (Chapter 4). As such, we may expect percent heterotrophic carbon gain (the percent of hemiparasite biomass that is made up of host-derived carbon) another potential quantitative metric of degree of parasitism, to be higher in annual hemiparasites than perennial hemiparasites.

The Castillejinae subtribe (Orobanchaceae) is a particularly interesting group in which to study correlations between life history and parasitic traits. Perenniality has long been thought by evolutionary biologists to be the ancestral condition in angiosperms, implying that the annual habit is derived (Stebbins, 1957; Friedman, 2020). However, the reverse trend is found in the Castillejinae, where perennial plants evolved from annual ancestors with a reversion back to the annuality (Figure 2.1). Though this is not the case in other hemiparasite clades, like the Rhinanthoid Orobanchaceae, life history seems to be a labile trait in Orobanchaceae (D. Olmstead, personal communication).

In this study I examined how particular morphological and physiological traits of hemiparasites vary with life history. I tested and hypothesized that:

i.) Annuals rely more on their hosts for resources and have higher heterotrophic percent carbon gain (hereafter "%H") than perennials.

ii.) Annuals invest less into their root system and have lower RMF and C:N ratios than perennials.

Methods

Quantifying heterotrophic carbon gain

Press et al. (1987) demonstrated how to quantify the proportion of host-derived carbon in hemiparasite biomass using natural abundance stable isotope values of ^{13}C of the hemiparasite *Striga hermonthica* attached to a C_4 host. Plants undertaking C_4 photosynthesis have a significantly enriched natural abundance $\delta^{13}C$ value compared to C_3 plants, owing to the differential discrimination of Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPCase), the first enzymes used in carbon fixation in C_3 and C_4 plants, respectively (Ehleringer, 1991). Těšitel *et. al* (2010) resolved the problem of a potential bias caused by re-fixation of soil-respired CO_2 using paired partner plants. Since all plants in the Orobanchaceae have a C_3 photosynthetic pathway, we can relate the excess of ^{13}C in hemiparasites attached to the C_4 host compared with those attached to the C_3 host to the difference in isotope composition between the C_3 and C_4 hosts themselves (Eq.1).

$$\%H = \left(\frac{\delta^{13}\mathrm{C}_{\mathrm{P(C_4)}} - \delta^{13}\mathrm{C}_{\mathrm{P(C_3)}}}{\delta^{13}\mathrm{C}_{\mathrm{H(C_4)}} - \delta^{13}\mathrm{C}_{\mathrm{H(C_3)}}}\right) \times 100[\%] \tag{1}$$

%H is the percent heterotrophic carbon in hemiparasite tissue. Data were collected as atom % ^{13}C and re-expressed as delta values relative to the Pee Dee Belemnite standard (ð) using Eq.2:

$$\delta^{13}C = (R_{Sample}/R_{Standard} - 1) * 1000\ \% \qquad (2)$$

where R_{Sample}= ^{13}C:^{12}C ratio in the sample and $R_{Standard}$ is the ^{13}C:^{12}C ratio in the Pee Dee Belemnite standard (Těšitel *et al.*, 2010) C_4 plants have much less negative ð^{13}C values than C_3 plants (Ehleringer, 1991). Thus, we would expect that hemiparasites attached to a C_4 host would have significantly less negative ð^{13}C values than unattached hemiparasites or hemiparasites attached to a C_3 host.

Research design

I used *Zea mays* as the C_4 host and *Achillea millefolium* as the C_3 host. *Achillea* is known to grow with all the hemiparasite species in natural conditions. Both plants were both sown from seed. As part of a pilot study in preparation for this experiment, I attempted to germinate and propagate twenty-six species in the Orobanchaceae (with the exception of *Comandra* , Santalaceae) including annual hemiparasites, perennial hemiparasites, and autotrophs (Table S2.1). Species were largely chosen based on the availability of seeds, which were sourced from independent collectors as well as nurseries. Seeds which germinated successfully were then grown with the host *Achillea* in identical conditions to this study to determine growth success. The final species list consisted of 13 species across four genera, though many were dropped from analysis due to low survivorship (Tables 2.1, 2.2). I included the perennial autotroph *Rehmannia* to have a baseline from which to compare autotrophic ^{13}C values.

The study was conducted at the Douglas Research Conservatory at the Center for Urban Horticulture, University of Washington. SunShine #2 media (SunGro Horticulture), which has mycorrhizae and a slight nutrient charge equivalent to one liquid feeding of a complete fertilizer, was used as the media in all pots. The average temperature was 20.8°C and the average relative humidity was 45.5%. Supplemental lighting was on between the hours of 6 AM and 8 PM, but was only illuminated if the outdoor light meter read a value of less than 500 μmol. Watering was performed as needed.

After the hemiparasite germinated in cold-moist stratification (Table S2.1) it was sown with a host seed. I did not germinate the host prior to planting the hemiparasite to try to avoid out-competition of a hemiparasite germinate by the host. Four hemiparasite individuals were planted normally and four others were placed in a root exclusion bag, which allows water and nutrients to pass through but prevents the parasite from making haustorial connections with the host (Figure 2.2). The root exclusion bags were made of nylon woven mesh of 35- and 45-micron diameter openings (Industrial Netting, MN). Fabric was cut to create 5x5 inch bags; seams were sealed with waterproof glue. The same pot design was repeated for the autotroph *Rehmannia*, although due its much larger size relative to the hemiparasites, only two individuals were planted with one sown inside the root-exclusion bag and one sown outside of the bag. For all pots, I initially planted more individuals and thinned, if necessary, to account for mortality. Following this procedure, I sowed 10 pots per host species. germinants that died within two weeks of planting were replaced. All plants were planted in 1.9 liter (half-gallon) pots with SunShine #4 media (Sungro Horticulture) which has mycorrhizae and a small nutrient charge equivalent to one feeding of a complete fertilize and trace elements at a normal rate. No additional fertilizer was applied to encourage parasitism and plants were watered as needed. I arranged the pots at arbitrarily on the bench, though without prior randomized design.

Due to differences in growth pattern, data collection was standardized by flowering time.

I harvested all plants within a host-hemiparasite combination when the first hemiparasite individual (from either parasitism treatment) began to bud, or at nine weeks (for perennials) and six weeks (for annuals), whichever came first. The cut-off harvest times were based on flowering times from my pilot germination and propagation study (Table S2.1). Host shoots and hemiparasites shoots and roots were dried for two days at 70°C. I measured the dry weight of host shoots (leaves and stems) and the dry weight of both roots and shoots (leaves and stems) for hemiparasites and *Rehmannia.* Only the mass of individuals used in isotope processing were included in biomass analyses.

Isotope processing

Leaf samples of hemiparasites, *Rehmannia*, and hosts were ground and sent to the University of California, Davis Stable Isotope Lab for natural ^{13}C and ^{15}N abundance analysis. of solids using an elemental analyzer isotope ratio interface to a continuous flow isotope ratio mass spectrometer. Total C and Total N values were also reported. For the hemiparasite, I sampled and submitted newly emerged leaves as they were the most likely to be made after connection to the host was established. I submitted leaves from the most robust looking hemiparasite individual within the parasitism treatment as increased growth is related to host attachment. For consistency, I also chose the largest individual in the no parasitism group for isotope processing.

Statistical analysis

All analysis was done in R 4.0.2 using an alpha level of 0.05 (RStudio, 2019). As an autotroph that is closely related to the hemiparasites, *Rehmannia* was removed from all statistical analysis but was included in graphics. To determine whether parasitism was successful, I evaluated the ð^{13}C values of the hemiparasites grown with *Zea*. I omitted pots in which ð^{13}C values for the no parasitism treatment were closer to the host average than the attached hemiparasite average, as this suggested that the hemiparasites did not attach to *Zea*. I could not confirm parasitism with *Achillea* based on $\delta^{13}C$ as *Achillea* and the hemiparasites use C_3 photosynthesis. However, I assumed equal parasitism rates between *Achillea* and *Zea;* therefore, if parasitism did not occur in more than half of the pots, I dropped the entire species from analysis.

RMF was calculated by dividing the root mass by the total mass (shoot and root) and biomass was calculated by summing the root mass and shoot mass. To test for differences between hemiparasites based on parasitism treatment for biomass and RMF, I used linear mixed models with the package *lme4* (Bates, 2015) in which parasitism treatment, life history and host identity were fixed effects and pot was a random effect. Biomass was log-transformed to improve normality. Interactions between parasitism treatment and life history, parasitism treatment and host, and life history and host were tested.

To test for differences in $\delta^{13}C$ values, I ran a linear mixed model with plant type (host, attached hemiparasite, and unattached hemiparasite) as a fixed effect and pot as a random effect. I included host in this model to confirm that *Zea* had significantly less negative values than *Achillea*. Since this test was used to confirm parasitism, I did not include life history in the model.

To calculate %H I combined all possible pots of *Zea* with all possible pots of *Achillea*, since both hosts are required for the calculation (Eq. 1). This calculation was performed for each hemiparasite species. This method resulted in violations of the homogeneity of variance. To account for this, I used the medians of the resulting distributions to test for a difference between annuals and perennials using a two-sample t-test.

Nitrogen concentration was calculated by dividing total nitrogen by the leaf mass of the individual. For total nitrogen, nitrogen concentration, and the carbon to nitrogen ratio, I ran a t-test for the host species to see if one had higher amounts of nitrogen than another. However, as I was most interested in how nitrogen concentration changes based on hemiparasite life history, I only ran linear mixed models for hemiparasite data and included duration (annual or perennial), type (attached hemiparasite, unattached hemiparasite) and host identity (*Zea* or *Achillea*) as fixed effects with pot as a random effect. Interactions between parasitism treatment and life history, parasitism treatment and host, and life history and host were tested.

Results

Survival and degree of parasitism

Castilleja campestris and *Castilleja coccinea* germinated successfully but experienced 100% mortality two weeks within transplanting (Table 1.2). For all hemiparasites parasitizing *Zea*, at least one pot was dropped due to lack of parasitism. Since the majority of hemiparasite individuals in *Castilleja hispida, Triphysaria versicolor* and *Triphysaria eriantha* did not successfully parasitize *Zea*, these species were dropped entirely from the analysis. This left 115 pots for the analysis (plus 28 *Rehmannia* pots which were not analyzed but were displayed graphically).

Biomass

When grown with *Zea*, unattached hemiparasites had significantly less biomass (0.035 g) than attached hemiparasites (0.069 g) With *Achillea*, the biomass of unattached hemiparasites (0.040 g) did not differ significantly from that of attached hemiparasites (0.045 g) Annuals

(0.034 g) had significantly lower biomass than perennials (0.061 g), and this effect did not differ with host identity (Table 2.3).

Root mass fraction (RMF):

Parasitism status affected RMF, but the significance depended on host identity. When grown with *Zea*, unattached hemiparasites had significantly higher RMF (0.182) than attached hemiparasites (0.095). With *Achillea,* the RMF of unattached hemiparasites (0.170) did not differ significantly from that of attached hemiparasites (0.138). Overall, the RMF of annuals (.151) did not differ from that (.132) of perennials. (Table 2.3, Figure S2.1).

δ^{13}C

Five attached hemiparasites had to be dropped from the analysis due to missing ð^{13}C data. The C_4 host plant *Zea* had significantly less negative δ^{13}C than the C_3 *Achillea.* For hemiparasites grown with *Zea* δ^{13}C differed by parasitism treatment ($p < 0.001$) with attached hemiparasites having less negative δ^{13}C and unattached hemiparasites having more negative δ^{3}C. This confirms that the root exclusion bags precluded parasitism. For hemiparasites grown with *Achillea*, there was no difference ($p = 0.604$) in δ^{13}C value between parasitism groups (Table 2.4, Figure 2.3).

Heterotrophic carbon gain (%H)

The average percent heterotrophic gain median value for annual hemiparasite (29%) did not differ from the average median (34%) of perennial hemiparasites ($p = .31$, $t = -1.28$) (Figure 2.4).

Nitrogen concentration, Carbon:nitrogen

Achillea had significantly higher nitrogen concentration (48.8 μg/g) than *Zea* (24.5 μg/g) but lower C:N ratio (mean = 8.26) than *Zea* (19.9).

Hemiparasites grown with *Achillea* had a significantly higher nitrogen concentration (50.8 μg/g) than hemiparasites grown with *Zea* (27.8 μg/g) but there was no interaction with parasitism type. The overall effect of parasitism status was not significant. However, the interaction between life history and parasitism type was significantly different between unattached annuals (45.7 μg/g) and unattached perennials (33.8 μg/g) (Figure 2.5, Table 2.5).

The carbon: nitrogen ratio was significantly higher for hemiparasites grown with *Zea* (15.9) than those grown with *Achillea* (7.8) however there was no interaction with parasitism type. Unattached annuals had a significantly lower C:N ratio (10.0) than unattached perennials (13.6), however there was no significant difference between attached annuals (11.1) and attached perennials (11.6). The overall effect of parasitism status was not significant. However, the interaction between life history and parasitism type was significant with attached perennials having a lower C:N than unattached perennials. There was no difference between attached and unattached annuals (Figure 2.6, Table 2.5).

Discussion

This study is the first to provide a quantitative assessment of the potential for annual and perennial hemiparasites in the Castillejinae to uptake and utilize heterotrophically derived carbon. I also demonstrate that these species decrease root investment upon the adoption of parasitism, in line with other hemiparasites tested. Contrary to expectations, I found that annual hemiparasites had similar RMF and greater heterotrophic carbon gain compared to perennial hemiparasites.

Attached hemiparasites reduce investment into root mass

Annuals had a lower biomass than perennials, which is expected given that they grow for shorter periods of time. Attached hemiparasites had significantly higher biomass than unattached hemiparasites and had lower values of RMF for both annuals and perennials (Figure S2.1). This is a common trend in hemiparasites as access to a host precludes the need for high investment into their own root system. Additionally, as hemiparasite biomass increases due to host access, its RMF continues to decline (Matthies, 2017). However, when unattached, the proportion of hemiparasite biomass allocated to roots (0.20) was still lower than that of the autotroph *Rehmannia* (0.40) and the accepted mean of herbaceous plants (0.30) (Poorter *et al.*, 2012). This could indicate that the unattached hemiparasites are still not behaving completely autotrophically with respect to root investment, even before attachment. However, investigating this line of thought would require further experiments.

Furthermore, host identity affected RMF, with hemiparasites attached to *Zea* substantially lowering RMF and those attached to *Achillea* showing no differences. Likewise, the biomass of attached hemiparasites was higher than unattached hemiparasites only for *Zea*. Given that we know *Achillea* is a good host, this is counter intuitive; generally, we would expect the RMF of a hemiparasite to decrease substantially and its biomass to increase. In this case, these results may be less to do with host performance and more with the experimental design. Since I sowed the hemiparasites at the same time as the host (to reduce the chance that the host would outcompete the hemiparasite), the hemiparasites had access to less host resources than they would if planted with an already established host. However, the lower RMF values with *Zea,* which has a fibrous root system generally preferred by hemiparasites, is in line with what we know about hemiparasite host preference (Mudrák *et al.*, 2016). Since *Zea* grew much faster than *Achillea*, it is plausible that the hemiparasites with *Zea* simply had a more pronounced response to parasitism due to host size, vigor, and access to host C. Additionally, since the differences between unattached and attached hemiparasites with *Zea* were significant yet attached hemiparasites had a higher average biomass with *Achillea* (0.12 g with *Achillea* and 0.09 with *Zea*), these trends may simply indicate that *Zea* was a superior competitor than *Achillea.* If the experiment were longer, I speculate that we would have seen concomitant decreases in RMF for attached hemiparasites with *Achillea*.

Although we show that attached hemiparasites increase their RMF in line with previous studies, there was no significant difference based on life history, contrary to the trends found in the literature (Matthies 2017, *cf.* Table 6). This may also be related to the short duration of the experiment, as I may not have captured the full range of root reduction in either group. However, additional studies should be conducted to see if the difference here represents a trend and that perennials depend on host carbon more than annuals. See "Study limitations" below for an additional discussion.

It is important to note that C:N varied only between unattached perennials and attached perennials, with attached perennials having lower C:N. Though the direction of change of C:N is in line with my hypothesis, it is interesting that there is no significant difference among annuals. However, since RMF is a more direct measurement of root investment, these findings do not negate the differences seen between attached and unattached annual hemiparasites in RMF. Additionally, perennial hemiparasites may have manifested more noticeable changes in C:N as they lived longer and hard more time to accumulate carbon in other tissues. Finally, although host identity significantly affected the C:N and nitrogen concentration of hemiparasites, this did not vary based on parasitism status (Table 2.5). Since the presence of the host affected unattached and attached hemiparasites equally, competition from the host for nitrogen may have played a more important role for the hemiparasites in their nitrogen concentration than parasitism. This suggests that both unattached and attached hemiparasites are poor competitors for nitrogen, though this needs additional experimentation with other types of host plants in natural systems.

Annual and perennial hemiparasites uptake host carbon

In contrast to my hypothesis, life history did not influence the amount of host carbon gained by the hemiparasite. Both annuals and perennials, however, showed significant variation in the percent of heterotrophic carbon gain, ranging from 9 to 48% for annuals and 5 to 45 % for perennials. Such variation is also described in the literature: the annual *Rhinanthus minor* gain varied from 6 to 90%; the annual *Euphrasia* averaged 21 to 29% gain; (Těšitel *et al.*, 2010) the perennial *Olax phyllanthi* had a mean gain of 30% (Pate *et al.*, 1990); the perennial *Castilleja linariifolia* had a mean gain of 40% (Ducharme & Ehleringer, 1996); and the annual *Striga hermonthica* had a mean gain of nearly 60% (Graves *et al.*, 1990). Given the differences in methods used to measure heterotrophic carbon, phenology of the species, plant tissue, host identity, and abiotic conditions, it is difficult to make direct comparisons. However, all hemiparasites that have been assessed show some capacity to utilize heterotrophic carbon. Here, I extend the number of species and genera (Figure S2.1) tested and thus provide further support to the idea that, although hemiparasites can photosynthesize, they also uptake host carbon.

The variation seen in the amount of heterotrophic carbon accumulated in hemiparasite biomass suggests that hemiparasites may be able to modify the amount of host carbon they uptake. This type of physiological plasticity in host carbon uptake would be adaptive for both annual and perennial hemiparasites. A perennial is more likely to encounter and need to react to a dynamic host environment over the course of its life. Thus, efficient photosynthesis would allow a perennial to tolerate periods of inadequate host supply. In the case of an annual, the host environment is unlikely to change dramatically over the course of a year. However, annuals have a very short time frame in which to locate a host, attach to it, grow, and reproduce. Autotrophic competency would give annuals at least some chance to grow and reproduce in the case that no good host is available. Whether this type of plasticity exists, and to what extent a hemiparasite can modify its carbon uptake in relation to its need, is a largely unexplored question with ecological implications concerning how the impact of a hemiparasite on the host may vary temporally.

Additionally, hemiparasites may be gaining carbon gain as a serendipitous byproduct of a parasitic attachment, and not related to carbon limitation of the hemiparasite. If carbon is not a limiting factor for hemiparasites, annuals and perennials may not be expected to differ. The fact that nitrogen concentration, which is correlated with photosynthetic output (Evans & Clarke, 2019) did not vary between annual and perennial hemiparasites (Figure 2.5, Table 2.5) may imply similar investment into photosynthetic machinery and, therefore, a potentially similar photosynthetic rate .A simultaneous analysis of photosynthetic gain and respiration output to assess the carbon budget of attached and unattached hemiparasites would allow us to better understand if unattached hemiparasites are limited by carbon (see Hodžić Chapter 4 for additional discussion)

Finally, although I standardized the harvest time by budding and harvested the newest leaves for isotope analysis, perennials still had longer to grow and accumulate host carbon.

Although the difference was not statistically significant, perennials did have a higher average median of heterotrophic carbon gain (34%) than annuals (29%). It is plausible that perennials were more demanding of heterotrophic carbon than annuals because they had a longer period between successful haustorial attachment and flowering from which to shift to a heterotrophic mode. However, though it is reasonable to assume that physiological mechanisms of a parasitic strategy require a period (and likely certain physiological and biochemical cues) to manifest upon initial host attachment, this has not been studied (Stewart & Press, 1990). Further research targeted at understanding the physiological, morphological, and genetic changes that are accompanied by hemiparasite attachment would shed light into the interplay between autotrophy and heterotroph in hemiparasites.

Study limitations

There are additional limitations in this study that makes it difficult to tease apart the role of life history. First, I had a relatively low sample size due to the difficulties in propagating hemiparasites. Also, it was difficult to standardize the study based on phenology and determine the optimal time to harvest; it is plausible that at a different life stage, a hemiparasite may demand carbon than a host.

Finally, and perhaps most importantly, by only measuring the perennials within their first growing season, I was not capturing the full range of their growth over time. Moreover, these plants may not have yet been exhibiting physiological characteristics of a perennial. This may have skewed both the RMF and %H results towards values more in line with annuality. However, whether perenniality is learned, inherited, and/or related to an environmental cue associated with dormancy, has not been studied extensively. Annual and perennial hemiparasitic plants could serve as an ideal study group with which to investigate such a topic given that their unique lifestyle makes it so we are able to generate solid physiological predictions, at least with respect to host resource acquisition. I suggest that a similar study be repeated in which the physiological traits of annuals and perennials, like heterotrophic carbon gain and transpiration rate, are measured many times with hosts of varying quality. In the case of perennials, this should be done for multiple seasons.

Conclusion

Individuals in the genera *Castilleja* and *Cordylanthus* conform to hemiparasite behavior with respect to their ability to uptake organic carbon from their host and reduce their investment into roots. Given that other resource related traits have been shown to vary with life history, we would expect that annuals and perennials differ with respect to their reliance on host carbon. Although our study did not demonstrate this, future research focusing on additional hemiparasitic species for longer periods of time may be better positioned to make comparisons based on life history.

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Table 2.1. Percent survival of species measured as number of individuals that survived divided by the number of individuals planted and multiplied by 100. Values are averaged across all pots per host-hemiparasite combination. P+ = allowed to parasitize; P- = parasitism excluded

Hemiparasite		**Host**					
		Achillea millefolium (ACMI)			*Zea mays* (ZEMA)		
		% Survival P+	% Survival P-	% Survival ACMI	% Survival P+	% Survival P-	% Survival ZEMA
Castilleja densiflora	Annual	83	83	100	60	65	90
Castilleja minor	Annual	85	95	100	70	88	100
Castilleja rubicundula	Annual	83	78	100	85	85	100
Cordylanthus pilosus	Annual	60	58	100	75	65	100
Triphysaria eriantha	Annual	48	35	100	20	30	100
Triphysaria versicolor	Annual	63	48	100	55	50	100
Castilleja foliolosa	Perennial	53	55	100	100	65	60
Castilleja hispida	Perennial	43	43	100	13	7.5	100
Castilleja levisecta	Perennial	70	58	90	43	45	100
Castilleja wightii	Perennial	43	55	90	40	63	100

Rehmannia elata	Perennial autotroph	38	35	50	7.5	23	100

Table 2.2. List of final species used in the analysis. Species codes listed here are used in the remaining tables and figures. *Rehmannia* is included in the graphics but not the statistical analysis.

Species	**Species Code**	**Life History**
Castilleja densiflora	CADE	Annual
Castilleja rubicundula	CARU	Annual
Castilleja minor	CAMI	Annual
Cordylanthus pilosus	COPI	Annual
Castilleja levisecta	CALE	Perennial
Castilleja foliolosa	CAFO	Perennial
Castilleja wightii	CAWI	Perennial
Rehmannia elata	REEL	Perennial autotroph

Table 2.3. Biomass and RMF linear mixed model results. DenDF =denominator degrees of freedom, F= F statistic, p = p-value. Note that the response variables are transformed. *Rehmannia* was not included in the analysis. Predictor values: Life history: annual or perennial; Host: *Achillea* or *Zea* ; Parasitism status: attached or unattached.

Response	***Predictor***	***DenDF***	***F***	***p***
Log (Biomass)	Life history	111.9	7.80	**0.006**
	Host	111.9	0.45	0.501
	Parasitism status	106.9	26.20	**<0.001**
	Life history * parasitism status	110.1	0.34	0.560
	Life history * host	111.9	0.02	0.904
	Parasitism status * host	106.9	13.40	**<0.001**
Log (RMF)	Life history	110.1	1.65	0.202
	Host	110.1	2.34	0.129
	Parasitism status	110.1	29.08	**<0.001**
	Life history * parasitism status	109.0	0.85	0.360
	Life history * host	110.1	1.23	0.271
	Parasitism status * host	110.1	7.23	**0.008**

Note: Pot was included as a random effect in all the models.

Table 2.4. Contrasts of linear mixed model evaluating the differences in ð̌^{13}C between attached hemiparasites, unattached hemiparasites, and their hosts. ZEMA =*Zea mays.* ACMI =*Achillea millefolium. Rehmannia* was not included in the analysis.

Host	*Contrast*	*Estimate*	*t*	*p*
ZEMA	ZEMA - ACMI	-21.1	-109.8	<**0.001**
	ZEMA – unattached hemiparasite	20.7	107.0	<**0.001**
	ZEMA – attached hemiparasite	14.2	74.0	<**0.001**
	Attached hemiparasite – unattached hemiparasite	6.5	33.6	<**0.001**
ACMI	ACMI – unattached hemiparasite	-0.1	-0.3	0.986
	ACMI – attached hemiparasite	-0.4	-2.2	0.243
	Attached hemiparasite – unattached hemiparasite	0.3	2.0	0.405

Note: Pot was included as a random effect in all the models.

Table 2.5. Nitrogen concentration and carbon:nitrogen linear mixed model results. DenDF =denominator degrees of freedom, F= F statistic, p = p-value. Note that the response variables are transformed. *Rehmannia* was not included in the analysis. Predictor values: Life history: annual or perennial; Host: *Achillea* or *Zea*; Parasitism status: attached or unattached.

Response	***Predictor***	***DenDF***	***F***	***p***
Nitrogen concentration (μg/g leaf tissue)	Life history	111.0	14.0	**<0.001**
	Host	110.0	91.7	**<0.001**
	Parasitism status	110.0	0.4	0.540
	Life history * parasitism status	109.7	5.3	**0.02**
	Life history * host	111.0	0.3	0.590
	Parasitism status * host	109.7	1.4	0.249
Carbon:nitrogen	Life history	114.12	12.0	**<0.001**
	Host	114.1	117.3	**<0.001**
	Parasitism status	113.2	0.9	0.326
	Life history * host	114.1	2.2	0.142
	Life history * parasitism status	113.2	8.5	**0.004**
	Parasitism status * host	113.2	0.1	0.927

Note: Pot was included as a random effect in all the models.

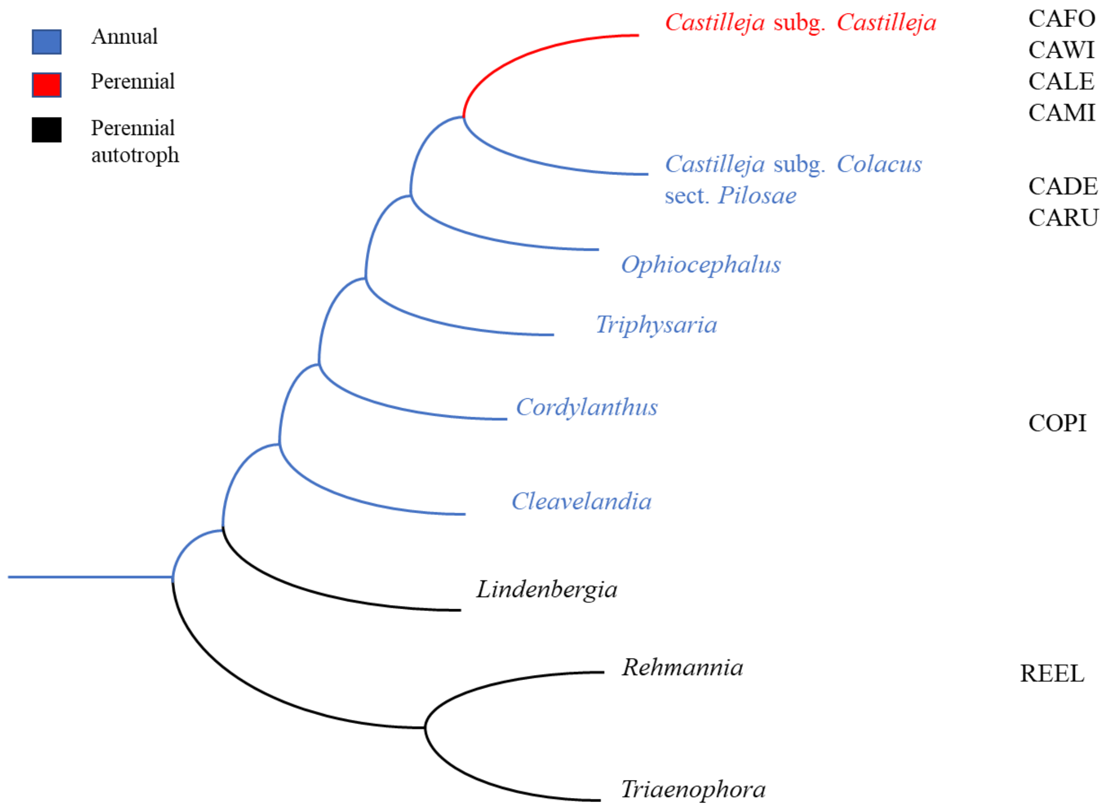

Figure 2.1. Phylogeny of Castillejinae (red and blue lines) and its relationship with the autotrophic members (black lines) of the Orobanchaceae. Phylogeny is highly simplified from Fig. 6 in Tank and Olmstead (2008) and Fig. 2 in Xia et al (2009) and does not include other tribes in the Orobanchaceae. Species codes (as in Table 2.2) represent those species used in the experiment and are aligned with their respective taxonomic classification.

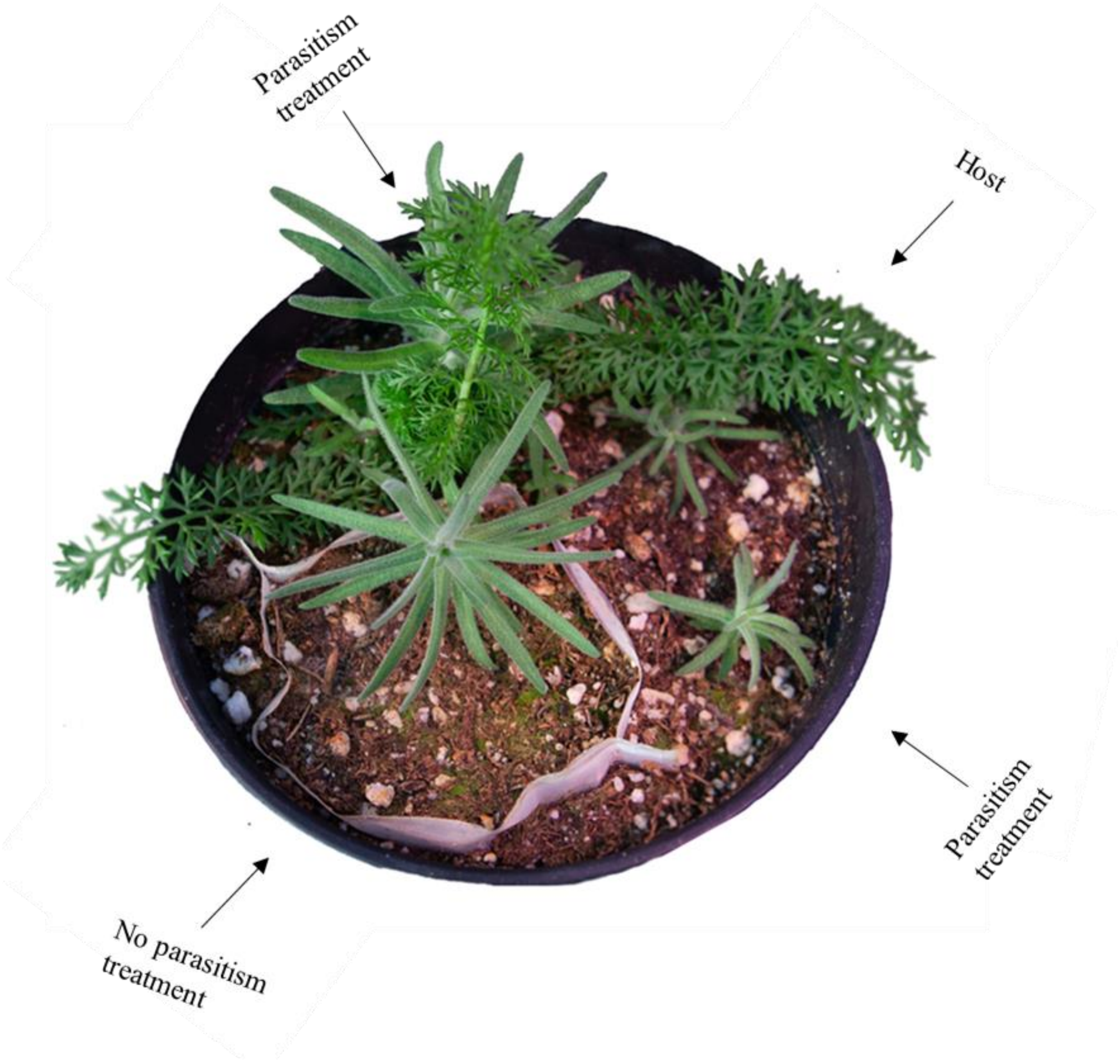

Figure 2.2. Photograph of an experimental pot showing the host plant and hemiparasites. Half of the hemiparasite plants are growing in a root exclusion bag that prevents them from parasitizing the host. In this image, the hemiparasite species is *Castilleja foliolosa* and the host is *Achillea millefolium*

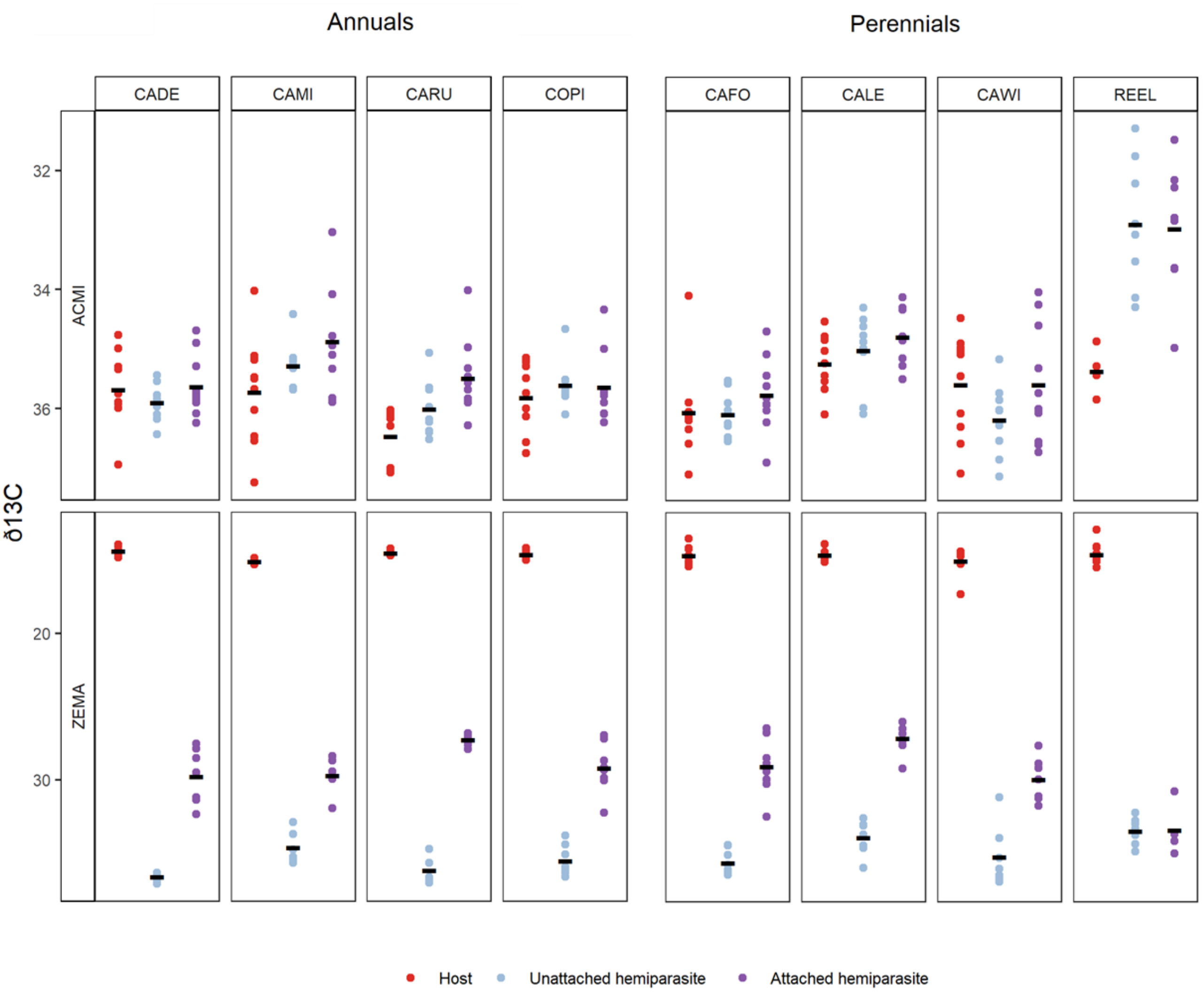

Figure 2.3. δ^{13}C values for unattached and attached hemiparasites with *Achillea millefolium* (ACMI, top row) and *Zea Mays* (ZEMA, bottom row). Black horizontal lines indicate the mean. Note extreme difference in y-axes. REEL (perennial autotroph) was included for comparison but not in statistical analysis. Attached REEL are individuals planted outside of root exclusion bags and unattached are those planted within bags. Species codes as per Table 2.2.

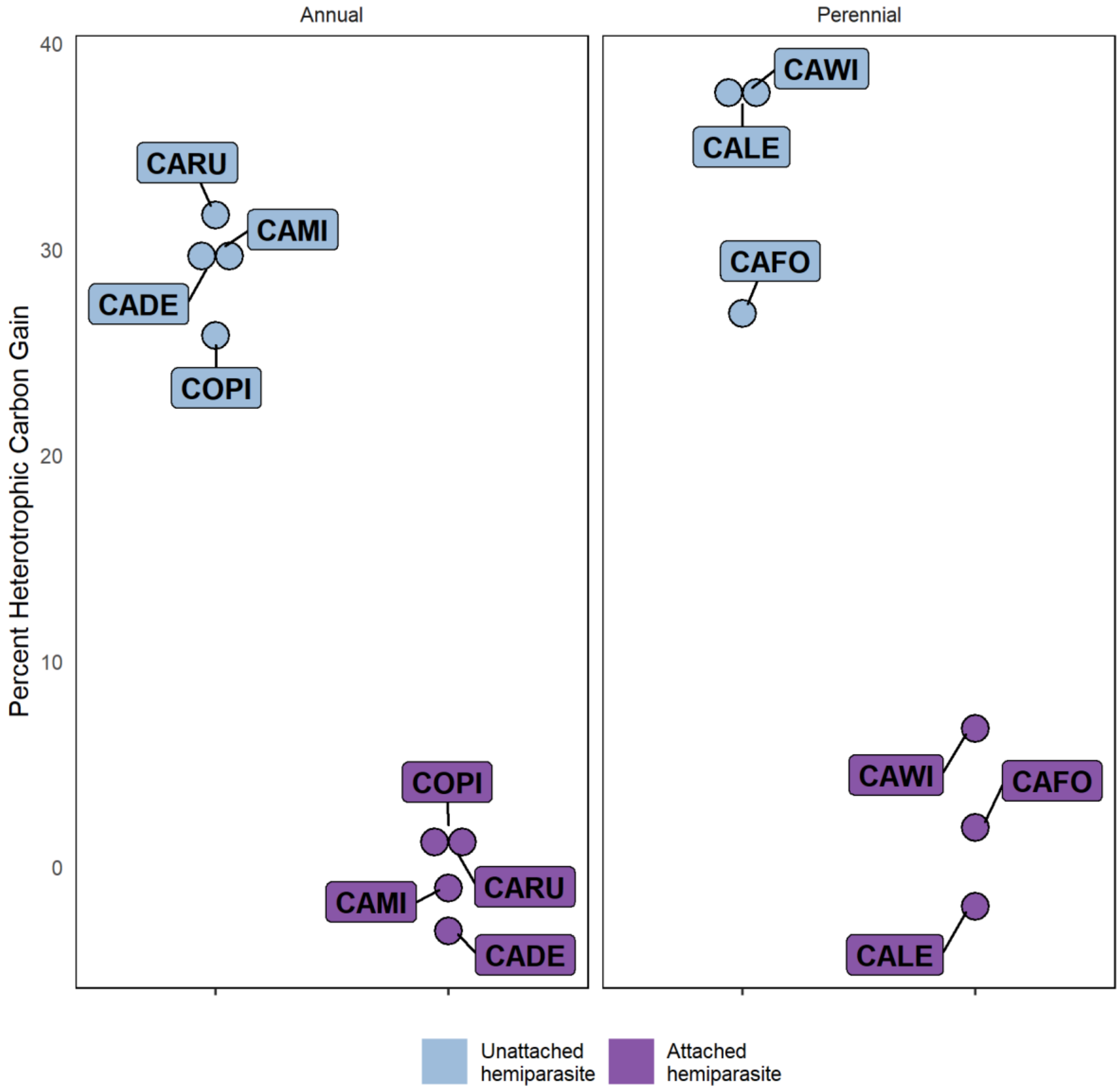

Figure 2.4. Dot plot showing the median percent heterotrophic carbon gain values for the seven hemiparasite species. Species codes as per Table 2.2.

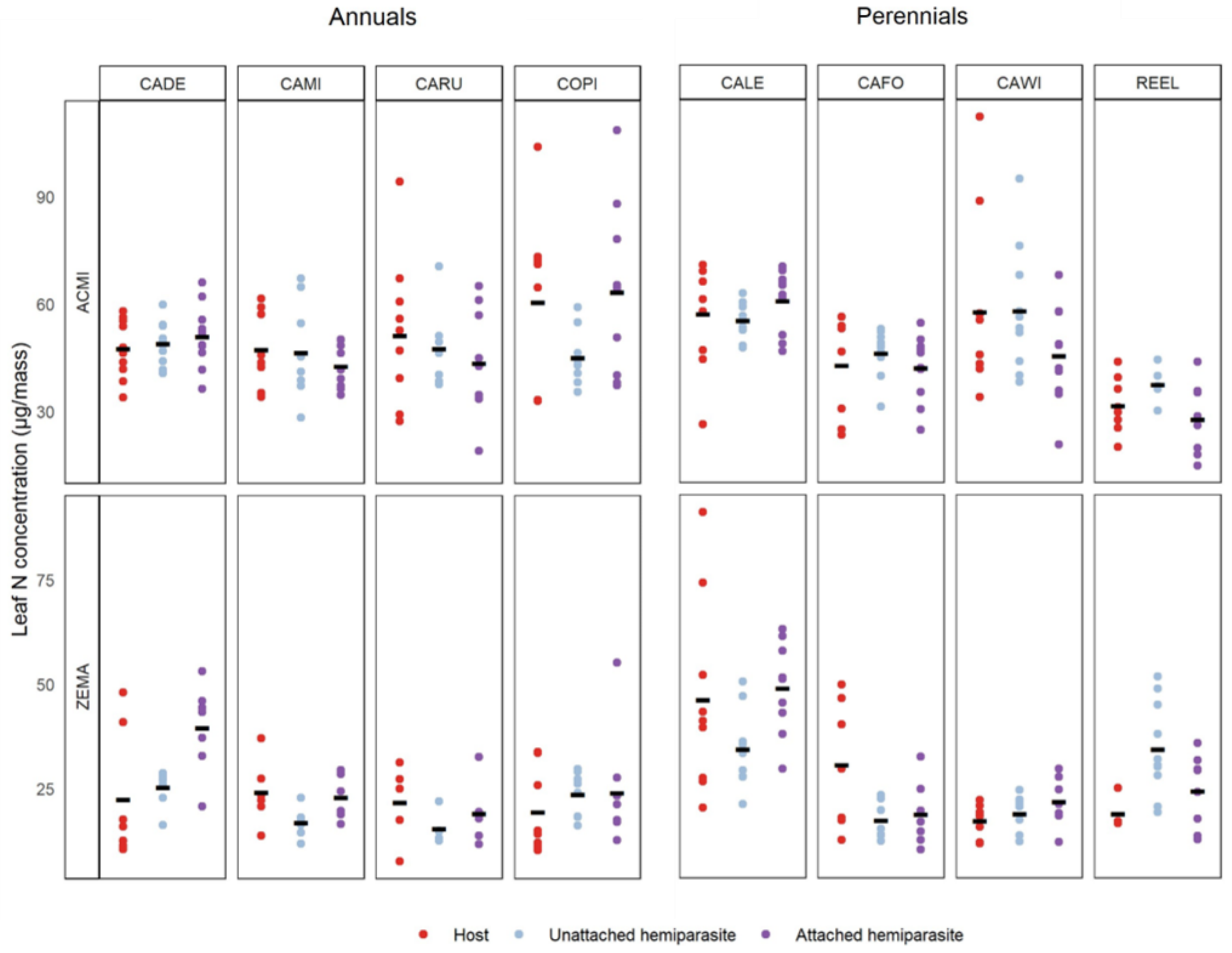

Figure 2.5. Leaf N concentration values for unattached and attached hemiparasites with *Achillea millefolium* (ACMI, top row) and *Zea Mays* (ZEMA, bottom row). Black horizontal lines indicate the mean. REEL (perennial autotroph) was included for comparison but not in statistical analysis. Attached REEL are individuals planted outside of root exclusion bags and unattached are those planted within bags. Species codes as per Table 2.2.

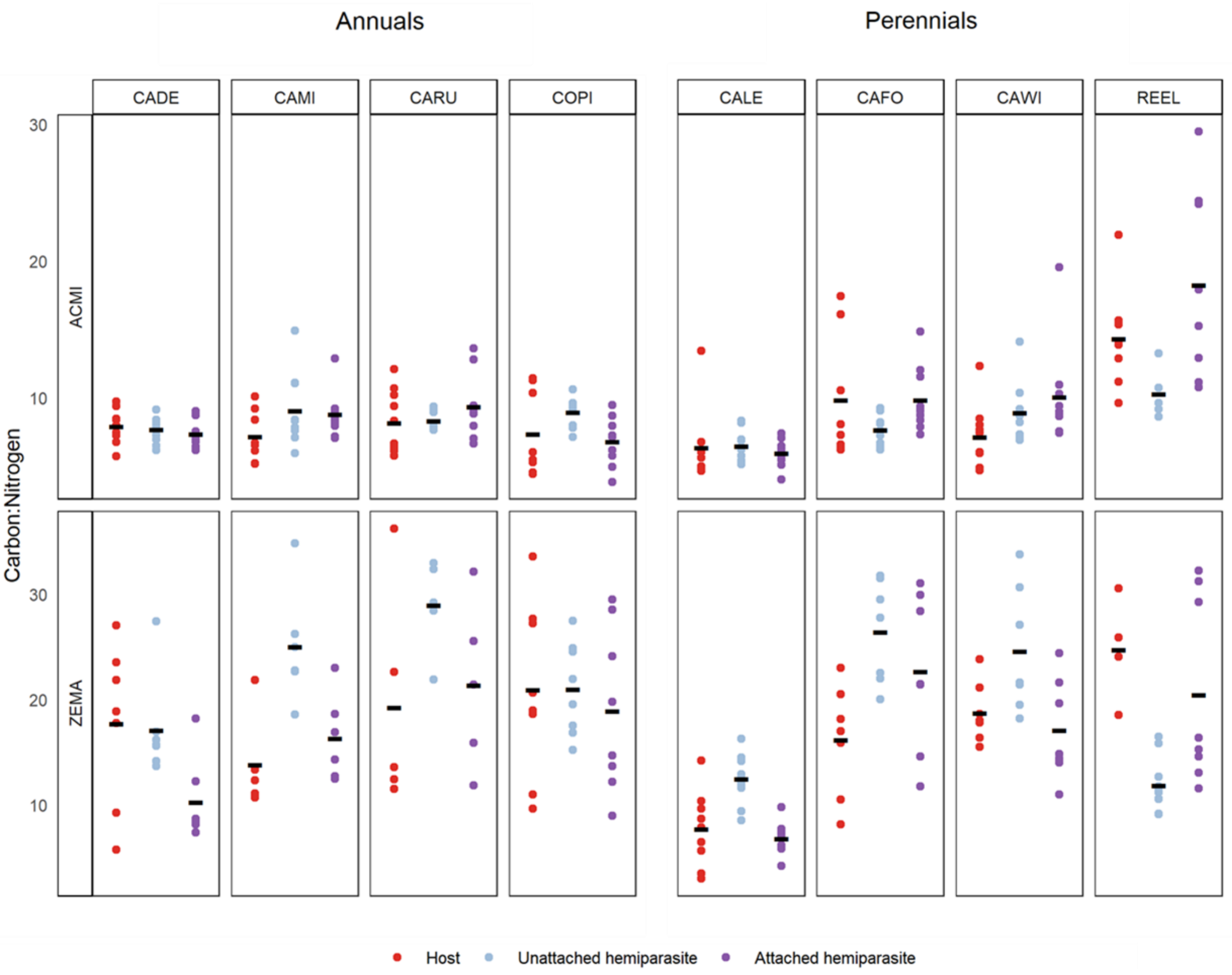

Figure 2.6. Total carbon to total nitrogen ratios for unattached and attached hemiparasites with *Achillea millefolium* (ACMI, top row) and *Zea Mays* (ZEMA, bottom row). Black horizontal lines indicate the mean. REEL (perennial autotroph) was included for comparison but not in statistical analysis. Attached REEL are individuals planted outside of root exclusion bags and unattached are those planted within bags. Species codes as per Table 2.2.

Chapter 2 **Supplement**

Table S2.1. Best results of time to germination and percent germination of a pilot study in which seeds were placed in winter and spring stratification for staggered periods of time. All species are in the family Orobanchaceae, except for *Comandra* (Santalaceae). Percent germination is measured as the number of seeds that germinated divided by the number of total seeds. "-" indicates species that never germinated in the final chamber. All species were left until they either germinated, or for up to 45 days. Asterisks denote species that were used in the final experiment. Winter condition is 5°C with 8 hours of light. Spring condition is 12°C with 12 hours of light. Summer condition is 22°C with 14 hours of light. All species were stratified on moist filter paper.

Species	**Days in winter stratification**	**Days in spring stratification**	**Days to germination**	**% germination**
Agalinis aspera	14	-	-	0
Agalinis auriculta	14	28	-	0
Agalinis tenuifolia	14	28	42	20
Aureolaria pedicularia	14	-	-	0
Bellardia trixago	11	11	22	50
Castilleja attenuata	11	7	18	75
Castilleja campestris	11	11	22	50
Castilleja coccinea	11	11	22	50
*Castilleja densiflora**	11	7	18	50
Castilleja exserta	11	7	18	60
*Castilleja foliolosa**	18	4	22	70
*Castilleja hispida**	23	16	39	60
*Castilleja levisecta**	23	6	29	75
*Castilleja minor**	7	10	17	70

*Castilleja rubicundula**	11	7	18	40
Castilleja wightii	18	4	22	45
Comandra umbellata	29	-	-	0
*Cordylanthus pilosus**	14	8	22	25
Dasistoma macrophylla	14	22	36	8
Lindenbergia philippensis [A]	10	10	20	0
Pedicularis canadensis	29	-	-	0
Pedicularis densiflora	29	-	-	0
Pedicularis lanceolata	-	-	14	85
*Rehmannia elata**[B]	0	0	14	75
*Triphysaria eriantha**	11	3	14	90
*Triphysaria versicolor**	11	3	14	80

Note: [A]*Lindenbergi*a was placed in summer, not spring conditions. [B] *Rehmannia* was directly sown without stratification.

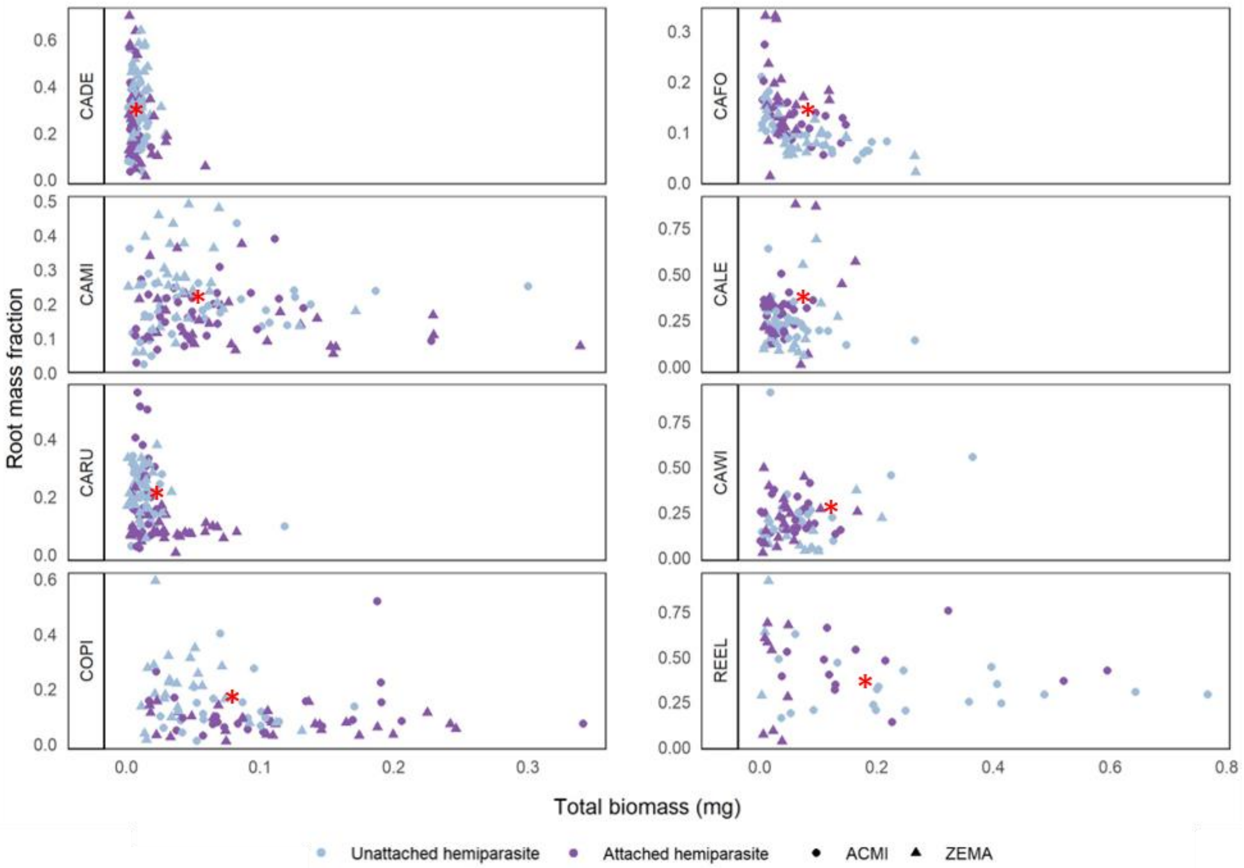

Figure S2.1. Root mass fraction (RMF), the proportion of total biomass represented by root biomass, for annual hemiparasites (left column) and perennial hemiparasites (right column) and the perennial autotroph (REEL) plotted in relation to total biomass. Shapes indicate host identity and colors indicate parasitism status (unattached or attached hemiparasite. ACMI=*Achillea millefolium*, ZEMA=*Zea mays*. Red asterisks indicate the mean. Species codes as per Table 2.2.

Chapter 3 : **Root hemiparasitic plants are associated with increased evenness in plant communities across North America.**

Abstract

Hemiparasites both compete with and extract resources from host plants. As a result, they have the potential to reduce the abundance of dominant plants and release subordinates from competitive exclusion, potentially impacting the diversity of plant communities. Most of the research findings supporting this notion have been experimental and have been conducted at relatively few sites with few hemiparasite taxa. Here, I ask whether patterns in natural plant communities corroborate the expectation that hemiparasites, by suppressing dominant plants, affect structure of plant communities.

I assessed whether hemiparasitic plants are associated with increased evenness and richness of their communities using data from 21,127 plots collected at 129 national parks across the United States. The majority of hemiparasite occurrences were from the genera *Pedicularis, Castilleja, Krameria* and *Comandra.* Increased species evenness in plant communities was strongly associated with hemiparasite presence and abundance, though hemiparasites were not associated with richer plant communities. Higher evenness was more strongly associated with the abundance of hemiparasites than it was with the abundance of other plant species that fell along the same portion of the rank-abundance curve, suggesting that the association between hemiparasite abundance and evenness is unique. The associations that I observed between hemiparasites and plant communities on a continental scale are consistent with evidence that the impacts of root hemiparasitic plants on evenness can be substantial and increase with hemiparasite abundance, but that the effects of hemiparasites on richness are less pronounced.

Introduction

Understanding the mechanisms that maintain and promote species coexistence and community diversity has long held the interest of ecological researchers (Chesson 2000). Both direct interactions (e.g., competition, predation, parasitism, mutualism) and indirect interactions (e.g., resource competition, abiotic resource mediation) are dynamic yet critical in determining how species coexist and whether such coexistence is stable. Additionally, there has been active discussion amongst ecologists centered on “functional plant ecology,” the notion that plants can affect community structure and ecosystem function through their morphological and physiological attributes (Violle et al. 2007). In this sense, specific taxa are described by certain traits, with a trait being defined as a physiological or morphological attribute that directly influences fitness (Adler et al. 2013). Additionally, there are certain specialized functional groups of plants that show similarities in both resource acquisition and responses to the environment. In some cases, these functional plant types can have disproportionate effects on their communities, affecting niche partitioning and species coexistence. One-well known example is the case of nitrogen fixing plants, which have been shown to facilitate nitrogen cycling and to stimulate growth and productivity of communities (Craine et al. 2002; Temperton et al. 2007).

Root hemiparasites, defined by their ability to photosynthesize while also acquiring resources from vascular host plants via parasitism, are another important functional group of plants (Těšitel 2016). Root hemiparasites tap host xylem sap via specialized organs known as haustoria (Yoshida et al. 2016). Hemiparasites are generalists, able to attach to and parasitize a wide variety of hosts simultaneously (Marvier 1998; Matthies 2017). However, host defense and tolerance can vary dramatically, as can intra and interspecific hemiparasite effect and dependance upon hosts (Hejduk et al. 2020, Li et al. 2012; Press and Seel 1996). Furthermore, hemiparasites have vestigial and shallow root systems and a large factor determining whether a host is parasitized is its below-ground dominance and proximity to the hemiparasite. Consequently, in natural communities, suitable host species that are also dominants are disproportionately parasitized by hemiparasites, simply by virtue of their relative abundance (Press and Phoenix 2005).

Variable performance with different hosts as well as disproportionate encounters with certain host species, position hemiparasites to alter community structure (Phoenix and Press 2005, Těšitel 2016). Based on hemiparasite addition and removal experiments we know that hemiparasites can increase the evenness and richness of their communities (Borowicz and Armstrong 2012, Grewell 2008, Těšitel et al. 2017). By reducing host growth and vigor, they can alter competitive dynamics between host and non-host species, potentially increasing the growth and abundance of subordinate species. They have even been shown to impact trophic levels beyond plants (Green et al. 2015; Haan et al. 2018) and even alter nutrient cycling (Fisher et al. 2013, Quested et al. 2003, Spasojevic & Suding 2011). Thus, due to their unique physiology, these plants can have a disproportionate effect on their ecosystem relative to their abundance and are considered to not only be keystone species but ecological engineers that change the availability of abiotic materials (Jones et al. 1994).

To date, there is strong experimental evidence in a few systems that indicates that hemiparasites impact their communities disproportionately relative to their abundance. However, it remains unclear whether what is observed in those systems represent a more universal dynamic between hemiparasites and plant communities. Notably, our understanding of a hemiparasite’s effect on its community is largely based on findings from robust experiments using annual hemiparasites in the genus *Rhinanthus* as study organisms. In general, these studies point to a positive association of hemiparasites with evenness and richness (Ameloot et al. 2006). However, exceptions are not uncommon (Callaway and Pennings 1998, Gibson and Watkinson 1992). Furthermore, our current knowledge of the effect of hemiparasites is based primarily on work conducted in grasslands and prairies (Těšitel et al. 2015). However, despite hemiparasites' general preference for poor ecosystems (e.g., alpine and, semi-arid, or grasslands), they are still found in habitat types where many co-occurring species (and potential hosts) are woody (Nickrent 2002, Těšitel 2016). Given our knowledge that hemiparasites more easily penetrate fibrous roots found mostly in herbaceous, not woody, plants (Mudrák et al. 2016), we might expect that a hemiparasite's effect would be more pronounced on communities dominated by herbaceous hosts than by woody hosts. However, this has not been previously tested. Additionally, growing evidence indicates that the effects of hemiparasites on evenness are likely linearly related to their abundance, whereas their effects on richness peak at intermediate abundances (Fibich et al. 2016, Těšitel et. al 2018, Hejduk et al. 2020). This suggests that the mechanisms underlying a hemiparasite's effect on evenness and richness differ. Since an increase in richness necessitates colonization by a previously absent plant species, it is more likely that a hemiparasite's impact is mechanistically related to evenness.

Observational studies that look for the signatures of experimentally demonstrated ecological processes can help researchers assess the scope of those processes (Adler et al. 2011, Beaury et al. 2020, Sofaer et al. 2018). In the case of hemiparasites, past experimental results are the basis for the subsequent expectations that hemiparasites can increase the evenness of plant communities by reducing the dominance of host species, and perhaps can also increase the richness of plant communities (Těšitel et al. 2017). It follows that we expect areas where hemiparasites are present and abundant to have higher evenness and richness than comparable areas without hemiparasites. hemiparasites. However, care must be taken when connecting processes to ecological patterns. First, we must be mindful that numerous aspects of the environmental factors affect plant community richness and evenness, and those must be accounted for when looking for assessing the nature of plant community associations with hemiparasites. Second, the fact that most hemiparasitic plant species are relatively uncommon in plant communities may be important to consider when determining our approach. That is to say, when assessing their hemiparasitic plant association with the evenness or richness of plant communities, the known associations of other types of plants that are relatively uncommon are a logical point of comparison. Since there are commonalities among species-abundance relationships that could result in spurious associations of an uncommon species with even or rich communities, comparing hemiparasites with non-hemiparasitic plants of similar abundance may be important in corroborating or rejecting the functional link between hemiparasites and plant community evenness and richness.

Here I analyze observations of hemiparasites and plant communities across North America, using 21,127 plots from the U.S. National Park Service (NPS) that span a variety of ecoregions. After accounting for differences in plant communities attribute to ecoregion characteristics and management unit practices, I aim to address:

(i) Whether the presence of hemiparasites is correlated with an increased richness of three groups of growth forms: all plants, herbaceous plants, and woody plants (hereafter I use the term “growth forms” to refer to all three of these groups)

(ii) Whether increased evenness and richness of these plant groups is associated with greater hemiparasite abundance

(iii) Whether associations of plant community evenness and richness with hemiparasite abundance are greater than expected for similarly uncommon plant species.

I demonstrate that high hemiparasite abundance is associated with more even herbaceous plant communities, but not more species rich plant communities, suggesting that some, but not all, experimentally demonstrated hemiparasitic processes have left their fingerprints at a continental scale.

Methods

Plot survey data:

These data come from a large-scale vegetation monitoring project undertaken to develop a uniform hierarchical vegetation classification standard for generating vegetation maps for national parks (Beaury et al. 2020; The Nature Conservancy 1994). Data for ach plot included a list of all observed plant species, as well as their percent cover and stratum class. Plot area ranged from $1m^2$ to $5400m^2$, depending upon the community type and park size, with 1,523 plots missing area size data. The median plot size was $400m^2$. Sampling years were between the early 1990s and at least 2008; due to data errors, the final year of sampling is not clear.

I assigned each plot to the level four ecoregion in which it was located (Omernik and Griffith 2014). Ecoregions are identified by “analyzing the patterns and composition of biotic and abiotic phenomena that affect or reflect differences in ecosystem quality and integrity” (Omernik 1987, 1995). There are four established ecoregion levels, with level four being the most detailed, allowing us to account for as much environmental variation as possible. Plots lacking latitude and longitude data (n= 4,024) were excluded, leaving 21,127 plots across 220 level four ecoregions and 129 national parks (Figure 3.1).

Our dataset included 8,620 plant species. We used the United States Department of Agriculture PLANTS Database (The PLANTS Database 2018) to identify the growth habit of each plant species. Nonvascular taxa were excluded from all analyses. We classified species as herbaceous or woody based on growth habit: herbaceous included the growth habits of “forb/herb”, “graminoid” and “sub-shrub”, while woody included the growth habits of “tree” and “shrub”. All hemiparasites were classified as herbaceous. We expressed abundance as the sum of the covers of all strata calculating this separately for all growth forms. Although we recognize that percent cover and abundance are not equivalent, we are assuming that increases in percent cover are correlated with increase in abundance. Finally, we calculated richness and the Shannon evenness index for all growth forms.

In our dataset, 2,430 plots contain one or more hemiparasites. Of these plots, 2,241 include herbaceous plants (richness range of 19–73) and 2,201 include woody plants (richness range of 5–37). There are 12 hemiparasitic genera in our dataset, which includes approximately 22 native and non-native genera found in the United States—*Aureolaria*, *Castilleja*, *Comandra*, *Cordylanthus*, *Dasistoma*, *Euphrasia*, *Geocaulon*, *Krameria*, *Orthocarpus, Triphysaria*, *Pedicularis*, and *Seymeria*—but the most abundant genera are *Pedicularis* (35% of total hemiparasite abundance, family Orobanchaceae), *Castilleja* (28%, Orobanchaceae), *Krameria* (15%, Krameriaceae), and *Comandra* (12%, Santalaceae). The remaining genera make up 2% or less of total hemiparasite abundance (Table 2.1).

Statistical analysis

All analyses were carried out in R 4.0.2 (R Studio Team 2019). To assess whether hemiparasite presence was associated with an increase in evenness and richness I considered pairs of plots in which one contained a hemiparasite and the other did not. I began by pairing each plot with the plot nearest to it using the package nngeo with the function *st_nn* (Dorman 2021). I then selected only those pairs in which one plot had a non-zero hemiparasite abundance and the other did not contain hemiparasites. Finally, we restricted our attention on pairs in which both plots were in the same level four ecoregion. This resulted in 1,138 pairs of plots. The mean distance between pairs of plots was 460 m with a maximum of 29,000 m. We analyzed the effect of hemiparasite presence on plot richness and evenness with a linear mixed model using the package lme4 (Bates et al. 2015), with pair ID specified as a random effect. Plot size varied between pairs in 352 of the 1,138 pairs. To account for this, we included plot size in all models as a covariate. Out of the 2,276 plots, 70 had no value for plot area and were therefore removed, leaving us with 2,206 plots for the analysis. For each response, we assessed the extent to which hemiparasite presence improved model fit by comparing the AIC from the full model to a null model without hemiparasite abundance.

To analyze the relationship of hemiparasite abundance with evenness and richness, I focused on plots in the full dataset that contained hemiparasites (n=2,430). Hemiparasite abundance and plant community richness were log transformed to improve normality, and evenness since evenness values are bounded between 0 and 1 (Lesaffre et al. 2007). In each model, hemiparasite abundance and plot size were specified as fixed effects, while ecoregion, sampling year and park identity were specified as random effects on the intercept. Out of 2,430 plots, 86 plots did not have a value for plot area and were removed, leaving 2,344 plots for analysis. I ran the models for the evenness and richness of all growth forms. For each response, we assessed the extent to which hemiparasite abundance improved model fit by comparing the AIC from the full model to a null model lacking hemiparasite abundance.

Finally, I considered whether hemiparasite abundance might be associated with increased community evenness or richness solely because of their position in the rank-abundance of plant species in communities. Often, hemiparasites are relatively uncommon at a small spatial scale. Because of commonalities in rank-abundance curves of plant communities, it is possible that uncommon plants might be associated with even or species rich communities irrespective of their role in those communities (Jenkins 2011, Iiher et al. 1998). To account for this, I used a null modeling approach to compare the effect of hemiparasites on plant community richness and evenness with the effect of equally rare plants on these community properties. First, I generated a rank abundance curve for all 8,620 plant species in the total dataset. The abundance of the 97 hemiparasite taxa fell within the range of ranks 792–8,538, with most clustered toward the lower ranks (Figure 3.2). I identified the non-hemiparasite plant species (n=3000) that fell within that range, and I randomly designated 97 of those species as null model focal species. I discarded plots that lacked any of the null model focal plants, calculated evenness and richness of each plot, and fit mixed models with the same structure as above, replacing hemiparasite abundance with the abundance of the set of null model focal species. I repeated this procedure 1000 times to create an empirical null distribution of model effects. I compared this empirical null distribution of model effects to the effects of hemiparasites to determine whether hemiparasite abundance had a greater association with plant community metrics than plants of comparable abundance. Since presence and absence has no bearing on the rank abundance curve, and I Ire unable to meaningfully repeat the paired plot analysis with the null model species, this analysis was only done for abundance models.

Results

Hemiparasite presence

Relative cover and richness of each growth form was comparable between paired and unpaired plots (Table 3.2). In the paired plots analysis, evenness in plots with hemiparasites present was significantly higher for all plants and woody plants than in plots without hemiparasites. Herbaceous evenness was not significantly different. Richness was greater for all growth forms in plots with hemiparasites (Table 3.3, Figure 3.3).

Hemiparasite abundance

Linear Mixed Models

Hemiparasite abundance was significantly correlated with increases in evenness for all growth forms. In contrast, hemiparasite abundance was not associated with richness for any of the growth forms (Table 3.3, Figure 3.4).

Null Analysis

Greater evenness of herbaceous plant communities was more strongly associated with hemiparasite abundance than it was with the abundance of comparably uncommon non-hemiparasites. Hemiparasite abundance model coefficients were significantly greater than the distribution of null model coefficients for herbaceous evenness. However, the effect of hemiparasites on the evenness of woody species and all plants fell within the null model range of effects of non-hemiparasites. The effects of hemiparasites on the species richness of communities of each growth form fell within the null distribution of effects of non-hemiparasites (Figure 3.5).

Discussion

I have demonstrated significant positive associations between the incidence and abundance of root hemiparasites and increased evenness in plant communities using data that extends over a large geographic scale. In particular, the patterns suggest that (1) hemiparasites are associated with even communities (2) this association is positively related with hemiparasite abundance, and (3) a hemiparasite's association with rich plots may be more attributable to their distribution patterns rather than a parasitic lifestyle.

North American hemiparasites can increase species evenness

Hemiparasites are thought to influence evenness and richness by disproportionately selecting dominant species as hosts and reducing these hosts' growth to such an extent as to mitigate competitive exclusion of other subordinate species. Several studies have shown that hemiparasites in *Castilleja* and *Pedicularis* have the potential to substantially damage their host, though host susceptibility varies (Kilgore 2017, Matthies 1997, Van Hoveln et al. 2011). Thus, when considered in the context of hemiparasite physiology, the results of this study show that North American genera can affect community evenness. Furthermore, these results also underscore those of the observational presence-absence study of McKibben and Henning (2018), which showed an association of *Castilleja* with evenness, as well as those of the observational study by Fibich et al. (2017), which demonstrated a correlation between the presence of 16 hemiparasite species—two of which were in the genus *Pedicularis*—and more diverse plots. Furthermore, the abundance-dependent effect of hemiparasites supports the idea that a hemiparasite's effect is additive, i.e., the greater the abundance of hemiparasites, the greater the effect on the community and, plausibly, on the dominant host species. Although I recognize that percent cover and abundance are not equivalent, I am assuming that increases in percent cover are correlated with increase in abundance. This is in line with the results of Těšitel et al. (2016) which found that community evenness and *Rhinanthus* abundance were linearly related. Mechanistically, this may be due to either a reduction in host abundance due to parasitism, or an increase in subordinate abundance as subordinate species are released from competitive exclusion. The relative importance of each of these mechanisms—i.e., a decrease in dominant abundance vs. an increase in subordinate abundance—will need to be elucidated through future research. In addition, it is important to note that for a root hemiparasite below-ground dominance is more important than above-ground dominance. These two are likely directly proportional in the open, nutrient-poor ecosystems typically occupied by hemiparasites, like grasslands, though the relationship of below-ground and above-ground abundance needs additional research (Hiiesalu et al. 2012).

Hemiparasites have unclear effects on richness

In contrast to evenness, this study showed no relationship with hemiparasite abundaunce and richness. These findings contradict most of the litreature, including studies incorporating density effects, where community richness was highest at intermediate densities of *Rhinanthus,* to then decline at high densities (Heer et al. 2018). Additionally, since hemiparasite associations with richness were the same as those of other species with similar distributions, any relationship between hemiparasites and richness, as found in the presence analysis, is likely more of a function of their distribution. It is important to note that a potential bias of the null models is that hemiparasites span such a large range of rank abundances yet are clustered predominantly towards the lower ranks (Figure 3.2). Thus, I may be artificially heightening their rarity by giving each rank equal weight. However, focusing on the narrow range defined by the most abundant hemiparasites did not change my inferences.

While an effect on evenness is predicated upon a stabilizing impact on competitive dynamics, to increase plant richness, a hemiparasite must faciliate the opportunity for colonization. Thus, the purported relationship of hemiparasites with richness may be more of a statistical artifact, whereas the relationship with evenness is more likely mechanistic.

Effects are centered on herbaceous communities

Although I was unable to specify ecosystem type in the analysis, the insignificant assocaition with woody plants makes it unlikely that hemiparasites are having pronounced effects in woodland or forest ecosystems. Furthermore, most hemiparasites tested exhibit some form of "host preference", selectively parasitizing only a subset of the hosts available to them and performing better with certain species (Gibson & Watkinson 1992; Matthies 1996; Shen et al. 2006; Těšitel et al. 2017). Moreover, hemiparasites more successfully penetrate herbaceous and graminoid hosts with fibrous, diffuse root systems (Mudrák et al. 2016). Thus, my results are in line with our understanding of hemiparasite plant biology. However, the fact that evenness trends were significant for hemiparasites across 107 ecoregions suggests that hemiparasites can have an effect in many herbaceously dominated eocsystems.

The role of life history

A hemiparasite's longevity informs its resource acqusition strategies. Life history theory suggests that annuals, which reproduce once and die, should prioritize resource acquisition while perennials, which live for more than one year, benefit most from resource conservation

(Friedman & Rubin 2015). Thus, whereas an annual hemiparasite may have a strong incentive to damage or even kill a host for rapid acquisition of resources, a perennial would benefit most by conserving host resources and inflicting less damage to hosts (Lepš and Těšitel 2015). Indeed, perennial hemiparasites have been shown to conserve more water and receive less host carbon than annuals (Press 1989). Unlike *Rhinanthus* and related genera, the dominant hemiparasitic genera in this study are mostly perennials (94% of total hemiparasite abundance in the dataset is from perennial hemiparasites). Given that the influence of a hemiparasite on its community begins with its relative impact on a host, a hemiparasite's life history should inform how it affects evenness and richness. Since annuals extract more host resources, they cause greater damage to their hosts and, therefore, their community effects should be more pronounced. Furthermore, while perennials grow longer and accumulate more biomass, they are less likely than annuals to reach the minimum abundance needed to promote evenness and richness. In addition, unlike an annual which, when it seneceses, leaves openings for colonization, a perennial does not provide annul regeneration gaps (Lepš & Těšitel 2015). On the other hand, perennials form multi-year relationships with their hosts, and thus, though their effects may be less pronounced, they could be more stable and longer lasting (Borowicz et al. 2019).

Additionally, hemiparasites are suggested to increase richness by lowering overall productivity and thereby facilitating the coexistence of grasses and forbs (Davies et al. 1997; Digiovanni et al. 2017; Těšitel et al. 2013). This is particularly applicable in grassland ecosystems, where most hemiparasitic plant research is conducted and in which declined producitivty is associated with increased richness due to resource limitation (Pywell et al. 2007), though this notion has been challenged (Adler et al. 2011). The literature suggests that annuals (predominantly *Rhinanthus*) damage host growth without replacing the lost biomass with their own, thereby decreasing community productivity (Ameloot et al. 2005). Even assuming an equal rate of host damage, a perennial needs to accumulate more biomass, particularly below-ground, for dormancy and multi-season longevity. Thus, we may not expect such a reduction in community biomass as is observed in annuals. Accordingly, Spasojević and Suding (2011) found a significant increase in community biomass in plots with the perennial *Castilleja occidentalis* and no significant difference in richness between plots with and without *Castilleja*. Moreover, in a grassland removal experiment, Demey et. al (2015) found no effect of the biennial *Pedicularis sylvatica* on community biomass, yet the *Rhinanthus angustifolius* significantly lowered community biomass. Given life history's theorized role in mediating the host-hemiparasite relationship as well as the obvious links between distribution and life history, investigating the link between a hemiparasite's longevity and its ultimate upstream effect on community productivity remains a promising area of future research.

Alternate hypotheses

It is important to note that this study is purely correlative. Hemiparasites may be more associated with even communities simply because hemiparasites may have an increased chance of colonizing more even communities. Furthermore, the factors that may decrease evenness in a community, like disturbance, could also decrease hemiparasite abundance. Future manipulative research with these genera is needed to clarify their relationship with community evenness. Finally, my results could be refined and made more predictive if there were more information on the host preference of the hemiparasites in this study. In fact, in contrast to research on *Rhinanthus*, whose ecological studies are bolstered by anatomical and physiological research, few experimental studies exist with the most abundant genera in North America.

Conclusion

This study improves our understanding of the role that hemiparasitic plants, as a functional group, play in structuring their communities. This is the largest scale analysis of its kind, spanning multiple ecosystems and hemiparasitic genera, and is the first to focus on North America. I hope the illustrated trends between hemiparasites and evenness prompt manipulative research into both the physiology and ecology of these plants to broaden our understanding of these genera.

Given that other research findings suggest that life history differences between hemiparasites and ecosystem type likely influence a hemiparasite's physiology, future research should investigate how such trends mediate a hemiparasite's ecological role. Furthermore, future research should prioritize determining the key traits of hemiparasitic species that affect communities, as well as the key traits of suitable hosts. Determining these functional traits will provide valuable insight into the nuances of the ecological role of hemiparasites.

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Table 3.1. Descriptive statistics on hemiparasitic genera found in the 2,430 plots containing one or more hemiparasite occurrence. “Other” indicates the following genera: *Cordylanthus, Aureolaria, Dasistoma*, *Euphrasia*, *Geocaulon, Orthocarpus, Triphysaria,* and *Seymeria*

Hemiparasite Genera	**Percent of total hemiparasite abundance**	**Prevalence (Percent of sites present)**	**Minimum Richness**	**Maximum Richness**
Pedicularis	35 %	30 %	0	3
Castilleja	29 %	43%	0	3
Krameria	16 %	13%	0	2
Comandra	11 %	17%	0	2
Other	10 %	4.7%	0	2

Table 3.2. Descriptive statistics for plots used in paired plot presence analysis (n =2206).

	Hemiparasite Presence	**Prevalence (Percent total plots present)**	**Mean Percent Cover**	**Minimum Richness**	**Maximum Richness**
Hemiparasites	Present	100%	1.10	1	3
Herbaceous Plants	Present	100%	48.3	18	72
	Absent	99%	45.0	0	56
Woody Plants	Present	92%	42.0	0	37
	Absent	91%	36.0	0	31
All Plants	Present	100 %	95.0	5	111
	Absent	100%	36.0	2	74

Table 3.3. Presence and abundance model results; note that the response variables are transformed. CI = confidence interval. ΔAIC is the difference between the null model (model excluding the listed predictor) and the full model (model containing the listed predictor). ΔAIC values equal or greater than two are bolded.

Predictor	**Response**	**n**	**Estimate**	**CI**	**Marginal R^2**	**Conditional R^2**	**ΔAIC**
Hemiparasite Presence	Logit All Plant Evenness	2206	0.110	0.06 - 0.16	0.01	0.42	**10.2**
	Logit Herbaceous Plant Evenness	2206	0.071	-0.01 - 0.15	0.01	0.28	-3.8
	Logit Woody Plant Evenness	2018	0.350	0.22 - 0.47	0.01	0.32	**21.7**
	Log All Plants Richness	2206	0.401	0.29 - 0.36	0.07	0.59	**321.0**
	Log Herbaceous Richness	2206	-0.031	0.36 - 0.44	0.08	0.53	**39.0**
	Log Woody Richness	2206	0.160	0.11 - 0.20	0.04	0.60	**35.0**
Log Hemiparasite Abundance	Logit All Plant Evenness	2344	0.100	0.08 - 0.12	0.03	0.59	**91.8**
	Logit Herbaceous Plant Evenness	2335	0.150	0.12 - 0.18	0.04	0.51	**91.2**
	Logit Woody Plant Evenness	1907	0.010	-0.03 - 0.05	0.01	0.26	**7.5**
	Log All Plant Richness	2344	-0.030	-0.04 - -0.01	0.01	0.67	1.8
	Log Herbaceous Plant Richness	2344	-0.030	-0.05 - 0.01	0.01	0.62	1.6
	Log Woody Plant Richness	2344	-0.001	-0.02 - 0.02	0.04	0.63	-9.2

Note: Plot area was included as a covariate but is not shown. Level four ecoregion, year and park identity were random effects. Woody plant evenness n is not equal to woody richness sample size as plots with n=1 had undefined evenness, but defined richness. Marginal R^2 shows variance only by fixed effects and conditional R^2 provides variance explained by the entire model.

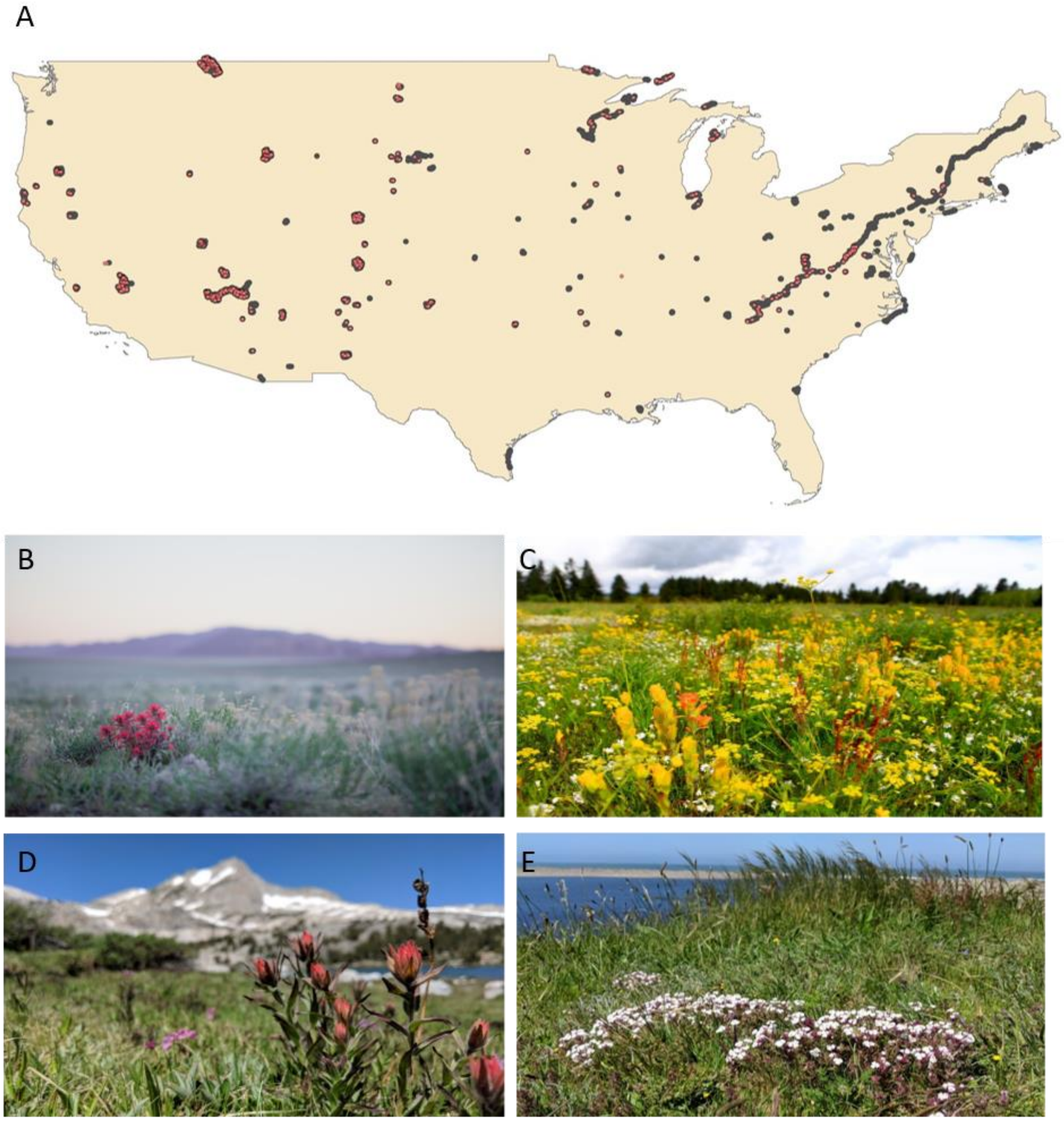

Figure 3.1. A) Map of the conterminous United States. Black circles indicate the location of all 21,127 plots and red circles indicate the location of 2,430 plots which contain one or more hemiparasite occurrences. Due to the large number of plots, the scale of plots with hemiparasites is slightly skewed, making it appear that they take up more of the total percent of plots than they do. B) *Castilleja chromosa* in the high sagebrush steppe of Nevada. C) *Castilleja levisecta* and *Castilleja hispida* in the Puget Sound prairies of Washington state. D) *Castilleja miniata* outside of Yosemite National Park, California. E) *Triphysaria floribunda* in Point Reyes National Park, California.

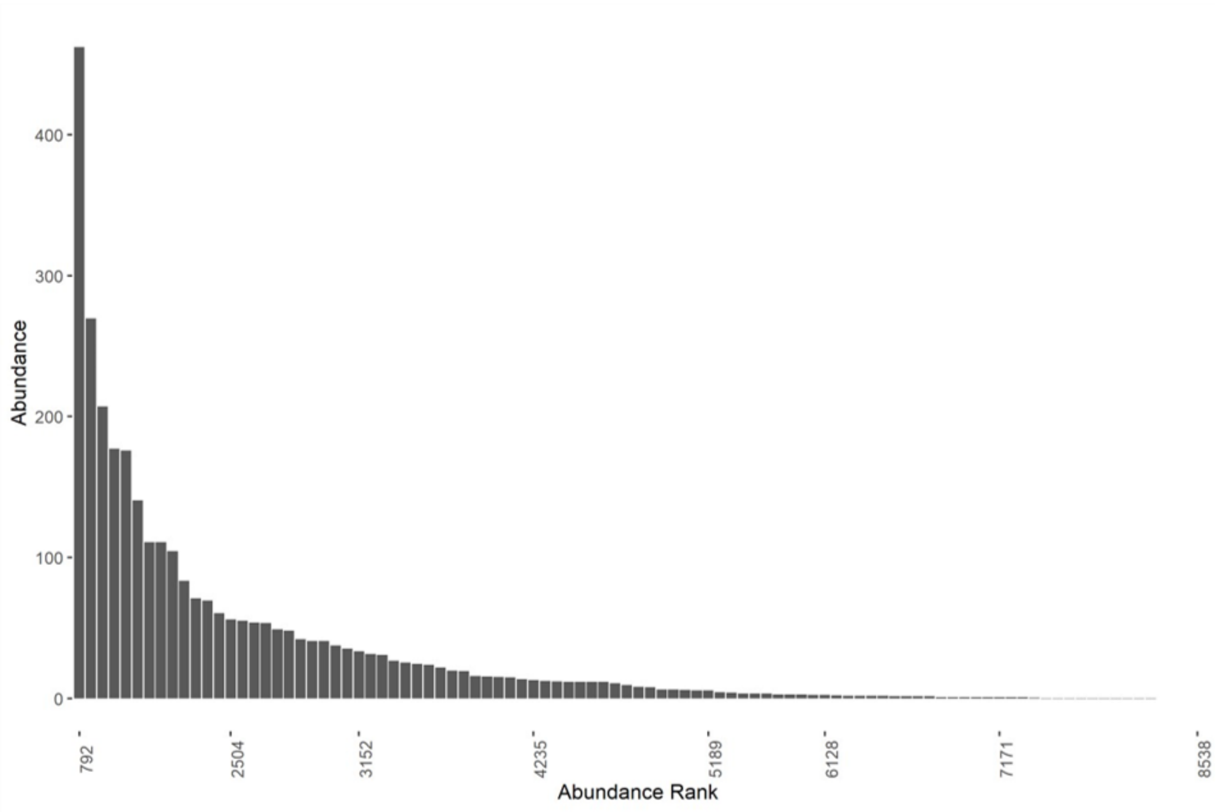

Figure 3.2. Rank abundance curve of hemiparasites within the dataset. Only hemiparasite taxa are shown, but the ranks are derived from the complete dataset.

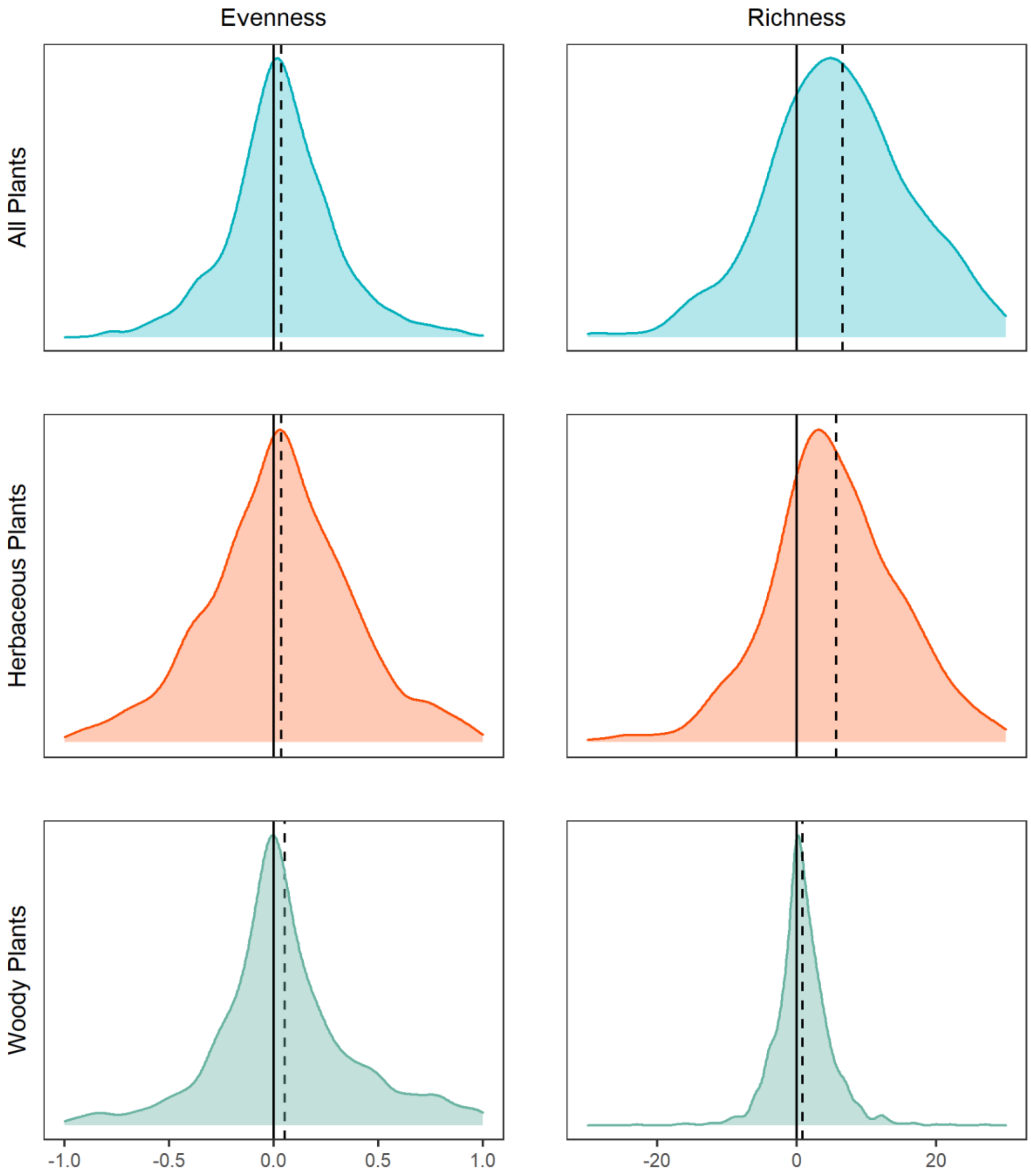

Figure 3.3. Density plots of differences in evenness (left) and richness (right) for all plants (top row) herbaceous plants (middle row) and woody plants (bottom row). Dashed lines indicate the mean and solid lines are placed at zero, indicating no difference between plots. Model results are shown in Table 3.3.

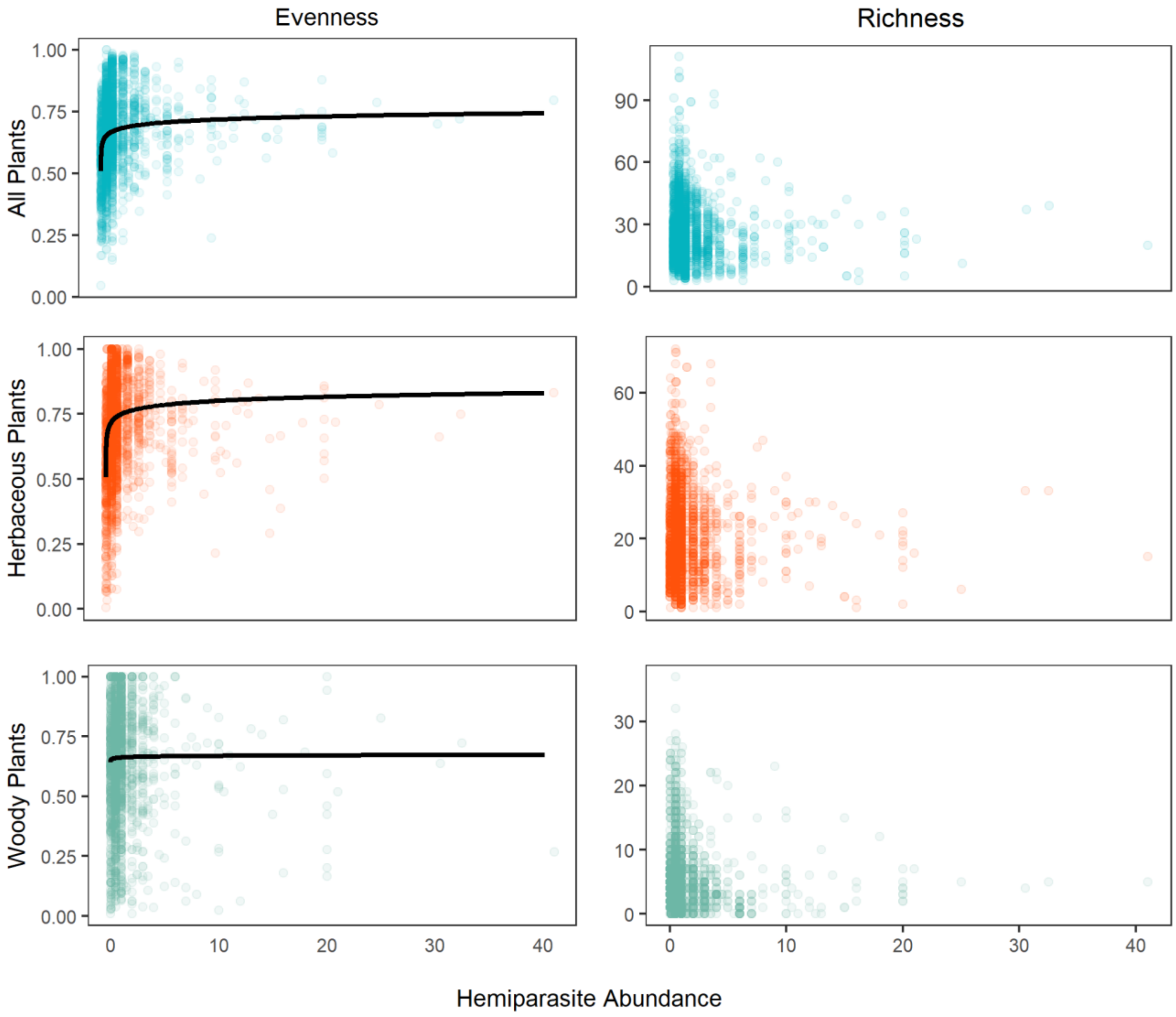

Figure 3.4. Relationship of hemiparasite abundance to evenness (left) and richness (right) of all plants (top row), herbaceous plants (middle row), and woody plants (bottom row). Model results are shown in Table 3.3. Note that scale of the y-axis varies among graphs. Fit lines are only shown for significant trends.

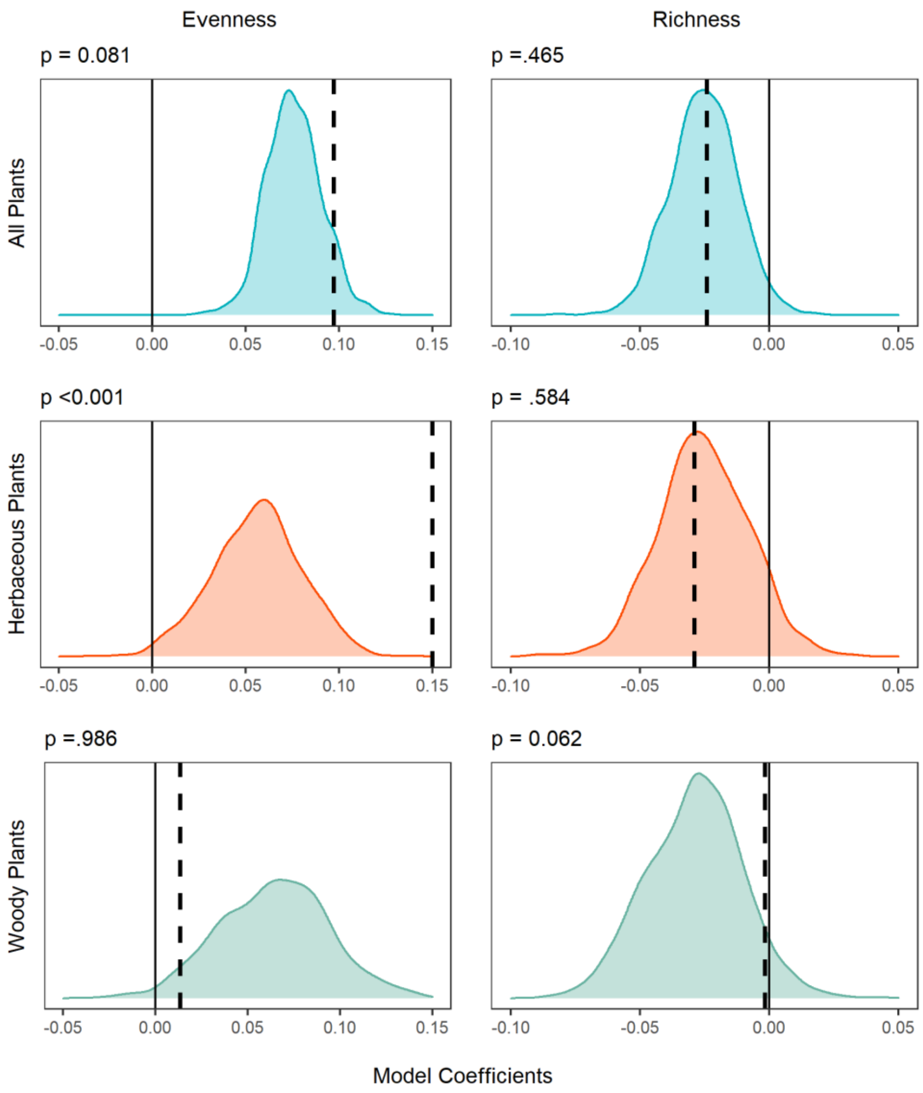

Figure 3.5. Density graph of model coefficients for 1000 null models created from plants in the same rank abunance as hemiparsaites for evenness (left) and richness (right) for all plants (top row) hebacoeus plants (midde row) and woody plants (bottom row). The y-axis limits are identical across rows. The dashed line indicates the hemiparasite model coefficient and the solid lineshows a coefficient of zero, indicating no relationship between abundance and the response. Positive coefficients indicate a positive relationship between abundance and the given metric; negative coefficients indicate a negative relationship.

Chapter 4 : **Root hemiparasitic plant ecology—what about the perennials?**

Abstract

All plants experience a fundamental tradeoff between the acquisition of resources to facilitate fast growth and the conservation of resources for longevity. Annuals, plants that have one reproductive event, have adapted physiologically for rapid resource uptake whereas perennials, which live for more than one year, tend to prioritize resource conservation. For root hemiparasitic plants, parasitic plants that have retained the ability to photosynthesize, host plants are an important resource. Consequently, these plants have developed a unique suite of eco-physiological traits adapted for host resource gain that also positions them to affect the structure of their communities. However, research shows that the degree and mechanism of hemiparasite community impact can vary greatly. Given the importance of longevity to resource physiology, integrating life history theory into hemiparasitic plant biology may improve our understanding of the ecological roles of hemiparasites.

Here, I argue that annual hemiparasites are adapted to prioritize resource gain by aggressively parasitizing the host, while host conservation confers greater adaptive value to perennials. Population dynamics and other ecological traits like density also vary with life history and influence the role a hemiparasite has on its community. I also consider how hemiparasitic longevity may relate to the evolutionary advantages conferred by a parasitic lifestyle. This type of framework has the potential to be predictive, allowing us to generalize as to if and how a particular hemiparasite will impact its community. This will facilitate the incorporation of this functional group of plants into ecological theory and models. I also

highlight many knowledge gaps in hemiparasitic plant biology and discuss technical limitations that are hindering the field.

Introduction:

Facultative root hemiparasites are one of the only groups of organisms (along with carnivorous plants) that can play the part of consumer and producer simultaneously (Watson 2009). Hereafter referred to as "hemiparasites," these plants retain the ability to photosynthesize yet also attach to the roots of other plants via a specialized structure called a haustorium (Figure 4.1), through which they redirect host resources dissolved in the xylem sap to their own vasculature (Těšitel 2016). Over the last several decades, there has been considerable interest in understanding whether and how native hemiparasites impact their natural communities. From this research, we know that hemiparasites can increase both community richness and evenness by attaching to and reducing the growth of dominant plants (Press & Phoenix 2005). Furthermore, they have been shown to affect nutrient cycling via the deposition of highly nutritious leaf litter (Fisher et al. 2013). Consequently, some hemiparasites are considered not only to be keystone species but also ecological engineers that can create shifts in the availability of abiotic resources (Jones et al. 1994).

However, despite the considerable attention given to the topic, the effect of a hemiparasite on its community is not easy to predict. Our understanding of the ecological roles of hemiparasites largely comes from robust theoretical and empirical work with *Rhinanthu*s, a genus of annual species found mostly in Central and Northern Europe. While studies focusing on *Rhinanthus* generally illustrate a positive association with the hemiparasite and community diversity (Ameloot et al. 2005) patterns are not always clear, particularly for other genera. Contradictory results are common, with some research finding neutral (Reed 2012, Schmidt 2016) or even negative effects of hemiparasites on community diversity (Gibson and Watkinson

1992, Demey et al. 2015). Furthermore, a large part of a hemiparasite's community impact begins with the host response to parasitism. The resource acquisition style of the hemiparasite, mediated by a unique suite of eco-physiological traits that all hemiparasites have adopted, greatly influence the degree of host damage (Phoenix and Press 2005). However, although hemiparasites are unified in adopting similar mechanisms to attach to and exploit hosts, there is a great deal of interspecific variation in their expression. Yet, these eco-physiological traits have only been assessed in detail for a few hemiparasitic genera.

Considering their diversity, it is not surprising that hemiparasitic plants do not always behave in the same way. Hemiparasites occur as herbaceous annuals, herbaceous perennials, and shrubs and can be found in all major biomes of the world, from xeric grasslands to the boreal forest (Atsatt 1973; Nickrent 2002; Těšitel 2016). Yet, most of the existing research has been conducted on annual herbaceous hemiparasites (Těšitel et al. 2020). However, even for well-studied plant families like the Orobanchaceae, most species are perennials (Table 4.1). Thus, although the functional nature of the host-hemiparasite relationship and the ecological role of a hemiparasite is related to growth form, life history has not been well integrated into hemiparasitic plant ecological theory (Press 1989).

In this review I discuss variation in hemiparasite physiology and ecology through the lens of life history theory (Figure 4.2). First, I summarize the main ways in which a hemiparasite can impact its hosts. I then consider how these mechanisms are largely contingent upon traits known to be influenced by longevity such as resource acquisition and allocation. I argue that the expression of these functional parasitic traits can be used to place hemiparasites on a spectrum between autotrophy and complete heterotrophy, with the impact on the host and community increasing with degree of heterotrophy. Additionally, I discuss how ecological correlates of life history, like population dynamics and density, influence the mechanisms of a hemiparasite's community impact. Throughout the text, I suggest research directions needed to fill in prominent knowledge gaps. Finally, I highlight technical limitations that hinder the advancement of parasitic plant biology.

The Ecological role of hemiparasites

Two well-understood mechanisms through which a hemiparasite can impact its community have been described: the "direct parasitism pathway" and the "indirect litter pathway," terms coined by Spasojević and Suding (2011) (Figure 4.2).

Direct parasitism pathway

Hemiparasites are generalists, able to attach to and parasitize a wide variety of hosts (Marvier 1998, Matthies 2017). However, host defense and tolerance can vary dramatically, as can intra and interspecific hemiparasite effect and dependance upon hosts (Hejduk et al. 2020, Li et al. 2012; Press and Seel 1996). Furthermore, hemiparasites tend to have a very shallow root system, so their zone of influence is limited (Press and Phoenix 2005, Yoshida et al. 2016). Thus, a large factor determining whether a host is parasitized is the below-ground proximity of the host to the hemiparasite. Consequently, in natural communities, suitable host species that are also dominant are disproportionately parasitized due to their higher root densities (Davies et al. 1997). By attaching to and reducing host growth, hemiparasites can alter competitive dynamics between host and non-host species, often increasing the co-existence of subordinate species, increasing evenness and potentially richness (Figure 4.2). Additionally, hemiparasites can reduce the biomass of hosts and often do not compensate with their own growth, reducing overall community productivity (Spasojević and Suding 2011, Westbury and Dunnett 2007) and, if the host is dominant, increasing diversity (Press and Phoenix 2005). There is growing evidence that a hemiparasite's impact on richness and evenness is density dependent, peaking at intermediate to high hemiparasite densities (Fibich et al. 2010; Heer et al. 2018; Těšitel et al. 2017, Hodžić, Chapter 3). Thus, along with the degree of host damage, a hemiparasite's relative abundance is an important factor influencing its direct community impacts.

Indirect pathway

Since hemiparasites act as a sink for host water and solutes their litter often contain higher concentrations of most xylem mobile nutrients than their hosts, particularly in nitrogen (N), phosphorus (P) and potassium (K) (Glatzel 1983; Jiang et al. 2010; Luo and Guo 2010; Stewart and Press 1990, also see Chapter 1). Many hemiparasites drop their leaves earlier and have litter that decomposes faster than that of co-occurring species due to low C:N ratio and high calcium (Ca) and P content (Berg 2000), stimulating the decomposition of recalcitrant litters of neighboring species and making resources available to plant individuals near the hemiparasite (Quested et al. 2003, Watson et al. 2011, Demey et al. 2013). In nutrient limited areas, this litter release may make up for productivity losses because of parasitism and may even temper any effects the hemiparasite has on diversity (Fisher et al. 2013).

Hemiparasites may have other impacts that have not received significant attention. For example, they have been shown to mediate interactions between host organisms and higher trophic levels, such as herbivores (Hartley et al. 2015, Haan et al. 2018). Finally, since these plants are also autotrophic, they simultaneously compete with their host plants for sunlight and below-ground resources, however, since they are often out-competed as primary producers, their impacts as competitors have mostly been shown to be negligible (Matthies 1997, Smith 2000).

Life history and hemiparasite physiology

Angiosperms primarily exhibit two broad categories of reproductive strategies, annuality and perenniality. Annuals have one reproductive event (semelparity) whereas perennials can either be semelparous or repeatedly cycle through vegetative and reproductive phases for multiple years (iteroparity). The relative advantage of either strategy is dependent upon the benefits of survivorship vs. future reproduction, which is determined by the constraints of a given environment (Stearns 2000). Accordingly, a plant's life history carries a genetic architecture that affects a variety of plant traits such as flowering time, growth rate, allometry and reproduction (Enquist et al. 1999; Aragón et al. 2009; Friedman 2020).

Traditional life history theory is based on optimization models that describe tradeoffs between traits and look for combinations that maximizes fitness (Stearns 1992). Life history theory suggests that annuals, whose fitness is completely dependent upon a single reproductive event, should maximize resource acquisition, whereas perennials should maximize resource conservation (Friedman and Rubin 2015). Given that resource strategy is thought to be genetically controlled (van Noordwijk and de Jong 1986, Friedman and Rubin 2015), we would expect that the life history of a hemiparasite should select for an expression of traits that maximizes rapid uptake of host resources or, alternatively, the conservation of host resources.

Here, I examine distinctive traits characterizing hemiparasitic plants that are related to resource acquisition and discuss how these traits may vary due to life history strategy, and how such differences can influence community-level effects. Additionally, I examine whether there is evidence that annuals and perennials have differential growing needs and prioritize uptake of distinct host material.

Method of resource gain

Water Relations

All parasitic plants ensure access to host resources via a unique “water-wasting” strategy. In fact, one of the consistent traits share by parasitic plants is a transpiration rate greater than that of the host, serving to create a sink for host material (Stewart and Press 1990, Press and Graves 1995, Světlíková et al. 2018). This implies functionally that stomatal conductance is greater in the hemiparasites than the hosts and that water potentials would be more negative in parasite than in host tissues. In some cases, these plants continue to transpire even under high water stress and also at night (Press and Graves 1995, Phoenix and Press 2005, Světlíková et al. 2018).

Consequences to the host and community

For a host, a parasitic plant is another sink which draws photosynthates, minerals, and water. The larger the sink the hemiparasite creates (i.e., the higher the transpiration rate), the greater the demand on the host. In some cases, though, the effect of the hemiparasite is disproportionate to its size, usually a result of the host responding to parasitism by altering its allometry or even its physiology (Press and Seel 1996, Shen et al. 2006). The most extreme example is the annual obligate hemiparasite Striga, which “bewitches” its host plants into believing they are undergoing drought conditions, causing the host to reduce transpiration, stomatal conductance, and photosynthesis, sometimes to the point of host death (Frosy et. al 1997, Runo and Kuria, 2018). Thus, the strength of a hemiparasite’s damage on a host should be directly related to the benefits conferred to the hemiparasite (resource gain) and the strength of the sink created by the hemiparasite (transpiration rate) (Mudrák et al. 2016).

Annuals are selected to parasitize aggressively

The water wasting strategy of hemiparasites presents a clear trade-off for perennial hemiparasites who maintain host relationships for several seasons: maximizing transpiration will increase host damage and, if host damage is substantial, could detrimentally impact parasite growth. Furthermore, a perennial hemiparasite which aggressively taps a host's resources via high transpiration rates could cause the xylem of the host itself to cavitate, a form of self-sabotage for a hemiparasite (Press and Graves 1995, Yoshida et al. 2016). Therefore, water regulation should be selected for. On the other hand, reducing transpiration rates will reduce host resource and autotrophic carbon gain. Annual hemiparasites, however, do not experience this trade-off since they are not concerned with long term survival and the maintenance of host competency.

Furthermore, reduced investment to a root system is one of the primary benefits of hemiparasitism in plants; by attaching to a host root mass, the hemiparasite is released from having to allocate as many resources as possible below-ground (Matthies 2017, Smith 2000). However, perennial hemiparasites, particularly in grasslands where they are abundant, go dormant every year. Thus, perennial herbaceous hemiparasites, whose root systems are the only plant parts that survive the winter, must invest considerably more into their root systems than do annual hemiparasites (Matthies 2017 *cf.* Table 6, though see Hodžić Chapter 1 for an exception). Thus, perennials may not be able to "cash in" as much on the evolutionary advantages of hemiparasitism, creating less of an incentive to completely adopt the water wasting strategy.

Finally, high transpiration rates are only adaptive for any hemiparasite if the parasite can exploit the host adequately before the onset of drought. Annual root parasites are likely to complete their life cycle before the onset of any drought; therefore, conservative water use is likely not to be of adaptive value. But for perennials, maximizing transpiration rate reduces the likelihood of long-term survival to reach a reproductive age.

Thus, life history theory suggests that a short life span selects for a heavy use of the water wasting strategy and an overall more aggressive deleterious relationship with the host, which will damage host growth and reduce its competitive vigor, allowing for other plants to flourish. In contrast, we may expect a perennial to be more risk averse and regulate their transpiration rates and water consumption at the cost of reduced host resource gain, leading to less pronounced impacts on the community. In this sense, a perennial likely exists physiologically between an autotroph and an annual hemiparasite (Figure 4.2).

Haustorial attachment

As the bridge which links hemiparasite to host, we might expect anatomical differences between hemiparasitic haustoria that reflect different resource uptake strategies. Indeed, some of the most aggressive hemiparasites, like *Rhinanthus* and *Striga* have been shown to have direct luminal continuity, ensuring access to all xylem mobile cations (Dörr 1997; Rümer et al. 2007). In some perennial hemiparasitic shrubs like *Olax* and *Santalum*, direct xylem-xylem links are not observed, and the uptake of solutes probably occurs through abundant parenchyma cells in the haustoria (Pate et al. 1990). However, the exact mechanism of solute transfer across the parenchyma walls has not been studied, though it likely is based on active transport, thereby not requiring a water potential difference. If this is true, it means that perennial hemiparasites do not need to maintain high transpiration rates, which has large implications for the water relations of perennial hemiparasites (Těšitel et al. 2010).

Many other research questions concerning haustoria remain unanswered. An anatomical assessment of annual herbaceous, perennial herbaceous and perennial shrub haustoria may provide insight into the methods through which xylem sap is accessed and give us insight into the strategies and priorities of these plants. Assessments of common host plants and their cellular defensive strategies would allow us to know if host defense is hemiparasite specific. Additionally, no research has been conducted investigating the longevity of haustoria. Thus, it is unclear if perennial haustoria remain for more than one growing season, though it seems likely. Finally, although it is unlikely that selective transfer (with the hemiparasite selectively uptaking resources) occurs at the haustorial interface, this topic is still an active research question. Though the presence of certain enzymes suggests this as a possibility, the sometimes-striking similarity between the xylem sap of host and hemiparasite provides counter evidence (Seel and Jeschke 1999).

Types of resource gain

The importance of host material gain to a hemiparasite is evident simply by observing that hemiparasite growth is rapidly stimulated upon attachment to a host plant (Matthies 1997, 2017, Press and Seel 1996). Hemiparasites can gain many types of material from the host plant such as carbon, mineral nutrients, mRNA and potentially bacteria (Yoshida et al. 2016). However, whether a hemiparasite is parasitic for a specific resource, and if this need is related to life history, is unclear. I focus on the acquisition of carbon and mineral nutrients below, given their fundamental importance to plant growth and survival and because inadequate research is available concerning the transfer of other materials like mRNA and bacteria.

Carbon gain

The ability of hemiparasites to acquire organic carbon (in the form of xylem-mobile organic compounds and amino acids) has been substantiated through several studies (Těšitel et al. 2010, see Hodžić Chapter 2). However, hemiparasites have been shown to differ widely in the extent to which they rely on their host for the supply of carbon (Press and Graves 1995). The annual *Rhinanthus minor* has been shown to receive anywhere between 6.2 to 89.5% of its carbon from its host with the annual *Euphrasia* varying between 20.9 to 28.7 % (Těšitel et al. 2010). *Striga*, on the other hand, has been shown to have, on average, 60% of its carbon as host-derived (Press et al. 1987) In my study with four annual and three perennial hemiparasites in the genera of *Cordylanthus* and *Castilleja*, annuals and perennials averaged 30% and 33 % of heterotrophically derived biomass, respectively (Chapter 3). Given the diversity in methods for abstracting the proportion of heterotrophic carbon in biomass, differences in environmental conditions as well as differences in types of tissue measured and phenology of the plants, direct comparisons are difficult to make. Still, it is clear hemiparasites –annual and perennial– effectively obtain host carbon.

Disentangling a reliance on host carbon from passive uptake is best investigated by examining nighttime transpiration and stomatal rates. In this scenario, high transpiration rates would only be beneficial for heterotrophic gain as no sunlight is available for photosynthesis and any host mineral nutrition gain could be conducted during the day, with simultaneous carbon dioxide uptake. Finally, a concurrent examination of respiration rate makes it clear whether the hemiparasite is losing more carbon than it can accumulate via its own photosynthesis. Surprisingly, few studies exist assessing the photosynthetic capacity of hemiparasites.

Press et. al (1988) showed that annuals (*Rhinanthus, Euphrasia, Pedicularis palustris*) kept their stomata open at night while perennials (*Pedicularis and Bartsia)* were more conservative in their water consumption, tending more towards stomatal control found in autotrophic plants. Perennials also received less host carbon than the annuals, which the authors link to their impact on the host, stating "annuals can inflict severe damage on their host, resulting in loss of productivity and reproductive capacity. Our data suggest that this may arise from loss of carbon as well as water and inorganic solutes" (Press 1988). In the same study photosynthetic rates of hemiparasites were not greater than those of their hosts, likely simply due to a reduced photosynthetic capability of the hemiparasite as well as high respiration rates (Stewart and Press 1990). Combined with a high transpiration rate, these low rates of carbon assimilation result in very low water use efficiency (WUE). Similarly high respiration rates leading to a low net carbon gain was found in the annual *Melampyrum*, suggesting a reliance on host carbon (Lechowski 1996). Thus, while annual hemiparasites can and do behave autotrophically we can interpret high transpiration rate as an adaptive strategy for host carbon gain.

Studies with perennials are also limited but offer insight. Luo and Guo (2010) showed that the perennial hemiparasite *Thesium chinense* lowered its nighttime transpiration rates to those like the host. The nighttime respiration rate of *Thesium* also declined, indicating that it made a net carbon gain and, therefore, only need rely on its host for water and solutes. Thus, like other perennials, *Thesium* was more restrictive of water consumption and less reliant on host carbon acquisition. However, in a study investigating the water relations of eight perennial species of *Castilleja* in the field, the nighttime stomatal conductance in the hemiparasite was 235% higher than in autotrophic taxa occurring near the hemiparasite, showing that these plants commonly transpire at night at rates higher than autotrophs (Haynes 2020). Although the respiration rate of the hemiparasites were not measured, this study does show that perennials can transpire greatly at night. Without these data, it is difficult to determine whether the hemiparasites are transpiring for access to host carbon, or for access to other resources.

Mineral nutrient gain

Given their partially autotrophic nature and vestigial root systems, access to host mineral nutrition has long been thought of as the primary evolutionary driver of hemiparasitism. (Press and Graves 1995). As a result of the uni-directional nature of xylem flow, root hemiparasites harbor large amounts of xylem mobile solutes, in particular potassium (Jiang et al. 2005; Phoenix and Press 2005, Smith and Stewart 1990, Chapter 1). Thus, mineral nutrition is closely coupled with water relations. Among the studies that exist, no patterns emerge correlating life history strategy with mineral nutrition status–all hemiparasites seem to accumulate large amounts of these solutes.

However, whether a hemiparasite is limited by a single nutrient is still unknown. Given that nitrogen is a limiting factor in many ecosystems, it was long thought that parasitism, like carnivory in plants, was a strategy to enhance nitrogen acquisition in limiting environments. Additionally, the N-parasitism hypothesis suggests that hemiparasites are particularly dependent on host-N, and that high rates of transpiration are used to access very dilute forms of N in the host xylem (in the form of amino acids). Research into this hypothesis has been dominated by stem parasites and has yielded mixed reports, with supporting evidence suggesting that N-fixing hosts cause increased growth rates in parasites and a decrease in WUE differences between the host and the parasite. Studies also show that most hemiparasites are richer in N post attachment; however, this does not indicate that N was growth limiting. Furthermore, N's prominent role in photosynthesis makes it hard to distinguish whether a hemiparasite 's change in WUE post is from an upregulation of its own photosynthesis or a downregulation of transpiration (Seel and Press 1994). Other research suggests that hemiparasites may be particularly reliant on their hosts for P as it tends to be very low in unattached hemiparasites to increase dramatically upon the onset of parasitism (Fer et al. 1994; Li et al. 2012; Hodžić Chapter 1). In fact, most plants rely on mycorrhizal networks for the uptake of P. A simple assessment of the presence or absence of mycorrhizal networks in hemiparasites would give us great insight into their P acquisition.

Furthermore, the literature on resource gain in hemiparasites may suggest that annuals rely on hosts for both mineral nutrient and carbon gain, whereas perennials primarily rely on the hosts for mineral gain. However, we still have very limited data on the resource gain of hemiparasites, and we cannot make any conclusive statements about whether the water loss economy of a hemiparasite is directly related to the uptake of a particular resource. Yet, the drivers of parasitism for annuals and perennials can be examined. The role of photosynthetic capacity relative to nighttime transpiration rate and respiration will elucidate a hemiparasite's dependence on the host for carbon and mineral nutrient growth studies (Chapter 1) will allow us to determine if hemiparasite growth is limited by a particular nutrient.

Plasticity in hemiparasite physiology

The ability of hemiparasites to respond to dynamic host environments or shifting abiotic conditions is poorly understood. There exists a spectrum between autotrophy and heterotrophy in which full reliance on a host (exhibited in its most extreme case by holoparasites, who cannot photosynthesize or forage below-ground) represents one end and full autotrophy represents another. Hemiparasites may oscillate their physiology between autotrophic and heterotrophic based on the relative advantages of either strategy. It is reasonable, though, to expect that this is a trade-off: shifting from one strategy to another demands resources and adaptations that make it more difficult to fully prioritize one strategy. However, maintaining parasitic traits in the absence of a host is clearly deleterious to the plant, as can be attaching to yet not utilizing a host plant effectively. In this sense, hemiparasitic annuals live fast and die young whereas perennials ensure longevity by potentially maintaining a balance between autotrophy and heterotrophy, though benefitting fully from neither. We might imagine a hemiparasite that is more autotrophically competent would have less of an impact on its host due to reduced demand for resources.

Water relations

An unattached hemiparasite's transpiration rate is closer to those normally found in autotrophs (Lechowski 1996, Sweatt 1997, Luo and Guo 2010). Evolutionary, this is logical; a high-water wasting strategy when unattached to a host is completely counter-productive. However, not enough studies exist comparing other traits such as stomatal conductance on attached and unattached hemiparasites, though some inferences can be made from the relative levels of K in leaf tissue. For example, some hemiparasites have been shown to maintain levels of K that are much higher than those of their autotrophic hosts when unattached (Luo and Guo 2010, Hodžić 2021) whereas, in other cases, K levels rise dramatically upon host attachment (Fer et al. 1994, Lechowski 1995). Since K is involved in the maintenance of abnormally high guard cell turgor in hemiparasites, this could imply that hemiparasites maintain high levels of stomatal conductance even when unattached (Smith and Stewart 1990, Press and Graves 1995). However, additional research is needed to determine whether elevated K levels are related to high stomatal conductance in unattached hemiparasites.

Growth and autotrophy

Observing how a hemiparasite performs without access to a host gives us great insight into its ability to grow and reproduce autotrophically. In general, it is thought that most hemiparasites need access to a host to maintain viable populations as, in greenhouse studies, most have limited growth and flowering when unattached (Klaren and Janssen 1978, Lechowski

1996). In a review of parasitic plant biology Těšitel (2016) claims "most of the species suggested as facultative hemiparasites are nevertheless unable to survive, produce flowers or their growth is largely reduced, and flower production is minute in host-free cultivation." Such a statement may be specious, though, as our understanding of the autotrophic abilities of hemiparasites are poor. For example, hemiparasites in the genus *Castilleja* have flowered successfully and quickly under non-fertilized greenhouse conditions, yet it is unknown whether this behavior would realistically be upscaled to a natural community (Hodžić, anecdotal observation). We also know that hemiparasites, both annuals and perennials, that are unattached invest more into root development than attached hemiparasites who, due to host access, have been released from developing their root systems (Matthies 2017). Thus, as would be expected, unattached hemiparasites invest more into building resources for autotrophic uptake, indicating some degree of autotrophic ability.

Manipulative field studies assessing the abilities of different hemiparasites to reproduce autotrophically would give us valuable insight into just how successfully they can maintain viable populations without a host. This would allow us to understand whether many of these plants could truly sustain populations without a host and clarify how much they invest into a parasitic lifestyle.

Life history and hemiparasite evolution and ecology

The tradeoffs of annuality and perenniality largely depend on the relative benefits of immediate reproduction balanced against survivorship and future reproduction, which are determined by ecological constraints. Thus, life history is correlated with certain environments, with annuality seen as a bet-hedging strategy in unpredictable habits (Stearns 2000). Perenniality has long been thought by evolutionary biologists to the be ancestral condition in angiosperms, implying that an herbaceous annual would be derived (Stebbins 1957, Friedman 2020). However, there is evidence that the evolution of annuality and perenniality in hemiparasites is more complex. Phylogenetic reconstruction of the Castillejinae subtribe (Orobanchaceae) describes the reverse of the norm–perennial plants evolved from annual ancestors with a reversion to annuality from the perennial clade (Tank and Olmstead 2008). Taxonomic analysis of the Rhinanthoid Orobanchaceae, however, shows multiple independent origins of annuality evolving from perennial ancestors (Malinov 2010). However, McNeal et al (2013), shows a clade containing *Agalinis* (perennial and annual herbs), *Seymeria* (perennial and annual herbs) *Macranthera* (semelparous annual/biennial), *Esterhazya* (perennial shrub), and *Phtheirospermum* (annual and perennial herbs) (Harley et al. 2004). These types of distributions strongly suggests that annual and perenniality is a flexible trait in Orobanchaceae (D. Olmstead, personal communication).

In this sense, it can be difficult to apply ecological theories related to life history to hemiparasites. By accessing a host, hemiparasites have released themselves from mineral nutrition needs, and much like autotrophic annuals, are able to survive and radiate in nutrient poor and unpredictable habitats, so long as a host is present. In fact, many annuals exist in perennial dominated grasslands, where they would typically be outcompeted. However, by attaching to a perennial, hemiparasites are essentially “acting” like belowground perennials (Strykstra et al. 2002).

Hemiparasitism should be most beneficial in nutrient-poor environments where low-cost acquisition of mineral nutrients is advantageous. In nutrient rich habitats, this advantage is diminished and competition for light is increased due to shading from plant growth (Těšitel et al. 2015). Moreover, some hemiparasites like *Castilleja* do exist in woodlands and forested ecosystems, though hemiparasites in these areas are understudied. One interesting avenue for research would be to determine if perennials, on account of presumed higher autotrophic competence, have wider habitat breadth than annuals.

Population Dynamics of Hemiparasites

Many annual hemiparasites do not have persistent seed banks and thus their populations can be erratic depending on the environmental conditions and seedling mortality each year (Borowicz et al. 2019). Studies suggest that while *Rhinanthus* can strongly affect community productivity, its effects do not persist over long periods of time, partly because *Rhinanthus* populations are particularly susceptible to drought (Ameloot et al. 2006). Perennial populations, on the other hand, should be more resilient as they invest more in root architecture and may not be as susceptible to drought (see water relations section above). In a removal experiment, Borowicz et. al (2019) showed that well-established populations of the perennial hemiparasite Pedicularis canadensis buffered their communities from large demographic swings, though their effects diminished after a decade. Thus, while managed populations of annual hemiparasites may provide stable community level impacts, in natural communities their effects are likely much more transient.

Community impacts

Given the density dependent effect of a hemiparasite's impact (see Ecological role of a hemiparasite section), annuals, in a favorable year, likely can reach such functional densities more quickly than perennials. Furthermore, since they senesce each year, annuals leave behind areas available for other plants to colonize, occupying a "regeneration niche" thought to be important in the maintenance of species rich communities (Grubb 1977). In this sense, an annual hemiparasite is mechanistically better suited to impact community richness (the number of unique species) than a perennial, whose effects are largely likely constrained, at least immediately, to impacts on evenness (relative abundance of species) because of prolonged damage to host plants. However, some hemiparasitic perennials, like *Pedicularis sylvatica* and *Bartsia alpina* are also clonal, with *Bartsia* reaching densities of almost 100 stems per m^2 (Quested et al. 2003), however these species represent anomalies in perennials with respect to their density (Press 1989). Additionally, Spajosević and Suding (2011) and Demey et. al (2013) point to the idea that perennials, due to larger biomass, increased leaf litter, and more stable populations, may have higher potential to impact communities via the indirect litter effect. Thus, both direct and indirect pathways must be jointly considered when integrating the population dynamics of a hemiparasite into its community impact.

Access to Hosts and Host Preference

There is a clear connection between host generality and longevity. Highly host-specific parasites, like mistletoes and other stem holoparasites, are rather long-lived and their life span depends primarily on the longevity of the host plant (Těšitel 2016). Most annual and short-lived hemiparasites, on the other hand, can parasitize a wide variety of host species (Press and Phoenix 2005). Furthermore, though this has not been assessed in root hemiparasites, it is reasonable to suspect that perennial hemiparasites may not select hosts that do not support overwintering because they would have to reinitiate another host association in the following spring (Marquardt and Pennings 2010). Since the hemiparasite with the greatest breadth of hosts will have the largest density dependent effect on the community, any constraint in host use would limit the impact of a perennial hemiparasite.

Host preference

Though hemiparasites are generalists, the impact of a hemiparasite on a host can be highly variable, with some hosts being seemingly unaffected by the parasite (Press et al. 1990). Furthermore, not all hosts confer the same benefits to the hemiparasite. One well-documented case is that *Rhinanthus* tends to perform better with grasses than with forbs (Cameron 2004). Much of this can be ascribed to host tolerance and defense with some hosts, including forbs in the case of *Rhinanthus,* being able to prevent parasitism by cellular-level resistance to the haustorium (Uction 2018). Additionally, most hemiparasites tested seem to parasitize only a subset of the range of hosts available to them and perform better with some hosts than others, a phenomenon termed “host preference” (Sandner and Matthies 2018). Although host selection, defense and preference are thought to be hemiparasite specific, many hemiparasite-host associations have not been assessed (Matthies 2017). Thus, many more studies of the mechanisms by which hosts tolerate or resist hemiparasites in natural communities are needed, particularly for other genera of hemiparasites. Additionally, whether host preference by the hemiparasite is directly related to host damage or is driven by the hemiparasite’s physiology and host selection mechanisms is poorly understood.

Limitations and technological needs

Phylogenetics

Questions relating to life history are ideally studied through a phylogenetic lens. Unfortunately, within parasitic plant families, we have limited scope due to the relatively few evolutionary transitions between life history strategies. However, we can use our phylogenetic understanding of hemiparasites to further recognize that life history may be an important and flexible trait in hemiparasites. The Orobanchaceae is the only parasitic plant family that has a co-occurrence of annual and perennial hemiparasites as well as holoparasitic and autotrophic genera

(Nickrent 2020). This, as well as the interesting evolutionary history of annuality in one of its subtribes, the Castillejinae, make it an excellent study system for which to conduct the various physiological and anatomical experiments described throughout this text.

Small size of hemiparasites limits the use of certain instruments

The small size of many hemiparasites makes the use of modern physiological instruments difficult. For example, in an ideal measurement with a steady state porometer (used to measure stomatal conductance) the leaf should fill the aperture of the porometer. Using a portable photosynthesis system like a LI-COR to measure photosynthetic rate, respiration and transpiration has similar issues. However, the typical small-plant chamber developed to mitigate small plant sizes cannot be used effectively to isolate hemiparasitic photosynthesis if the hemiparasite is being grown with a host. In these cases, stomatal conductance and water use efficiency could be inferred from relative abundances of ^{18}O and ^{13}C as done by Těšitel et al. (2018). Haynes (2021) used a steady state porometer and for any leaves which did not fill the leaf area the measurement was scaled up by marking where each leaf area entered the porometer sensor head (Haynes 2021 *cf.* Chapter 2). Still, given the importance of water relations and gas exchange to hemiparasitic research, this technical limitation can truly hamper the progression of the field.

Lack of Propagation Protocols

Given the importance of the host plant to parasitic plant performance, it can often be difficult to propagate hemiparasitic plants, particularly perennials. Although there has been increased interest and attention to the propagation of genera that have horticultural value, like *Castilleja* (Luna 2005), many genera still have not been grown successfully in a greenhouse. Poor success in propagating hemiparasites has likely contributed to our murky understanding of whether they can grow autotrophically. To do much of the manipulative experiments proposed here, we need propagation protocols for these species in both soil and, ideally, agar. Germination information for unstudied genera that I attempted to propagate is included as a supplement to Chapter 2.

Conclusion

A hemiparasite's effect on its community will always be difficult to predict. Dynamic factors such as host environment, host resistance, host age, abiotic factors, and the level of hemiparasite competition all contribute to the host-hemiparasite and host-community relationship.

Although a plant's life span is a large determinant of its resource acquisition strategy, life history is ultimately the outcome of complex responses to environmental variation and is not categorical. However, as most of hemiparasite effects are influenced by physiological mechanisms related to host resource acquisition, we can use life history theory and functional plant ecology in a predictive way. Thus, we predict that annual hemiparasites are adapted to rely more aggressively on their host when compared to their perennial counterparts. Annuals likely depend on hosts for heterotrophic carbon, water and mineral nutrition, whereas perennials may primarily rely on hosts for mineral nutrition and water. Consequently, annuals have an increased capacity to damage their hosts and their direct parasitism effects are more pronounced. However, perennials may be ecologically better positioned to create longer-lasting effects via longer-lasting and more stable effects on hosts as well as sustained litter deposition.

Experimental studies connecting the proposed physiological differences of annuals and perennials – as well as ecological differences – are required to substantiate these trends. Given that hemiparasites are a common component of the world flora, understanding their diverse roles in communities in a functional way will also us to incorporate hemiparasites into community theory and climate change models. Finally, in light of their physiology, evolution, and ecology, these plants can be a model group for studying mechanisms of community assembly and life history evolution.

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Table 4.1. Subset of most species-rich and studied genera in the Orobanchaceae. All species numbers are approximations from most recent sources. Genera in annual category are all or mostly annual. Genera in the perennial category are all or mostly perennial.

Genus	Number of Species	Annual	Perennial	Distribution
Agalinis	62	x		Temperature tropical America
Alectra	33	x		Africa and Asia
Aureolaria	8		x	Eastern United States and Mexico
Bartsia	1		x	United Kingdom
Buchnera	230		x	Temperate areas in New World
Castilleja *	200		x	North and Central America, South America, Eurasia
Chloropyron	4	x		Western United States
Cordylanthus	17	x		Western North America
Cycnium	21		x	Africa
Euphrasia	450	x		Europe, Asia, Australia, North and South America
Melampyrum	21	x		Northern temperate regions
Odontites	31	x		Temperate Eurasia
Orthocarpus	8	x		Western North America
Pedicularis	500	x		Central and Eastern Asia
Rhamphicarpa	21		x	South Africa, India, tropical Australia
Rhinanthus	42	x		Europe, Northern Asia, North America
Seymeria	24	x		Southern North America and Mexico
Striga	40	x		Tropical Old World
Triphysaria	5	x		Western North America

* *Castilleja* has ~ 20 annual and 180 perennial species. Compiled from Sánchez (2016), Nickrent (2020), Tank et. al (2009)

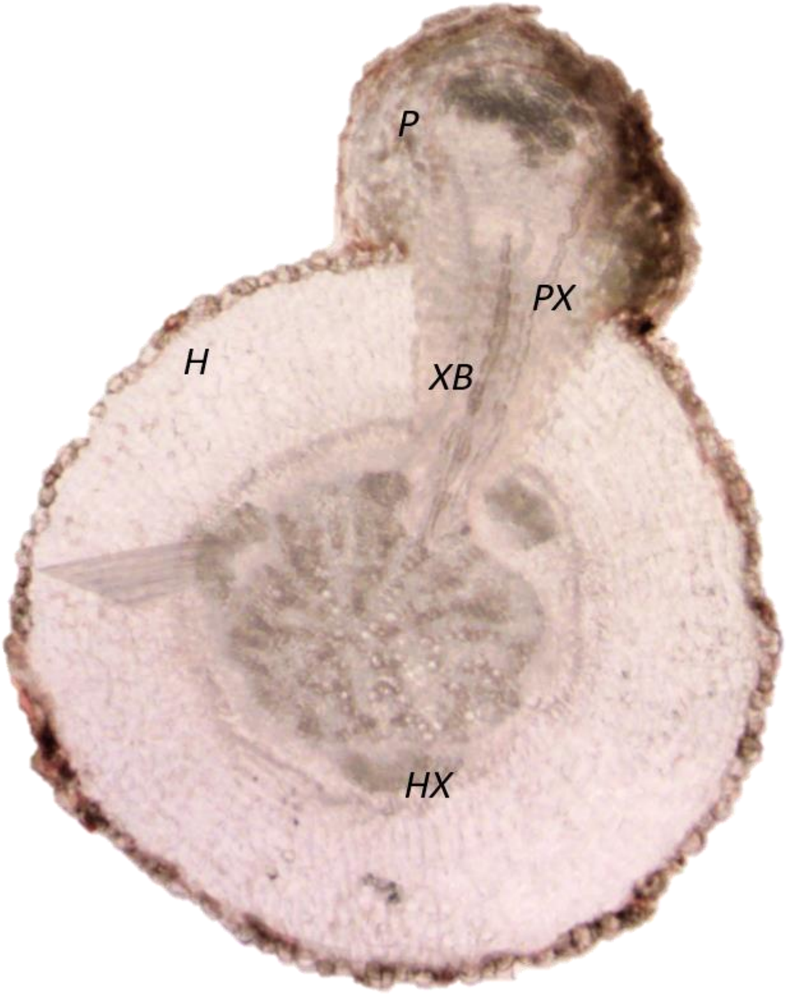

Figure 4.1. Root cross section of the hemiparasite *Castilleja levisecta* parasitizing *Achillea millefolium*. A fully formed xylem bridge is seen connecting the host to the parasite. (P- parasite, PX- parasite xylem, H- host, HX- host xylem, XB- xylem bridge). Photo and cross section by Wai Pang Chan.

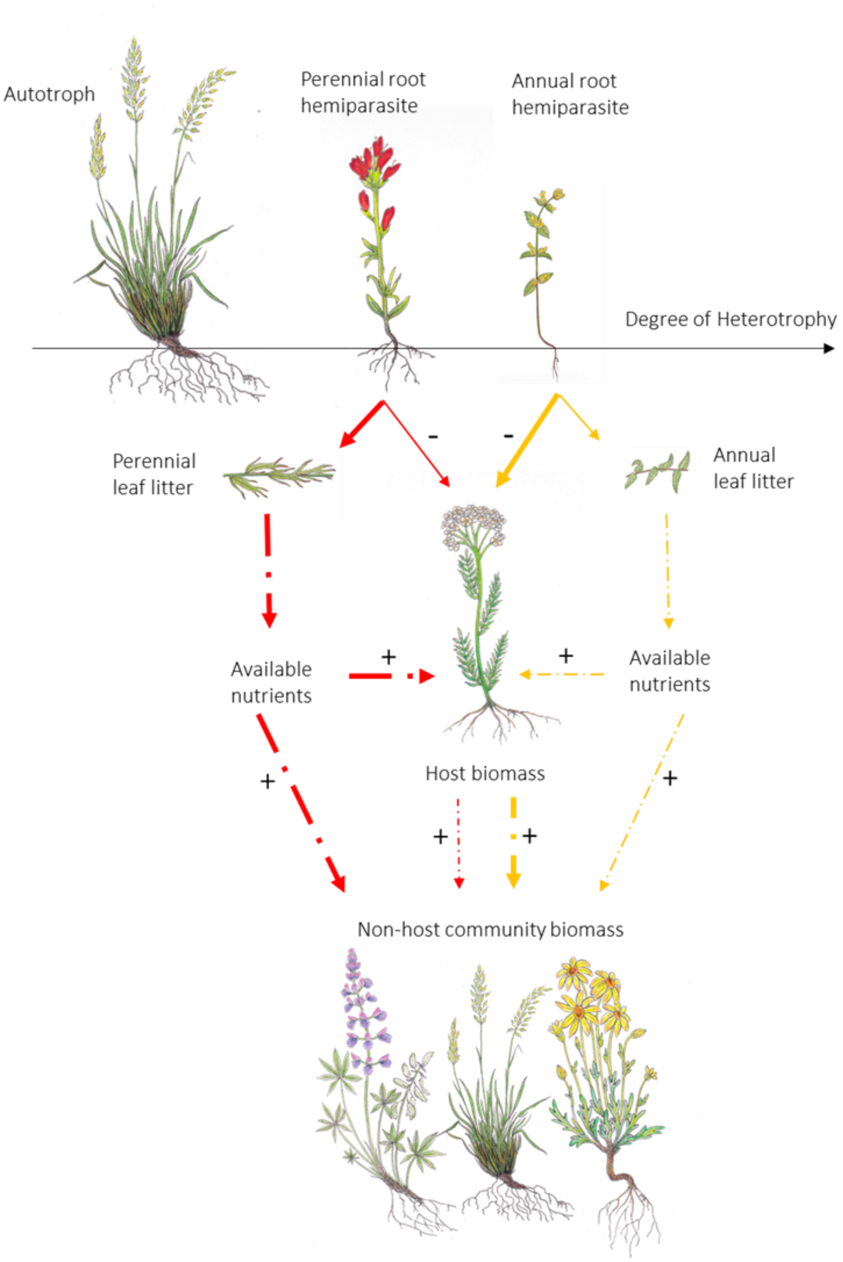

Figure 4.2. Conceptual model illustrating how differences in hemiparasite life history can impact a hemiparasite’s physiology (degree of heterotrophy) and the plant community at large. Dashed arrows represent indirect effects and solid arrows represent direct effects. Red arrows demonstrate a perennial effect (and yellow show the annual effect. The width of the arrow corresponds to the relative size of the effect. Illustrations by Sage Stowell. Model based party on Demey (2013).

Chapter 5 **: Synthesis and future research questions**

The research presented in Chapters 1–4 describe the physiology and ecology of root hemiparasites with a particular focus on the genera *Castilleja*, *Cordylanthus*, and *Pedicularis.* This work enhances our understanding of hemiparasitic plant biology by showing that these genera conform to broad patterns associated with hemiparasitism. I also demonstrate and discuss variation in the nuances of the interactions between hemiparasites and their communities that should be of interest to ecologists and biologists. Here, I provide a summary of my findings and connect my work to broader research topics and long-standing ecological questions.

Summary

In Chapter 1 and Chapter 2 I show that previously unstudied species in *Castilleja* and *Cordylanthus* are like other hemiparasites in regard to host resource acquisition. I was also able to pinpoint resource needs of these hemiparasites. First, hemiparasites in the genera *Castilleja* and *Cordylanthus* had higher concentrations of Ca, K, Mg, S and P than their hosts. Interestingly, the hemiparasites when unattached also had higher concentrations of these nutrients, except for P. In fact, P and K were the only nutrients that increased in both concentration and content of unattached and attached hemiparasites. When taken together, the fact that the concentration of P in unattached hemiparasites was comparable to that of the host, and that P increased in both content and concentration in attached hemiparasites, suggests it limits the growth of unattached hemiparasites. In another study (Chapter 2), I quantified host carbon gain, showing that, on average, 30% of the biomass of these hemiparasites were derived from host carbon. Finally, attached hemiparasites invested less into their roots than attached hemiparasites, in line with our expectations.

In Chapter 3, I ask whether these, and other North American genera, play similar ecological roles as other hemiparasites. By using a null model approach, I was able to demonstrate that hemiparasites were associated with increases in plant community evenness. I also showed that this trend was related to abundance, i.e., as hemiparasite abundance increased, so did community evenness. However, hemiparasites had no significant effect on richness when compared to non-hemiparasitic species of similar distribution. This result contrasts with much of the literature, which illustrates density-dependent relationships between hemiparasites and abundance, suggesting that hemiparasites can impact their communities via various mechanisms. Given that most of the genera in my study are perennials, and that the literature is largely dominated by studies of annuals, this mechanistic variation may be related to growth form. In Chapter 4, I break down why we may see such a variation in ecological role by taking a step back and focusing on the eco-physiological suite of traits that allow hemiparasites to influence their community. In this way, I hope to have connected Chapters 1-3 with a testable theory.

Future Research Questions

Much of the research questions raised by my doctoral work are discussed in depth in Chapter 4. Additionally, throughout my dissertation I point to a general lack of physiological, anatomical, genetic, and ecological studies of perennial hemiparasites. Some questions are obvious—for example, what are the mechanisms by which hemiparasites harbor such high amounts of mineral nutrients, like K? How do parasitic plants locate a host and how do hosts defend themselves? Do most hemiparasites have direct xylem to xylem connections with the host, and is there active selection for resources at the host interface? These types of mechanistic biological questions have long been of interest and are actively being researched. Here, I highlight more broad research topics related to this dissertation and discuss how hemiparasites could be used as a model system to study a myriad of biological and ecological questions.

What do hemiparasites want?

Whether hemiparasitism has evolved to facilitate the uptake of resources is an underlying question that is woven throughout this research. Though many studies, like my own in Chapter 1, speculate as to the answer–be it that a hemiparasite needs a host for N, or P, or C, –these theories often tend to be offshoots from a principal research question asked in the paper. Given that hemiparasites have two sources for mineral nutrients (below-ground resources and host resources) and two for carbon (photosynthetic output or host carbon), studying host-hemiparasite resource flow can be complicated. A large-scale greenhouse, common garden, or even field experiment in which hemiparasites are grown with and without hosts of varying qualities, and in which particular resources are manipulated, would be very useful in enhancing our knowledge of the host-hemiparasite relationship. Additionally, isotopic tracer studies could help elucidate the source of materials. Any additional knowledge into understand what , if anything, a hemiparasite is precisely parasitic for would certainly provide additional evolutionary context to plant parasitism as whole.

The role of phosphorous

The fact that P limited hemiparasitic plant growth (Chapter 1) is not surprising given that most plants cannot access forms of P most commonly found in the soil. For this reason, mycorrhizal associations are common among plants, as these fungi can convert P to a plant mobile form. However, to my knowledge there has never been a comprehensive assessment of the mycorrhizal status of hemiparasites. Such a review would allow us to understand whether hemiparasites form other associations that would negate the need of a host plant, or, if perhaps hemiparasitism is simply another means for plants to form a relationship with another organism to uptake P. This could be assessed by manipulating the parasitism status of a hemiparasite and tracking its mycorrhizal status. In other words, do hemiparasites have mycorrhizal associations to then lose them upon attachment to a host plant?

The role of P could also be assessed through a large-scale habitat model that seeks to elucidate whether hemiparasites are more abundant in P-depleted areas. If so, this would suggest that they hemiparasitism may have evolved partly as a strategy for P uptake.

The plasticity of hemiparasite physiology

Our understanding of the ecological role of hemiparasites is only as good as our understanding of their ecophysiology and ability to adapt to changing abiotic and biotic resources. Do hemiparasites regulate their transpiration rate under severe drought? If a hemiparasite is grown with a host, and then the host is removed, do they revert to more autotrophic rates of photosynthesis and transpiration? Do annual hemiparasites have a "time limit" after which they begin to behave fully autotrophically if a host is not located? These types of questions all will inform the temporal and spatial variability of a hemiparasite's ecological impact and will make it easier for their incorporation into predictive models and community ecological theory. Given the variation we see in a hemiparasite's ecological role (Chapters 3,4), which we know to be linked to its ecophysiology, we can expect that such traits should vary. However, the mechanisms and extent of this physiological plasticity has not been researched.

Life history

Hemiparasites are an ideal study group for investigating life history theory. Since hemiparasites are united by a unique lifestyle, we can make very specific physiological hypotheses based on how they should act, i.e.,; attached hemiparasites should always maintain a transpiration rate higher than that of the host to ensure access to host resources. Thus, if a physiological or a morphological trait of annual hemiparasites differs from perennial hemiparasites in ways that would be expected based on life history theory, it would provide a solid experimental foundation to substantiate the relationship between longevity and the expression of physiological traits. Additionally, though it is well understood that life history is genetic (Friedman, 2020), little research has investigated how a plant's life history manifests at an ecological scale. For example, does a first-year perennial (that can flower and set seed in its first year) conform to morphological and physiological expectations of a perennial before or after its first year in dormancy? In the case of hemiparasitic plants, the answer to this question will have real implications for the host-hemiparasitic relationship.

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Appendix A: Anatomy of *Castilleja* haustoria

I isolated haustoria of *Castilleja* growing with various hosts for a variety of anatomical imaging. Wai Pang Chan in the Department of Biology at the University of Washington was highly involved in all stages of each project. These pilots never yielded the precise images we desired yet could still be useful. Select images and methodology is presented below:

Structural Electron Microscopy

I prepared haustoria samples of *Castilleja levisecta* attached to *Danthonia californica* and *Achillea millefolium* for scanning electron microscopy. The purpose was to get high resolution images of the exterior structure of haustoria and determine if there were any morphological differences between haustorium attached to *Achillea* and to *Danthonia*.

Preparation:

I grew *Castilleja* with the hosts *Achillea millefolium* and *Danthonia californica* in the greenhouse of the Center for Urban Horticulture. I harvested the plants, washed the roots, identified haustoria, and cut the haustoria. I dehydrated the haustoria in ethanol and fixed in formaline-acetic acid-alcohol (FAA).

Scott Braswell in the Molecular Analysis facility mounted the specimens using aluminum stubs with a flat surface and performed sputter coating. A subset of mages is shown below.

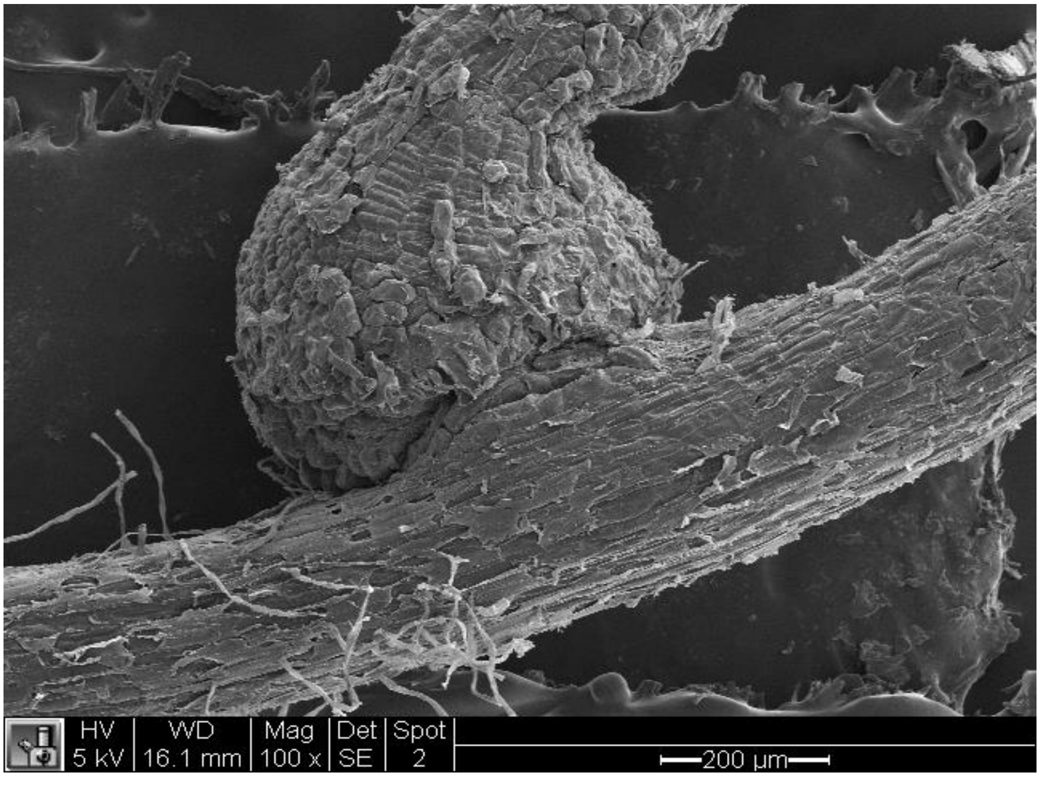

Figure A.1. *Castilleja levisecta* (top) parasitizing *Achillea millefolium* (bottom).

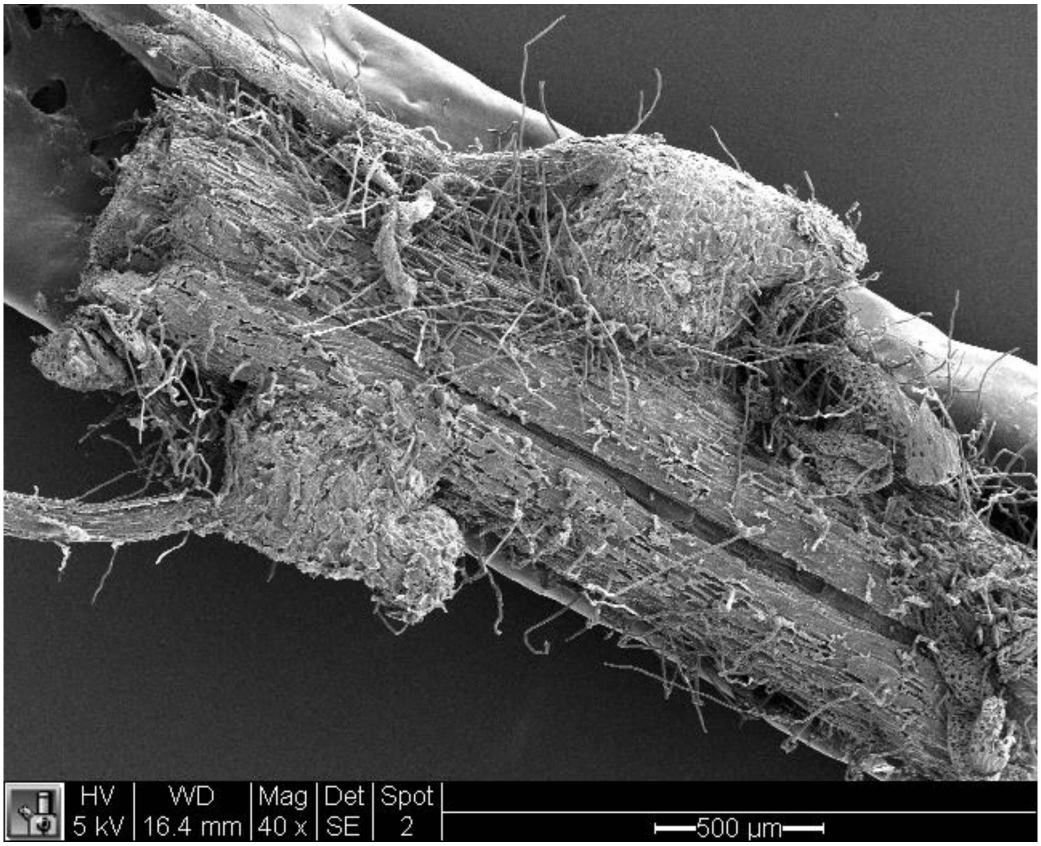

Figure A.2. Two haustoria (top right, bottom left) attached to the roots of *Danthonia californica* (center).

Transmission Electron Microscopy:

For stained anatomical images, I worked with the Department of Pathology, University of Washington. Plants were grown as above. Dr. Kelly Hudkins and Ying-Tzang Tien, embedded, sectioned, and stained the haustoria with paraffin. Staining was done using Johansen's method (Johansen, 1940). We hoped to use the staining method to see if hosts showed resistance to haustorial invasion (usually identified by the presence of lignin, which appears bright pink). Due to difficulties of sectioning, we were never able to yield a cut that showed the xylem connection between host and hemiparasite. Sample images are provided.

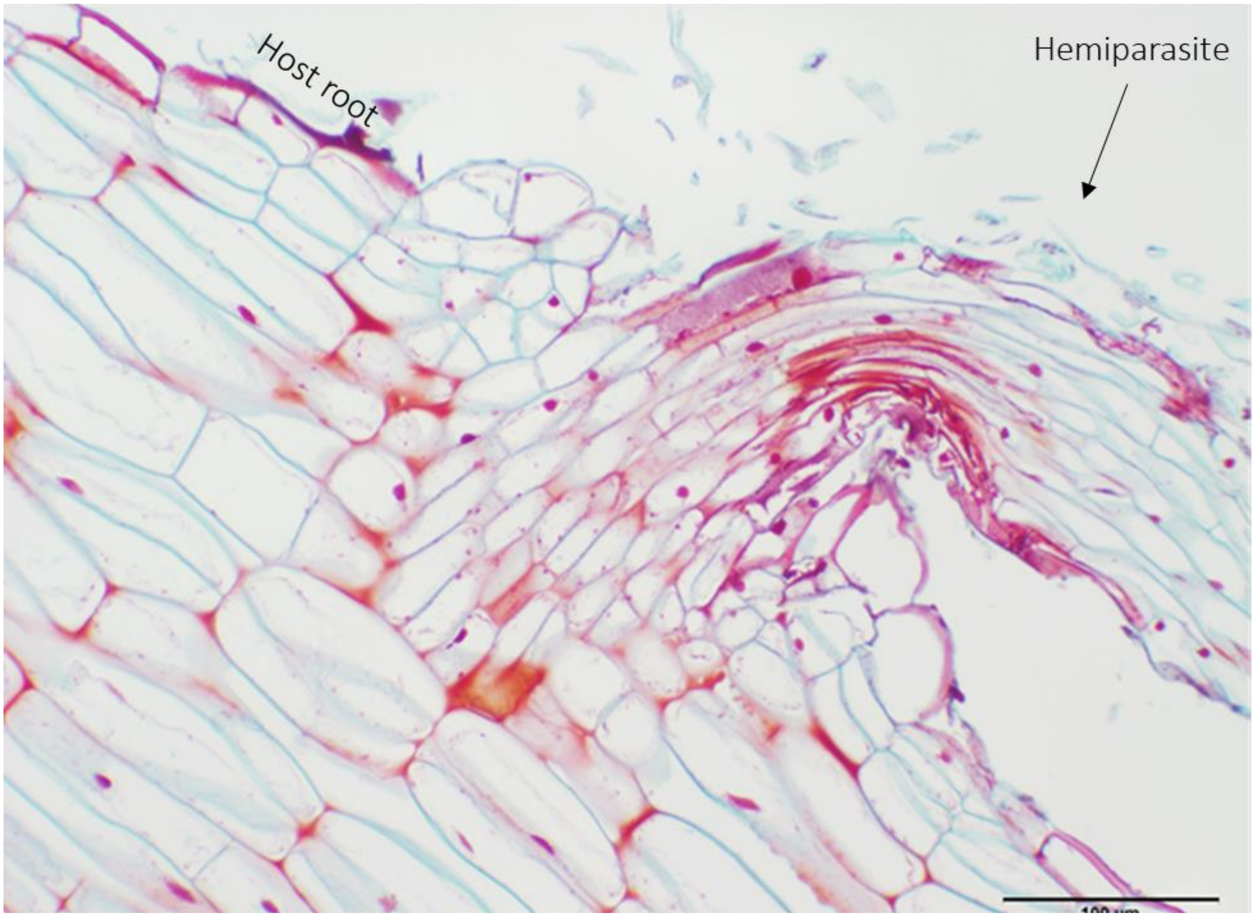

Figure A.3. *Castilleja levisecta* parasitizing *Achillea millefolium.*

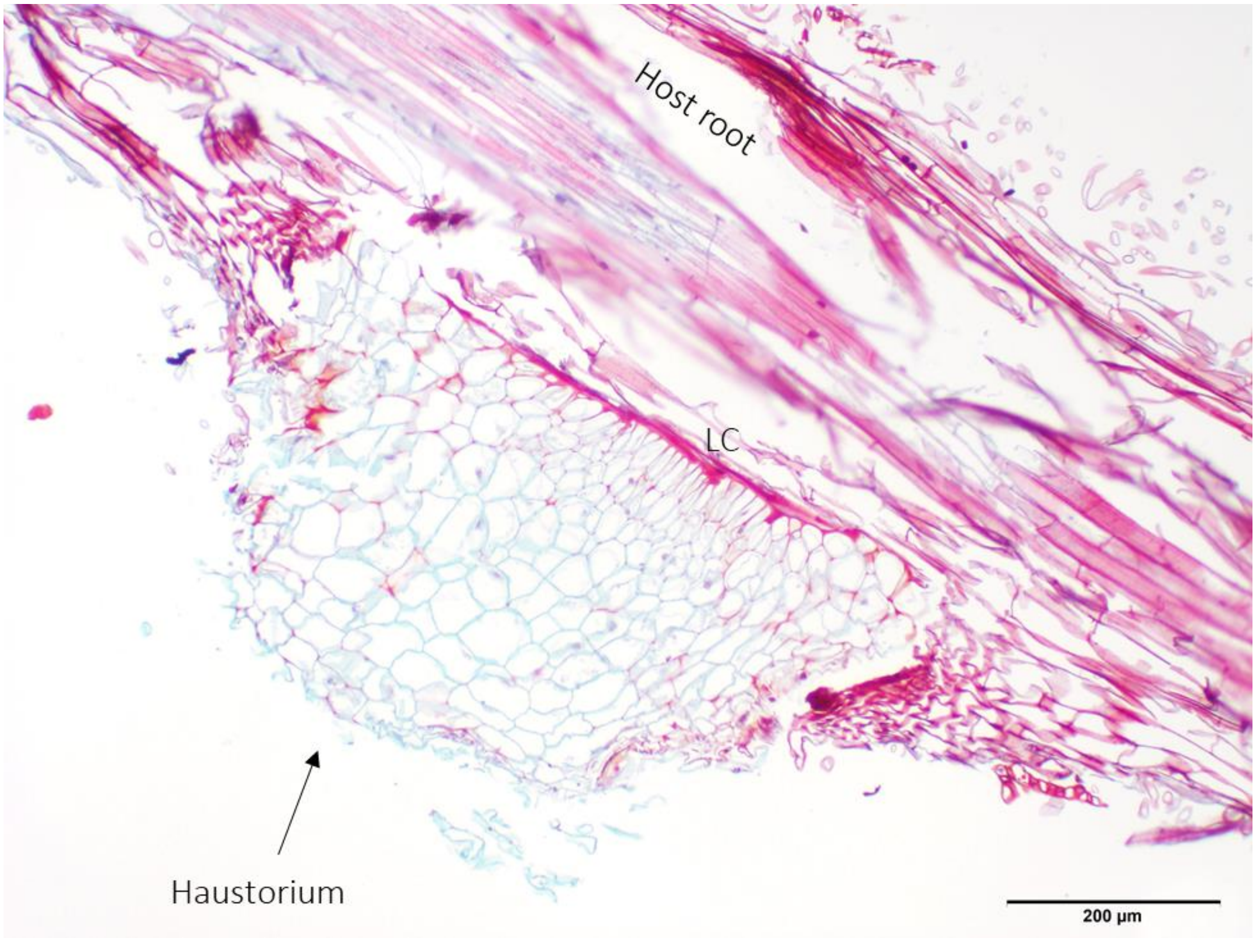

Figure A.4. *Castilleja levisecta* parasitizing *Festuca roemeri*. LC = lignified cells. The presence of lignified cells at the host-haustorial interface suggests potential host resistance to haustorial penetration. The elongated haustorial cells at the host-haustorial interface are typical of parasitic plants.

Appendix B: Assessing nitrogen movement between hemiparasites and hosts using foliar isotope labeling

Labelling plants with ^{15}N and ^{13}C stable isotopes usually require gas-tight labelling chambers, making it difficult to investigate *in situ* resource flow between hemiparasite and host.

In this greenhouse experiment, I tested a labelling method (Světlíková, 2016) in which dual-labelled (^{15}N, ^{13}C) urea solution is brushed directly onto leaves of plant species. I grew *Castilleja hispida* and *Castilleja levisecta* with seven different host species –*Achillea millefolium, Clarkia amoena, Danthonia californica, Deschampsia cespitosa, Eriophyllum lanatum, Festuca roemeri* and *Plectritis congesta.*

I applied the same width and height streak of urea to the leaves of the host species using a thin paintbrush. Samples were harvested after the hemiparasite flowered. Leaf samples were submitted to the University of California, Davis, Stable Isotope Lab (see Methods, Chapter 2). Linear mixed model analysis (with pot as a random effect) showed no relationship between host identity and the amount of ^{15}N in the tissue. Still, I was able to confirm the presence of ^{15}N in all individuals tested, showing that the label was successful. However, difficulty in standardizing the amount of urea applied due to differential leaf tissue types in hosts seriously confounded the results. Furthermore, the hemiparasites experienced high mortality with *Clarkia* and *Plectritis,* suggesting they are not suitable hosts for these species. Overall, this method seems highly useful for assessing relationships between hemiparasites and the same host, however, its utility is questionable for multiple host mixtures.

References:

Světlíková P., Blažek P., Mühlsteinová R., Těšitel J. (2016) Tracing nitrogen flow in a root-hemiparasitic association by foliar stable-isotope labelling. Plant Ecology and Evolution 149 : 39-44.

Appendix C: Detecting endophyte transfer between *Castilleja* and its host

In 2018, with the collaboration of Dr. Sharon Doty and Pierre Joubert, I designed an experiment to see if bacterial endophytes could move through the haustorium of a parasitic plant and into the host. This would show that bacteria could be shared between plants. Given the importance of the plant microbiome to plant health and wider community ecosystem services, these results would have wide-spread ecological implications.

Methods

We chose to inoculate hemiparasites in the species *Castilleja hispida* and *Castilleja levisecta* with green (GFP) and red-fluorescent bacteria (RFP) and verify movement via microscopy. We also verified inoculation of the bacteria via plating. Pierre Joubert led the microbiological lab work.

Given the thin delicate roots of the hemiparasitic plant germinates, we were initially worried that inoculation would be too stressful and cause high mortality. However, most recovered quickly. We allowed the plants to grow for 9 weeks with the hosts *Achillea millefolium* and *Festuca roemeri* before harvesting for verification by plating and by microscopy.

Results

Verification by plating

The sterilization protocol used to kill other bacteria was too strong for the thin roots and we were unable to verify inoculation of *Castilleja.* We did verify inoculation into both host plants.

Verification by microscopy

Initially, microscopy suggested that the experiment was successful; we saw high fluorescence in both the red and green spectrum around the haustoria and within the host and hemiparasite tissues. However, upon evaluation of a control hemiparasite that was not inoculated, we observed that the haustorium showed significant auto-fluorescence like that of the endophytes. Thus, the RFP (Figures B.1, B.2) and GFP control signal was as strong as that in the experimental slides (Figure B.3). This made it very difficult to determine if the fluorescence was coming from the bacteria or the hemiparasite tissue.

Wai Pang Chan at the Department of Biology attempted to determine the spectral signature (emission wavelength histogram) of the haustoria to compare this to the signature of the added endophytes via “spectral mixing” to tease apart the signal of the haustoria from that of the bacteria. Unfortunately, this proved to be too complicated for this study but may have better results with additional preparation.

Future research and conclusions.

A future study could use ddPCR/DNA probes for verification. Improved plating methods to verify colonization would be required. Furthermore, methods still need to be developed to prevent bacteria leaking into the soil and inoculating the host via the soil. One potential solution is to grow the plants in agar, though no protocols exist for growing parasitic plants in agar for long enough periods of time to allow for haustorial attachment and growth.

This pilot itself is novel for showing that these haustorium do fluoresce at such high intensities, though precluded microscopy as a method for analyzing the transfer of endophytes between parasitic plants and their hosts.

References:

Johansen, D.A. (1940). Plant Microtechnique. McGraw-Hill,New York. pp. 1-52

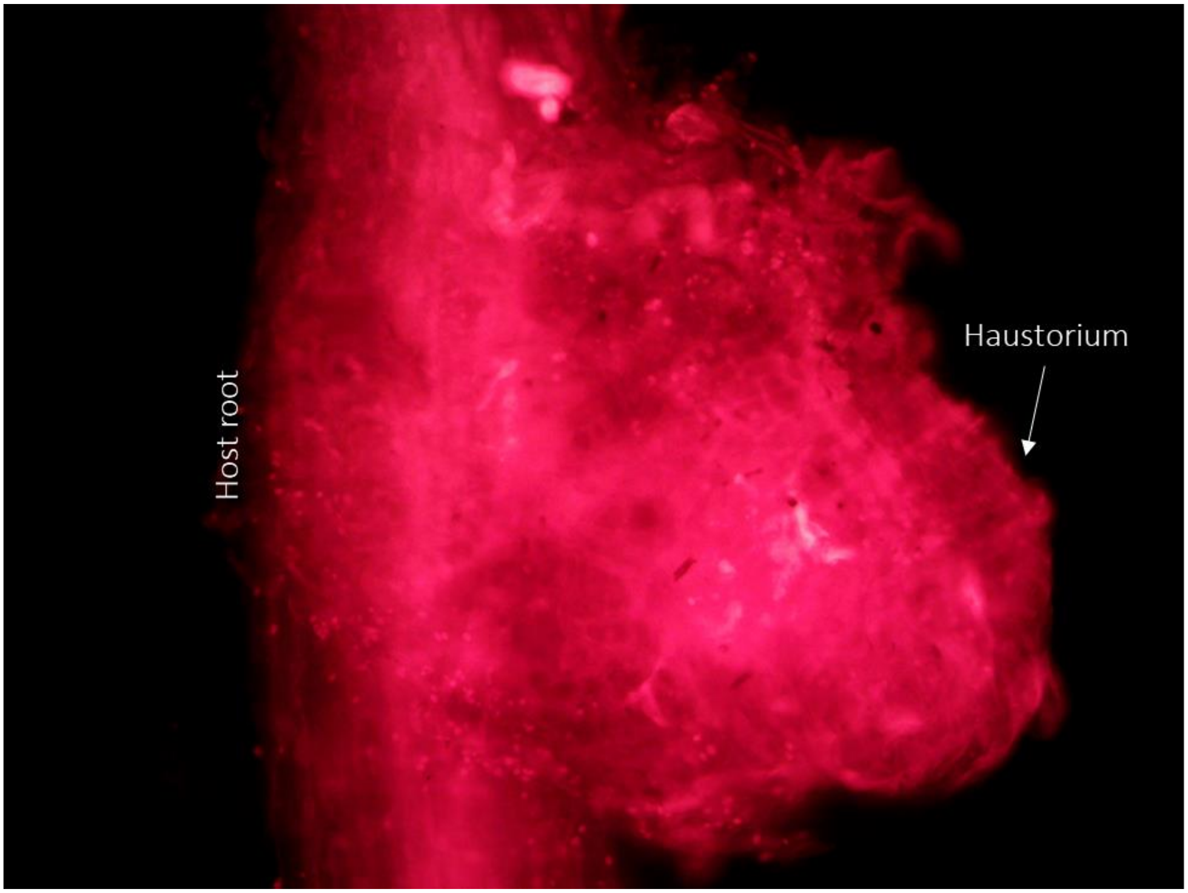

Figure C.1. Fluorescence in the red spectrum of inoculated *Castilleja levisecta* haustorium and host root

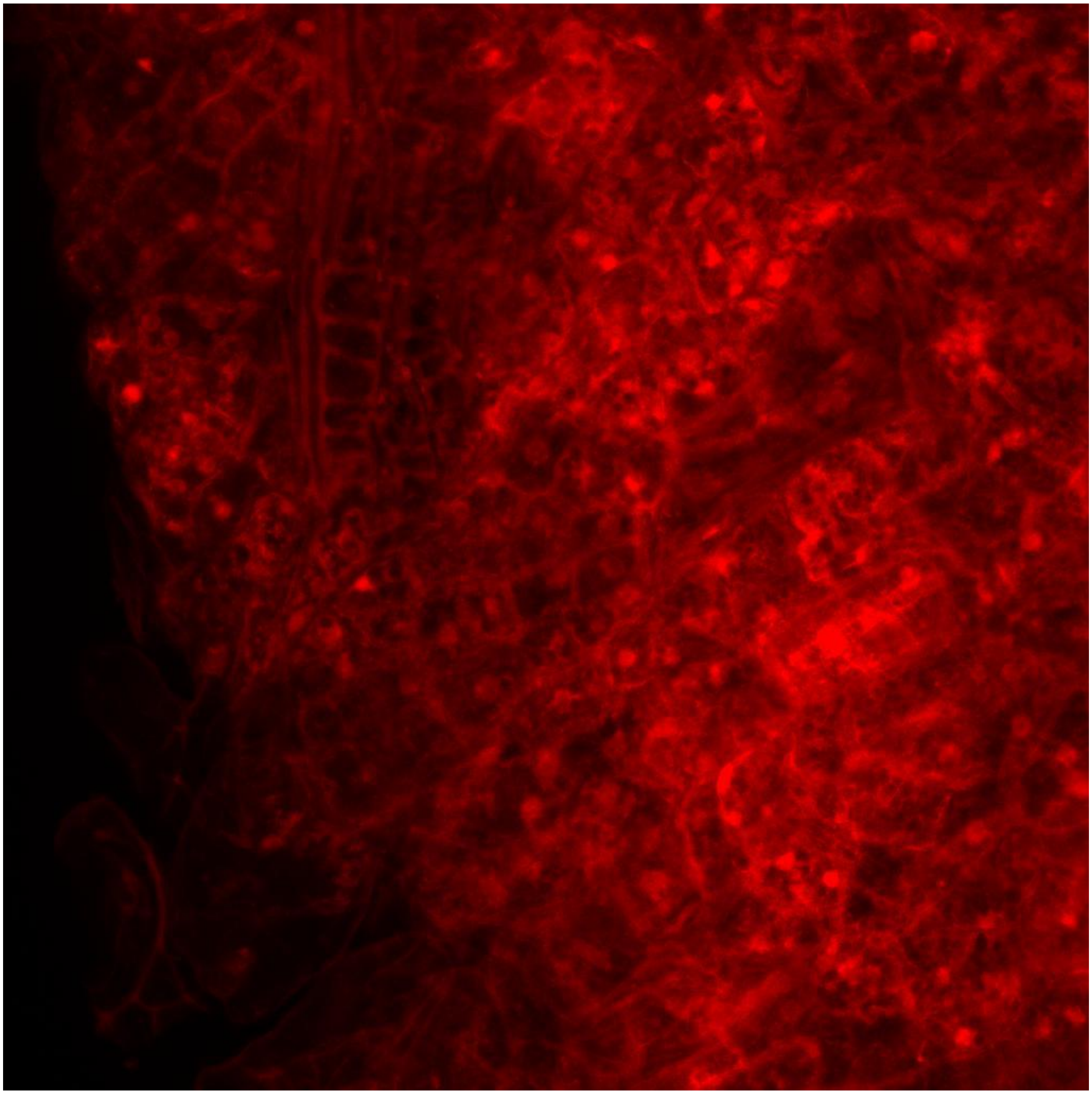

Figure C.2. Confocal close-up of the auto-fluorescence of the haustorium of uninoculated (control) *Castilleja levisecta.*

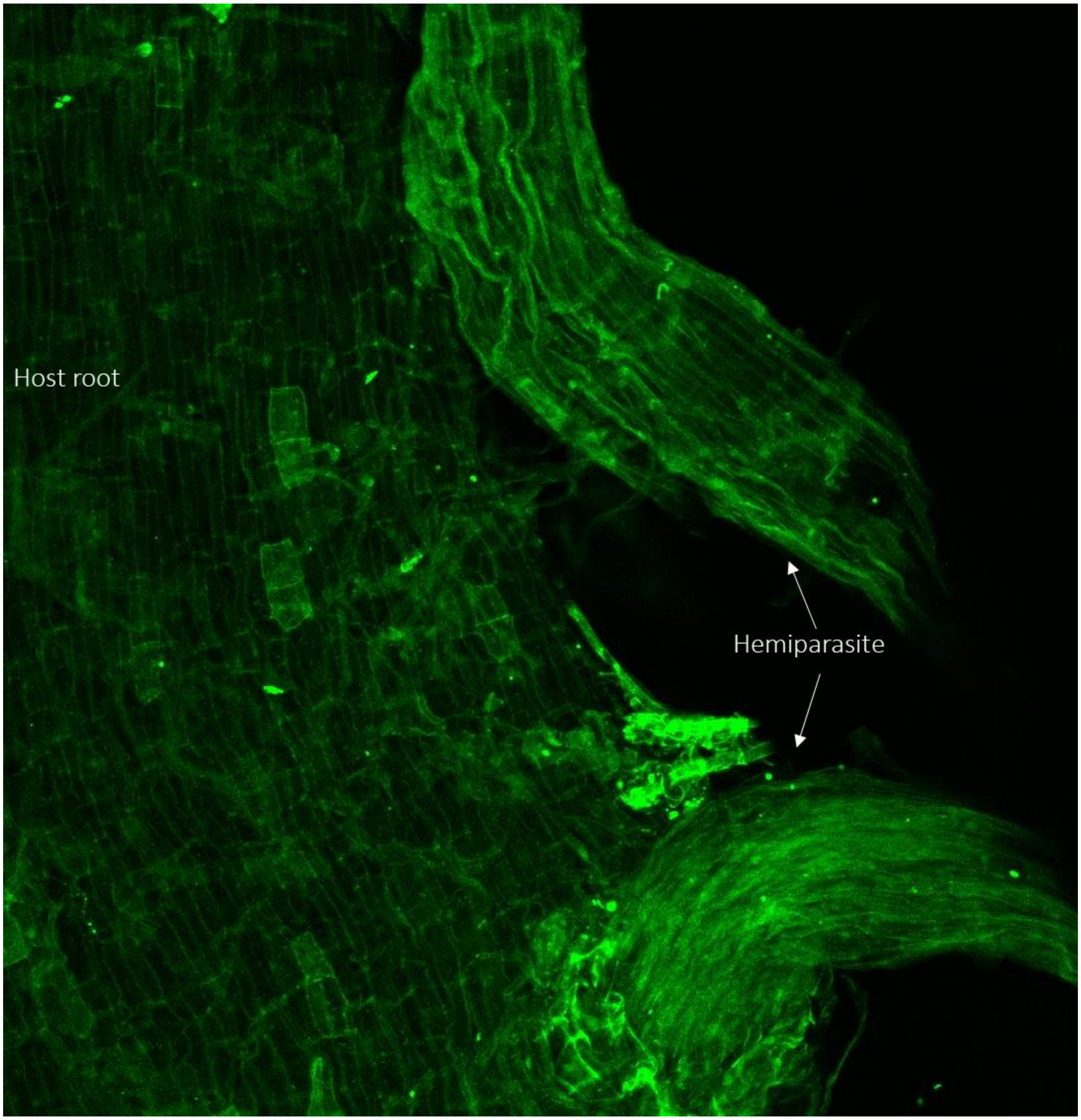

Figure C.3. Auto-fluorescence in the green spectrum of uninoculated (control) *Castilleja levisecta* roots attached to the host *Achillea millefolium.*

Appendix D: Photographs of *Castilleja* in its natural communities

These photos are provided with the intention of illustrating a small slice of the diverse ecosystems in which *Castilleja* occurs. All photos by Jasna Hodžić.

Figure D.1. *Castilleja chromosa*, Golden Gate Range, NV.

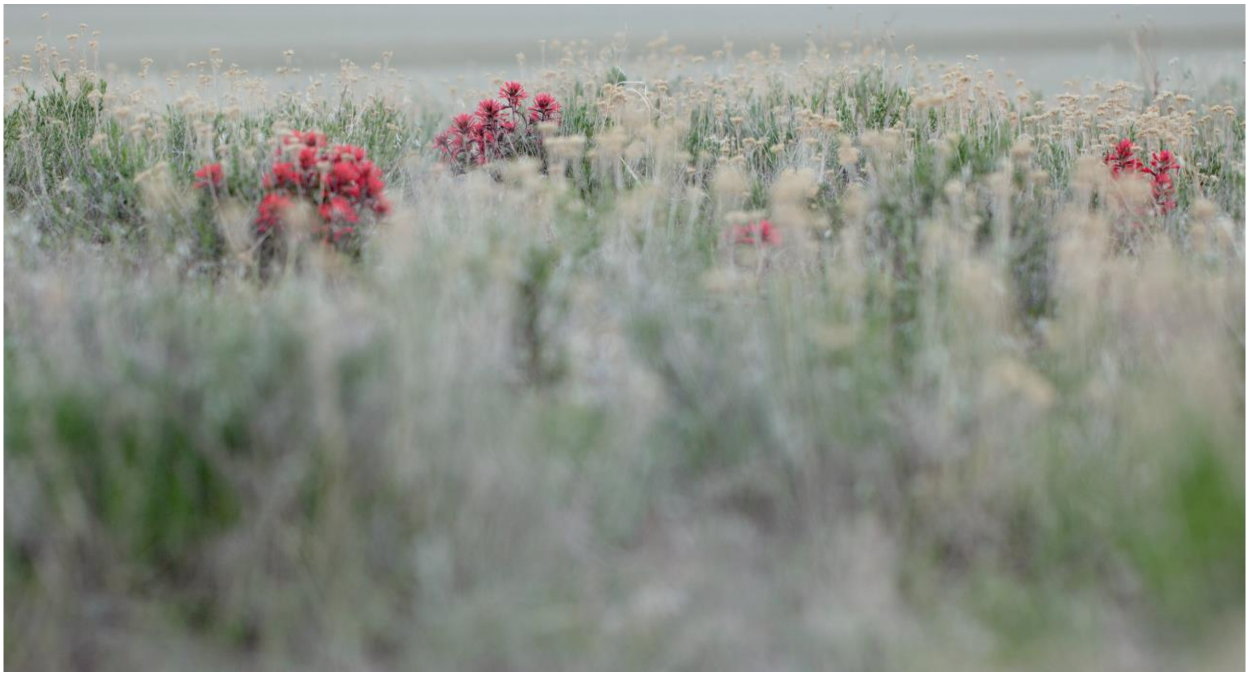

Figure D.2. *Castilleja chromosa*, Golden Gate Range, NV.

Figure D.3. *Castilleja chromosa*, Golden Gate Range, NV.

Figure D.4.*Castilleja chromosa*, Golden Gate Range, NV.

Figure D.5. *Castilleja levisecta*, Glacial Heritage Preserve, WA.

Figure D.6. *Castilleja levisecta* and *Castilleja hispida*, Glacial Heritage Preserve, WA.

Figure D.7. *Castilleja linariifolia*, Rifle Mountain Park, CO.

Figure D.8. *Castilleja angustifolia*, Padre Canyon, UT.

Figure D.9. *Castilleja chromosa*, Padre Canyon, UT.

Figure D.10. *Castilleja chromosa*, Zion National Park, UT.

Figure D.11. *Castilleja chromosa*, Zion National Park, UT.