Soil, Growth, Arthropods, and Competition: Native parasitic vine Cassytha pubescens and its indirect ecological effects on native and invasive host species.

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B. Sc. (Hons)

Submitted for the degree of Doctor of Philosophy

December 2021

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6/12/2021

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COVID-19 PANDEMIC IMPACT STATEMENT:

The pandemic outbreak in March 2019 had a negative effect on this thesis by hindering work in the laboratory and in the field. Although many contingency plans were in place for the loss of plants and, for example, alternative field sites in case of bush fires, it would not have been possible to foresee the degree of impact the pandemic had on my studies. The greatest disruptions during the pandemic prevented me accessing laboratory equipment or working in the field, or being unable to be in the same laboratory as my volunteers. The disruption of COVID 19 prevented having different parts of the project done in the best possible order. I had to perform certain studies in tandem to save time, instead of doing one after the other as originally planned.

The impact of the pandemic on the mental health of myself and all my postgraduate cohort was substantial. Trying to adjust to working from home without all our necessary equipment or materials, including books or hardware only available on campus, made the progress very difficult during lockdowns. While the university gave us two more months of funding, to make up for time loss during the pandemic, this was not sufficient to compensate the severe impact of the pandemic on my studies. Since student support from the university funding was halved, I was not able to afford as many chemical analyses as I had originally planned. This may have had an impact on the reliability of some nutrient analyses in my studies.

The retrieval of samples from the field was very difficult during the pandemic, given the government's isolation and social distancing mandates. Unfortunately, this was made even more difficult because the University of Adelaide changed its fieldwork policy by not allowing researchers to undertake fieldwork by themselves. This had a negative impact on how

frequently I could retrieve samples in the field for chapter 2. I had originally planned to identify all captured arthropods for chapter 4 to family level at the very least, but only a limited number of people could work simultaneously in a lab given COVID 19 restrictions.

In June 2020, during a COVID 19 outbreak in South Australia the University of Adelaide considered a total campus shutdown that would not have allowed anyone on campus – not even for essential work such as watering plants in the glasshouse. My supervisory team and myself made the decision to minimize the risk and harvest the plants in the chapter 5 experiment far earlier than originally planned (harvested after 71 days, originally planned to harvest after 8 months). This may have hindered a more accurate assessment of the physiological impacts of parasitic plants on hosts in direct competition. The early harvest of experimental plants help explain the lack of negative effects on the foliar nutrients and photosynthetic parameters that were expected.

General Abstract:

In spite of negative perception, parasites are keystone organisms because of their indirect benefits on biotic and abiotic environments. In spite of their abundance, parasites have not been fully incorporated into current understanding of ecological communities; this represents a massive gap in current understanding of community ecology.

This project focused on the indirect effects of an Australian-native parasitic plant, *Cassytha pubescens*. This project identified whether these effects differed when the parasite infected native or invasive plants, and whether these effects benefit natives or invasives. *Cassytha pubescens* has strong negative effects on invasive host species, particularly on one of the most invasive plant species in the world, *Ulex europaeus*. In contrast, *C. pubescens* has negligible impacts when infecting native species, therefore *C. pubescens* has biocontrol applications.

I assessed whether *C. pubescens* modified litterfall of native and invasive hosts, and whether this influenced soil nutrient returns. Using plastic pots capturing litterfall in the field and using plant and soil nutrient analyses, I found that invasive host *U. europaeus* had decreased litterfall, but infection had no effect on native host litterfall. Infected plants had minor differences in soil composition compared with uninfected plants. This demonstrated that *C. pubescens* may have little impact on soil nutrient returns, unlike other parasitic plants, because it is leafless.

I assessed how *C. pubescens* litter and soil under infected shrubs influenced seedling emergence and growth. In a glasshouse study, seeds of native and invasive species were sown in soil taken beneath infected or uninfected shrubs, adding *C. pubescens* litter or not. Native and invasive species had decreased emergence under parasite litter – with stronger effects on

invasive *U. europaeus*. Only *U. europaeus* grew larger in soil from under infected shrubs, probably because of its greater resource use efficiency. This study demonstrated that *C. pubescens* litter, which can be substantial in mass upon the death of host, can decrease invasive species recruitment around hosts.

 I assessed whether *C. pubescens* influenced abundance and composition of arthropod communities. I used pitfall traps in the field to trap arthropods over one year, beneath infected and uninfected native and invasive shrubs. I found slight differences for beetles, being less abundant under infected shrubs than uninfected shrubs. *Cassytha pubescens* did not increase arthropod abundance, unlike other parasitic plants, but we found no negative effect on arthropod communities. These results suggest *C. pubescens* has negligible effects on arthropod communities.

I assessed whether *C. pubescens* modified competitive interactions between native and invasive host species. Using glasshouse studies I assessed whether growth of native and invasive plants differed when grown alone *vs*. grown with a competitor. I also assessed how the native and invasive grew, in a single large pot, when both were uninfected, only invasive infected, and only native infected. Invasive *U. europaeus* had less growth when infected, whether or not *A. paradoxa* was infected. However, native *A. paradoxa* grew equally well competing with an infected invasive. These results suggest that *C. pubescens* will decrease the competitive ability of invasive *U. europaeus,* but not that of natives.

Chapter 1: General Introduction

Parasites are organisms that feed on live hosts. While animal parasites such as fleas or ticks feed by sucking blood from hosts, parasitic plants suck nutrients from host plants. Parasitic plants vary in form, ranging from small herbs to large trees (Watling and Press 2001). They have independently evolved at least 11 times (Barkman *et al*. 2007; Westwood *et al*. 2010), and they occur in all biomes from the tropics to the Arctic (Press 1998). They may rely for their nutrition entirely (holoparasitic) or partially (hemiparasites) on host plants, the latter being capable of photosynthesis (Watling and Press 2001). Depending on how parasitic plants attach to their hosts they can be further divided into root-parasitic, attaching haustoria (suckers) to a hosts' roots, or stem-parasitic if haustoria attach to stems.

While parasites are broadly perceived negatively, this view of parasites may be biased. The study of plant parasites has not been fully incorporated our present understanding of community ecology, vastly underestimating their benefits and influence beyond their direct effects on individual hosts.

The focus of this thesis is the Australian-native hemiparasitic *Cassytha pubescens* R. Br. 1810. *Cassytha* L., 1753 is the only parasitic genus in the family Lauraceae, consisting of *c*. 23 species, of which the majority are native to Australia (Press and Graves 1995). When mature, *C. pubescens* is a perennial obligate parasite. It is rootless and leafless, forming coils with haustoria around the stems of its hosts (Prider *et al*. 2009). The indeterminate growth habit of *C. pubescens* allows it to infect multiple hosts simultaneously (McLuckie 1924). *Cassytha pubescens* is a generalist parasite, infecting a wide range of species (Facelli *et al*. 2020), including invasive and native leguminous shrubs in the South Australian Mt. Lofty Ranges (Cirocco *et al*. 2017).

Cassytha pubescens has a strong negative impact on host physiology when it infects invasive plant species, decreasing seed output host growth and photosynthetic rate. However, when *C. pubescens* infects native plant species, it has negligible effects on host physiology (Prider *et al*. 2009, 2011; Cirocco *et al*. 2017). Thus, *C. pubescens* may be a potential biocontrol agent against invasive weeds in South Australia, such as *Ulex europaeus* L., *Cytisus scoparius* Link., and *Rubus fruticosus* L. sp. aggregate. A common invasive host species of *C. pubescens* is *U. europaeus* (Fabaceae), a leguminous spiny shrub (Lee *et al*. 1986; Broadfield and McHenry 2019). *Ulex europaeus* is considered one of the 100 most invasive plant species (Lowe *et al*. 2000; Atlan *et al*. 2015), and is a major problem in many parts of the world, including Australia.

As parasitic plants extract water and nutrients from their hosts, they have deleterious effects on them (Watling and Press 2001), decreasing host performance, lowering host growth rate, and decreasing host competitive ability (Matthies 1996; Tennakoon and Pate 1996; Cameron *et al*. 2008; Yu *et al*. 2009). In spite of these negative effects on their hosts they can have indirect effects on the biotic and abiotic environment that may outweigh the negative effects of infection, resulting in benefits to ecological communities. Hence, parasites are increasingly recognised as functionally important organisms in their communities, despite comprising only a minor component of any ecological community (Press and Phoenix 2005; Hartley *et al*. 2015). Parasitic plants can play important roles in several ecosystem processes (Mathiasen *et al*. 2008) and may modify community structure and dynamics (Press and Phoenix 2005). In this introductory chapter I will focus on the indirect effects of parasitic plants and potential ecological benefits parasitic plants exert beyond direct interactions with their hosts.

Indirect effects of parasites at the community level:

Until recently, parasites remained largely excluded from food web studies and ecological community theory (Marcogliese and Cone 1997; Press and Phoenix 2005; Quested 2008). This is surprising, as approximately 40-50% of all living organisms engage in some form of parasitism – perhaps making parasitism the most common of all consumer strategies (Poulin 1999; Dobson *et al.* 2008; Lafferty *et al.* 2008). Consequently, there is a large knowledge gap regarding the effects parasites on their communities, particularly in comparison to what we know about the effects of top-predators and competitively dominant species (Lafferty *et al.* 2008; Angelini and Silliman 2014; Hartley *et al.* 2015). However, in the last few decades, considerable research efforts have revealed a disproportionately large effect of parasites in community-level processes (Pennings and Callaway 2002; Ameloot *et al.* 2005; March and Watson 2007; Wood *et al.* 2007; Fisher *et al.* 2013; Ndagurwa, Dube, and Mlambo 2014; Hartley *et al.* 2015; Mellado *et al.* 2019).

Parasitic plants can have far-reaching ecological effects beyond their hosts by modifying processes that drive ecological community species composition and diversity maintenance. They can alter the recruitment and establishment of host and non-host species (Marvier 1998), change the spatial distribution of resources (Quested, Press, *et al.* 2003; Bardgett *et al.* 2006; March and Watson 2007, 2010; Ndagurwa, Dube, and Mlambo 2014; Ndagurwa *et al.* 2016), alter competitive hierarchies within, and between, species (Matthies 1996; Marvier 1998; Niemelä *et al.* 2008). By modifying these ecological processes parasitic plants can have strong effects on ecological community composition. Ultimately, the 'cost' of infection in communities may be outweighed by indirect community-level 'benefits' (Watson 2009).

Indirect effects of parasitic plants on resource distribution:

Parasitic plants may have strong impacts on soil nutrient cycling which in turn may influence the growth of plants (hosts and non-hosts) in the surrounding of their hosts. Parasitic plants withdraw mineral nutrients from their host and accumulate these resources in their foliage (Seel and Press 1993; Quested, Cornelissen, *et al*. 2003) and, unlike most plants, prior to leaf abscission parasite reabsorption of nutrients is minimal (Quested *et al*. 2002; 2005). Thus, their tissues decompose readily and locally increase soil fertility (Spasojevic and Suding 2011; Fisher *et al*. 2013). Mistletoes (Loranthaceae, Santalaceae) thus enhance nutrient cycling by shedding nutrient-rich litter at high rates, drastically increasing input of quantity, and quality (as assessed by nutrient content and decomposing potential) of litter compared with uninfected trees (March and Watson 2007; 2010). By increasing soil nutrient concentration in patches around hosts through decomposing nutrient-rich litter, parasitic plants re-distribute resources for non-host plants. This effect has been called 'the Robin Hood hypothesis' (Press 1998) and may be a ubiquitous property of parasitic plants (Quested, Press, *et al*. 2003; Ameloot *et al*. 2005; Bardgett *et al*. 2006; Yu *et al*. 2009; March and Watson 2010; Demey, Staelens, *et al*. 2013; Fisher *et al*. 2013; Ndagurwa *et al*. 2013; Mellado *et al*. 2016).

While high leaf turnover may enhance nutrient returns under mistletoes, other parasitic plants seem to produce similar effects through different mechanisms. For root-hemiparasitic *Rhinanthus* spp. (Orobanchaceae), high decomposition rates have been reported to enhance soil nutrient returns (Quested, Cornelissen, *et al*. 2003; Spasojevic and Suding 2011). For leafless holoparasitic vines, it seems that soil nutrient levels may be modulated via host effects (Yu *et al*. 2009); however, the decomposition or litter contribution of any parasitic vine has not been studied. These modifications to soil nutrient returns under infected plants may have ecological consequences, and these effects may be more pronounced in nutrient-poor systems (Press 1998;

Quested, Press, *et al*. 2003; Watson and Herring 2012). By enhancing nutrient returns parasitic plants may increase the productivity of local plant assemblages. Beneath mistletoes the productivity of undergrowth is greater than the productivity beneath uninfected trees (March and Watson 2007; Ndagurwa *et al*. 2016; Mellado and Zamora 2017). Parasitic plants can therefore strongly affect co-occurring plant species other than their current host, which may have consequences for ecological community species composition.

There are major gaps in our understanding of the role of parasitic plants in soil nutrient returns. While we know that plants like mistletoes increase soil nutrient returns by drastically increasing litter deposition or by shedding nutrient-rich litter that decomposes rapidly, we do not know whether leafless parasitic plants alter soil nutrient returns (but see Yu *et al*. 2009). Furthermore, we do not know the extent to which parasitic vines alter soil nutrient returns through litterfall quality and quantity or how quickly their tissues decompose.

Indirect effects of parasitic plants on plant assemblages:

The direct negative effects of parasitism are the most obvious concern to the conservation of other species; thus, these effects are relatively well understood. How parasitic plants indirectly affect the emergence and growth of co-occurring plant species by increasing litter input and increasing soil nutrient returns is less understood. The increased nutrient returns beneath infected hosts may not benefit all plants species equally. Some fast-growing, competitively dominant species may exploit better the enhanced resource availability than subordinate species (Funk and Vitousek 2007; Demey, Staelens, *et al*. 2013; Funk 2013). The effects of parasites on local plant assemblages ultimately depend on how various species are disadvantaged by parasite infection (determined by degree of parasitism and tolerance to infection) and how they differentially benefit from nutrient increases (Demey, Staelens, *et al*.

2013; Fisher *et al*. 2013). To the best of my knowledge, no studies have assessed whether parasitic plant litter can affect the seedling emergence of co-occurring plant species, and few studies have investigated how nutrient inputs affect different species in the plant community. Of particular importance is whether invasive species would benefit, given their ability to exploit better increased resource availability (but see Demey, Staelens, *et al*. 2013).

Plant litter at high densities may act as a mechanical barrier to seedling emergence, impeding seedling emergence as well as growth and development (Facelli and Pickett 1991; Facelli *et al*. 1999; Donath and Eckstein 2008; Asplund *et al*. 2018; Campanella and Bisigato 2019). Furthermore, secondary metabolites in parasitic plant tissues (Johns and Lamberton 1966; Johns *et al*. 1966; Wu *et al*. 1997; Brophy *et al*. 2009) may have further negative effects on seedling emergence by inhibiting seed germination and seedling growth (Wink 1983; Roberts and Wink 2013). Considering that some parasitic plants drastically increase host-parasite litter input, how that litter may influence the emergence of seedlings of co-occurring species is a major gap in the literature (but see Mellado and Zamora 2017). Changes to seedling emergence, if different for various species, may have important consequences for species composition. While several parasitic plants drastically increase litterfall beneath infected hosts, increasing soil nutrient returns and increasing the growth of co-occurring species, we do not know whether leafless parasitic vines can do this. Leafless parasites can have substantial contributions to litter input upon the death of their host (Fig. 1).

Fig. 1: Dead *Cassytha pubescens* stem litter smothering a dead *Ulex europaeus* shrub, approximately 1.5 m tall. Picture taken in Belair NP, SA (BJ O'Connor).

Indirect effects of parasitic plants on arthropod communities:

Parasitic plants directly withdraw resources from hosts, which may become available for animals (Watson and Herring 2012; Mellado and Zamora 2016; Hódar *et al*. 2018). Some stem-hemiparasitic plants provide nectar and pollen, nutritious foliage, and fruits (Canyon and Hill 1997; March and Watson 2007; Watson *et al*. 2011). These resources may be exploited by various vertebrate and invertebrate visitors, making parasitic plants potential keystone resources in ecological communities (Watson 2001; March and Watson 2010; Watson and Herring 2012; Rowntree *et al*. 2014). In this manner, parasitic plants may have indirect effects

on arthropod communities. Arthropods are important components of ecological communities, regulating carbon and nutrient cycling by changing the quantity, quality as well as the timing of plant detritus inputs (Mattson and Addy 1975; Yang and Gratton 2014). Changing the abundance of arthropods has consequences for other organisms, such as birds and other insectivores (Watson *et al*. 2011).

Relatively few studies (Room 1972; Whittaker 1982; Anderson & Braby 2009) have investigated the differences in arthropod communities between infected vs. uninfected conspecifics (Burns *et al*. 2011, 2015). Even fewer studies have investigated how the strong influence that parasitic plants have on plant litter accumulation and decomposition can affect ground arthropods. Parasitic plants may have indirect effects on arthropods by modifying plant assemblage structure (Hartley *et al*. 2015) and modifying ecological processes driven by the host litter deposition (*e.g*. microhabitat structure, litter quantity and litter quality)(Ndagurwa, Dube, Mlambo, *et al*. 2014; Hartley *et al*. 2015; Mellado *et al*. 2019). By altering microhabitat availability and litter quantity/quality, parasitic plants can greatly modify the abundance and diversity of ground arthropods. Mistletoes can form dense layers of litter (March and Watson 2007) that, in turn, can have a drastic increase in abundance and diversity of arthropods (Ndagurwa, Dube, Mlambo, *et al*. 2014; Mellado *et al*. 2019). Via input of nutritious flowers and foliage litter quality may influence detritivores and other decomposers, parasitic plants can have direct implications for predatory arthropods (Bultman and Uetz 1984; Mellado *et al*. 2019) and their linked trophic levels (Watson and Herring 2012).

Nothing is known about whether these patterns may differ for infected native *vs*. invasive host species of a parasitic plant. Arthropod communities may also differ between native and invasive plant species, with invaded communities having decreased arthropod abundance

(Samways *et al.* 1996; Litt and Steidl 2010; Foster *et al.* 2021). While native and invasive plant species may differ in the arthropod communities they can support, it is not known whether parasitic plants infecting natives and invasive species would alter these patterns – to the best of my knowledge this has not been previously investigated.

Indirect effect of parasitic plants on host competitive interactions:

Parasites engage in interactions with multiple species across all trophic levels, and may mediate intraspecific and interspecific interactions amongst host species and other organisms (Hatcher *et al.* 2012; Sotomayor and Lortie 2015). Furthermore, parasites can afflict some host species more than others, altering the fitness of hosts differently, modifying the competitive interaction between host species, and ultimately altering community structure (Marvier 1998; Poulin 1999; Hatcher *et al.* 2006; Prider *et al.* 2011). Parasites can thus have a net beneficial effect on one host species at the expense of another. Consequently, some plant species in the local plant assemblage may indirectly benefit from decreased competition from species vulnerable to infection (Cameron *et al.* 2005, Cameron *et al.* 2006, Cameron *et al.* 2009). Therefore, the presence of a parasite affects the abundance or distribution of the two host species relative to each other (Price *et al.* 1986).

This phenomenon, called parasite-modified competition, may have important consequences in the structure of ecological communities (Holt 1977; Price *et al.* 1986; Ruggieri and Schreiber 2005). In Californian salt-marshes, stem holoparasitic *Cuscuta salina* Engelm. (Convolvulaceae) led to shifts in vegetation cover by preferentially infecting dominant competitor *Salicornia virginica* L., increasing the cover of less preferred host *Arthrocnemum subterminale* (Parish) Standl. (Callaway and Pennings 1998). Root-parasitic *Melampyrum arvense* L. (Orobanchaceae) may influence the competitive balance between hosts, because it

reduced the growth of leguminous host more strongly than the other host species in binary mixtures (Matthies 1996). Other root-hemiparasitic species (*Rhinanthus* spp, Orobanchaceae) indirectly increase herb layer cover and prevent the competitive exclusion of subordinate species by supressing dominant grasses (Těšitel *et al.* 2017; Těšitel *et al.* 2018). Similarly, *Pedicularis palustris* L. (Orobanchaceae) can also supress dominant sedges (*Carex acuta* L.) and increase local species richness (Decleer *et al.* 2013). In China, *Cuscuta campestris* Yuncker can supress the invasive *Mikania micrantha* H.B.K., allowing native plant species to recover (Yu *et al.* 2008). Through such affinity for a particular groups or species, parasitic plants may facilitate competitive release of non-host species with low competitive ability (Pywell *et al.* 2004; Mudrák *et al.* 2016; DiGiovanni *et al.* 2017).

Studies on how parasitism affects competitive interactions, due to differential host vulnerability, have focused mainly on root-hemiparasites. These differ vastly from stem-hemiparasites, as stem-hemiparasites have no direct access to soil resources and may have a stronger impact on host nutrient content and related processes (*e.g.* photosynthetic rates, carbon budget). Furthermore, there are a many examples of parasitic plants affecting native and invasive species differently (Prider *et al.* 2009; Yu *et al.* 2009; Shen *et al.* 2010; Cirocco *et al.* 2016, 2017; Cirocco, Watling, *et al.* 2020). While it is clear that root parasites modify competitive interactions between hosts (Marvier 1998; Bullock and Pywell 2005; Yu and Liu 2011; Li *et al.* 2019), it is not known whether a native parasitic plant may modify competitive interactions between vulnerable invasive hosts that generally outcompete native species that are less affected by parasite infection.

Invasive plants and their effects in invaded ranges:

Invasive plants are a major problem globally, affecting biodiversity by interfering with several ecosystem processes, but parasitic plants may be viable long-term solutions to supress overabundant species and environmental weeds (Yu and Liu 2011; Těšitel *et al.* 2017, 2018; Cirocco, Watling, *et al.* 2020; Těšitel *et al.* 2020). Plant invaders may disrupt several ecological processes as they become increasingly abundant. Invasive plants are known to locally decrease plant species diversity (Vilà *et al.* 2006; León Cordero *et al.* 2016; Těšitel *et al.* 2017), alter nutrient cycling and soil properties (Dewar *et al.* 2006; Weidenhamer and Callaway 2010), leading to positive feedbacks benefiting their establishment (Mangla *et al.* 2011) which further their competitive advantage over native species (Fogarty and Facelli 1999; McAlpine *et al.* 2008). In new ranges, invasive species may escape their natural enemies that regulate their abundance in their ranges of origin, giving invasive species an advantage over native competitors (Enemy Release hypothesis) (Parker and Hay 2005). The Enemy Release hypothesis has been the framework for many biological control efforts; introducing specialist enemies into the invaded range. In this manner however, further alien species are introduced into an already invaded range (Prider *et al.* 2009; Těšitel *et al.* 2020). Such practices have had varying degrees of success (Hill *et al.* 2008). On the other hand, introduced species may gain enemies through strong interactions with enemies in the invaded range (Biotic Resistance hypothesis) (Levine *et al.* 2004; Parker and Hay 2005; Prider *et al.* 2009). Therefore, as an alternative to introducing specialist enemies from the invasive species original range, native generalist parasites in the invaded range can be exploited (Yu *et al.* 2008, 2009; Yu and Liu 2011).

Parasitic plants as biocontrol agents for invasive species:

If a parasite affects invasive hosts more severely than it does native hosts, the native hosts can gain a competitive advantage against the invader (Prider *et al*. 2009; Dunn *et al*. 2012). Such can be the case for *U*. *europaeus*. This species is a nitrogen-fixing shrub native to northern Europe, and it was introduced to Australia as fodder for livestock, hedging, and other uses (Atlan *et al*. 2015). However, *U*. *europaeus* is now considered as one of the world's worst weeds because it spreads quickly, modifies soil properties, and displaces native species (Broadfield and McHenry 2019). While spraying, mechanical removal and other methods have had some success in supressing *U*. *europaeus* in Australia, these approaches are costly for agricultural industries (*c*. $7 million per annum., Ireson and Davies 2012). At least five biological control agents have been introduced into Australia to control this weed, including sap-suckers and foliage-feeders, but these have had limited success (Partridge *et al*. 2003; Ireson and Davies 2012).

An alternative to traditional methods of weed control may rely on parasitic plants, either alone or with complementary biocontrol and traditional methods. In China, the holoparasitic vine *Cuscuta campestris* suppresses invasive *Mikania micrantha*: where the parasite occurs there is higher species richness and diversity of native plants (Yu *et al*. 2008; Yu and Liu 2011). In European grasslands, *Rhinanthus* species can supress dominant grasses that outcompete other groups of plants, with effects similar to that of selective herbicides. This suppression of dominant grasses facilitated the regeneration of other species, restoring natural communities of high diversity (Pywell *et al*. 2004; Těšitel *et al*. 2017, 2018). In South Australia, *C*. *pubescens* often infects several invasive species, in addition to native hosts. By efficiently withdrawing nutrients from invasive hosts, *C*. *pubescens* has a strong impact on growth, photosynthetic rate, transpiration, and seed output of invasive host species (Prider *et al*. 2009;

Shen *et al*. 2010; Prider *et al*. 2011; Cirocco *et al*. 2016; 2017; Cirocco, Facelli, *et al*. 2020). In Australia, *C. pubescens* has a broad host range including at least three Weeds of National Significance– invasive plants that have major economic, environmental, and social impacts. Based on this observation a research program (Cirocco *et al*. 2018) was established to determine the possibility of using *C. pubescens* as a biological control of these weeds. Results so far clearly indicate that *C. pubescens* can have much stronger negative effects on invasive than on native species.

Knowledge gaps:

All studies of the indirect effects of parasitic plants on nutrient cycling, plant assemblage composition, arthropod abundance, and the modification of competitive interactions, have been conducted on root-hemiparasites or stem-hemiparasites which are vastly different from *C. pubescens*. *Cassytha pubescens* is unable to survive without a host, and does not derive any nutrients directly from the soil when mature unlike those well-studied root-hemiparasites. *Cassytha pubescens* can infect multiple hosts simultaneously unlike well studied stem-hemiparasites (namely mistletoes). Unlike those parasites, *C. pubescens* is leafless. While some root-hemiparasitic (*Rhinanthus*) and stem holoparasitic (*Cuscuta*) plants may also infect multiple host species simultaneously, the literature largely consists of annual species that do not sustain ecological effects over many years, unlike *C. pubescens* may. Research is needed to understand how leafless vines that infect multiple host species simultaneously can influence nutrient cycling, plant assemblage composition, arthropod abundance, and the modification of competitive interactions.

General question:

The general question in this thesis was: Does *C. pubescens* have differential ecological effects when it infects native and invasive host species? I attempted to answer this question by assessing: Ch. 2) how does *C. pubescens* influences native and invasive host litterfall and how that may produce difference in soil composition, Ch. 3) how does *C. pubescens* litter and soil under hosts influences seedling emergence and growth, Ch. 4) how *C. pubescens* influence ground arthropod communities under native and invasive hosts, and lastly, Ch. 5) how does *C. pubescens* modify the direct competitive interactions between native and invasive host species?.

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Statement of Authorship

Title of Paper	Native parasitic plant decreases invasive host litterfall, but increases soil nutrient returns.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted fieldwork, labwork, statistical analyses and wrote the manuscript.

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Name of Principal Author (Candidate)	Bernardo J. O'Connor
Contribution to the Paper	Conducted fieldwork, labwork, statistical analyses and wrote the manuscript.
Overall percentage (%)	80
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Co-Author Contributions

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ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Chapter 2: Native parasitic plant decreases invasive host litterfall, but increases soil nutrient returns.

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Abstract:

While parasites have negative effects on their hosts, they can have indirect beneficial effects on their biotic and abiotic background. The influence of parasitic plants on soil nutrient cycling has been studied with diverse groups of hemiparasites (*e.g.* mistletoes and root-parasitic plants). However, no studies have been done on leafless parasitic plants and how their influence soil nutrient availability. In this study, we assessed the contribution of *Cassytha pubescens*, an Australian-native hemiparasitic vine, on soil nutrient levels beneath a native (*Bursaria spinosa*) and an invasive (*Ulex europaeus*) hosts species. First, we quantified the total litterfall under hosts with and without the parasite using leaf-litter traps for one year to assess how *C. pubescens* contributed to litterfall. Secondly, we used fibreglass mesh bags to assess how rapidly *C. pubescens* stem litter lost nutrients. Lastly, we took soil samples under infected and uninfected shrubs, native *B. spinosa* and invasive *U. europaeus*, to assess whether *C. pubescens* enriched soil nutrient levels under infected shrubs. We found that *C. pubescens* decreased the litterfall rate of *U. europaeus*, but not native *B. spinosa*. However, when considering total litterfall input, *C. pubescens* compensated for the decreased litterfall with fruits and flowers – cancelling out the decrease of host litterfall. We also found that *C. pubescens* stem-litter loses nutrients relatively slowly, possibly because of the secondary metabolites present in tissues of *Cassytha* species. Lastly, we found that under infected shrubs there is greater soil C and soil K returns compared with uninfected shrubs of the same species. In this study we demonstrated

that a leafless parasitic plant can influence soil nutrient levels, something that has not been previously documented for a leafless hemiparasitic plant.

Introduction:

Parasites are often perceived negatively because of their negative impact on host species. However, in ecological communities the indirect positive effects of parasites may outweigh the negative effects of host infection. Parasitic plants are increasingly recognised as 'keystone' species (Pennings and Callaway 2002; Watson 2009; Watson and Herring 2012; Mellado *et al.* 2019), because of their large impacts on their community despite their relatively low abundance (Watson 2001). Parasitic plants can control soil nutrient cycling (Quested, Press, *et al.* 2003; Spasojevic and Suding 2011) and differentially affect the growth and abundance of various plant species consequently affecting coexistence and diversity in the community (Ameloot *et al.* 2005; March and Watson 2007; Demey, Staelens, *et al.* 2013; Mudrák *et al.* 2016; Těšitel *et al.* 2017). By altering local soil fertility parasitic plants can alter the performance of other plants (Quested, Press, *et al.* 2003; Demey, Staelens, *et al.* 2013) and consequently species composition (Spasojevic and Suding 2011; Těšitel *et al.* 2017). Through these effects parasitic plants can have cascading effects on arthropods and their vertebrate predators (Canyon and Hill 1997; Watson *et al.* 2011; Mellado *et al.* 2019). While several studies have assessed the influence of stem-hemiparasites mistletoes and root-hemiparasitic rattles, little is known about rootless, leafless perennial parasites and how they may influence soil nutrient returns. In this study, we focus on the role of an Australian-native hemiparasitic vine, *Cassytha pubescens* R. Br. and whether it can indirectly increase soil nutrient levels, and whether it achieves this quantitatively (change in total litterfall beneath host), or qualitatively of litter (input of nutrient-rich litter).

Parasitic plants play key ecological roles by regulating the cycling of limited resources in ecological communities, as well as modifying the spatial distribution of these resources. Parasitic plants enhance soil nutrient concentrations via nutrient-rich litter that forms spatially heterogenous, fertile soil patches which increase understorey plant biomass. This is because, in parasitic plants, nutrient reabsorption is minimal prior to leaf abscission (Těšitel *et al*. 2021); thus, their tissues readily decompose and fertilise the soil, increasing understory productivity (March and Watson 2007). This effect has been called 'the Robin Hood hypothesis' (Press 1998) and it appears to be a widespread property of parasitic plants (Quested, Press, *et al*. 2003; Ameloot *et al*. 2005; Bardgett *et al*. 2006; March and Watson 2007; Watson 2009; Yu *et al*. 2009; March and Watson 2010; Demey, Staelens, *et al*. 2013; Fisher *et al*. 2013; Ndagurwa *et al*. 2013; Demey *et al*. 2014; Mellado *et al*. 2016; Ndagurwa *et al*. 2016). By shedding nutrient-rich litter that fertilises soil patches parasites can re-distribute resources. However, unlike the Robin Hood story, the redistributed resources may benefit competitively superior plants (*e.g.* invasive weeds), rather than poor competitors (Demey, Staelens, *et al*. 2013). Furthermore, it is not known whether leafless parasitic plants can alter litterfall and soil nutrient beneath their hosts. Ultimately, how parasitic plants affect community structure depends on the equality with which nutrients benefit co-occurring species (Quested, Press, *et al*. 2003; Demey, Staelens, *et al*. 2013; Fisher *et al*. 2013) and how strongly parasitism supresses dominant species (Prider *et al*. 2009; Yu *et al*. 2009; Spasojevic and Suding 2011; Mudrák *et al*. 2016; Cirocco *et al*. 2017). Therefore, it is essential to determine what effects parasitic plants have on native and invasive hosts in terms of litterfall and soil nutrients returns.

In particular, it is necessary to understand if and how parasitic plants can produce soil nutrient differences and potential positive feedbacks in growth for invasive species. In Australia, the native hemiparasite *C. pubescens* afflicts invasive host species, such as *Ulex europaeus* L.

(gorse), more strongly than native host species. *Ulex europaeus* is on one of the 30 most noxious weed species in the world, now invading 50 countries (Christina *et al*. 2020) and declared a noxious environmental weed in Australia. *Cassytha pubescens* frequently infects *U. europaeus* in southern Australia and infected *U. europaeus* individuals have stunted growth and decreased photosynthetic potential (Cirocco *et al*. 2017, 2018; Cirocco, Facelli, *et al*. 2020). These changes in photosynthetic rate seem to be due to the withdrawal of nitrogen (N) by the parasite, which can reduce foliar N by up to 10% (Cirocco, Watling, *et al*. 2020). In contrast, the negative effects on native hosts are minimal. Consequently, *C. pubescens* is currently being trialled as a biocontrol agent against these weeds of national significance (see Cirocco *et al*. 2018). If the parasite is indeed introduced to new areas or its abundance increased where present, it could produce changes in the spatial and temporal patterns of nutrient availability. Hence, we need to understand how *C. pubescens* modifies nutrient levels under infected hosts.

Here we present results of a series of studies investigating whether *C. pubescens* infection can produce differences in soil nutrients under infected and uninfected shrubs of a native and an invasive species. We investigated the litterfall response of invasive *U. europaeus* and native *B. spinosa* to *C. pubescens* infection. We also present results from nutrient analyses of soils collected under invasive *U. europaeus* and native *B. spinosa* infected or not uninfected by *C. pubescens*. We evaluate two pathways through which a parasitic plant drives change in soil nutrient concentrations. Firstly, via litter changes in host litter deposition, and secondly via parasite litterfall. We asked: is there a difference in litterfall among infected and uninfected shrubs? If so, does the relationship differ between native and invasive host species? We expected that native and invasive shrubs infected with *C. pubescens* would have increased total litterfall under their canopies, due to the parasite shedding stems, fruits, and flowers. Secondly,

we asked: How much mass and nutrient content is lost over one year by *C. pubescens* litter decomposing in the field? Lastly, we asked: Is there a relationship between litterfall differences among infected native and invasive shrubs, and soil nutrient concentration? To answer these questions we quantified the litterfall in *Bursaria spinosa* Cav. and *U. europaeus* for one year on individuals infected or not by *C. pubescens*. Furthermore, we investigated whether changes in litterfall due to infection could explain differences in soil nutrient levels under hosts – and whether this differed between native and invasive species. We also assessed the mass loss and nutrient concentration of *C. pubescens* stem litter around every three months for one year

Methods*:*

Study species:

Cassytha pubescens (Lauraceae) is a generalist hemiparasite, native to Australia. It is a rootless and leafless vine that attaches to the stems of its hosts, allowing it to infect multiple hosts simultaneously. *Ulex europaeus* (Fabaceae) is an evergreen leguminous shrub that is considered one of the 100 most invasive plant species globally by the International Union for Conservation of Nature (Lowe *et al.* 2000; Atlan *et al.* 2015). In *U. europaeus*, adult leaves are modified into spines (Lee *et al.* 1986; Broadfield and McHenry 2019). *Bursaria spinosa* (Pittosporaceae) is an evergreen shrub (3 -5 m) endemic to Australia. It has elongated oval leaves and the stems have short (5-7 mm) spines. *Bursaria spinosa* is a common host of *C. pubescens*. *Acacia pycnantha* Benth. (Fabaceae) is a leguminous tall (3- 8 m) shrub that can often be infected by *C. pubescens*, it is native to south-eastern Australia. *Acacia pycnantha* has compound leaves as a seedling, but in adult form *A. pycnantha* has large phyllodes.

Study sites:

We surveyed Belair National Park, in Mount Lofty ranges of South Australia, for *U. europaeus* and *B. spinosa* hosting *C. pubescens* (-35.02123° N, 138.67355° E). The area has Mediterranean-type climate with cold, wet winters and hot, dry summers, with *c.* 900 mm per annum, with max mean temp = 22.6°C, min mean temp = 5°C, (Bureau of Meteorology 2021). The area is an open forest woodland dominated by *Eucalyptus obliqua* L'Hér, with abundant medium shrubs and diverse understorey vegetation. The ground in the area is largely covered by *E. obliqua* leaf litter, with small grass cover, and some bare ground. The soil composition is mainly sandy clay with a of pH *c.* 6.5.

Study 1: Litterfall from infected and uninfected shrubs:

To determine whether the litterfall produced by infected and uninfected plants were different, 96 litter traps were deployed under shrubs. We selected 16 *U. europaeus*, and 16 *B. spinosa* shrubs, each with eight infected and eight uninfected plants. Infected and uninfected shrubs were interspersed in the study area, and occurred within 1 km. Overlapping canopies were avoided. We had planned to include *A. pycnantha* in this experiment, but we could not find enough infected individuals interspersed in the vicinity of the other two species. Each shrub had three littertraps placed under the canopy, consisting of plastic pots (4.7 L, 20 cm diam. x 20 cm H). Cones of fibreglass mesh (~15cm depth) were glued onto the lip of the pots. To ensure that all traps were upright, and consistently capturing litterfall, a spirit level and metal pegs were used to pin pots to the ground. Between October 2019 and November 2020, we retrieved litterfall samples from each shrub every 2 to 3 months. Litter captured throughout the year was collected and dried at 60° C for 48 hours and weighed. Any litter that did not belong to hosts or parasite was excluded. After drying, we sorted the litter into species (host or parasite), and weighed it.

Study 2: Parasite litter decomposition

To estimate the decomposition rate of *C. pubescens* litter, and thus how quickly it may return nutrients into soil we collected c. 15 g of fresh *C. pubescens* stems in the field from each host shrub and air dried them for 48 h in the laboratory. We collected *C. pubescens* stems from the three host species in which soil was sampled: *U. europaeus*, *A. pycnantha*, and *B. spinosa* of similar sizes and in the same area as Study 1. *Cassytha pubescens* stems were taken from eight individuals of each species, each with similar parasite loads. The air-dried material was then weighed and placed in 10 cm x 10 cm mesh bags made of fibreglass (2 mm openings) and tie-sealed. Each set of bags was then placed back on the soil surface underneath the shrubs from which the *C. pubescens* material was collected. After 92, 147, 231, and 364 days, we randomly selected a subset of the litterbags to be collected using a random number generator in Microsoft Excel. Samples were then taken to the lab and weighed after air-drying for 48 h. To obtain the percentage of dry mass remaining we used the formula below:

$$\frac{M_0 - M_t}{M_0} \times 100$$

Where M_0 = initial litter mass at 0 days, M_t = mass at last day of assay (Coleman, Crossley and Hendrix 2004, pg. 305). To calculate the percentage of dry mass remaining we weighed the contents of litterbags on a fine-scale balance after oven-drying for 48 hrs at 60° C. To compare how *C. pubescens* decomposed through time, we measured total N% (DUMAS method), total C% (organic), K%, and total P% for each retrieval period. To calculate percentage remaining nutrients in decomposed litter after each retrieval period, we used the following formula from O'Connell (1988):

$$\% \, Remaining = \frac{[X] \; in \; decomposed \; litter}{[X] \; in \; fresh \; litter} \times \% \, Dry \; weight \; Remaining$$

Study 3: Soil nutrient concentrations:

We took soil samples beneath individuals of three shrubs species, *A. pycnantha*, *U. europaeus*, and *B. spinosa* infected or not by *C. pubescens* in May 2021. We firstly located *C. pubescens*-infected shrubs of all three species, then located uninfected shrubs of the same species and of similar height and orthogonal width. Single ~400 g soil samples were taken from each shrub, 10 cm deep, within *c*. 10 cm from the centre of shrubs. Soil testing was conducted in APAL Agricultural laboratories, Adelaide SA. Major and trace elements were quantified using microwave digestion and ICP-COES analysis. To assess differences in soil pH we sampled $n = 14$ shrubs per species, seven infected and seven uninfected, and quantified pH with a testing kit in the laboratory.

Statistical analysis:

All analyses were performed in R statistical software version 3.5.3 (R Core Team 2016). To analyse the effects of *C. pubescens* infection on host litterfall, decomposition rates of litter, and soil nutrient levels, we used linear models (LMs) with host species and infection as fixed factors. We tested homogeneity of variances in data using Bartlett's test function (*bartlett.test* function) and Shapiro-Wilk test (*shapiro.test* function) to assess normality of residuals. When appropriate, we used squared-root transformations. To conduct post-hoc analyses, comparing soil nutrient levels, litterfall, and nutrient content of litter within species, we used Tukey's pairwise comparisons with a 95% confidence level in *emmeans* function in the *emmeans* package (Lenth 2019). To compute effect sizes, we used the Cohen's *d* function in the *effsize* package (Torchiano 2018). Plots were made in R using the *ggpubr* package (Kassambara 2018).

Results:

Study 1: Litterfall in infected and uninfected shrubs

Comparisons of annual and seasonal means of litterfall showed that infection by *C. pubescens* slightly reduced the litterfall of both *B. spinosa* and *U. europaeus*, but the effect was larger in the latter, over the year of sampling (Fig. 1, Table 1, $p < 0.05$, Cohen's $d = -0.41$). Annually, infected *B. spinosa* individuals had 11.0% ± 0.25% less litterfall ($p > 0.05$, Fig. 1 A, Table 1), while infected *U. europaeus* had 37.6% ± 0.25% less litterfall than uninfected conspecifics ($p > 0.05$, Fig. 1 A, Table 1). Although host shrubs had decreased litterfall when infected, there was no difference in total litter input between uninfected and infected shrubs, *i.e.* when considering also the parasitic input ($p > 0.3$, Table 2). Overall, shrubs shed more litter in warmer months, having the greatest litterfall rate in summer (November-January), followed by spring, and then autumn (Supp. Fig. 1). Litter produced by uninfected hosts was mostly foliage and a few seed pods. Below infected shrubs, *C. pubescens* litter was largely composed of dried flowers, with a small proportion being made up of fruits – particularly in summer.
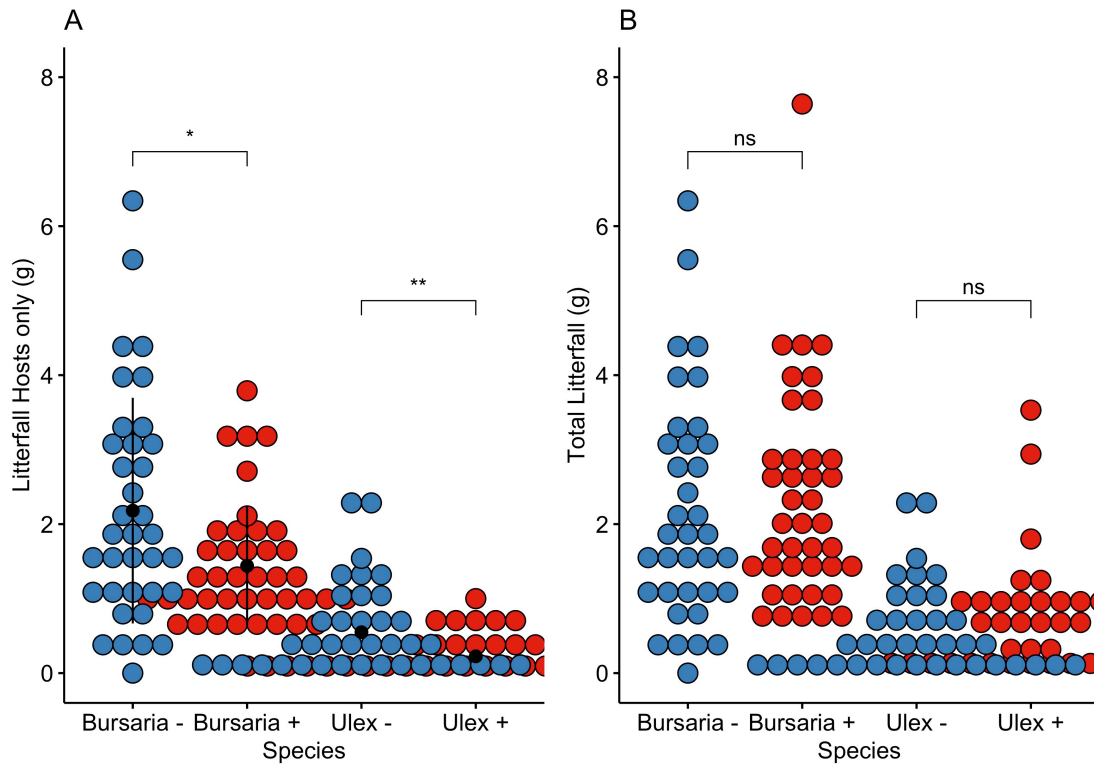
Fig. 1: A: Shrub litterfall mass under the canopies of native *Bursaria spinosa* and invasive *Ulex europaeus*, when *Cassytha pubescens*-infected (red +) or not (blue -). **B:** Total litterfall mass of host and parasite, when infected (red +) or not (blue -). Black dots show means and error bars show SD.

Table 1: Summary of linear model of host litterfall, Adj. R^2 = 0.55, F-statistic: 18.13 on 11 and 140 DF, Alpha = 0.05.

Factor	DF	Sum Sq	F-value	*P*-value
Infection	1	1.580	11.070	**0.00112**
Species	1	22.881	160.302	**2e^{-16}**
Season	2	2.437	8.537	**0.00031**
Infection * Season	2	1.147	4.019	**0.020**
Infection * Species	2	0.015	0.106	0.7450
Residuals	140	19.983		

*sqrt(host litterfall g) ~ species * infection * season*

Table 2: Summary of linear model of total (host + parasite) litterfall, $R^2 = 0.56$, F-statistic: 16.83 on 11 and 140 DF, alpha = 0.05.

Factor	DF	Sum Sq	F-value	*P*-value
Infection	1	0.124	0.719	0.3978
Species	1	23.063	133.700	**$2e^{-16}$**
Season	2	6.778	19.648	**$3.01e^{-08}$**
Season*Species	2	0.359	1.042	**0.3556**
Infection * Season	2	1.285	3.726	**0.0265**
Residuals	146	21.544		

*sqrt(total litterfall g) ~ species * infection * season*

Study 2: Decomposition of *Cassytha pubescens* litter *in situ*:

After 231 days in the field, *C. pubescens* litter had lost approximately half of its mass (Fig. 2, 54.3 ± 1.8% SE). We also compared nutrient concentrations of *C. pubescens* litter after different decomposition time intervals. Mobile nutrients were readily lost in decomposing *C. pubescens* stem litter, with the concentration of K (Fig. 3) sharply dropping in the first three months. In contrast, N and C fluctuated in concentration between days 0 and 364. Mean P% dropped in the first three months of decomposition, but then fluctuated between days 92 and 364.
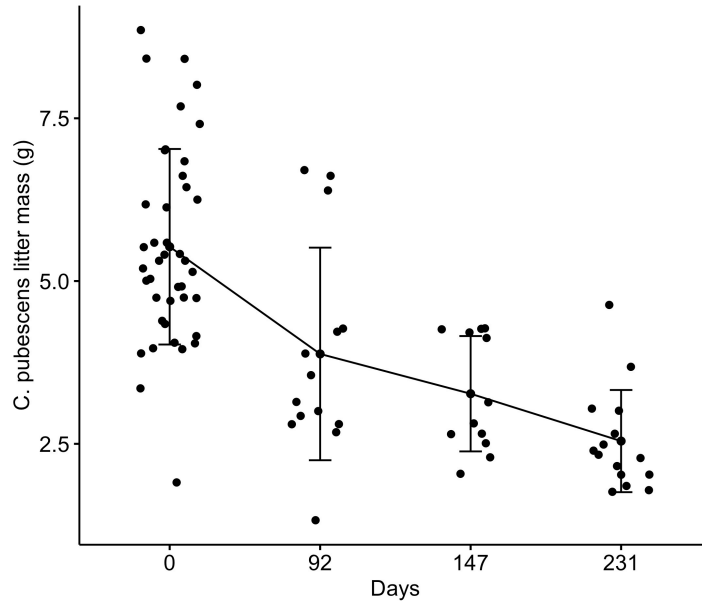
Figure 2: *Cassytha pubescens* litter mass (g) remaining in the field over 231 days, in fiberglass mesh bags. Error bars show SD.
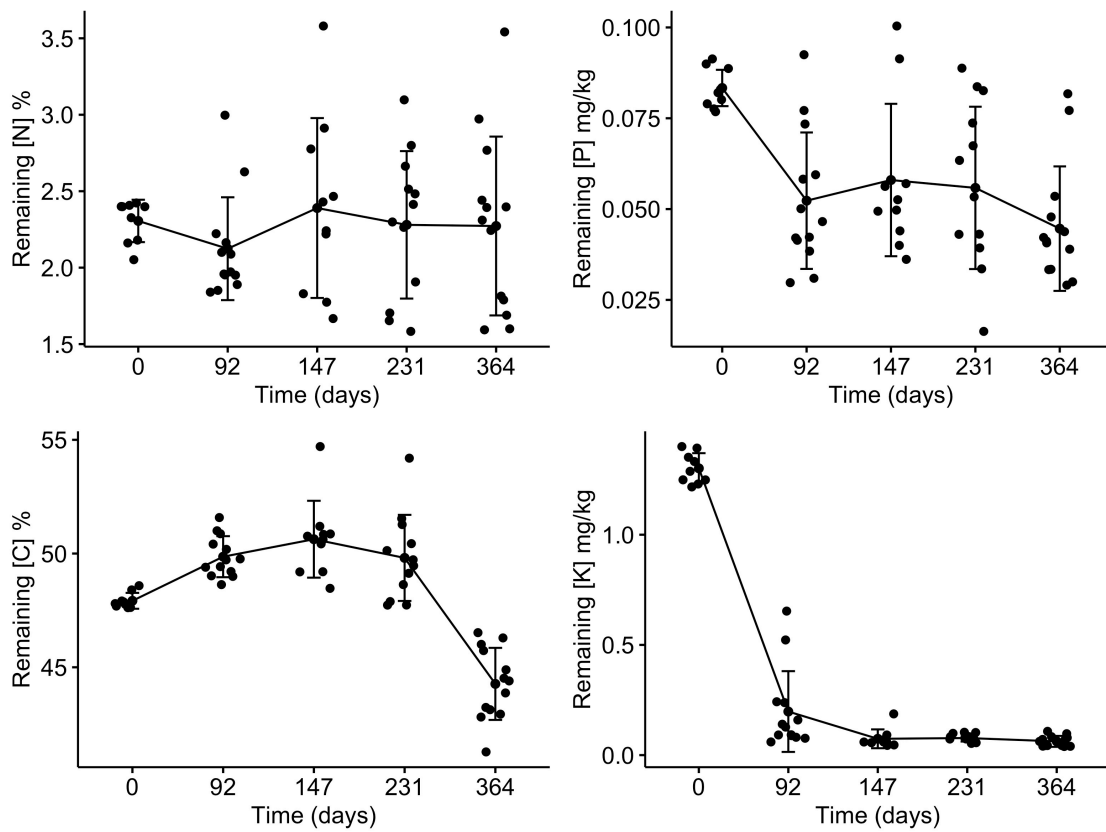
Figure 3: Nutrient contents of *Cassytha pubescens* litter decomposing over 12 months in the field. Error bars = SD.

We calculated C:N ratio for *C. pubescens* at each time period to assess litter quality and how it changed among seasons. The ratio of C to N can be highly informative of the quality of plant material (Seneviratne 2000; Kirschbaum *et al*. 2001). *Cassytha pubescens* litter retained a relatively high C:N ratio (~ 20-25) throughout the year that decomposition was measured (Fig. 4).
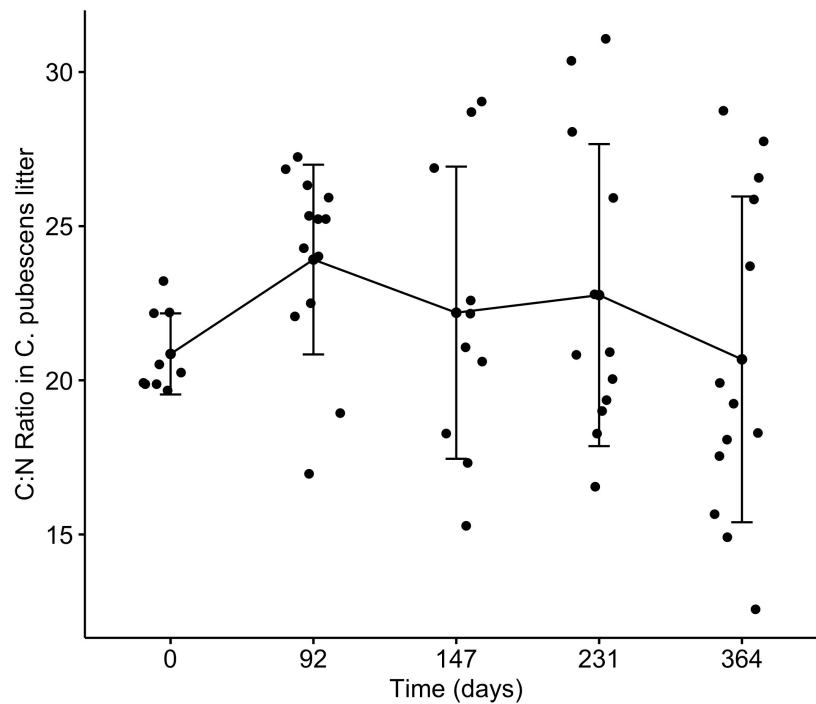
Figure 4: Plot of C:N ratio of *Cassytha pubescens* stem litter decomposing over 12 months in the field. Error bars = SD.

We calculated nutrient content of N and C during the first 8 months of *C. pubescens* decomposition (Tables 3, 4). Total N content in *C. pubescens* stem litter decreased by ~40% in N content in the first three months. Between 3 and 8 months after being left in the field, decomposition slowed down with N content dropping only by ~4% over this time. After 8 months in the field, *C. pubescens* litter had lost ~60% of the initial N content in litter. Similarly, for total organic C in *C. pubescens* litter, there was a marked decrease (~22%) in C content in the first 3 months of decomposition in the field.

Table 3: Nitrogen content loss in *Cassytha pubescens* litter in the field over 12 months, at various time intervals. Error on [N]% = sum of fractional error from terms.

	Day 0	Day 92	Day 147	Day 231
[N]%	2.50 ± 0.79	2.12 ± 0.34	2.39 ± 0.58	2.19 ± 0.47
% Weight Remaining	100 - 0.50	70.46 ± 6.86	58.3 ± 6.90	45.66 ± 7.4
% N Content Remaining	100 - 0.62	59.69 ± 0.57	55.58 ± 0.68	39.89 ± 0.70

Table 4: Carbon content loss in *Cassytha pubescens* litter in the field over 12 months, at various time intervals. Error on [C]% = sum of fractional error from terms.

	Day 0	Day 92	Day 147	Day 231
[C]%	44.7 ± 0.73	49.87 ± 0.90	50.66 ± 1.80	50.0 ± 1.85
% Weight Remaining	100 - 0.5	70.46 ± 6.86	58.3 ± 6.90	45.66 ± 7.4
% C Content Remaining	100 - 0.03	78.47 ± 0.13	65.95 ± 0.17	50.99 ± 0.22

Soil properties under infected and uninfected shrubs:

We found that soil under the three species had different pH, and that there was no significant interaction between species and infection (Table 6, $p > 0.3$). When infected, shrubs had more acidic soil ($p < 0.03$, Cohen's $d = 0.77$, Table 6). Overall, *B. spinosa* and *U. europaeus* had slightly less acidic soil (5.05 ± 0.44, 5.05 ± 0.28, respectively), and *A. pycnantha* had slightly more acidic soil (4.7 ± 0.26). We found mixed results in soil nutrient levels under infected and uninfected shrubs of the three species. Infected shrubs had greater soil C% levels ($p < 0.05$, Cohen's $d = 0.64$, Fig. 5, Table 5) than uninfected shrubs, with all three species responding similarly. We found no differences in soil N% between infected and uninfected shrubs ($p > 0.2$), nor between species ($p > 0.3$, Table 5). For soil P%, we found a significant interaction term (infection*species, $p < 0.05$) that accounted for 10.4% of soil P variance in our models. Soil under *A. pycnantha* had much lower P% when infected ($p < 0.05$, Cohen's $d = 1.2$, Table 5). In contrast, *U. europaeus* and *B. spinosa* did not differ in soil P when infected or not with *C. pubescens* ($p > 0.5$; $p > 0.4$, respectively). In soil Mg% levels, we only found differences in *U. europaeus,* which, when infected, had much greater soil Mg% levels than uninfected

individuals ($p < 0.05$, Cohen's $d = 1.2$). Similarly, we found only a marginal increase in soil K% in infected compared with uninfected shrubs ($p > 0.08$). However, *U. europaeus* had much greater soil K% when infected ($p < 0.05$, Cohen's $d = 1.2$). Differences among species accounted for 17% of the soil K % variance in our models.
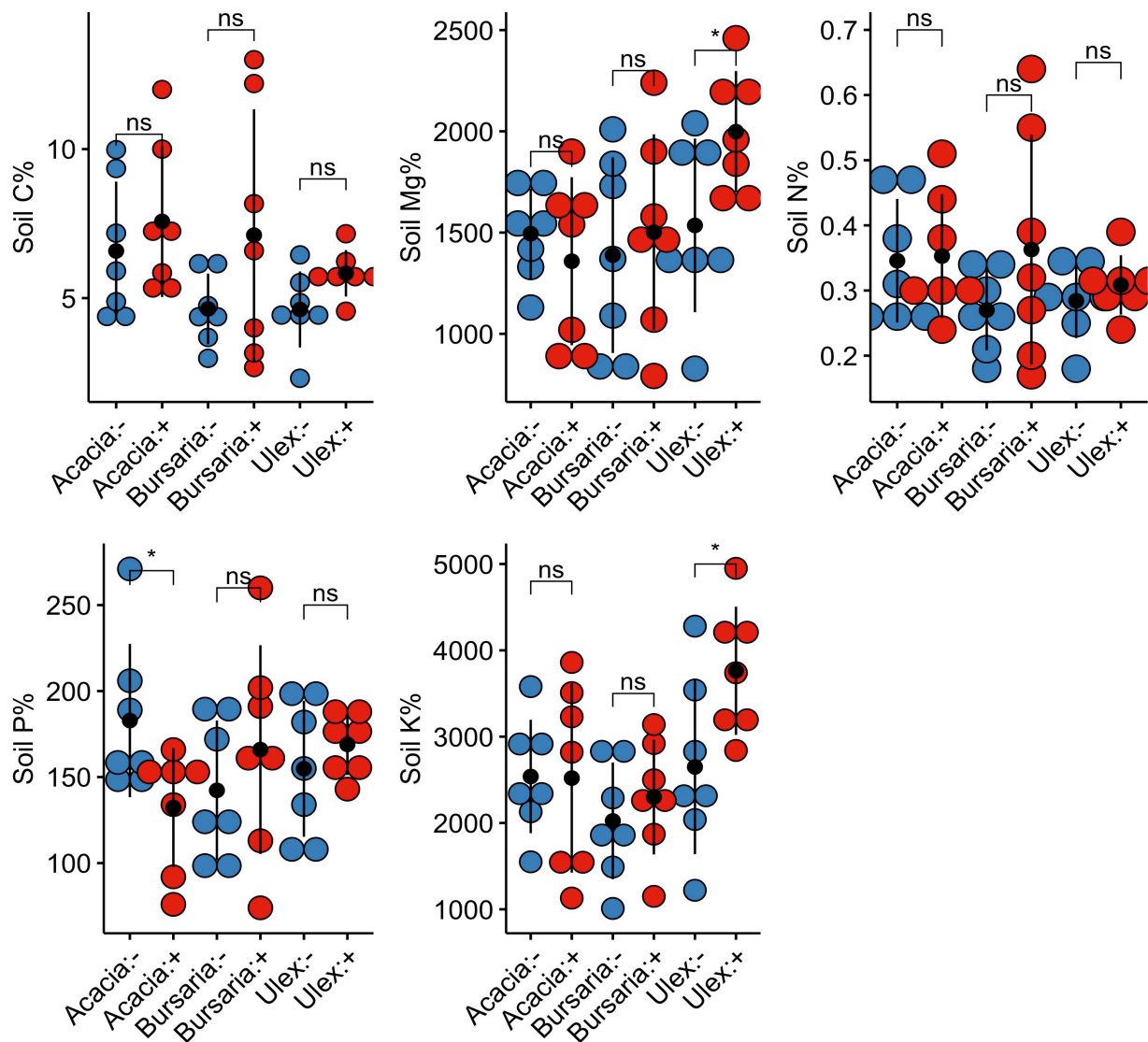
Figure 5: Soil nutrient levels of carbon (C %), magnesium (Mg %), nitrogen (N %), phosphorus (P %), and potassium (K %) under *Acacia pycnantha*, *Bursaria spinosa*, and *Ulex europaeus*, infected with *Cassytha pubescens* (red +) or uninfected (blue -). Comparisons display Tukey's adjusted *p*-values within species only. Black dots show means, error bars = SD.

Table 5: Linear model outputs for effects of host species and *Cassytha pubescens* infection on soil nutrients, under native *Bursaria spinosa* and invasive *Ulex europaeus*.

	DF	Sum sq.	*F*-value	*P*-value
Nitrogen				
Infection	1	0.0242	1.497	0.229
Species	2	0.0374	1.160	0.325
Infection * Species	2	0.0129	0.398	0.674
Residuals	36	0.5809		
Carbon				
Infection	1	0.579	4.260	**0.0463**
Species	2	0.684	2.516	0.0949
Infection * Species	2	0.040	0.148	0.8631
Residuals	36	4.891		
Potassium				
Infection	1	2194286	3.208	0.08168
Species	2	7846490	5.736	**0.00688**
Infection * Species	2	2418700	1.768	0.18514
Residuals	36	24621886		
Magnesium				
Infection	1	226748	1.413	0.2424
Species	2	1026750	3.199	**0.0526**
Infection * Species	2	633502	1.974	0.1537
Residuals	36	5777497		
Phosphorus				
Infection	1	189	0.109	0.7434
Species	2	426	0.123	0.8847
Infection * Species	2	11431	3.297	**0.0484**
Residuals	36	62407		

Table 6: LM output of soil pH of the three studied species. $R^2 = 0.40$, F-statistic: 3.27 on 5 and 24 DF. Alpha = 0.05.

Coefficients	DF	Sum Sq	*F*-value	*P*-value
Species	2	0.816	4.26	**0.0261**
Infection	1	0.533	5.56	**0.0268**
Species * Infection	2	0.216	1.13	0.3395
Residuals	24	2.300		

*pH ~ Species * Infection*

Discussion:

Host and Parasite Litterfall:

Cassytha pubescens enhances soil returns of K and C under native and invasive hosts. This is the first investigation into whether leafless, rootless hemiparasitic vines can enhance soil nutrient return rates, a trait seemingly ubiquitous to mistletoes globally, as well as root-parasites. We did not find evidence to support our hypothesis that *C. pubescens* increases litterfall rates beneath host canopies. In fact we found that *C. pubescens* decreased invasive host litterfall but compensated by maintaining an equal overall mass of litterfall by shedding mainly fruits and flowers. Invasive *U. europaeus* had a strong reduction in annual litterfall (-37.6 ± 0.25%, Fig. 1 A, Table 1) while, the effect was weaker in annual litterfall in native *B. spinosa* (11.0% ± 0.25%, Fig. 1 A, Table 1). This most likely reflects the stronger negative effects *C. pubescens* has on invasive species compared with native species. We expected litterfall to be reduced in *U. europaeus* when infected because *C. pubescens* strongly decreases its growth (Cirocco *et al*. 2016; 2017). When considering the litter contribution from *C. pubescens* and its hosts, *U. europaeus* shrubs had no difference in total litterfall when infected or not (Tukey' *adj. p* = 0.9, Fig. 1), and neither did *B. spinosa* (Tukey's adj. *p* = 0.5, Fig. 1 B). These results contrast with those found by March and Watson (2007) who found that the mistletoe *Amyema miquelii* Tiegh. drastically increased total litterfall rates beneath eucalypt hosts. However, eucalypts hosting *A. miquelii* and uninfected eucalypts did not differ in leaf turnover rates. Therefore, the input solely from mistletoe led to increased total litterfall and increased soil nutrient returns (March and Watson 2007, 2010). However, Mellado *et al*. (2016) found that *Viscum album* (Wiesb.) infection decreased host litterfall in pines, but total litter input was greater for infected pine trees. While mistletoes may decrease host litterfall or not, they appear to consistently increase total litterfall (March and Watson 2007; Ndagurwa *et al*. 2013; Ndagurwa, Dube, and Mlambo 2014; Mellado *et al*. 2016). We suspect this is largely due to the high leaf-turnover rates of mistletoes, whereas *C. pubescens* is leafless and

does not readily shed tissues other than fruits and flowers (pers. observation). Thus, we can conclude that *C. pubescens* does not contribute to soil nutrient returns by increasing litter input, unlike other parasitic plants – as can be expected due to its leafless nature in comparison to high-rate leaf-turnover of mistletoes.

Cassytha pubescens field decomposition:

In our study assessing *C. pubescens* decomposition rate in the field, we found that *C. pubescens* stems may decompose more slowly than tissues from other parasitic plants. This suggests that upon the death of host and parasite, the large mass of litter from *C. pubescens* may return soil nutrients relatively slowly. We found that *C. pubescens* litter lost 44.42% ± 0.68% of initial N content after 4.8 months (Table 3). In contrast, Demey, Staelens, *et al.* (2013) found that within two months of decomposition, litter of *R. angustifolius* and *Pedicularis sylvatica* released ~45% of total N. After 231 days in the field, *C. pubescens* litter had lost approximately half of its mass (Fig. 2, 54.3% ± 1.8% SE), whereas *R. angustifolius* and *P. sylvatica* lost > 80% and > 55%, respectively after similar timeframes (Demey, Ameloot, *et al.* 2013). Litter quality, as approximated by C:N ratios, does not explain these differences in mass-loss rates. *Cassytha pubescens* had a similar, if not lower, C:N ratio (22.14 ± 3.6 SD, annual mean) as the rapidly decomposing *R. angustifolius* and *P. sylvatica* (*R. angustifolius* = 22.1 - 27.2; *P. sylvatica* = 24.6 - 27.3, Demey, Ameloot, *et al.* 2013). Compared with other parasitic plants or its host species (e.g. *U. europaeus*, Magesan *et al.* 2012) the relatively slow decomposition rate of *C. pubescens* is likely due to the abundance of secondary metabolites in the tissues of *Cassytha* species inhibiting bacterial breakdown. *Cassytha pubescens* contains alkaloids and phenolic compounds (Johns and Lamberton 1966; Johns *et al.* 1966) that inhibit or decrease breakdown by decomposer microorganisms (Anderson 1973; Ormeño *et al.* 2006; Palm and Sanchez 2016). It appears that unlike other

parasitic plants, *C. pubescens* litter may not contribute significantly to soil nutrient returns by shedding high-quality litter, given its slow decomposition relative to other parasitic plants, at least within one year of decomposition. While we found that *C. pubescens* decomposes more slowly than other parasitic plants, environmental factors (temperature, annual rainfall) may also account partially for those differences. Nonetheless, *C. pubescens* litter may contribute to soil nutrient returns by augmenting the diversity of organic matter input (Muvengwi *et al*. 2015; Mellado *et al*. 2016), potentially accelerating decomposition rates and enhancing nutrient cycling, when in combination with other litter (Quested *et al*. 2002; Spasojevic and Suding 2011).

Soil nutrients:

We found evidence to support our hypothesis that *C. pubescens* can increase soil nutrient levels beneath native and invasive host species, but these effects were less dramatic than those reported for other parasitic plants (Quested, Press *et al*. 2003*;* March and Watson 2007; 2010; Mellado *et al*. 2016). This results also suggest that upon the death of host and parasite, the large mass of litter from *C. pubescens* may slowly return soil nutrients. *Cassytha pubescens* infection only changed the soil nutrient levels of C, P, K and Mg, but we found no effect on soil N (Fig. 5, Table 5). Most of these effects, however, were host-specific. These results support the generalisation of parasitic plants forming fertile and spatially heterogeneous soil patches that may have substantial consequences in arid, oligotrophic systems.

Overall, we found infected shrubs had higher soil C levels than uninfected shrubs (Fig. 5), regardless of host species. The observed increase in soil C levels under *C. pubescens* infected shrubs may be due to increased input of *C. pubescens* flower and fruit litter (Fig. 1). The flowers of *C. pubescens* had C levels of 45.25%, and given flowers made up the majority of litterfall contributions from parasites, this may explain the increase soil C under infected

plants. In agreement with our results, the soil beneath eucalypts infected with *A. miquelii* had greater soil C levels than uninfected eucalypts (March and Watson 2010). Similarly, pine trees infected by *V. album* had greater soil C levels than uninfected trees (Mellado *et al.* 2016). In both studies, mistletoes increased total litterfall beneath their hosts. In contrast, we did not observe a statistically significant increase in total litterfall beneath infected shrubs. However, *C. pubescens* infection may have increased the frequency of extreme litterfall events in both host species (Fig. 1), contributing to increased soil C returns. Alternatively, the presence of *C. pubescens* may produce a more complex structure that favours the accumulation of materials transported towards the patch.

We did not find any difference in soil N levels between infected and uninfected shrubs (Fig. 5). This differs from studies on mistletoes (Santalaceae, Loranthaceae), rattles (Orobanchaceae) and morning-glories (Convolvulaceae) (Quested, Press, *et al.* 2003; Yu *et al.* 2009; March and Watson 2010; Ndagurwa, Dube, and Mlambo 2014). Although N is not enriched in the foliage of *A. miquelii*, its high leaf turnover rate can increase soil N returns (March and Watson 2010). However, similar to our results, Mellado *et al.* (2016) did not find greater amounts of total N, under pines parasitised by *V. album*, despite increasing litterfall (Mellado *et al.* 2016). In comparison with mistletoes, *C. pubescens* stem litter had N levels greater than leaves of either *A. miquelii* (0.76% ± 0.032%, Supp. Info, March and Watson 2007) or *V. album* (1.47% ± 0.17%, Mellado *et al.* 2016), with a mean of 2.3% ± 0.54% for fresh and senescent litter (Table 3, Fig. 3). The flowers of *C. pubescens* had similar N concentration to that of stems, N = 2.88%. This suggests that parasitic plants may also increase soil N returns by depositing high quantity, rather than just high-quality litter. However, the holoparasitic vine *Cuscuta campestris* (Convolvulaceae) is similar to *C. pubescens* in life form – both being leafless vines. However, the soil beneath *Cu. campestris*-

infected individuals had greater soil N after two years, which may be due to decreased root mass, and consequently decreased resource capture potential (Yu *et al*. 2009). While it appears *Cu. campestris* can enhance soil N returns via host effects, *C. pubescens* does not. This may be due to the difference in N-budgets for holoparasitic *Cuscuta* spp. versus hemiparasitic *Cassytha* species, requiring N for photosynthesis. These differences in parasite traits (leafless *vs*. high leaf turnover) may have ecological consequences for community structure (Demey, Staelens, *et al*. 2013; Fisher *et al*. 2013).

We found species-specific effects in soil P for one of the studied species. *Acacia pycnantha* shrubs had lower soil P levels when infected; however, *U. europaeus* and *B. spinosa* did not differ in soil P whether infected or not (Fig. 5). This contrasts with patterns observed in mistletoes having P-enriched tissues which substantially increase soil P returns (March and Watson 2010; Ndagurwa, Dube, and Mlambo 2014; Muvengwi *et al*. 2015; Mellado and Zamora 2016). Fresh stem litter of *C. pubescens* has P levels of 0.083% ± 0.05% (Fig. 3), which is much smaller than P levels in mistletoe tissues (~ 0.25% ± in *Viscum album*, Mellado *et al*. 2016; 0.1% ± in *A. miquelii*, March and Watson 2010). While intrinsic parasite P-levels may account for the difference in soil P returns between mistletoes and *C. pubescens*, host effects may also play a role in differences of P returns. However, as we found that *C. pubescens* does not readily shed stem-tissues, these are unlikely to contribute to soil fertility. Nitrogen fixation by rhizobia in root-nodules is a P-demanding process (Magesan *et al*. 2012). It is possible that infected *A. pycnantha* shrubs had lower soil P levels because of increased P-demand than uninfected shrubs because of parasite removing resources (Cirocco *et al*. 2021). However, *B. spinosa*, in contrast, does not fix nitrogen and may not have increased P demand when infected. While this does not explain why *U. europaeus* did not have any difference in soil P when infected or not, this may be because P becomes immobilised beneath

U. europaeus canopies (Dewar *et al*. 2006). P can be a strong limiting factor in the growth of Australian plants, particularly in sclerophyllous habitats (Vitousek *et al*. 2010). Furthermore, increasing P supply may have important consequences for legumes, as they are not limited by N but may be by P (Fisher *et al*. 2013). This suggests that *C. pubescens* may have some indirect effects on soil nutrients by reducing soil P returns beneath infected *A. pycnantha* shrubs.

Ultimately, these changes in soil nutrient returns by parasitic plants may have consequences for the structure of ecological communities. In the case of *C. pubescens*, the parasite appears to have small effects on soil nutrients and may have little impact on soil nutrient cycling. Although parasitic plants may have less influence on nutrient cycling than herbivores (Pennings and Callaway 2002), parasitic plants sustain these returns over their lifetime (March and Watson 2010). Also, combined with their patchy distribution, parasitic plant may increase the small-scale spatial heterogeneity of resources and other processes (Watson and Herring 2012; Mellado and Zamora 2014; Opoku *et al*. 2020).

Conclusion:

Contrary to our expectations, *C. pubescens* did not significantly increase total litterfall under native or invasive host species. We attribute this to the different life form and traits of this leafless parasitic plant which do not enable it to shed litter *en masse* like mistletoes. Nonetheless, we still found that it decreased litterfall rates beneath its invasive hosts, but not its native host. While *C. pubescens* did not increase the total litterfall rate and did not contribute nutrient returns of the same magnitude as mistletoes, substantial amounts of nutrients, however, may leech into the soil after the parasite's death, which occurs often after the death of the host. Soil nutrients are highly limiting for plant growth in Australia. Cycling rates determine

productivity, function, and other factors. *Cassytha pubescens* appears to increase the frequency of litterfall extremes in hosts, with important long-term consequence. Changes in litterfall are important for undergrowth (Demey, Staelens, *et al.* 2013; Ndagurwa, Dube, and Mlambo 2014; Mellado *et al.* 2016) and can have bottom-up effects (Watson 2009; Watson and Herring 2012). While we did not find the expected contribution to soil fertility from dead stems, we have observed that after host death, massive amounts of dead *C. pubescens* stem material are present. In these patches litter deposition may be substantial and may form patches of high nutrient levels. This should be addressed in future studies better to understand nutrient dynamics and responses of different plant species (native and invasive) in these patches.

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Supplementary Information:
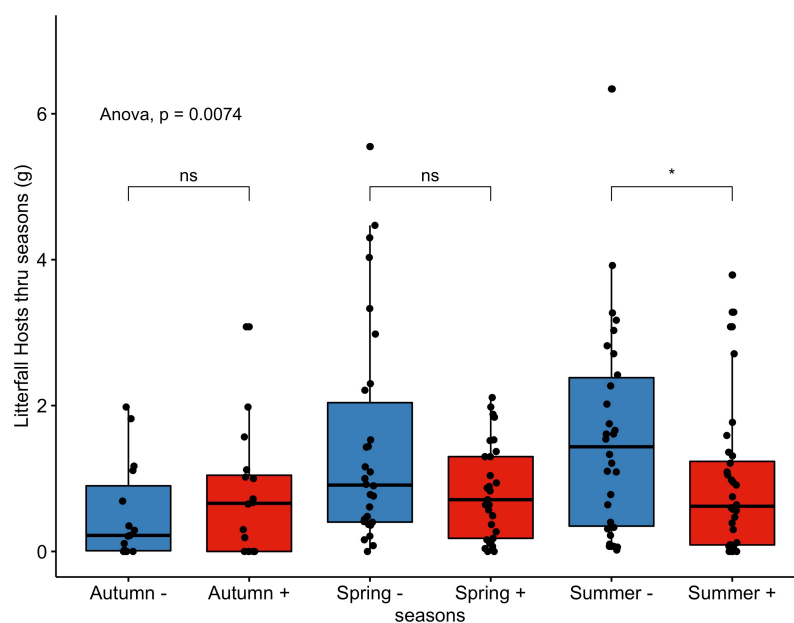
Supp. Fig. 1: Litterfall of infected (red) and uninfected (blue) shrubs, infected (+) or not (-) in summer, autumn and spring. Winter sampling could not be completed due to COVID-19 and protocols in place for the school.
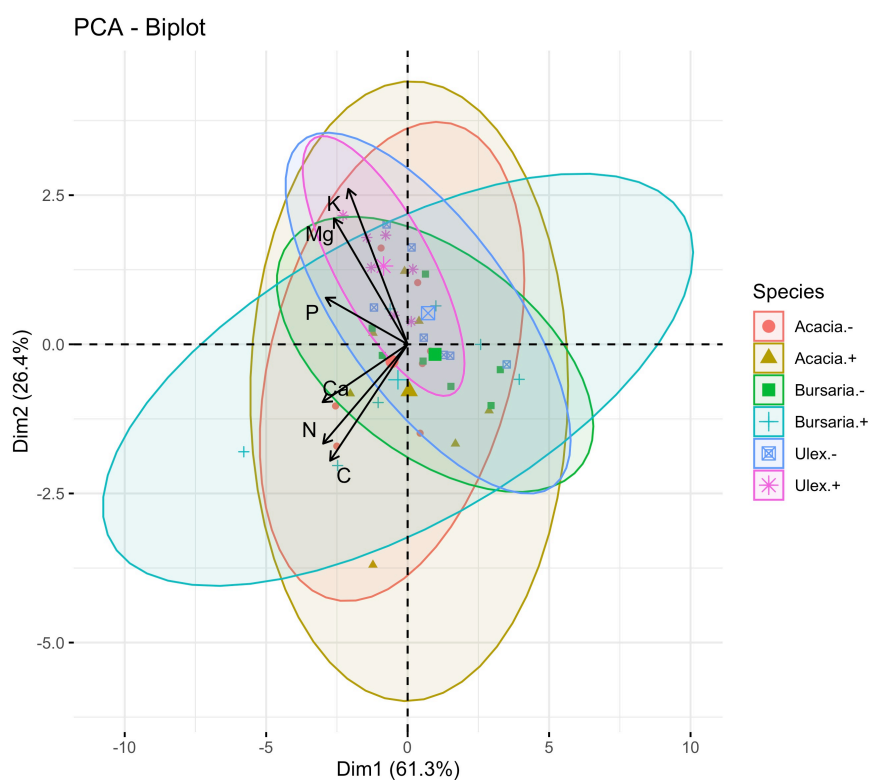
Supp. Fig. 2: Principal Component Analysis biplot of soil nutrients, of soil sampled under native plant species *Acacia pycnantha*, *Bursaria spinosa*, and invasive species *Ulex europaeus*. Soils were sampled under plants infected by *Cassytha pubescens* as well as uninfected plants. displaying the two first principal components. PC 1 on X axis accounted for 61.3% of variance in soil nutrient concentration, PC 2 accounted for 26.4% of variance. This plot shows that all three species had similar nutrient profiles when infected or not by *C. pubescens*.
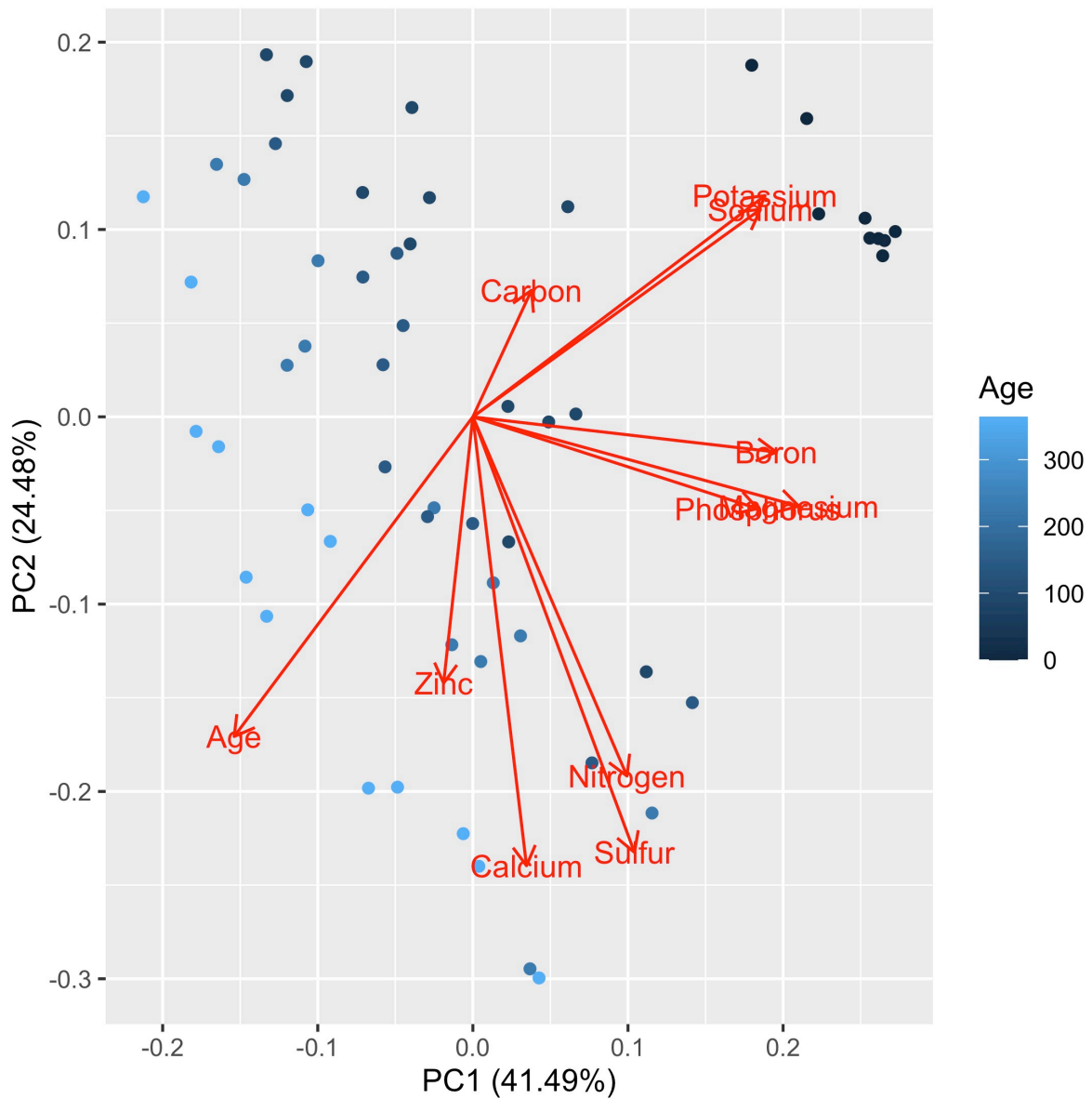
Supp. Fig. 3: Principal Component Analysis of *Cassytha pubescens* litter nutrient content and its relationship with age of litter. PC 1 accounted for 41.5% of all variance in data, and PC 2 accounted for 24.5% of variance in data. Generally, small cations levels in litter (K, Na) decreased with increasing age of litter. In contrast, less mobile elements (N) changed less drastically with age.

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Statement of Authorship

Title of Paper	Australian native parasitic plant soil indirectly increases growth of invasive species, but its litter decreases invasive seedling emergence.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted all fieldwork, all labwork, all statistical analyses and writing.

Principal Author

Name of Principal Author (Candidate)	Bernardo J. O'Connor
Contribution to the Paper	Conducted all fieldwork, all labwork, all statistical analyses and writing.
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 20/11/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Adviced on experimental design, statistical analysis, and helped write the manuscript.
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Contribution to the Paper	Helped with drafting process, data interpretation, and supervised development of work.
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Chapter 3: Australian-native parasitic plant soil indirectly increases growth of invasive species, but its litter decreases invasive seedling emergence.

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Abstract:

Parasitic plants can have disproportionately large ecological effects on their biotic and abiotic environment, despite being minor components of ecological communities. *Cassytha pubescens*, a hemiparasitic vine native to Australia, forms large masses of growth on invasive hosts. We tested whether *C. pubescens* indirectly influenced seedling emergence and growth via soil effects and/or its litter. We performed a factorial glasshouse experiment, with seeds of six species sown in soil taken under infected or uninfected shrubs, and adding *C. pubescens* litter or not. We found that the hemiparasite had an indirect negative effect on seedling emergence of some native and invasive species; and only the invasive *U. europaeus* grew larger in infected shrub soil. *Cassytha pubescens* litter reduced the emergence rates of the leguminous species studied, having the strongest effect on the invasive seedlings. These findings reflect the effect of *C. pubescens*, which like other parasitic plants, modifies soil conditions beneath hosts. Litter effects may result from allelopathic properties, however they seemingly are species specific. In this study we demonstrated that the effects of parasites can extend beyond their host, highlighting their potential to affect ecological structure. Understanding the influences of *C. pubescens* directly applies to its use for biocontrol of invasive plants, which is currently being trialled.

Introduction:

Parasitic plants can have disproportionately large effects on ecological community processes and function, compared to other plants (Pennings and Callaway 2002; Wood *et al.* 2007; Ameloot *et al.* 2008; Hatcher and Dunn 2011; Fisher *et al.* 2013; Hartley *et al.* 2015). Parasitic plants therefore play prominent roles in several ecosystem processes (Press and Phoenix 2005; Mathiasen *et al.* 2008), which is remarkable for small, subordinate components of ecological communities (Hartley *et al.* 2015). Parasitic plants may alter trophic energy flow (Lafferty *et al.* 2006), the distribution of resources (Bardgett *et al.* 2006), and competitive hierarchies among host species (Pennings and Callaway 2002; Demey, Staelens, *et al.* 2013; Těšitel *et al.* 2017, 2018). While the direct effects of parasitic plants onto hosts are relatively well understood, the effects of their litter input and soil changes on local plant assemblages are not understood. In this study, we focused on the indirect effects a parasitic plant may induce on seedling emergence via litter and soil modifications.

Parasitic plants can drastically increase litter input beneath their hosts, and this increased amount of litter can in turn form fertile and spatially heterogeneous soil patches. Plant litter can have beneficial effects on vegetation, providing microhabitats (Facelli and Pickett 1991), increasing habitat complexity (Mooney *et al.* 2006), and enhancing soil nutrient returns (Quested, Press, *et al.* 2003; March and Watson 2007; Demey, Staelens, *et al.* 2013). Tissues of parasitic plants typically have relatively high nutrient contents in their tissues (March and Watson 2007, 2010; Demey, Staelens, *et al.* 2013). Root and stem hemiparasites (*e.g.* rattles, mistletoes) tissue deposition and decomposition can enhance soil nutrient returns and enhance understorey productivity (Quested, Press, *et al.* 2003; Bardgett *et al.* 2006; March and Watson 2010; Demey *et al.* 2014; Ndagurwa, Dube, Mlambo, *et al.* 2014). The local increase in soil fertility by litter deposition is a key pathway through which parasitic plants indirectly affect

plant community composition (Demey, Staelens, *et al*. 2013; Demey *et al*. 2014). Augmentation of soil nutrient inputs may have greater consequences on plant assemblages in oligotrophic systems (Quested 2008; Spasojevic and Suding 2011; Ndagurwa, Dube, and Mlambo 2014). Studies of hemiparasitic litter feedbacks on coexisting plant species are few, and those on leafless stem hemiparasites are even more limited. Yet, there is compelling evidence of parasitic plants driving change in soil nutrients from glasshouse and field experiments (Quested, Press, *et al*. 2003; March and Watson 2007; Demey, Stealens, *et al*. 2013; Ndagurwa *et al*. 2013).

Parasite litter production can be substantial, and that litter can decompose rapidly, contributing to nutrient enrichment in localised patches. Litter accumulation can drastically increase under infected plants compared with uninfected plants (March and Watson 2007; Ndagurwa *et al*. 2013; Mellado *et al*. 2016). Trees infected with mistletoe can have drastically increased litterfall beneath their canopies, increasing soil returns of N and P (March and Watson 2007; Ndagurwa *et al*. 2013). As well as adding large amounts of nutrients, these additions may occur in short term timescales as parasitic plants tissues may decompose rapidly, increasing nutrient cycling rates (Demey, Staelens, *et al*. 2013; Demey *et al*. 2014). Pot based bioassays and field studies suggest that parasites accumulating litter, and therefore soil nutrients, may increase the growth of coexisting plants (Quested, Press, *et al*. 2003; March and Watson 2007; Demey, Staelens, *et al*. 2013). Trees with greater mistletoe biomass also may have greater understorey plant biomass (March and Watson 2007; Ndagurwa *et al*. 2016). Similarly, the nutrient-rich litter of hemiparasitic *Bartsia alpina* L. increased the growth and nutrient content of coexisting plant species by up to 51% in *Betula nana* L., compared with pots that had shrub litter added (Quested *et al*. 2003). How holoparasitic vines influence soil nutrients is less studied (Yu *et al*. 2009), but we know nothing about hemiparasitic vines and their influence on soil nutrients.

Cassytha pubescens R. Br. does not share the same traits as other parasitic plants that enhance nutrient cycling. Unlike mistletoes, it does not have leaves, and therefore has less potential to contribute to litterfall. Unlike other leafless parasites (*e.g. Cuscuta* spp.), *C. pubescens* is capable of photosynthesis. Furthermore, unlike root-hemiparasites (*e.g. Rhinanthus* spp.) *C. pubescens* has no contact with soil and cannot derive soil nutrient, since it has no roots when mature. The combination of these traits and its effects on soil nutrients has not been previously investigated.

Parasitic plants can benefit coexisting plant species by soil nutrient enrichment and strongly parasitising superior competitors, which may results in changes in local species assemblage composition (Těšitel *et al*. 2017, 2018). Furthermore, parasitic plant litter may also induce indirect negative effects on plant seedlings. The presence of litter can enhance water availability by reducing evaporation and reducing thermal amplitude, forming microhabitats beneficial to germination (Facelli and Pickett 1991; Rotundo and Aguiar 2005). However, plant litter can also act as a mechanical barrier to emerging seedlings, particularly under trees with quick leaf turnover preventing establishment (Facelli and Pickett 1991). By withdrawing solutes from their host, parasitic plants may accumulate or produce abundant secondary metabolites (Schädler *et al*. 2005). These biologically active secondary metabolites in plant litter may also negatively affect other plants by inhibiting seed germination (Wink 1983), presumably decreasing recruitment. Secondary metabolites sequestered from host solutes and these compounds may thus have ecological significance (Bouwmeester *et al*. 2003).

Parasitic plants may be useful in the biocontrol of dominant and overabundant species, such as invasive weeds because of their negative effects on hosts. However, before attempting to manipulate their abundance in the field we must understand their direct and indirect effects on

the recruitment of local plant assemblages. For instance, greater levels of soil nutrients under infected plants may increase the growth of fast growing species (Demey, Staelens, *et al.* 2013), including the growth of *Ulex europaeus* L. (Hartley and Thai 1982) aiding its establishment. However, fertile soil may lead to lower survival of *U. europaeus* seedlings by increasing competitive intensity from other plants (Thompson 1974; Ledgard 2006). Small reductions in seedling density of *U. europaeus* may not be beneficial since it can re-establish from a few individuals and establish dominance quickly (Hartley and Thai 1982; Ledgard 2006). Increasing soil nutrients may increase ground cover of grasses and other vegetation, which in turn can supress *U. europaeus* seedlings by root-competition (Ivens and Mlowe 1980; Davies *et al.* 2005; Delerue *et al.* 2018) and shading (Salisbury 1929). *Cassytha pubescens* R. Br. 1810 (Lauraceae) is a hemiparasitic vine native to Australia. In both glasshouse and field settings, *C. pubescens* can negatively afflict invasive host physiology more severely than it does native host species (Prider *et al.* 2009; Cirocco *et al.* 2017). Infected invasive plants infected have less photosynthetic biomass, lower transpiration rates, lower photosynthetic rates as well as smaller root nodule biomass. While these effects are severe for invasive plants, native plants have minimal to no impact from *C. pubescens* infection (Prider *et al.* 2009; Cirocco *et al.* 2016, 2017). However, *C. pubescens* may also negatively affect coexisting plant species via allelopathic effects of alkaloids and secondary metabolites (Johns and Lamberton 1966; Johns *et al.* 1966; Wu *et al.* 1997; Brophy *et al.* 2009) that may inhibit germination even at low concentrations (Wink 1983; Roberts and Wink 2013). If we deploy *C. pubescens* in the field to target invasive plants, we need to understand the positive and negative indirect effects of *C. pubescens* on coexisting host and non-host species. Indeed, when the effect of the parasite overwhelms the host and it dies, the parasite attached to the host also dies, producing a substantial amount of stem litter. It is not clear what role this parasitic litter may plays in the succession of native and invasive plants; both native and invasive plant species could be

hindered by litter in the patches. To assess the indirect effects of the parasite via litter and soil nutrient enrichment we tested the prediction that seedling emergence and growth will be lower from soil from infected shrubs compared to uninfected shrubs.

Methods:
Study site:

Soil sampling for the study took place in Belair National Park (35.02123° S, 138.67355° E), in the Mount Lofty Ranges of South Australia. The Range has a Mediterranean-type climate with cold, wet winters and hot, dry summers (Rainfall 990 mm per annum, max mean temp = 22.6°C, min mean temp = 5°C, Bureau of Meteorology 2021). The area is a woody shrubland dominated by *Eucalyptus obliqua* (L'Her) in the overstorey, an understorey including small and large shrubs (Fabaceae, Sapindaceae, Pittosporaceae), and diverse low-lying vegetation (Asteraceae, Droseraceae). Invasive species can be locally prominent, particularly *U. europaeus* (Fabaceae) which forms dense monospecific patches. All fieldwork for this study occurred in October 2018.

Soil sampling:

To assess whether *C. pubescens* indirectly changed the growth of native and invasive seedling via soil effects, we took soil samples under infected and uninfected shrubs in the field. These samples were taken directly below uninfected and infected individuals of the native species *Acacia pycnantha* Benth., *Bursaria spinosa* Cav., and the invasive species *U. europaeus*, within a 1 km stretch in Belair NP. All shrubs were approximately equal in size and infected shrubs had approximately equal *C. pubescens* load. To obtain soil beneath shrubs, rocks and litter were cleared within 20 cm from host stem and 10 cm deep samples were extracted. In the laboratory, individual shrub samples were further cleared of organic debris, arthropods, and rocks. Soil samples from under infected or uninfected shrubs were pooled and homogenised to

remove species-specific effects and focus only on the broader effects of the presence/absence of *C. pubescens*. The two soil types (infected or uninfected hereafter) were then used to fill aluminium trays for the emergence and growth bioassay.

Plant species:

The six species chosen for the bioassay co-exist with *C. pubescens*, including both native and invasive plants. Some of these are immune to *C. pubescens* infection (*e.g. Acacia myrtifolia* Willd., Fabaceae). *Eucalyptus obliqua* L. Her. (Myrtaceae) probably cannot be infected by *C. pubescens*, however it is included in this study given its dominance and importance in Mt. Lofty Ranges. *Acacia pycnantha* was chosen as it is a common host of *C. pubescens* in the Mt. Lofty Ranges, as is *Bursaria spinosa* (Pittosporaceae) is a medium-sized shrub that can host large loads of *C. pubescens* in the field. *Dodonaea viscosa ssp. angustissima* J.G. West (Sapindaceae) is a native non-leguminous host of *C. pubescens*. The invasive host species chosen were *U. europaeus* and *Cytisus scoparius* L. Link, both are nitrogen fixers (Fabaceae) and often host *C. pubescens* in Mt. Lofty Ranges. *Ulex europaeus* has negative economic and environmental impacts in Australia, and globally, making *U. europaeus* one the world's 100 worst invasive weeds (Lowe *et al*. 2000) and are their eradication is of high priority.
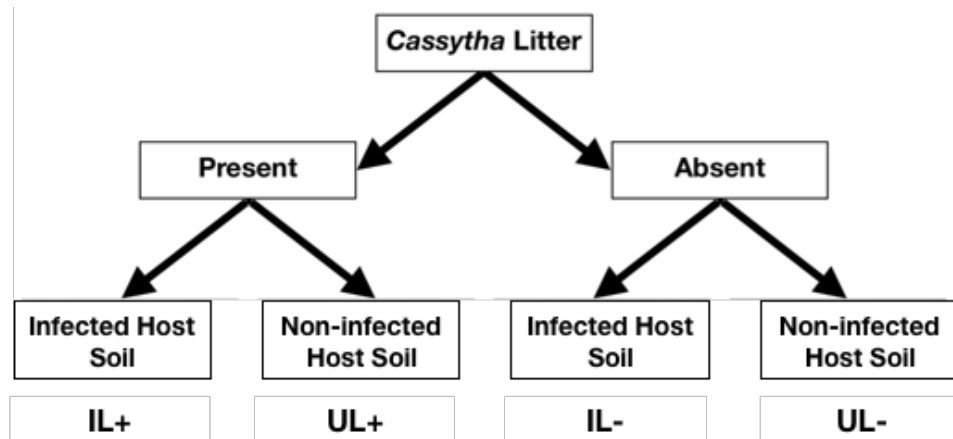
Figure 1: Diagram showing fully-crossed factorial design, treatments consisted of **IL+** = infected host soil with litter, **IL-** = infected host soil without litter, **UL+** = uninfected host soil with litter, and **UL-** = uninfected host soil without litter.

Emergence and Growth bioassay:

The experiment was conducted at the Benham glasshouses, at The University of Adelaide. The emergence and growth bioassay used 40 aluminium trays (17.5 x 9.5 x 4.5 cm) with holes in the bottom for drainage and filled with infected or uninfected soil and covered or not with *C. pubescens* litter, according to randomly assigned blocks and treatments (10 trays per treatment in 10 blocks, see Fig. 1). We randomly assigned treatment into blocks to control for small differences in light and irradiance conditions within the glasshouse. Live *C. pubescens* masses had been collected from infected *U. europaeus* in the field, air dried for 7 days, and then cut ~5 g to fit into trays for litter present (L+) treatments. Soil was brought to field capacity and seed sown into rows with approx. 3 cm apart. Each plant species was sown in its own row with five sub-plots, in the same arrangement for all trays. For all species, we sowed 3-5 seeds per sub-plot, except for *E. obliqua* for which we sowed approximately ~ 0.5 g of seed. As required, the seeds of some seed species, to break dormancy, were soaked in recently boiled water the day before sowing or overnight.

The emergence of any new seedlings was recorded at the same time daily for all trays. An emergence event was defined as the day of penetration of a stem or seed head through the soil. All seedlings were marked and identified and labelled with species identification, and the day emerged. Unwanted species (*i.e.* seedlings emerging from the soil seed bank, rather than sowed seeds) were removed upon identification (*e.g.* blackberry, *Rubus sp.*). Trays were watered daily to keep the soil moist. The glasshouse was air-conditioned at maintain a temperature below 26°C during summer.

Biomass measurements:

After 189 days from the start of the experiment, seedlings were gently removed from trays and washed using water, retaining as much intact root biomass as possible. Seedlings were then put on a tray and placed in an oven at 60° C for 24 hours, after which dry weight biomass was measured. Out of the 939 seedlings that emerged during the bioassay, 780 were included in these analyses. The remaining 159 seedlings were excluded due to age uncertainty, root/shoot damage, or death prior to harvest.

Statistical analyses:

The analyses of data from biomass and seedling emergence were performed through ANOVA and linear model (LM) fits in R studio statistical software (R Core Team 2016). To assess differences in biomass, we used dry biomass (g) as a response variable, with soil origin and litter presence, and age (no. days between emergence and harvest) as explanatory factors and blocking as a random error term. The normality of residuals in linear models and ANOVAs were assessed through Shapiro-Wilk test function. We assessed homogeneity of variances through and F-test and Bartlett's test functions. When data did not satisfy normal distribution assumptions, square-root transformations were applied due to negative-skew and checked

again using the Shapiro-Wilk test. Effect sizes were calculated as Cohen's *d*, a standardised effect size calculation for comparing mean differences between treatment and control groups, standardised by dividing mean differences by pooled standard deviation ('*effsize*' package, Torchiano 2020). To conduct Tukey's HSD posthocs, we used the pairwise comparisons on the R package *emmeans* (Lenth 2019). Plots were made using package *ggpubr* (Kassambara 2020).

To assess differences in emergence patterns, we used generalised linear model functions using quasi-poisson log-link functions (*glm*) in R. The cumulative seedling count was used as the response variable, with soil origin (under infected host or not) and litter (present or absent) used as explanatory factors. For post-hoc comparisons within species, we used Tukey contrasts (generalised linear hypothesis testing function). We used the package '*DaBestR*' (Ho *et al*. 2019) to compute bootstrapped 95% confidence interval, set with random set-seed and 5000 iterations.

Results:
Seedling emergence:

We did not find an interactive effect of soil origin and litter on seedling emergence (GLM: $p > 0.2$, Table 1). Similarly, soil origin did not affect the emergence of any of the species studied (GLM: $p > 0.9$, Table 1). There was a negative effect of the litter on emergence: fewer seedlings emerged in treatments with added *C. pubescens* litter (GLM: $p < 0.001$, Table 1). However, these effects were species-specific. *Ulex europaeus* had a decrease of 27% in seedling emergence when *C. pubescens* litter was added (posthoc GLM: $p < 0.05$). *Acacia pycnantha* had a 29% decrease in emergence, and *A. myrtifolia* had a 36% decrease in emergence due to *C. pubescens* litter (Fig. 2). In contrast, the emergence of *C. scoparius* and *E. obliqua* was not affected by presence of *C. pubescens* litter.
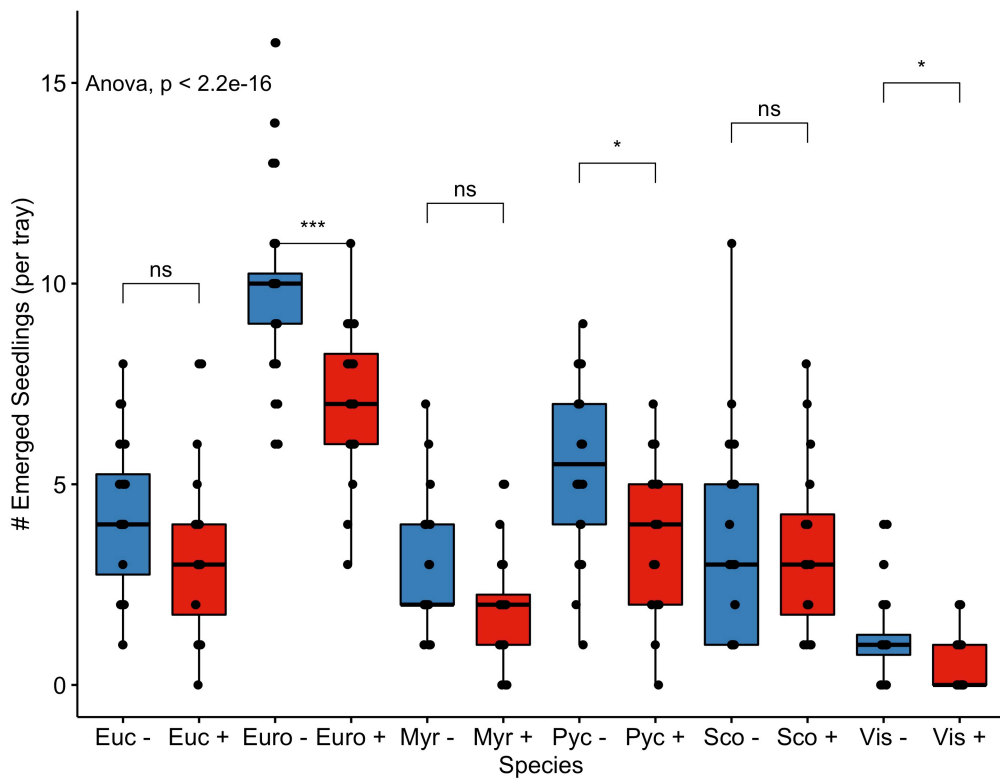
Fig. 2: Seedling emergence of six species; *Eucalyptus obliqua (*Euc)*, Ulex europaeus* (Euro)*, Acacia myrtifolia* (Myr)*, Acacia pycnantha* (Pyc)*, Cytisus scoparius* (Sco)*, and Dodonea viscosa* (Vis). Emergence was assessed in trays filled with soil from under infected or uninfected hosts (not shown in here), with added parasitic *C. pubescens* litter (**red**, **+**) or not (**blue**, **-**). Seedling emergence per tray did not differ by soil origin, but parasite litter decreased the emergence of invasive seedlings (see Table 1). Global *p*-value shown on top left corner.

Table 1: Analysis of deviance on seedling emergence. GLM, N = 240, Quasi-Poisson family, log Link function. $\chi^2(11) = 393.06$, p < 0.0001, Pseudo-R^2 (Cragg-Uhler) = 0.80, Pseudo-R^2 (McFadden) = 0.30. Significant effects in bold.

	Df	Deviance	Residual Df	Residual Deviance	$P(\chi^2)$
Null			239	618.38	
Litter	**1**	**23.74**	**238**	**594.63**	**1.213e^{-06}**
Soil	1	0.00	237	594.63	0.9741
Species	**5**	**346.46**	**233**	**248.28**	**2.2e^{-16}**
Soil*Species	5	7.06	221	235.58	0.2202
Litter*Species	5	4.44	226	242.64	0.4932
Litter * Soil	1	1.20	231	247.07	0.2749
Block	**1**	**6.69**	**231**	**241.59**	**0.009064**

*No. of seedlings ~ Litter * Soil * Species + block*

Seedling biomass:

We found no significant interactive effects between soil origin and litter on seedling dry mass (GLM: $p > 0.3$, Table 2). Seedlings grew larger in infected shrub soil (LM: $F = 13.029$, df =1, $p < 0.005$). However, post-hoc tests showed only seedlings of invasive *U. europaeus* grew larger in infected-shrub soil (Tukey's Adj. $p < 0.05$). *Ulex europaeus* grown in infected-shrub soil grew x1.18 times larger than *U. europaeus* grown in uninfected soil (Cohen's $d = 0.39$). Native and one invasive (*C. scoparius*) species did not differ in total dry biomass when grown in infected or uninfected shrub soils ($p > 0.05$, Fig. 3, Table 2). In the biomass of seedlings, the effect of *C. pubescens* litter was contingent on species (LM: $F = 1.767$, df = 5, $p < 0.005$). However, no single species had a differential response in biomass when litter was present or absent. As we were not interested in between-species differences in biomass (since different species are expected to grow differently), we did not consider this biologically significant. We used the number of seedlings per tray as an indicator of a competitive effect. In our model, seedling density accounted for less variance in biomass than did seedling age (LM: $F = 23.245$, df = 1, $p < 0.0005$).
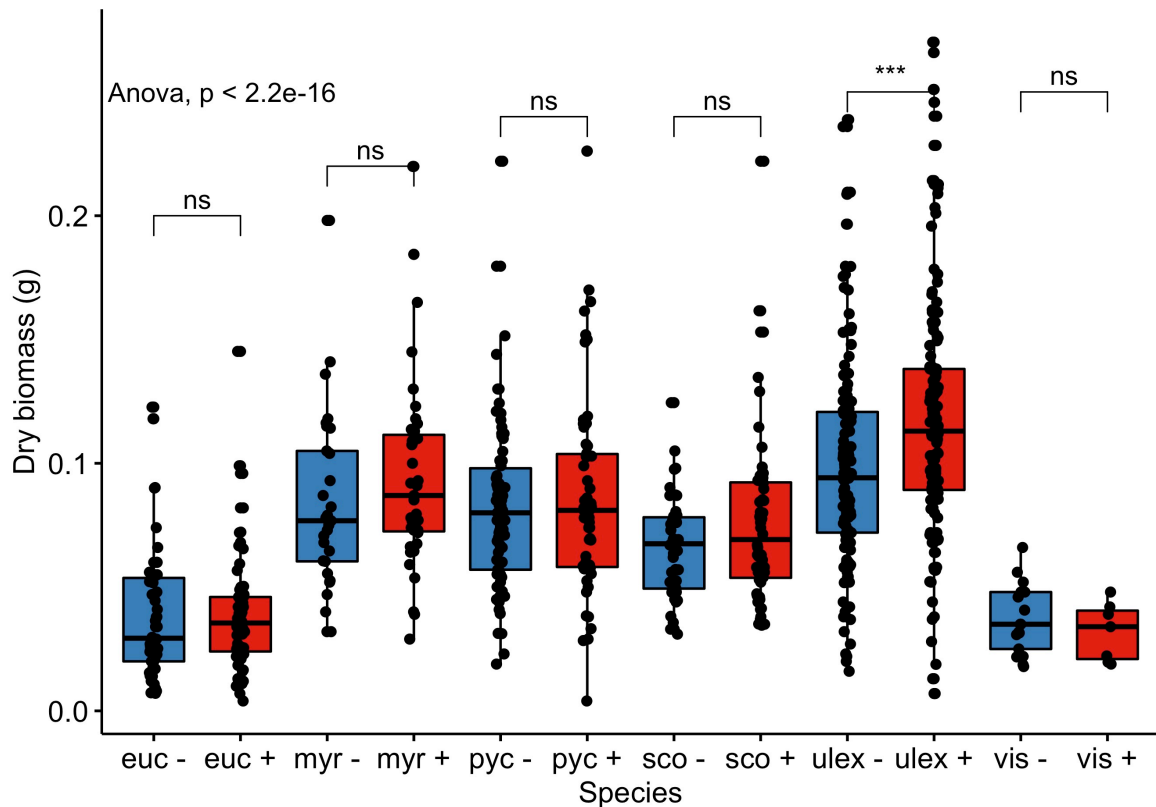
Figure 3: Plot of dry mass (g) for each species growing in infected shrub soil (red), or uninfected shrub soil (blue), in aluminium trays with (+) or without (-) added *Cassytha pubescens* litter. Black bars show median, error bars show SD.

Table 2: GLM output for seedling biomass (OLS). N = 714, *F*-statistic: 22.07 on 25 and 688 DF, R^2= 0.42, $p < 0.005$, Alpha = 0.05. Significant terms denoted in bold.

Sqrt(biomass)	Df	Sum sq	F-value	P-value
Soil	**1**	**0.0487**	**12.648**	**0.000329**
Litter	1	0.0066	1.767	0.184237
Species	**5**	**1.7102**	**91.575**	**< 2e-16**
Age	**1**	**0.1300**	**34.795**	**< 5.74e-09**
Count	**1**	**0.0868**	**23.245**	**1.76e-06**
Soil*Litter	1	0.0027	0.725	0.394958
Soil*Species	5	0.0167	0.897	0.432834
Litter*Species	**5**	**0.0441**	**2.364**	**0.038476**
Soil*Litter*Species	5	0.0152	0.815	0.539432
Residuals	689	2.6511		

*sqrt(dry mass) ~ Soil * Litter * Species + Age + Count*

Discussion:

Our results suggest that *C. pubescens* may alter plant assemblage composition not only through the direct effects of parasitism but it also through the indirect effects of seedling emergence and growth of other species via changes in soil nutrients and its litter. These results suggest that seedling emergence may be lower beneath and around infected shrubs (Fig. 2). There have been few studies on the emergence and growth of *U. europaeus*. However, even fewer studies have quantified and compared the biomass growth of *U. europaeus* in different conditions. In our study, only *U. europaeus* responded to the infected shrub soil (Fig. 3). Its larger biomass in this soil is in the presence of other seedlings, which is in stark contrast to other studies that found competitors supress the growth and emergence of *U. europaeus* (Ivens and Mlowe 1980; Davies *et al*. 2005; Ledgard 2006; Delerue *et al*. 2018). Although *C. pubescens* litter decreased overall seedling density per tray (Fig. 2), differences in the soil nutrients and early emergence contributed more to *U. europaeus* biomass than did seedling density per tray (Table 2). Invasive plant species may exploit small increases in nutrients better than native species (Demey, Staelens, *et al*. 2013). Thus, the difference in our results to those of other studies may be due to the life-forms of plants in competition with *U. europaeus*, since other studies used similarly aged *U. europaeus* seedlings. Grasses, the main competitors in the mentioned studies, tend to have vigorous growth and dense root systems that may outcompete *U. europaeus* seedlings (Ivens and Mlowe 1980), and generally, shrubs seedlings do not grow as rapidly as grasses. Alternatively, since *C. pubescens* litter decreased seedling emergence of several species, a reduction in interspecific and intraspecific competition may also explain the increase in growth of *U. europaeus* in treatments with fertile infected-shrub soil and *C. pubescens* litter. Taken together, these results suggest that under infected shrubs, *U. europaeus* seedlings emergence will decrease. However, they may have better growth due to small increases in nutrient levels (see chapter 2).

Not all species were affected equally by soil beneath infected shrubs or by *C. pubescens* litter. In contrast to *U. europaeus*, the other invasive species in our study, *C. scoparius,* grew no larger in infected-shrub soil and number of seedlings emerged did not differ with or without *C. pubescens* litter. This may be due to the greater resource use efficiency of *U. europaeus* compared the other species in our study. *Cassytha pubescens* litter supressed seedling emergence for some species, but the smallest seedlings (*E. obliqua*) were unaffected by the litter (Fig. 2, Fig. 3), which suggests that reduced emergence may have been due to chemical rather than physical effects. *Cassytha* species have diverse and abundant secondary metabolites (Brophy *et al*. 2009), including several groups of alkaloids (Johns and Lamberton 1966; Johns *et al*. 1966) that can inhibit the germination of seeds (Wink 1983). While our results suggest that the litter of *C. pubescens* inhibits seedling emergence, our treatments may underestimate its physical effects, as *C. pubescens* can form dense patches of litter upon its death.

Generally, nutrient inputs from parasitic plants tend to increase understorey diversity and biomass (March and Watson 2007; Ndagurwa, Dube, and Mlambo 2014; Hódar *et al*. 2018). However, the large volumes of litter input from parasitic plants, as well as biologically active secondary metabolites from parasite tissues, may supress seedling emergence. Although *C. pubescens* litter decreased *U. europaeus* emergence, the *U. europaeus* seedlings grew larger in infected-shrub soil. Unchecked, *U. europaeus* may have increased establishment rates due to faster seedling growth in these nutrient-rich patches, further exacerbating its spread.

To the best of our knowledge parasitic plant litter influencing seedling emergence was a largely unexplored effect prior to this study. Much like other parasitic plants, *C. pubescens* infection may indirectly increase the levels of some nutrients through host-effects. However, few previous studies had considered whether native and invasive species respond differently to soil enrichment or parasite litter (but see Demey *et al*. 2013). Our results suggest that *C. pubescens*

may have indirect effects as a biocontrol agent, besides its strong direct effects on the growth, photosynthetic biomass, photosynthetic rate, and seed output of invasive species (Prider *et al.* 2011; Cirocco *et al.* 2016; 2017). While the direct negative effects of *C. pubescens* on invasive species are highly promising for decreasing the vigour, recruitment, and range expansion of invasive weeds of national significance in Australia. Furthermore, the effects of *C. pubescens* on soil and litter accumulation do not have any drastic negative effects on native species.

Conclusion:

While the negative effects of *C. pubescens* on invasive host physiology make it a viable biocontrol agent against Weeds of national significance in Australia, this study demonstrated negative indirect effects of *C. pubescens* on both native and invasive plant species that coexist with the parasite in its native range. While we found negative effects of *C. pubescens* litter on native species, effects were stronger on invasive plants. We believe that this also demonstrates the conservation value of often ignored, or vilified parasitic plants. *Cassytha pubescens* has already been shown to be a cost-effective conservation tool by decreasing the vigour of invasive plants, but it may be most effective when used with complementary weed-control methods.

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Statement of Authorship

Title of Paper	The leafless plant parasite *Cassytha pubescens* has only minor effects on arthropod communities under invasive and native hosts
Publication Status	☐ Published ☐ Accepted for Publication
	☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted all fieldwork, all labwork, all statistical analyses and writing

Principal Author

Name of Principal Author (Candidate)	Bernardo J. O'Connor
Contribution to the Paper	Conducted all fieldwork, all labwork, all statistical analyses and writing.
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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By signing the Statement of Authorship, each author certifies that:

i.   the candidate's stated contribution to the publication is accurate (as detailed above);

ii.   permission is granted for the candidate in include the publication in the thesis; and

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Chapter 4: The leafless plant parasite *Cassytha pubescens* has only minor effects on arthropod communities under invasive and native hosts.

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Abstract:

Parasitic plants may have important roles in ecological communities by modifying several ecological processes. They may also have indirect effects on arthropods by forming layers of litter and increasing the abundance of arthropods. This is important to understand because of bottom-up effects on arthropod abundance may impact vertebrate predators (*e.g.* birds). In this paper, we estimated how a native parasitic plant, *Cassytha pubescens*, may influence the abundance and composition of arthropod communities around native and invasive shrub species, using pitfall traps. We found that *C. pubescens*, unlike other parasitic plants that have been studied, does not increase the total abundance of arthropods. Some groups differed in abundance between infected and uninfected shrubs: we found fewer beetles under infected shrubs, during summer months. *Cassytha pubescens* and other parasitic plants differ in how they influence arthropod communities around their hosts. The main difference in their influence may be due to *C. pubescens* being leafless, whereas other parasitic plants (*e.g.* mistletoes) have rapid leaf turnover rates.

Introduction:

The importance of parasites in ecological communities, in spite of their small size and low relative abundances, is increasingly being recognised (Watson 2001; Watson and Herring 2012; Hatcher *et al*. 2014; Hartley *et al*. 2015). The disproportionately strong ecological effects parasitic plants can have on co-occurring plants and animals may qualify them as 'keystone' species in some cases (Watson 2001; Watson and Herring 2012). While the direct effects of host-infection are relatively well understood, the indirect effects of parasitic plants on arthropods are not well known and they may be hard to predict. This is important since arthropods effect key ecological roles, from herbivory to pollination. Parasitic plants may affect arthropod communities, as they compete with herbivores for host nutrients (Bass *et al*. 2010; Ewald *et al*. 2011) and may weaken host defence responses to herbivore attacks (Lehtonen *et al*. 2005; Runyon *et al*. 2008). However, they may also provide resources for pollinators, herbivores and detritivores (Canyon and Hill 1997; March and Watson 2007; Watson *et al*. 2011). Parasitic plants may have indirect effects on all trophic levels of arthropods by modifying plant assemblage structure and litter quantity and quality inputs (Ndagurwa, Dube, Mlambo, *et al*. 2014; Hartley *et al*. 2015; Mellado *et al*. 2019). This may change resource availability for various consumer which can then cascade to higher trophic levels. Whether all parasitic plants in general can indirectly affect arthropod community abundance and diversity is not known. The vast majority of existing such studies focus on stem-hemiparasitic mistletoes with high leaf turnover rates, while leafless parasitic plants have not been studied in, to the best of my knowledge. Unlike mistletoes leafless parasites do not increase litter deposition. On the other hand they alter litter quality and produce changes in the physical structure of the site. In this study, we assessed the indirect effects of *Cassytha pubescens* R. Br., an Australian-native leafless hemiparasitic vine, on ground arthropod communities to assess the generality of

parasitic plants influencing ground arthropods via litterfall, particularly for leafless parasitic plants.

Available research indicate that parasitic plants may have positive and negative effects on different arthropod groups by providing microhabitats, resources, and changing litterfall quantity and quality (Lázaro-González *et al*. 2019; Mellado *et al*. 2019). For ground arthropods the increased litter input from parasitic plants may increase microhabitat availability and resource availability. In Australia, mistletoes can increase total litterfall under *Eucalyptus* hosts by up to 189 % (March and Watson 2007), forming dense litter mats. In accumulated litter beneath mistletoe-infected trees, arthropods are more abundant (up to 47.1 %) and have greater biomass than arthropods in litter beneath uninfected eucalypts (Mellado *et al*. 2019). In semi-arid Zimbabwe, mistletoe-infected trees had both greater arthropod abundance and greater diversity, with *c*. 28-34 % more arthropod species beneath their canopies than under uninfected trees (Ndagurwa, Dube, Mlambo, *et al*. 2014). Whether all parasitic plants can influence arthropods in this manner is unknown, as not all parasitic plants can increase litterfall beneath hosts and their effects on epigeic arthropods are unknown. Parasitic plants may also influence arthropod abundance by altering vegetation structure. *Rhinanthus minor* may induce changes in plant assemblages or structural changes in sward height, which in turn drive change in arthropod abundance (Hartley *et al*. 2015).

While some parasitic plants with high leaf turnover rates (*e.g*. mistletoes) can form dense layers of litter, other parasitic plants may only alter the composition of litter input rather than quantity. In chapter 2, I reported that *C. pubescens* infection did not alter total litterfall under its hosts. Infection decreased host litterfall, and while parasite compensated by dropping fruits and flowers, total litterfall was not different from that under uninfected hosts. However, litter composition was altered. While increasing arthropod abundance under infected host species may primarily be due to increased litter input beneath mistletoes (Ndagurwa, Dube, Mlambo,

et al. 2014), changes in litter quality may also have important effects. The contribution of nutritious flowers and fruits (*e.g.* low carbon : nitrogen ratio, high micronutrients) by *C. pubescens* may change litter quality and possibly palatability for detritivores and other decomposers, which has potential consequences for predatory arthropods (Bultman and Uetz 1984; Mellado *et al.* 2019). Although leafless parasitic plants may not contribute substantially to litter input, their contributions may still be important as arthropods may be most abundant in heterogenous mixtures of litter (Mellado *et al.* 2019). This idea is congruent with the current understanding of decomposition of litter and nutrient release, since decomposition is greatest in mixtures where no one nutrient is limiting for decomposition.

The influence parasitic plants may exert on arthropod communities is important to understand, particularly if parasitic plants are used for habitat restoration. Parasitic plants may be useful in habitat restoration by supressing dominant plant species thus increasing plant diversity (Pywell *et al.* 2004; Decleer *et al.* 2013; Těšitel *et al.* 2017, 2018), or by biological control of invasive species (Yu *et al.* 2008, 2009; Yu and Liu 2011; Cirocco *et al.* 2018). While the existing research indicates that there is little potential for negative effects of increasing parasite abundance on the plant community, little is known about possible effects on other ecosystem components. Potential changes to abundance and diversity of detritivores, decomposers and their predators may have implications for higher trophic levels and nutrient cycling in ecological communities (*e.g.* predatory arthropods, Hartley *et al.* 2015; birds, Watson 2009). Few studies have investigated the effects of the litter of parasitic plants on ground arthropods, and most of these studies have been done in systems where parasites produce large amounts of leaf litter (Ndagurwa *et al.* 2014; Mellado et al 2019). This paper investigates the effects of a leafless parasitic vine that alters litter quality, but not quantity (chapter 2), on ground arthropod abundance and diversity. *Cassytha pubescens* is a hemiparasitic vine native to southeastern

Australia and being leafless, it does not deposit large amounts of litter as do mistletoes and rattles (chapter 2). However, *C. pubescens* litter may alter the decomposition and palatability of detritus for decomposers given it is rich in potassium, phosphorus, and carbon (chapter 2). We tested the predictions that total arthropod abundance different under infected shrubs would be higher than under uninfected shrubs. We also tested the prediction that arthropod assemblage composition under native and invasive host species would differ.

Methods:
Study species:

We used two species as hosts. The first was *Ulex europaeus* L. (Fabaceae), a leguminous evergreen shrub, reaching growing up to 7 m tall, and living for *c.* 30 years. *Ulex europaeus* is considered one of the 100 most invasive plant species globally by the International Union for Conservation of Nature (Lowe *et al.* 2000; Atlan *et al.* 2015). It is a major problem in Australia, invading pastures, forestry plantations and natural vegetation. The second host was *Bursaria spinosa* Cav. (Pittosporaceae), a spiny shrub native to Australia, which grows up to 4 m in height, and it has elongated oval-shaped, slightly sclerophyllous leaves. *Cassytha pubescens* can grow densely on both *U. europaeus* and *B. spinosa*.

Study site:

The study was conducted at Belair National Park, in Mount Lofty ranges of South Australia (-35.02123° N, 138.67355° E). The Mt. Lofty ranges area has Mediterranean-type climate with cold, wet winters and hot, dry summers, with ~ 900 mm per annum, with summer max. mean temperature = 22.6°C, and winter min. mean temperature = 5°C. In summer the mean rainfall is *c.* 36 mm and in winter *c.* 152 mm (Bureau of Meteorology 2021). The area used in this study is an open forest woodland with a canopy dominated by *Eucalyptus obliqua* L'Hér.,

diverse understorey vegetation, including some grass cover in between. The ground cover is mostly *E. obliqua* leaf and bark litter and some bare ground.

Ground arthropod sampling:

We used pitfall traps to assess whether the abundance of different arthropod taxonomic groups differed between infected and uninfected shrubs of both host species. The study ran for one year, sampling arthropods between July 2020 and July 2021. In the study site we located individuals of *B. spinosa* and *U. europaeus* infected or not with *C. pubescens*. We placed the traps under eight infected and eight uninfected shrubs of each species (N = 32). Individual shrubs were interspersed and had similar heights and cover (as the largest diameter and its orthogonal diameter). They were at least 10 m apart, but no more than 20 m apart. The study was done over an area of *c*. 1.45 ha.

To ensure a flush surface around the holes of pitfall traps, we drilled 20 mm diameter holes on the lids of 80 mm diameter x 80 mm tall plastic containers. Eight pitfall traps per shrub were placed in a circular fashion, within a 2/3rd radius from the centre of the canopy and filled with *c*. 75 ml of a non-flammable and non-toxic aqueous solution of 1:5 aqueous propylene glycol solution. We chose this over ethanol mixes because of their potential fume ignition and extreme bushfire danger during that year. After one week of trapping arthropods at each sampling period, we retrieved the pitfall and sorted the samples in the laboratory. We identified arthropods as far as possible, however, due to time constraints brought on by COVID-19 most arthropods were identified only to order level but the number of putative morphospecies determined per order. We excluded winter samples from 2020 and 2021 because of inadequate sample sizes (many zero-captures) for comparisons between factors (order, species, infection)

because generalised linear models were rank-deficient for winter data (for years 2020 and 2021).

Statistical analysis:

To determine whether there were differences in ground arthropods between infected and uninfected shrubs, we used generalised mixed effects linear models (GLMMs) in R statistical software version 3.5.3 (R Core Team 2016). We employed the package *lme4* (Bates *et al*. 2015) to fit GLMMs using a Poisson log link for all fits. To assess the validity of models we tested homogeneity of variances using Bartlett's test function (*barlett.test*) and residual distribution with Shapiro-Wilks test (*shapiro.test*). When data did not satisfy the assumptions, we computed lambda values with the greatest likelihood using the *BoxCox* function, to compute appropriate power transformations in the *MASS* package (Venables and Ripley 2002). To conduct post-hoc analyses between infected and uninfected shrubs, within species and within seasons, we used estimated marginal means (least-square means) in the package *emmeans* (Lenth 2019) and Z-tests. We used the package *vegan* (Oksanen *et al*. 2019) to conduct a principal component analysis (PCA), to assess the similarity of arthropod communities between infected and uninfected shrubs. We refrain from making statements about diversity, firstly because we could not identify arthropods to species level, but also since many of these indices (*e.g*. Shannon) are meant to be used at ecological community level only and have serious statistical issues when misused (see Barrantes and Sandoval 2009).

Results:

We found a total of 2,524 arthropods, in 21 orders, across all the pitfall traps for all seasons (spring 2020 and late summer 2021; winter data not shown). More arthropods were caught in spring 2020 (n = 1566) than in summer (n = 960). For total arthropods, we found no differences

in abundance when shrubs were infected or not, regardless of shrub species or season (Table 1, Supp. Fig. 1). The most abundant orders in our samples were: Hymenoptera (n = 948)(most of which were ants, Formicidae), followed by Diptera (n = 389), Coleoptera (n = 260), Entomobryomorpha (springtails) (n = 208) and Araneae (n = 205). Some terrestrial crustaceans were also present; Amphipoda n = 16, and Isopoda n = 46. The rarest orders in our pitfalls included Scorpiones, Zygentoma, and Orthoptera which were each represented by one specimen. Principal component analysis (Fig. 1) shows that infected shrubs are clustered within uninfected shrubs. This suggests that infected and uninfected shrubs are not dissimilar in arthropod community composition. The first five principal components explained 66.08 % of all variance in the dataset.
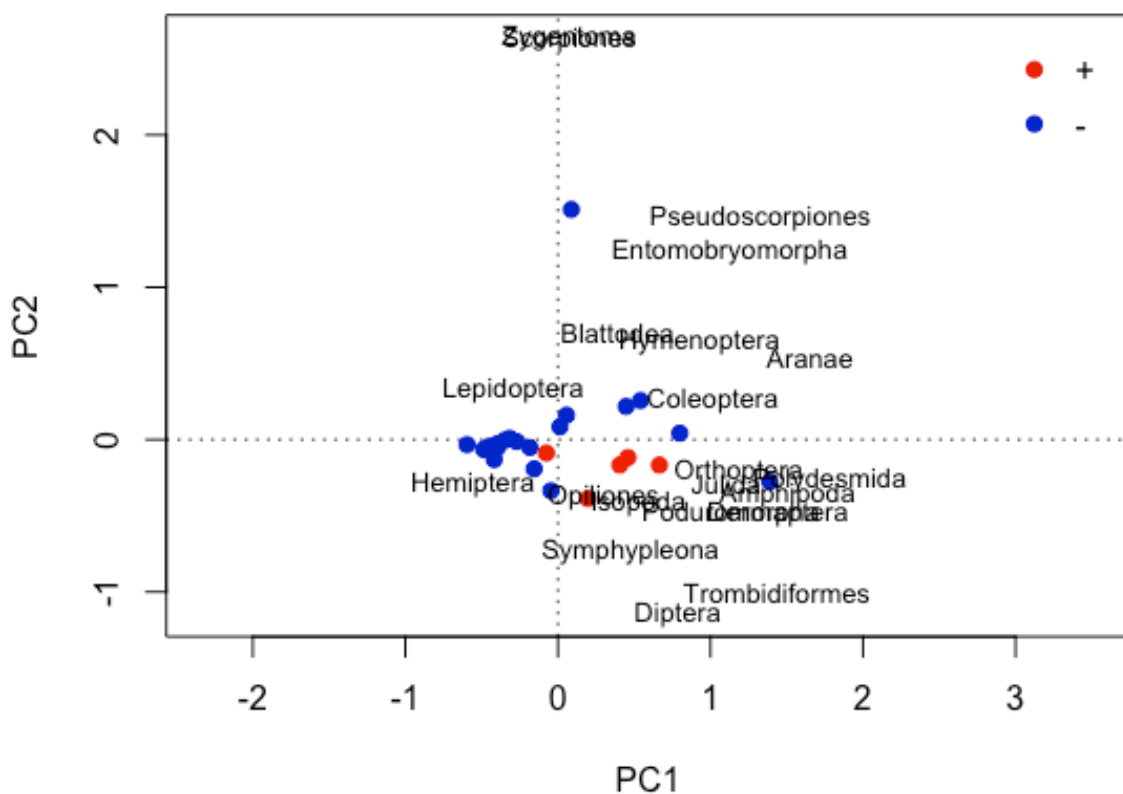
Fig. 1: Principal Component Analysis of all arthropod orders found under native *Bursaria spinosa* and invasive *Ulex europaeus*, with and without parasitic *Cassytha pubescens*. PC 1

explained 25.09 % of variance and PC 2 explained 13.3 % of variance in the dataset. Infected

shrubs are represented in red dots, and uninfected shrubs are represented in blue dots.

Table 1: Mean number ± SE of arthropod orders captured per infected vs uninfected shrub. Data include only arthropods captured in spring 2020 and summer 2021, arthropods trapped in winter 2020 and winter 2021 are not included.

Order	Total Uninfected	Mean shrub^{-1}	± SE	Total Infected	Mean shrub^{-1}	± SE
Amphipoda	8	2	0.577	8	1.6	0.6
Araneae	84	7.63	1.94	121	8.64	1.99
Blattodea	6	3	1	9	3	2
Coleoptera	106	10.6	1.05	83	5.92	1.15
Dermaptera	16	2.28	0.993	28	3.5	1.22
Diptera	213	19.3	4.95	176	13.5	3.76
Entomobryomorpha	116	10.5	2.36	92	9.2	1.69
Hemiptera	19	3.16	1.44	12	3	0.912
Hymenoptera	435	36.2	11.8	513	36.6	15.51
Isopoda	18	1.63	0.278	28	3.11	0.715
Julida	47	4.7	0.895	87	6.21	1.24
Lepidoptera	7	2.33	0.881	9	3	1.52
Opiliones	13	2.6	0.871	10	1.66	0.421
Orthoptera	4	1	0	1	1	0
Poduromorpha	6	6	0	1	1	0
Polydesmida	20	2.22	0.521	32	3.55	0.765
Pseudoscorpiones	34	6.8	1.46	27	3.37	1.17
Scorpiones	1	1	0	0	0	0
Symphypleona	8	2	0.408	1	1	0
Trombidiformes	24	3	0.823	31	4.42	0.922

When comparing total arthropod abundance between shrubs, we found no interaction between

infection and shrub species (Table 2, $p > 0.9$). In our models we did not find evidence of any

infection, shrub species, or seasonal effects (Table 2, $p > 0.9$, $p > 0.8$, $p > 0.4$, respectively).

Table 2: Analysis of deviance table on GLMM of total arthropod abundance. $R^2 = 0.52$, Alpha = 0.05.

	Chi sq (c^2)	Df	P-value
Infection	0.0041	1	0.9489
Species	0.0406	1	0.8403
Season	0.5415	1	0.4618
Infection * Species	0.0038	1	0.9509

*Total arthropod abundance$^{-0.5}$ ~ Infection * Shrub species + Season +(1|Shrub)*

When comparing Araneae trapped in our study we found no interactive effects of infection and species (Table 3, $p > 0.3$). We also found no infection or species effects (Table 3, $p > 0.7$, $p > 0.4$, respectively). We found marginally significant seasonal effects on Araneae abundance (Table 3, $p < 0.08$), suggesting spiders may have been more abundant in spring than in summer (Fig. 1).

Table 3: Analysis of deviance table on GLMM of Araneae abundance. $R^2 = 0.63$, Alpha = 0.05.

	Chi sq (c^2)	Df	P-value
Infection	0.1479	1	0.70053
Species	0.5723	1	0.44937
Season	3.2383	1	0.07194
Infection * Species	0.8000	1	0.37110

*Total spider abundance$^{-0.4}$ ~ Infection * Shrub species + Season +(1|Shrub)*

Figure 2: Spider (Araneae) abundance in pitfall traps placed under shrubs, infected (+) by *C. pubescens* or not (-), during spring (red) and summer (blue).

The abundance of Coleoptera around shrubs had an interactive effect with infection and season (Table 4, $p < 0.05$). We found that in spring, there were more beetles on uninfected shrubs than on infected shrubs (Fig. 3). Generally, there were more beetles present in spring than in summer (Fig. 3, Table 4.5).

Table 4: Coleoptera analysis of deviance table, n = 75 observations.

	Chi sq (c²)	Df	P-value
Infection	6.0291	1	**0.01407**
Species	0.0605	1	0.80573
Season	**27.8064**	**1**	**1.341e⁻⁰⁷**
Infection * Species	0.3049	1	0.58084
Infection * Season	4.4296	1	**0.03532**

*Log Abundance ~ infection * species * season + (1|shrub).*

Table 4.5: Z-test *post-hoc* analysis, testing difference in Coleoptera abundance between infected and uninfected shrubs, within seasons. Holm adjusted *p*-values. Poisson distribution family, log link.

	Estimate	Std. Error	Z ratio	Pr(>|z|)
Spring: – vs. +	**0.775**	**0.265**	**2.930**	**0.0034**
Summer: – vs. +	-0.281	0.556	-0.506	0.6130
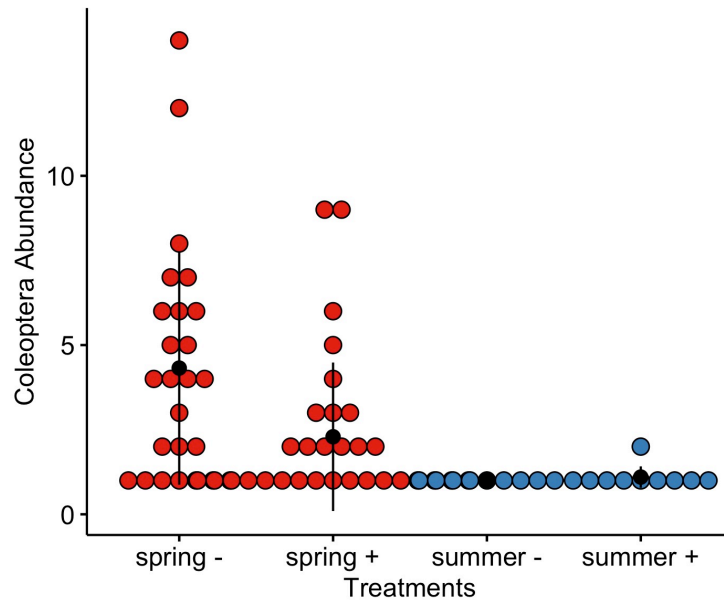
Figure 3: Coleoptera (beetles) abundance capture using pitfall traps in spring (red) and summer (blue), under shrubs infected (+) or not (-) by *Cassytha pubescens*.

With the abundance of Formicidae between shrubs, we found no interaction between infection and species (Table 5, $p > 0.9$). We did not find significant differences between infected shrubs (Table 5, $p > 0.9$), nor between shrub species (Table 5, $p > 0.9$), or season (Fig. 4, Table 5, $p > 0.8$).
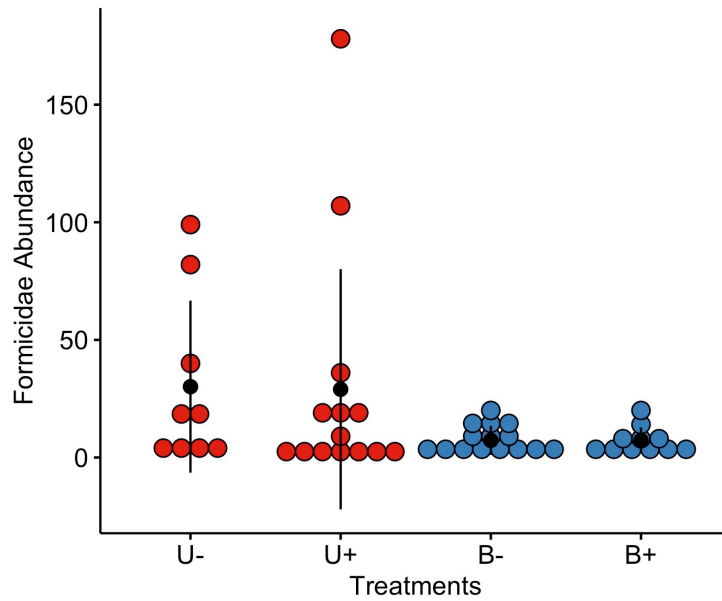
Figure 4. Ant (Formicidae) abundance captured with pitfall traps under the canopies of *Ulex europaeus* (red) and *Bursaria spinosa* (blue) when shrubs were infected (+) or not (-) by *Cassytha pubescens*.

Table 5: Analysis of deviance on GLMM of ant (Formicidae) abundance. $R^2 = 0.55$, Alpha = 0.05.

	Chi sq (c^2)	Df	*P*-value
Infection	0.0002	1	0.9898
Species	0.0187	1	0.8912
Season	0.0140	1	0.9059
Infection * Species	0.0016	1	0.9683

*Ant abundance$^{-0.1}$~ Infection*Species + Season +(1|Shrub)*

When comparing diplopods (incl. native and introduced millipedes) we found no difference in abundance between infected and uninfected shrubs (Table 6, $p > 0.9$), and no interactive effects between shrubs species and infection (Table 6, $p > 0.9$). Furthermore, we did not find any difference of diplopod abundance between seasons either (Fig. 5, Table 6, $p > 0.9$).

Table 6: Analysis of deviance on GLMM of Diplopoda abundance. $R^2 = 0.57$, Alpha = 0.05.

	Chi sq (c^2)	**Df**	**P-value**
Infection	0.00001	1	0.997
Species	0.00073	1	0.978
Season	0.02602	1	0.871
Infection * Species	0.00003	1	0.995

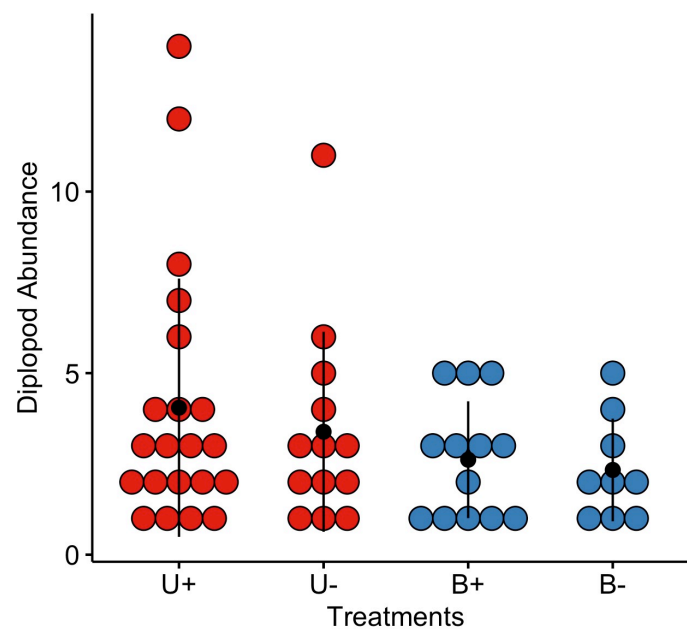*Diplopoda abundance$^{-0.1}$~ Infection*Species + Season +(1|Shrub)*

Figure 5: Diplopod abundance (millipedes) abundance captured with pitfall traps under the canopies of *Ulex europaeus* (red) and *Bursaria spinosa* (blue), when shrubs were infected (+) or not (-) by *Cassytha pubescens*.

Discussion:

Unlike previous studies which reported substantial effects on arthropod communities, we only found minor differences. We found few differences at order level between infected and uninfected shrubs. For total arthropod abundance, we found no difference between infected and uninfected shrubs, regardless of host species or season of sampling (Table 2). Our results differ from those found in the only two similar studies on stem hemiparasitic plants (Ndagurwa, Dube, Mlambo *et al*. 2014; Mellado *et al*. 2019). In southeastern Australia, the abundance of all arthropods was 47.1% higher beneath mistletoe-infected trees than under uninfected trees (Mellado *et al*. 2019). Similarly, during the rainy season in Zimbabwe, arthropod diversity was greater by 28% more species beneath trees infected with *Erianthemum ngamicum* (Sprague) Danser and 34% when infected by *Plicosepalus kalachariensis* (Schinz) Danser. While Ndagurwa, Dube, Mlambo *et al*. (2014) and Mellado *et al*. (2019) found 5 and 17 orders of arthropods, respectively, we found a total of 21 orders (Table 1). In contrast to these studies, we found similar abundances of arthropods in total beneath infected and uninfected shrubs (Table 1).

The differences in our results from those in previous studies are most likely due to fundamental differences between mistletoes and *C. pubescens*. While mistletoes and *C. pubescens* are both aerial hemiparasites, *C. pubescens* is a leafless vine, which limits its potential to contribute to litterfall. Mistletoes, in contrast, have high rates of leaf turnover (March and Watson 2007; Ndagurwa *et al*. 2014), forming thick layers of organic material that provides resources and microhabitats for arthropods (Seastedt and Crossley 1981; Ndagurwa *et al*. 2014). Other hemiparasites may also contribute to changes in arthropod abundance not through litter, but by modifying vegetation structure. While mistletoes may influence arthropod abundance via indirect litter-structure effects, root-parasitic *R. minor* may induce changes in plant

assemblages or structural changes in sward height, which in turn drive change in arthropod abundance (Hartley *et al.* 2015). While we did not find evidence of increased total arthropod abundance, we found order-specific differences. Furthermore, since *C. pubescens* has little influence on litterfall and soil nutrients, it may not enhance the growth of understorey vegetation as mistletoes and rattles do (March and Watson 2007; Hartley *et al.* 2015). Therefore, *C. pubescens* does not have an effect on arthropod community composition via structural effects, unlike *R. minor* (Hartley *et al.* 2015). It is surprising that *C. pubescens* had little effect on arthropod communities since it altered litter quality input under infected hosts (see chapter 2). In addition, we expected that arthropods could have responded to the dense mass of stems of the parasite that cover the infected shrubs, forming a complex structure, which most likely changes the microenvironment in ways that could alter invertebrate abundance.

We found only some order-specific differences between infected and uninfected shrubs. We found spiders (Araneae) to be equally abundant in spring and summer beneath infected and uninfected shrubs (Fig. 2, Table 3). These results differ from those found by Mellado *et al.* (2019) where almost twice as many spiders occurred beneath infected trees compared with uninfected trees (Mellado *et al.* 2019). In contrast, Araneae/Opiliones abundance were equal whether root-parasitic *R. minor* was present or absent (Hartley *et al.* 2015). *Cassytha pubescens* and *R. minor* do not have high tissue turnover rates as mistletoes do. This may explain why we did not find greater spider abundance beneath infected shrubs, as spiders may respond in abundance to ground-level structure complexity rather than biotic resources (Bultman and Uetz 1984).

The effect of infection on Coleoptera abundance was contingent on season. We found that only in spring coleopterans were more abundant beneath uninfected shrubs than beneath infected

shrubs, regardless of shrub species (Table 4, Table 4.5, Fig. 3). Our results also differ from those found under mistletoes, where there is a greater abundance of coleopterans beneath infected trees than uninfected trees (Mellado *et al*. 2019). However, in agreement with our results, some beetles were more abundant beneath uninfected trees (Ndagurwa, Dube, Mlambo, *et al*. 2014). The patchy distribution of some gregarious beetles in our samples (*e.g*. dung beetles, Bolboceratidae) may account for some of these differences in Coleoptera as a group. Alternatively, fewer beetle are present around infected shrubs because of the altered litter input from the parasite (see chapter 2). Interestingly, we found no difference in coleopteran abundance in summer, when moist-retaining microhabitats may be more important for invertebrates (Fig. 2). One potential reason why we found more beetles under uninfected shrubs and in certain seasons may be due to flowering. Infected *U*. *europaeus* may have fewer flowers when infected by *C*. *pubescens* due to decreased C-budget (Cirocco *et al*. 2017), which may decrease pollen, seed, and detritus availability for generalist and specialist beetles. During summers, particularly in the year of sampling when heatwaves were frequent, any litter may provide moisture-retaining microhabitats.

The most abundant hymenopteran group was Formicidae. Our study found no difference in their abundance between infected and uninfected shrubs, regardless of shrub species and season (Table 5, Fig. 4). In contrast, Ndagurwa *et al*. (2014) found that *Dorylus* and *Crematogaster* ants were more abundant beneath infected trees than uninfected trees. In agreement with our results, there was no difference in ant abundance between infected or not by *A*. *miquelii* (Mellado *et al*. 2019). Similarly, there was no difference in ant abundance regardless of *R*. *minor* presence or absence (Hartley *et al*. 2015). While it is not clear why some mistletoes have greater ant abundance and why others do not, *C*. *pubescens* did not have a negative effect on ant abundance when present. This is important as ants are key components of dispersers of

some Australian plant species (Hughes and Westoby 1992). Ants are reliable ecological indicators of habitat quality (Brown 1997; Andersen *et al*. 2004) and the lack of difference between infected and uninfected shrubs demonstrates that *C. pubescens* may not have negative effects on arthropod communities.

We found no difference in Diplopoda abundance among infected or uninfected shrubs, regardless of season or plant species (Fig. 4, Table 5). This group was expected to have large differences if litter accumulated beneath infected shrubs. During winter, diplopods are abundant and consume detritus. Mellado *et al*. (2019) in contrast, found greater Diplopoda abundance beneath infected trees. Diplopods, particularly the Portuguese millipedes (*Ommatoiulus moreleti* Lucas 1860, Julidae), occur abundantly on *C. pubescens* dead tissues that remain attached to host – feeding on wet litter after rains (pers. obs.).

The potential negative effects of parasites are the main concern when using them as biocontrol agents. Our results suggest that *C. pubescens* has little effect on overall arthropod community abundance or order diversity and this study demonstrates that not all parasitic plants can have equal effects on their biotic background.

Conclusion:

Unlike other previously studied parasitic plants, *C. pubescens* seems to have little effect on arthropod communities. *Cassytha pubescens* does not form dense layers of litter that create dense microhabitats and provide resources, but through small changes in the type of litter may have provided more resources for certain groups of arthropods (particularly beetles). Other parasitic plants may increase the abundance of several groups of arthropods by increasing resource availability or changing habitat and vegetation structures. Nonetheless, *C. pubescens*

does not appear to have negative effects on ground arthropod communities in Southern Australia. This is probably due to its lack of leaves, and therefore its small contribution to soil nutrient returns. Our results indicate that the use of *C. pubescens* as a biocontrol agent in South Australia to supress invasive *U. europaeus* would not have any negative effect on the ground arthropod community.

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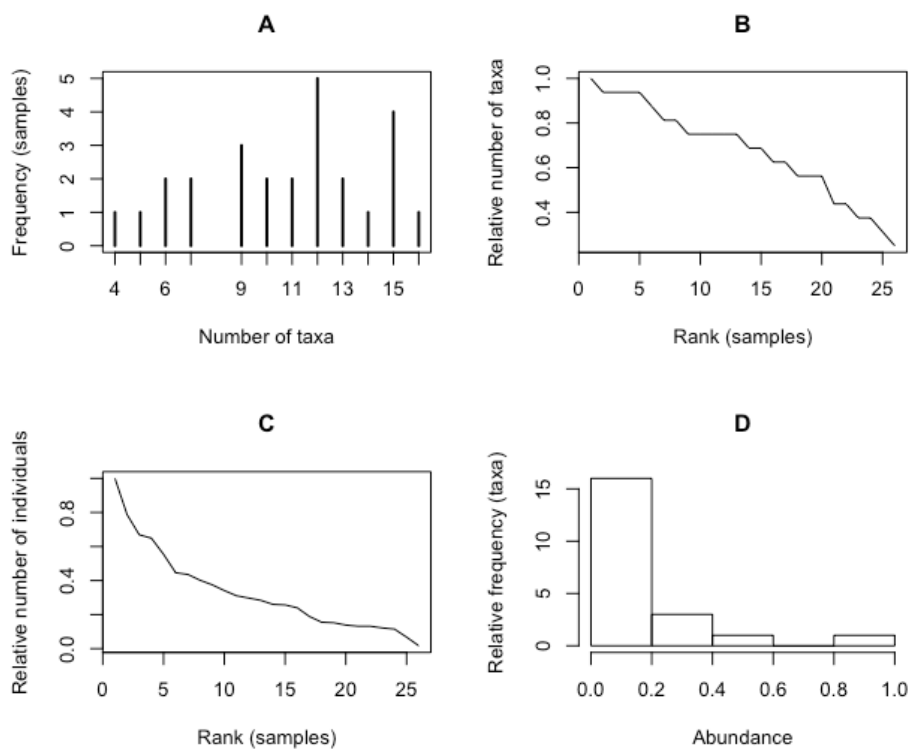
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Supplementary Information:

Supp. Fig. 1: Summary of characteristics of arthropod samples trapped under infected and uninfected *Bursaria spinosa* and *Ulex europaeus*, infected by *Cassytha pubescens* or not. Arthropods were captured between June 2020 and June 2021. **A)** Arthropod species richness, **B)** samples ranked by relative number of taxa, **C)** samples ranked in order of relative number of individuals, and **D)** the relative frequency of taxa.
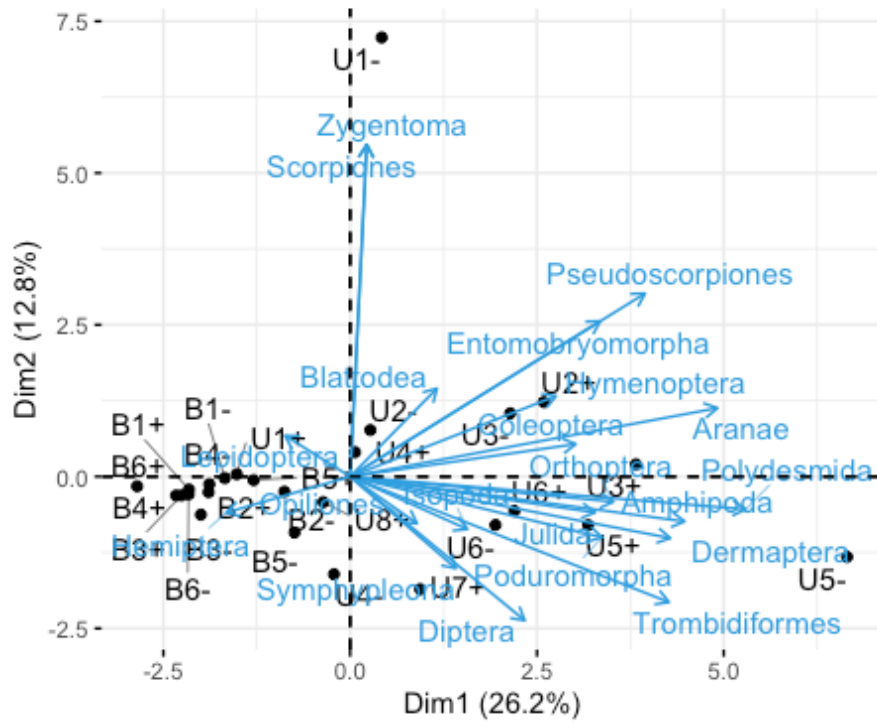
Supp. Fig. 2: Principal component analysis of arthropod order-level abundance, for arthropods captured in pitfall traps under native *Bursaria spinosa* and invasive *Ulex europaeus*, when infected or not by *Cassytha pubescens*.

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Statement of Authorship

Title of Paper	The native parasitic plant Cassytha pubescens impairs the competitive ability of invasive hosts, but not of native hosts.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted fieldwork, labwork, statistical analyses and wrote the manuscript

Principal Author

Name of Principal Author (Candidate)	Bernardo J. O'Connor
Contribution to the Paper	Conducted all fieldwork, all labwork, all statistical analyses and writing.
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 21/11/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

     i.   the candidate's stated contribution to the publication is accurate (as detailed above);

     ii.   permission is granted for the candidate in include the publication in the thesis; and

     iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Robert M. Cirocco
Contribution to the Paper	Adviced on experimental design, helped with fieldwork, data collection, and drafting.
Signature	Date 21/11/2021

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Contribution to the Paper	Adviced on experimental design, statistical analysis, and helped write the manuscript.
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Statement of Authorship

Title of Paper	The native parasitic plant Cassytha pubescens impairs the competitive ability of invasive hosts, but not of native hosts.
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☒ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Conducted fieldwork, labwork, statistical analyses and wrote the manusc

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Name of Principal Author (Candidate)	Bernardo J. O'Connor
Contribution to the Paper	Conducted all fieldwork, all labwork, all statistical analyses and writing.
Overall percentage (%)	80 %
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
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Co-Author Contributions

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    ii.   permission is granted for the candidate in include the publication in the thesis; and

    iii.   the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew D. Austin
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Chapter 5: The native parasitic plant *Cassytha pubescens* impairs the competitive ability of invasive hosts, but not of native hosts.

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Abstract:

Parasitic plants may be important mediators of interactions among their hosts in ecological communities. In invaded ranges, invasive species can be competitively superior to native species – locally displacing native plants. Parasitic plants may be useful in habitat restoration and biological control of invasive plant species because they have stronger negative effects on invasive hosts *cf*. native hosts. In this paper, we conducted two glasshouse studies. One study assessed the growth of each *A. paradoxa* (native) and *U. europaeus* (invasive) when grown individually in pots or paired with a competitor. We found that under our experimental conditions *A. paradoxa* and *U. europaeus* did not strongly compete as both gained no more mass when grown alone vs. when grown in interspecific competition. In our second study, we set up a full-factorial glasshouse study, where one *A. paradoxa* and one *U. europaeus* of roughly the same size were planted in a single large pot. These pairs were randomly assigned to one of four treatments: uninfected pairs (A-U-), infected pairs (A+U+), infected *U. europaeus* only (A-U+), and infected *A. paradoxa* only (A+U-). We found that *C. pubescens* consistently decreased the biomass and growth of *U. europaeus* by *c*. 50%, but not that of *A. paradoxa*. This study demonstrates that the negative effects of *C. pubescens* diminish the competitive ability of *U. europaeus* when in competition with a native plant. This study supports the use of *C. pubescens* as a biocontrol agent against invasive weeds like *U. europaeus*.

Introduction:

Interactions between pairs of species do not occur in isolation but are almost always modulated by interactions with other species. Parasites may be important mediators of interactions among their hosts in ecological communities (Price *et al*. 1986; Dunn *et al*. 2012). There are many empirical examples of competition modified by animal parasites (Park and Frank 1948; Schall 1992; Tompkins *et al*. 2003) and parasitic plants (Alexander and Holt 1998; Bullock and Pywell 2005; De Castro and Bolker 2005; Li *et al*. 2019). Some parasitic plants are unique, however, in their ability to infect several hosts simultaneously. When the same parasite infects two competing host species, parasite-modified (altering host traits) and parasite-mediated (altering host population density) competition can occur (Holt 1977; Holt and Pickering 1985; Yan 1996). Depending on which host species can tolerate the greater parasite load, the parasitic plant may indirectly enhance or reverse the outcomes of direct competition between its hosts (Holt 1977; Matthies 1996; Yan 1996; Bowers and Turner 1997).

Parasitic plants may be useful in habitat restoration and biological control of invasive plant species in invaded ecological communities (Těšitel *et al*. 2020). This is because parasitic plants can have differential effects on the performance of competing plant species, interfering with traits directly related to their competitive success (Těšitel *et al*. 2017). Invasive and overabundant species are a worldwide issue affecting biodiversity, often outcompeting and displacing native species (Lee *et al*. 1986; Bateman and Vitousek 2018). Invasive and dominant species may be highly successful due to higher resource-use efficiency, fast growth rates, high fecundity, and the ability to create soil microbial assemblages that promote further invasion (Bever *et al*. 2010; Yelenik and D'Antonio 2013). Parasitic infection may diminish those advantageous traits for invasive host species without substantially affecting traits of native hosts, relieving native species from the competitive effect of invasive species (Yu *et al*. 2008;

Yu and Liu 2011; Těšitel *et al.* 2017, 2018). For example, In European grasslands, *Rhinanthus* species (Orobanchaceae) negatively affect the underground storage of the dominant grass *Calamagrostis epigejos* (L.) Roth, which indirectly increases the herb layer cover and abundance of subordinate species (Těšitel *et al.* 2017; 2018). Through such affinity for a particular species, *Rhinanthus* species may facilitate the competitive release of non-host species with low competitive ability (Pywell *et al.* 2004; Mudrák *et al.* 2016; DiGiovanni *et al.* 2017). Similarly, in European meadows, *Pedicularis palustris* L. (Orobanchaceae) can also supress *Carex acuta* L., a dominant sedge, increasing species richness (Decleer *et al.* 2013), and in China, *Cuscuta campestris* Yuncker (Convolvulaceae) can supress the invasive *Mikania micrantha* H.B.K. (Yu *et al.* 2008). In their native ranges, parasitic plants may thus supress invasive hosts more than native hosts, indirectly altering the outcomes of competitive interactions. In Australia, the native parasitic vine *Cassytha pubescens* R. Br. has also been found to have a much greater effect on invasive than native species studied (Prider *et al.* 2009, 2011; Cirocco *et al.* 2016, 2017). Common native hosts of *C. pubescens* are relatively slow-growing, drought-tolerant species adapted to nutrient impoverished soils of temperate Australia. As a result, in a high-fertility, disturbed habitat, native plants can be expected to be inferior competitors to common invasive species such as *Ulex europaeus* L., which has high growth rates, high fecundity (Ivens and Mlowe 1980), and long-lasting seeds with high germination rates (Lee *et al.* 1986). Considering that *C. pubescens* can strongly hinder the advantageous traits (*e.g.* high photosynthetic rate) that make invaders successful (Prider *et al.* 2009; Prider *et al.* 2011; Cirocco *et al.* 2016; 2017) it can be expected that this parasite may alter the competitive interactions between invasive and native hosts in favour of the more tolerant native species. Many studies on the competitive ability of *U. europaeus* against other species, globally, have focused on the effects on the emergence and survival of *U. europaeus* seedlings (Salisbury 1929; Hartley and Thai 1982; Popay *et al.* 1990; Ledgard 2006). However,

few studies have actually quantified and compared the biomass growth of *U. europaeus* in mixed vs. monocultures (Ivens and Mlowe 1980; Delerue *et al*. 2018). At present, there have been no studies evaluating the impact of *C. pubescens* on the competitive outcomes between native and invasive hosts.

Moreover, although the strong direct competitive effects of invasive plants on native plants are well documented (Lee *et al*. 1986; Richardson and Hill 1998; Fogarty and Facelli 1999; Hill *et al*. 2008; Broadfield and McHenry 2019), we know nothing how stem hemiparasites mediate these competitive interactions. This is a major gap in the literature considering that stem hemiparasites constitute 25% of all known parasitic plants and may have profound effects on the competitive release of native plants from invasion, thereby restoring biodiversity.

This study aims to test whether *C. pubescens* infection modifies the interspecific competitive balance between native and invasive host species. Understanding how *C. pubescens* modifies the intensity and outcomes of these competitive interactions has direct applications in assessing the benefits or drawbacks of its use as a biocontrol agent. Furthermore, indirect effects such as apparent competition can be strong drivers of plants community structure (Pennings and Callaway 2002). While there is clear evidence of the advantage of using this parasite in monocultures of invasive species, it is not clear what the potential effects of its use in mixed (*i.e.* native and invasive species growing together in the field) may be.

Here we present results from two experiments. Firstly, we assessed the intensity of competitive interactions between a native (*Acacia paradoxa* DC) and an invasive species (*U. europaeus*) without the parasite. We hypothesised that the growth of the invasive *U. europaeus* would be relatively unaffected when growing with *A. paradoxa*, whereas the growth of *A. paradoxa* would be adversely affected when growing with *U. europaeus*. We tested this hypothesis by

assessing changes in growth (dry shoot and root mass) in *A. paradoxa* and *U. europaeus* when grown in mixed vs. monocultures. Second, we assessed whether *C. pubescens* modified the intensity of competitive interactions between those two host species. We hypothesised: 1) Native *A. paradoxa* individuals would perform better and gain more biomass when competing with infected *U. europaeus*, regardless of the infection status of *A. paradoxa* and 2) *U. europaeus* would perform poorly and gain less biomass when infected, regardless of the infection status of competing *A. paradoxa*. To test these last two hypotheses we measured photosynthetic performance, total organic C, total N, and dry shoot, root, and nodule biomass on infected and uninfected pairs of *A. paradoxa* and *U. europaeus*, including *C. pubescens* in infected treatments.

Methods:
Study species:

Cassytha pubescens (Lauraceae) is an Australian-native perennial hemiparasitic vine that attaches to the shoots of its hosts. Its growth habit allows *C. pubescens* to infect multiple hosts simultaneously. It is an obligate parasite with a wide range of hosts, including native and invasive plant species (Prider *et al.* 2009; Cirocco *et al.* 2018; Facelli *et al.* 2020). *Acacia paradoxa* (Fabaceae) is an evergreen leguminous shrub native to Australia, with spines on stems and phyllodes instead of true leaves. It is often found in eucalypt-woodlands, growing to ~2.5-4 m in height (Harden 1991). *Ulex europaeus* (Fabaceae) is an evergreen leguminous shrub, reaching up to 7 m in height. In *U. europaeus*, adult leaves are modified into spines (Lee *et al.* 1986; Broadfield and McHenry 2019). It is considered one of the 100 most invasive plant species (Lowe *et al.* 2000; Atlan *et al.* 2015), and is a major problem in many parts of the world, including Australia.

In this paper, we report the results from two glasshouse studies: an assessment of plant competitive interactions between the two target species, and an assessment of how *C. pubescens* modifies these interactions. In both experiments, all plants were well-watered throughout and fertilised with Nitrosol (Rural Research Ltd, Auckland, New Zealand; NPK 8:3:6) monthly at manufacturer's recommended maximum rates. To mitigate pests present in the glasshouse we sprayed Yates Natrasoap (DuluxGroup Pty Ltd, Victoria, Australia) Veggie and Herb spray, every 5 days at 30 mL/L to kill gorse spider mites (*Tetranychus lintearius* Dufour). The experiments were conducted in an evaporatively cooled glasshouse at The University of Adelaide's North Terrace Campus.

Experiment 1: Mixed and monocultures growth assay

To assess the intensity of competitive interactions between *A. paradoxa* and *U. europaeus,* we set up a mixed and monocultures productivity assay in the glasshouse. We dug up several young *U. europaeus* (~15-20cm height) in Belair NP (N -35.020, E 138.673) in August 2020 and transplanted them into 4.7 L pots containing 80/20 sandy loam. To account for this transplant shock, we waited one month before we transplanted *A. paradoxa* of similar size (purchased from a nursery) into the 4.7 L pots. After transplanting *U. europaeus* into 4.7 L pots, we allowed them to recover from transplant shock for a month before we planted together *A. paradoxa* with *U. europaeus*. We estimated the initial biomass of *A. paradoxa* and *U. europaeus* using the sampling unit method (Andrew *et al*. 1979), with $n = 7$ for each *A. paradoxa* and *U. europaeus*. Then, in September 2020, we randomly assigned individual plants into three treatments and 10 blocks: **AU**, *A. paradoxa* and *U. europaeus* growing together in a single pot; **A**, *A. paradoxa* only a single plant per pot; **U**, *U. europaeus* only a single plant per pot. We had 40 plants in total in this experiment ($n = 20$ *A. paradoxa*; $n = 20$ *U. europaeus*), each treatment had 10 replicates (total of 10 plants in the **A** treatment, 10 in the **U** treatment,

but a total of N = 20 in the **AU** treatment). After 95 days we harvested and oven-dried plants at 60° C for 96 hrs before weighting them.

Experiment 2: Effects of Parasite and competition between native and invasive hosts

We set up a fully-crossed factorial experiment to assess whether *C. pubescens* modifies direct interspecific interactions between competing native and invasive hosts. We dug up *U. europaeus* (*c*. 30 cm tall) from Belair NP, in June 2019, planted them in 80/20 sandy loam, in 4.7 L pots and kept them in moist conditions for eight weeks to recover from transplant shock. We sourced *A. paradoxa* from a nursery. After a recovery period of one month, pairs of *A. paradoxa* and *U. europaeus* were planted together in 30 L pots and filled with 80/20 sandy loam (pH ~6.5).

We took several precautions in experiment 2 to ensure that *U. europaeus* and *A. paradoxa* pairs were as closely matched in size as possible to avoid purely size-related competitive advantage. Before planting pairs together, we used the sampling unit method (see Andrew *et al*. 1979) to estimate the initial biomass of all plants. To estimate the initial mass of host plants in experiment 2, we had *n* = 7 for *U. europaeus* and *n* = 10 for *A. paradoxa*. We then discretised size ranges into three groups; larger, medium, and smaller. Then, *A. paradoxa* individuals were randomly assigned into pairs, blocks, and treatments with *U. europaeus*. Each block had a total of four replicates per block, one each from the four different treatments. After planting, the larger *A. paradoxa* individuals were trimmed to match the size and shape of their competitor. If there was a disparity of more than 15% in estimated mass, then the pair was excluded. Our experimental set-up had four treatments (see Fig. 1): uninfected *A. paradoxa* and uninfected *U. europaeus* in a single pot (**A-U-**), infected *A. paradoxa* and uninfected *U. europaeus* in a single pot (**A+U-**), uninfected *A. paradoxa* and infected *U. europaeus* in a single pot (**A-U+**), lastly, we had both species infected in a single pot (**A+U+**). Out of the planned 15 blocks we harvested only 6, as some plants had too much size disparity, did not get infected, or died. For

the actual study, we had a total of 7 replicates per treatment and four treatments consisting of

n = 24 *A. paradoxa*, n = 24 *U. europaeus*, and n = 12 *C. pubescens*.
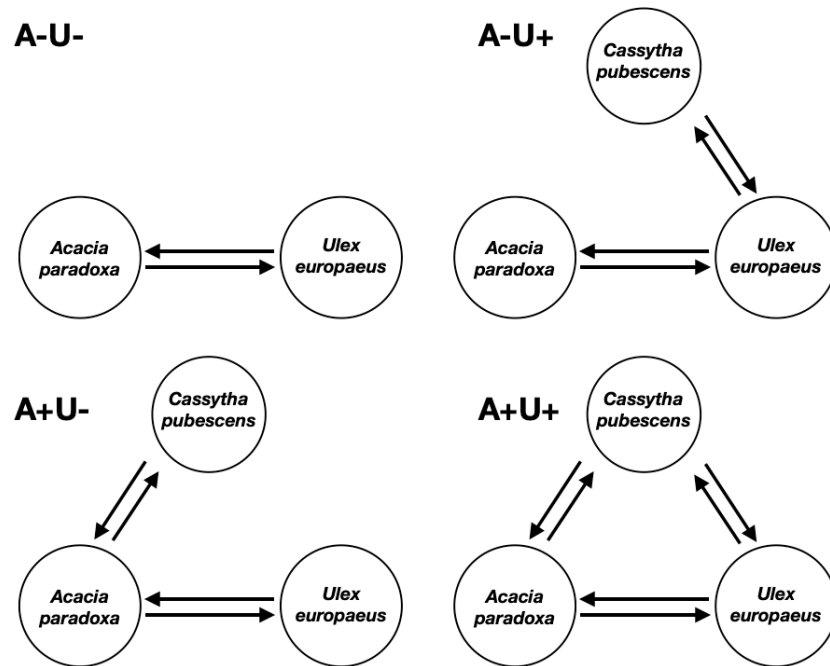

Figure 1: Module diagrams of experimental set-up for experiment 2. The modules represent interspecific competitive interactions amongst a native host *(Acacia paradoxa),* an invasive host (*Ulex europaeus)* and parasitic *Cassytha pubescens*. Arrows denote interactions.

We used the technique of Shen (*et al*. 2010) to infect the two plants species with *C. pubescens*. The experimental pots designated for infection were arranged around parasite-donor plants already infected with the parasite. *Cassytha pubescens* stems were placed around the foliage of hosts to be infected. The infection process began in early September 2019, and was completed in mid-January 2020, after which the stems of the parasite connecting the donor and recipient plants were severed. Prior to severing the connection, parasite and hosts were visually assessed for the infection status and their vigour. The least vigorous plants, or plants sustaining insufficient infection with *C. pubescens* were excluded from the experiment. To keep plants in

uninfected treatments parasite-free during the infection process and during the experiment, we manually untangled the parasite from plants not to be infected twice a week.

Host and parasite photosynthetic performance, N and C relations and growth:

In March 2020, we used a portable chlorophyll fluorometer (MINI-PAM; Walz, Effectrich, Germany) equipped with a leaf clip (2030-N; Walz) to measure pre-dawn quantum yield (F_v/F_m) 71 days after the infection process ended (DAT) and midday quantum yield (ϕ_{PSII}) after 72 DAT for each of the three species. Quantum yield is a measure of photosystem II efficiency. This allowed us to compare whether *C. pubescens* infection influenced the performance of each species and whether this was related to their competitive ability. We measured F_v/F_m between 04:30 and 05:30 hrs and ϕ_{PSII} between 11:50 and 14:10 hrs, on a warm, clear sunny day. Photon flux density was 931 ± 11.01 μmol m^{-2} s^{-1} for measuring ϕ_{PSII} ($n = 76$). To measure F_v/F_m and ϕ_{PSII} of *C. pubescens*, we used the leaf clip ~10 cm from a growing tip. In both *A. paradoxa* and *U. europaeus*, we measured the youngest fully expanded phyllode or spine, respectively. We measured F_v/F_m and ϕ_{PSII} on n = 24 *U. europaeus*, n = 24 *A. paradoxa*, n = 12 for *C. pubescens* (see Supp. Fig. 2).

After 77 DAT, we harvested shoots and roots (including nodules) of uninfected and infected hosts, and the parasite shoot; the material was harvested and oven-dried at 60° C for 96 hrs. To harvest nodules, the roots of each plant was gently washed in water above a large fine sieve (1.5mm) to capture fallen nodules after separating nodules from roots. Nitrogen and carbon concentration of host foliage was determined with EA-IRMS (Elemental Analyser Isotope Ratio Mass Spectrometry) by Mawson Analytical Spectrometry Services (The University of Adelaide).

Statistical analysis:

For both experiments differences in mean biomass, growth rate, nutrient/elemental concentrations were tested using ANOVA and linear mixed effect models in R statistical software (R Core Team 2016), using base functions and *lme4* package (Bates *et al*. 2015). For experiment 2, physiological responses (F_v/F_m at dawn, ϕ_{PSII} at noon) were analysed using linear mixed models (*lmer* function in *lme4* package). We planned *a priori* comparisons for both experiments. These comparisons were made within species only and using planned orthogonal contrast. We planned to compare responses in parameters within species between uninfected pairs (**A-U-**) to the three other treatments (**A-U+**, **A+U-**, **A+U+**). Species pairs (*e.g.* pot effects) and randomly allocated blocks were considered as random effects in our models. Infection status and treatments were used as explanatory factors in our models.

For both experiments, we tested the normality of variances using the Shapiro-Wilks test of normality in R Studio (*shapiro.test* function). When Shapiro-Wilks revealed variances were not normally distributed, we employed appropriate transformations and checked again with Shapiro-Wilks tests. Homogeneity of variances was checked using Bartlett's test function in R Studio '*bartlett.test*', or Levene's test when data were transformed. To perform *post-hoc* comparisons, we used the package '*emmeans*' (Lenth 2019) for Tukey's adjusted least-square means pairwise comparisons. To assess effect sizes, we used Cohen's *d*; a standardised effect size calculation for comparing mean differences between treatment and control groups, standardised by dividing mean differences by pooled standard deviation ('*effsize*' package, Torchiano 2020).

Results:

Experiment 1: Mixed and monocultures productivity assay

In our first experiment we assessed growth reduction lost by each species when growing alone in a pot or growing with another species. We found no difference within species among treatments in shoot or root mass (g) (Tables 1 & 2, Fig. 2).
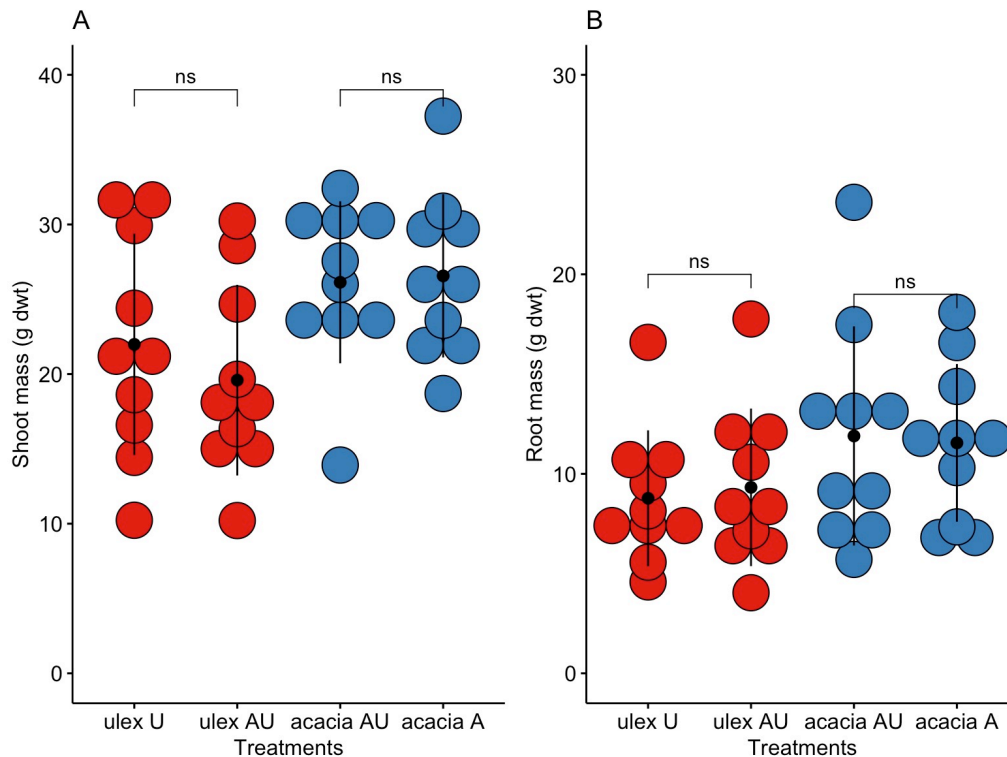

Figure 2: A) Shoot mass (g dwt) and **B**) root mass (g dwt) of *Ulex europaeus* (**red**) and *Acacia paradoxa* (**blue**) when grown in monocultures (**A**, **U**) vs. mixed cultures (**AU**), showing mean (black dots) ± SD. n = 10 plants per treatment, N = 40.

Table 1: Experiment 1, shoot mass (g dwt) linear model results. Multiple $R^2 = 0.259$, $p = 0.0497$. F-statistic 2.135 on 12 and 27 Df. Residual standard error = 5.732 on 27 Df.

Shoot mass (g)	Sum sq.	Df	F-value	P-value
Species	**309.1**	**1**	**9.410**	**0.00487**
Treatment	29.5	1	0.449	0.642 NS
Block	503.1	9	1.702	0.137 NS
Residuals	887.0	27		

Shoot mass ~ Species + Treatment + block

Table 2: Experiment 1, root mass (g dwt) linear model results. Multiple $R^2 = 0.29$, $p = 0.53$. F-statistic 0.923 on 12 and 27 Df. Residual standard error = 0.654 on 27 Df.

Sqrt(Root mass) (g)	Sum sq.	Df	F-value	P-value
Species	1.661	1	3.886	0.059 NS
Treatment	0.032	2	0.038	0.963 NS
Block	3.040	9	0.790	0.628 NS
Residuals	11.544	27		

Sqrt(root mass) ~ Species + Treatment + block.

Experiment 2: Parasite infection and competing pairs

In our second experiment, we found that in competing pairs of native and invasive species, native *A. paradoxa* attained the same final shoot biomass (g) in all treatments, whether *A. paradoxa* or competing *U. europaeus* were infected with *C. pubescens* or not (Table 3, Fig. 3, A). Although no statistical difference in mean shoot mass was detected, we found a slight reduction in shoot mass in *A. paradoxa* (Cohen's $d = -0.78$, $p = 0.185$) when infected. In contrast, we found that in invasive *U. europaeus, C. pubescens* infection greatly diminished shoot biomass growth (Table 3, Fig. 3 B, $p < 0.001$). Infected *U. europaeus* individuals had ~50% smaller shoot biomass than uninfected conspecifics (Cohen's $d = -2.74$), whether competing *A. paradoxa* was infected or not.
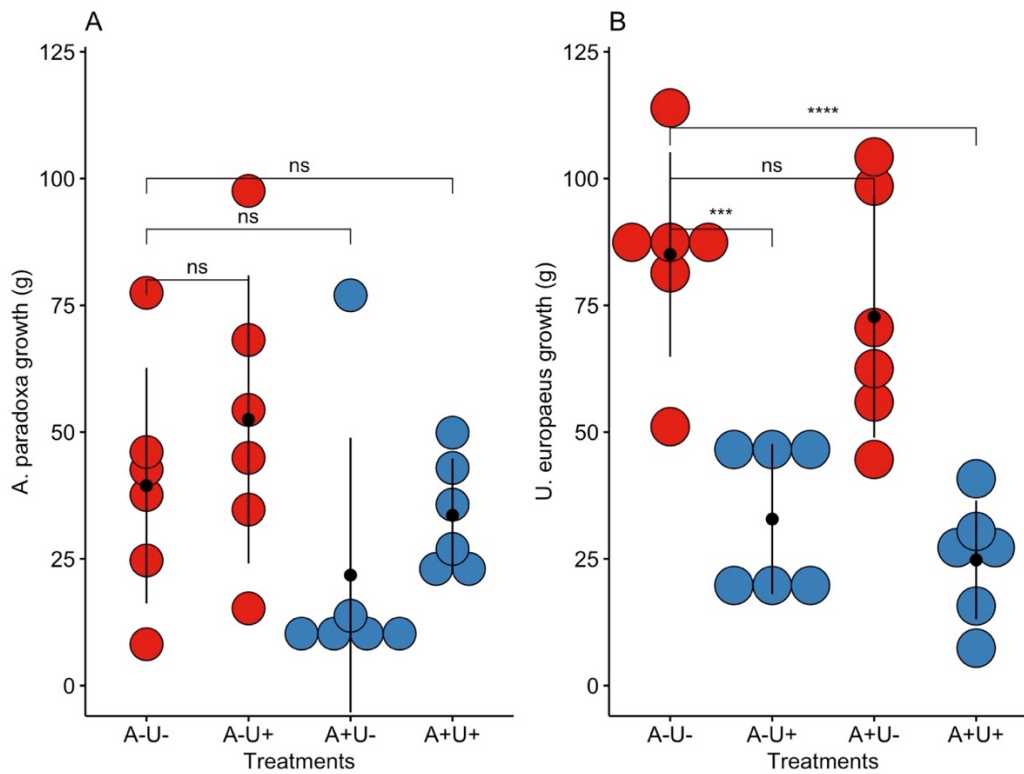
Figure 3: Growth of *Acacia paradoxa* and *Ulex europaeus* shoot mass; defined as the final mass minus the initially estimated mass of each individual. **Red** = uninfected, **Blue** = infected. **A**: Growth of shoot mass in native *Acacia paradoxa,* **B**: growth of shoot mass in invasive *Ulex europaeus*. Tukey's HSD pairwise comparisons, showing *adj. P* value are made against uninfected pairs (**A-U**-). **A-U+** = uninfected *A. paradoxa*, infected *U. europaeus*; **A+U-** = infected *A. paradoxa*, uninfected *U. europaeus*, **A+U+** = both infected. Black dots show means, with SD. N = 24.

Table 3: ANOVA output table for growth (g dwt) in Experiment 2. Growth was defined as the final mass minus the initial estimated mass of individuals. Alpha = 0.05, R^2 adj. = 0.54, F = 5.51 on 12 and 35 df. Residual SE = 1.503 on 35 df.

	Sum Sq.	df	F-value	P-value
Species	19.54	1	8.651	**0.00576**
Treatment	32.08	3	4.734	**0.00710**
Block	25.12	5	2.224	0.07370 NS
Species *Treatment	72.61	3	10.715	**3.83 e^{-5}**
Residuals	79.06	35		

*Sqrt(Growth ~ treatment * species + block)*

Experiment 2: Hosts shoot mass:

When comparing final shoot mass, we found a marginally significant effect for species (p = 0.08), but this effect was contingent on treatments ($p < 0.0005$, Table 4). *Ulex europaeus* had about 50% less shoot mass when infected by *C. pubescens,* regardless of the infection status of competitor (Fig 4, Cohen's d = 2.39). In contrast, *A. paradoxa* individuals did not statically differ in mass whether infected or not, regardless of the infection status of the competitor (Fig. 4, Cohen's d = 0.66). In our pre-planned post-hoc comparisons we found that *A. paradoxa* did not differ in shoot mass whether it was infected or not (**A-U-** *vs.* **A+U+** p = 1.0, **A-U-** *vs.* **A+U-** $p > 0.7$), however *U. europaeus* individuals had less shoot mass when infected (**A-U-** *vs.* **A+U+** $p < 0.0033$; **A-U-** *vs.* **A-U+** $p < 0.035$).
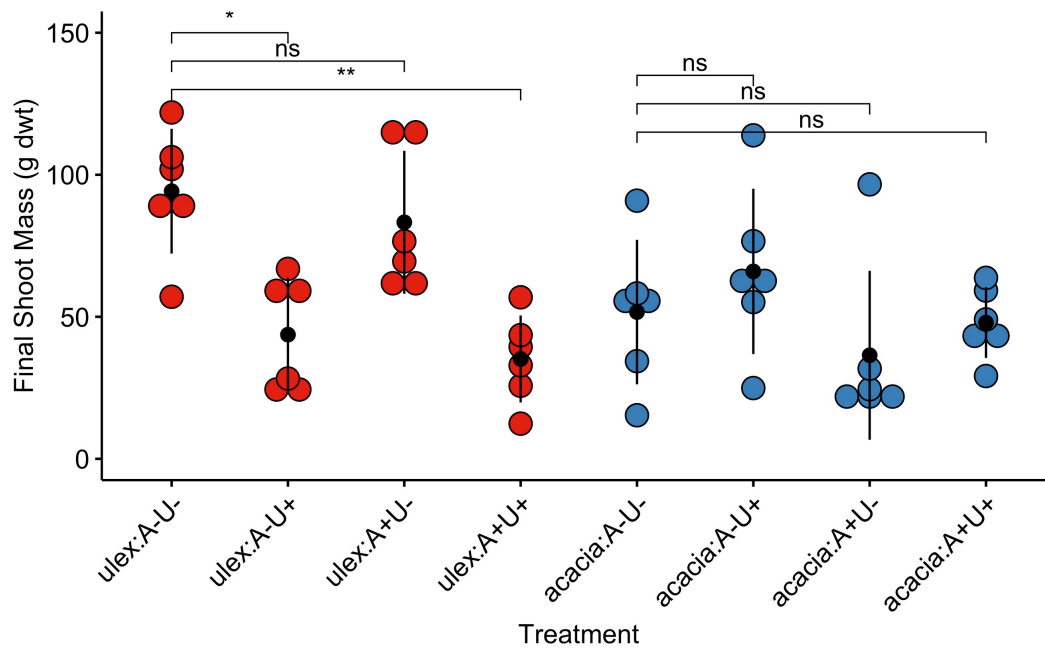
Figure 4: Final shoot mass of *Ulex europaeus* (red) and *Acacia paradoxa* (blue) across four treatments: **A-U-** = uninfected pairs, **A-U+** = infected *U. europaeus* only, **A+U-** = infected *A. paradoxa* only, **A+U+** = both species infected with *Cassytha pubescens*. Lines denote planned contrasts among **A-U-** and other treatments, within species. Black dots show mean for each treatment, with SD. N = 24.

Table 4: Linear model output table for log of final shoot mass, hosts only. Alpha = 0.05, R^2 = 0.581, R^2 *adj.* = 0.43, N = 48.

	Sum Sq.	df	F-value	P-value
Species	0.583	1	3.147	0.0847
Treatment	1.609	3	2.892	0.049
Block	2.119	5	2.286	0.067
Species *Treatment	4.697	3	8.445	**0.000235**
Residuals	6.488	35		

*Log (Final mass) ~ treatment * species + block*

Experiment 2: Root mass

We found no difference in the root mass of *A. paradoxa* or *U. europaeus*, whether infected or not (Fig. 5, A; $p > 0.05$, Cohen's $d = -0.3$). However, we found a negative effect of *C. pubescens* infection on *U. europaeus* root mass (Fig. 5 B, Cohen's $d = -1.2$), although it was not statistically significant for any planned comparison of interest to this study (Tukey's Adj. $p > 0.05$).
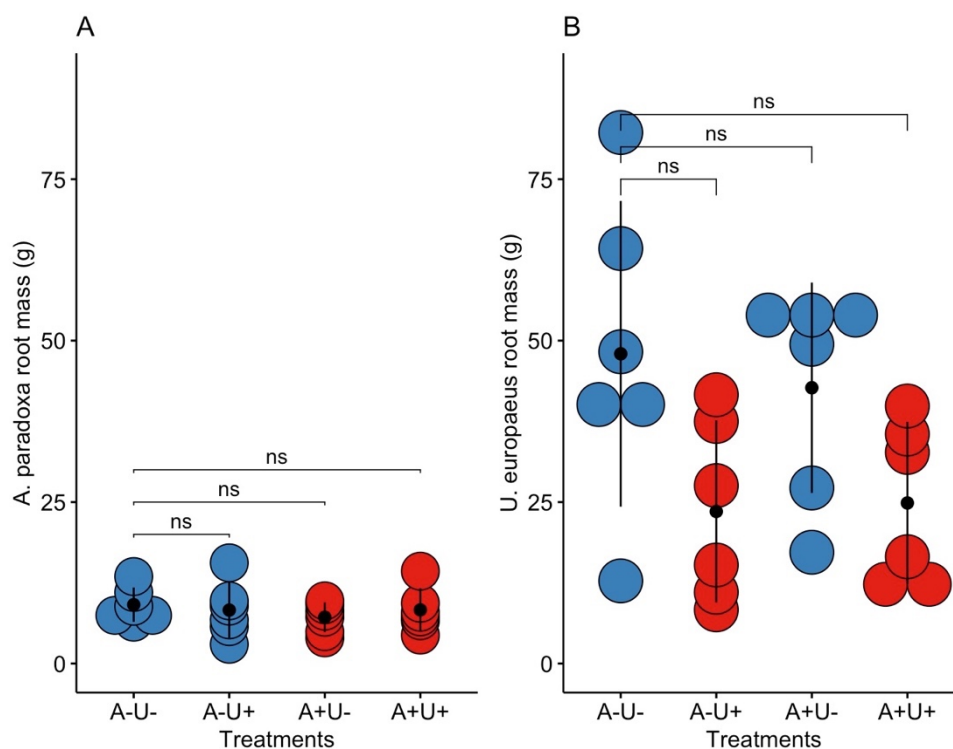

Figure 5: Root mass of **A:** *Acacia paradoxa* and **B:** *Ulex europaeus,* when uninfected (blue) and infected (red) with *Cassytha pubescens*. Black dots show mean and SD, N = 6 per treatment.

Table 5: ANOVA table of root mass (g dwt) in expt. 2; R^2 Adj.= 0.29, $p = 0.012$.

Final Root Mass:	Sum sq.	Df	F-value	P-value
Treatment	1.605	3	1.972	0.136 NS
Species	**21.383**	**1**	**78.841**	**$1.73e^{-10}$**
Block	0.838	5	0.618	0.687 NS
Treatment * Species	1.271	3	1.562	0.216 NS
Residuals	9.492	35		

*Log(Dry root mass) ~ species * treatment + block*

Quantum Yield study:

We found no difference in F_v/F_m and ϕ_{PSII} in either species when infected or not. We found no significant differences among our pre-planned contrasts within species, across treatments in pre-dawn F_v/F_m measurements (Table 6, $p = 0.17$) or noon ϕ_{PSII} measurements (Table 7, $p = 0.53$). We found a significant interaction effect between species and treatment (Table 6, $p = 0.049$), but these differences were not the focus of this study. We measured F_v/F_m and ϕ_{PSII} on n = 28 *U. europaeus*, n = 28 *A. paradoxa*. We found no difference in parasite F_v/F_m nor ϕ_{PSII} Supp. Tables 4 & 5).

Table 6: Analysis of deviance table pre-dawn QY (F_v/F_m), Alpha = 0.05. $R^2 = 0.57$, $n = 56$ (hosts only) from linear mixed model effect estimates.

	Chi sq.	df	P-value
Treatment	4.9987	3	0.1718 NS
Species	49.0256	1	**$2.526e^{-12}$**
Treatment*Species	7.8296	3	**0.04967**

*Predawn QY ~ treatment * species + block.*

Table 7: Analysis of deviance table for midday QY. Alpha = 0.05, $R^2 = 0.07$. $n = 56$ (hosts only). From linear mixed model effect estimates.

	Chi sq.	df	P-value
Treatment	2.2055	3	0.5309 NS
Species	0.2646	1	0.6070 NS
Treatment*Species	1.2238	3	0.7473 NS

*Midday QY ~ treatment * species + block.*

Nutrients:

We found no significant differences in N or C in our *a priori* contrasts within species, between infection treatments ($p < 0.37$, Table 8). While we found some marginally significant differences, these comparisons were not of interest in our study
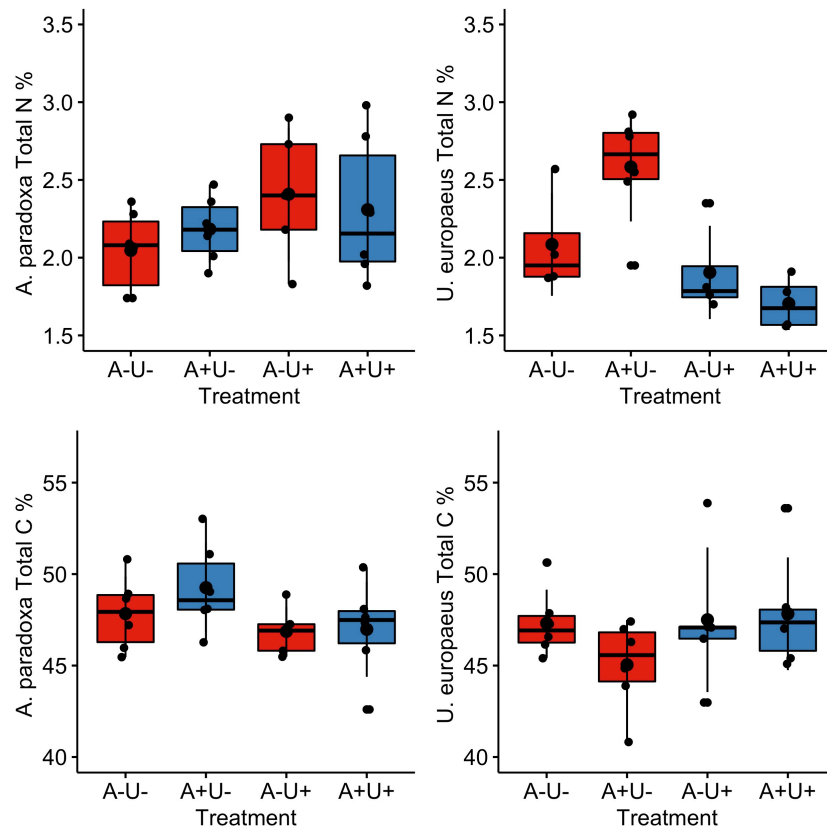

Figure 6 : Total N% (top) and total organic C% (bottom) in *Acacia paradoxa* (left), and *Ulex europaeus* (right). We found no differences within species, across treatments, in either total N% or total C%. Black dots = mean, error bars = SD.

Table 8: ANOVA table for N concentration among infected and uninfected *A. paradoxa* and *U. europaeus*. R^2 Adj. = 0.45, F-statistic: 4.57 on 15 and 49 DF. Alpha = 0.05.

Nitrogen:	Sum sq.	Df	F-value	P-value
Species	4.840	2	15.087	**7.84e⁻⁶**
Treatment	0.510	3	1.059	0.3751 NS
Block	0.631	5	0.786	0.564467 NS
Species * Treatment	5.015	5	6.253	**0.000147**
Residuals	7.859	49		

*Nitrogen% ~species * treatment + block*

Table 9: ANOVA table for C concentration among infected and uninfected *A. paradoxa* and *U. europaeus*. R^2 Adj. = 0.52, F-statistic: 5.794 on 15 and 49 DF. Alpha = 0.05.

Carbon:	Sum sq.	Df	F-value	P-value
Species	4.840	2	15.087	**2.22e^{-05}**
Treatment	0.510	3	1.059	0.0833
Block	0.631	5	0.786	**3.88e^{-05}**
Species * Treatment	5.015	5	6.253	**0.0109**
Residuals	7.859	49		

*Carbon% ~species * treatment + block*

Nodules: Host specific nodule mass

We found no difference between the mass of nodules per gram root in *A. paradoxa* or in *U. europaeus* (Table 10). We did not harvest block 7 for nodules, due to time constrains, n = 48 instead of 56.

Table 10: Analysis of deviance table for nodule dry mass of *Ulex europaeus* and *Acacia paradoxa*. Alpha = 0.05. R^2 = 0.50, n = 48.

Nodulation:	Sum sq.	Df	F-value	P-value
Treatment	0.2450	3	1.073	0.3730
Species	1.33	1	17.501	**0.0000183**
Block	0.8086	5	2.120	**0.08599**
Treatment * Species	0.0163	3	0.071	0.974
Residuals	2.6635	35		

*Sqrt(nodule mass) ~ treatment * species + block*

Discussion:
Expt. 1: Mixed vs. monocultures:

The aim of experiment 1 was to determine the intensity of competition between *U. europaeus* and *A. paradoxa*, while our second experiment determined whether their competitive balance would change when infected by *C. pubescens*. In our first experiment, surprisingly, we found that *U. europaeus* and *A. paradoxa* lost no productivity when grown in monocultures vs. mixed cultures, suggesting these species did not compete intensely under our experimental conditions. This evidence supports the null hypothesis that there would be no change in growth for either species when alone vs. in the presence of the other. We had expected a large decrease in the growth of *A. paradoxa* since *U. europaeus* was assumed to be the superior competitor. This inference was primarily due to the fast growth (Atlan *et al*. 2015), and greater photosynthetic capacity (Supp. Fig. 2) of *U. europaeus* compared with native Australian plants (Supp. Fig. 2), and the ability of *U. europaeus* to locally exclude native species (Lee *et al*. 1986; Dewar *et al*. 2006). The fact that there was no sign of competition can be ascribed to an ample resource supply, that even in the mix culture provided resources at a rate at least equal than the rate of extraction by the two plants together. We cannot rule out, however, that low irradiance during Autumn limited the growth, and consequently the use of water and nutrients of the plants. Priority effects may also account for lack of difference in *U. europaeus* growth between **U** and **AU** treatments. While we tried to minimise size disparity in pairs by quantifying their initial mass, small differences in initial conditions can lead to substantial differences in mass (May and Leonard 1975; Cameron *et al*. 2009). Since *A. paradoxa* individuals were generally larger than the *U. europaeus* in our first experiment (Fig. 2), perhaps *U. europaeus* was always at a disadvantage. Future studies should consider comparing growth patterns of native and invasive species at a range of size differences and their consequences for growth patterns.

Other studies on the competitive success of *U. europaeus* suggest it may be outcompeted, as seedlings by root-root competition and shading effects (Ivens and Mlowe 1980; Delerue *et al.* 2018). The results of Ivens and Mlowe (1980), Davies *et al.* (2005), and Delerue *et al.* (2018) disagree with the results observed in our first experiment. In our experiment, we did not see *U. europaeus* gain any more mass when grown in the absence of competitors. However, to the best of our knowledge, our study is the first study to assess the competitive ability of *U. europaeus* at stages larger than seedlings. Within our study, at later growth stages, *A. paradoxa* and *U. europaeus* seem to be in stable competitive balance under our experimental conditions.

Expt. 2: Competition and Cassytha pubescens infection

In our second experiment we found the presence of *C. pubescens* changed the competition outcomes from those observed in experiment 1. We found that *C. pubescens* infection did not affect native and invasive hosts growing together in the same way (Fig. 3); *U. europaeus* had up to *c.* 50% less shoot mass when infected, but *A. paradoxa* had similar shoot mass when infected or not. This evidence supports our third hypothesis that *U. europaeus* would have less growth when infected regardless of the infection status of competing *A. paradoxa*. We found that *C. pubescens* had a greater effect on the presumed superior competitor. In agreement with our results, root-hemiparasitic *Odontites litoralis* (Orobanchaceae) decreased the competitive advantage of a superior competitor, although the inferior competitor was most vulnerable to parasitism (Niemelä *et al.* 2008). Similarly, root-hemiparasitic *Melampyrum arvense* (Orobanchaceae) growing in mixed cultures of three host species did not alter the competitive hierarchy among hosts, but consistently decreased the growth of leguminous *Medicago sativa* much more strongly than the other host species in binary mixtures (Matthies 1996). These studies suggest that vulnerable host species will have decreased competitive advantage when competing with species less vulnerable to the same parasite. In contrast to our results, when

Triphysaria pusilla (Orobanchaceae) was grown in combination with grasses and dicots, authors expected the competitive release of dicot hosts from dominant grasses, instead they found that the combination of *T. pusilla* and grasses led to marked reduction in performance in dicots (Marvier 1998). The author suggested these results were due to the parasite competing for water with dicots, or alternatively that the parasite performed better when infecting grasses and therefore may have been able to infect nearby dicot species (Marvier 1998). Taken together, these studies suggest three-way interactions cannot be predicted on the basis of two-way interactions. Nonetheless, our results suggest that *C. pubescens* can modify competitive interactions between native and invasive hosts by having stronger negative effects on invasive hosts.

In our study, the greater negative effect on *U. europaeus* seems related to negative parasite effects on host photosynthetic biomass and resource removal, considering we found no effects on the photosynthetic performance of this host (Table 6 & 7, Supp. Figure 2). In our study, *A. paradoxa* did not increase in growth rate by competing with infected *U. europaeus* (Fig. 3, Fig. 4) as we had hypothesised. There may have been a negative effect in *A. paradoxa* growth due to photosynthetic area reduction by the parasite, by potentially reducing C budget (Cirocco *et al.* 2017). However, growth was not significantly different in *A. paradoxa* whether infected or not (Fig. 3), but was marked in *U. europaeus* (Fig. 3, 4).

How host species respond to resource availability may account for the differential responses to *C. pubescens* infection. By growing and performing similarly on both species (Supp. Table 5, Supp. Figure 3), the parasite likely removed similar relative amounts of resources from each host species. *Cassytha pubescens* infection can decrease the foliar nutrient content of *U. europaeus*, but not the foliar nutrient content of native hosts (Cirocco *et al.* 2017; Cirocco, Watling, *et al.* 2020). To explain our results, we developed a conceptual model (Fig. 7). In this

model, *C. pubescens* infection removes resources from hosts, decreasing effective resources available to hosts from R (supply in pot) to R_p (resources available for host after extraction of resources by the parasite), triggering a reduction in biomass in *U. europaeus* but not in *A. paradoxa* (Fig. 7). Generally, Australian native plants have adaptations commonly associated with nutrient-poor soils (Handreck 1997; Wright *et al.* 2004) – and thus, being adapted to relatively low resource availability compared with *U. europaeus*, *A. paradoxa* may not have been negatively affected in biomass by *C. pubescens* infection removing resources– and likely more resilient to changes at relatively higher resource availability levels. In contrast, *Ulex* species may readily respond to increasing resources (O'Toole *et al.* 1991; Augusto *et al.* 2005; Cavard *et al.* 2007), but may also consistently requires higher levels of resources, as suggested by their relatively high assimilation rate, as indicated by higher QY than *A. paradoxa* (supp. Fig. 2).
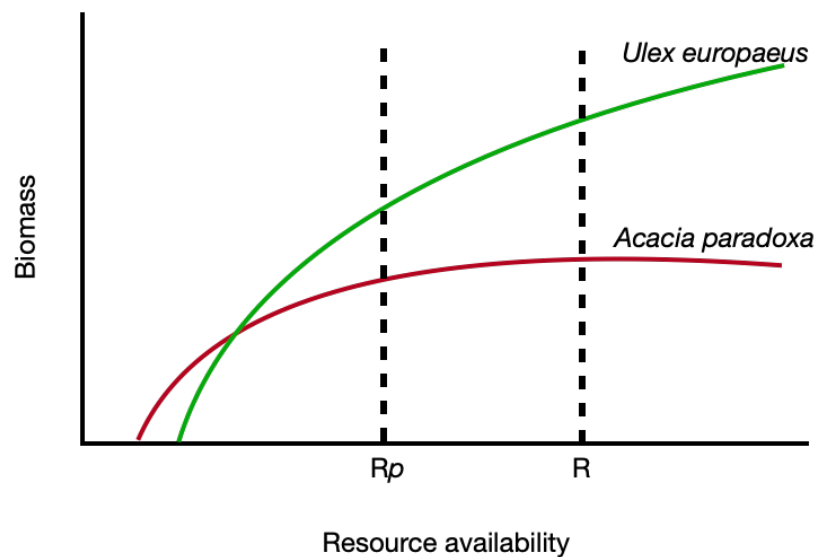

Figure 7: Conceptual model of biomass reduction in *Ulex europaeus* (green) and *Acacia paradoxa* (red), when infected by *Cassytha pubescens*. When each plant is infected, the parasite removes resources from host solutes. Thus, infection decreases effective resource availability for hosts from *R,* resource availability when uninfected to *Rp*, resource availability when

infected. Since *A. paradoxa* has lower photosynthetic rates than *U. europaeus*, it is possible that *U. europaeus* has a greater nutrient demand and is more negatively affected by the parasite removing resources.

Our results agree with previous studies on the effects of *C. pubescens* on native and invasive hosts; *C. pubescens* consistently has more substantial negative effects on the performance of invasive hosts species, but not on native hosts species (Prider *et al.* 2009; Shen *et al.* 2010; Prider *et al.* 2011; Cirocco *et al.* 2016; 2017; 2020). Our study, however, is the first to demonstrate that *C. pubescens* can alter competitive interactions between a native and an invasive host species. These results are important in demonstrating *C. pubescens* is unlikely to be harmful to native vegetation if deployed in the field for *U. europaeus* biocontrol. We know of numerous distantly related species that are immune or resistant to *C. pubescens* infection (Facelli *et al.* 2020). This makes sense considering that *C. pubescens* has coexisted with native Australian plant species for millions of years (Prider *et al.* 2009; 2011). Native host species to *C. pubescens* may act as infection reservoirs, facilitating the spread of *C. pubescens* onto pest species without greatly harming native hosts. While *C. pubescens* does not give a net benefit to the native host, as we hypothesised, the invasive host is consistently disadvantaged in growth rate. The main concern with increasing *C. pubescens* abundance in the field to control invasive species is the direct negative effects on native host physiology. We have demonstrated that when the parasite attacks native species, they are unlikely to be disadvantaged when competing with invasive species.

This paper demonstrated how a native parasitic plant modifies competitive outcomes between a native and an invasive competitor. While native *A. paradoxa* and *U. europaeus* do not appear to intensely compete (Fig. 2), *C. pubescens* may indirectly mediate population-level

competitive interactions over long timescales. Population dynamics of 'prey' species (*e.g.* hosts) may become linked by the effects of shared enemies through apparent competition. Competing species need not come into contact but by independently altering population dynamics (*e.g.* parasite-induced mortality, reduced fecundity), apparent competition occurs (Holt and Pickering 1985; Hatcher and Dunn 2011, pp. 33).

Conclusion:

We have demonstrated that a native parasitic plant can modify the competitive effects between native and invasive species. Previous studies suggested that the physiological impairment in *U. europaeus* by *C. pubescens* infection may reduce its competitive ability against native species resistant/tolerant of infection. We have demonstrated, via glasshouse experiments, that *C. pubescens* can modify the outcome of competitive interactions between a vulnerable invasive and resistant/tolerant native species. Organisms that mediate the coexistence or exclusion of other species may play keystone roles in ecological communities (Holt *et al.* 2003; Hatcher and Dunn 2011; Dunn *et al.* 2012).

Parasitic plants may be pests in agriculture and are thus perceived negatively in general for biodiversity. However, this perspective has been challenged with an increasing body of evidence from across the globe in the last two decades. Parasitic plants can have disproportionately strong and beneficial effects in ecological communities. This paper demonstrates that a native parasitic plant in Australia negatively impacts only invasive host species even when co-infecting native and invasive hosts. In a glasshouse setting, *C. pubescens* greatly decreased the growth of *U. europaeus*, an invasive plant considered a global pest, with no negative effect on the growth, nutrient concentration, or photosynthetic performance of a native host. Our results continue to support the potential use of *C. pubescens* is a viable

biocontrol agent in southeastern Australia against *U. europaeus*, one of the world's 100 worst invasive species.

Future studies should incorporate intraspecific as well as interspecific competition mediated by parasitic plants. Furthermore, future studies should assess how interactions may be modified in high vs. low nutrient conditions.

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Supplementary Information:
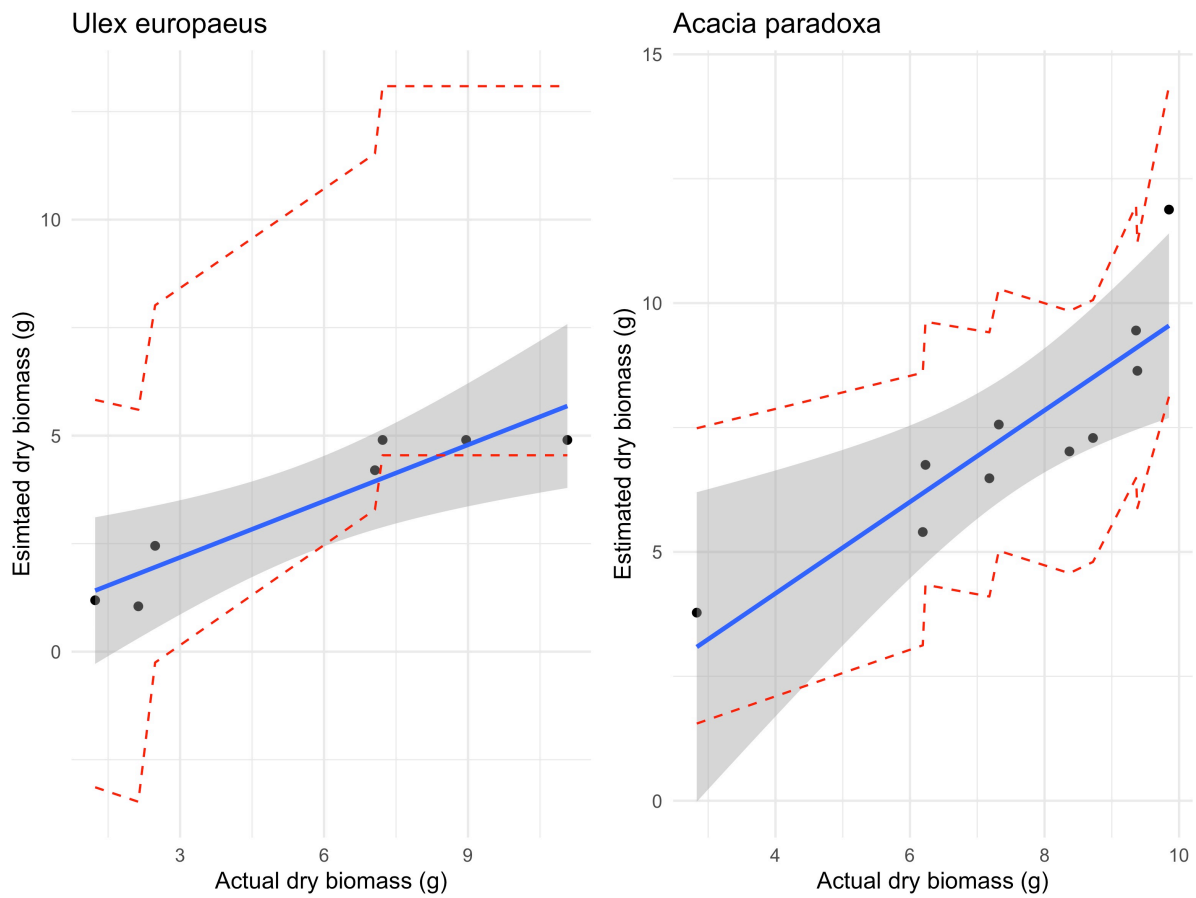
Estimating initial biomass:

We estimated the initial size of plants prior to randomly assigning them into blocks and treatments, for both experiment 1 and experiment 2. Using initial size estimates enabled us to calculate growth rates and minimise mass disparity among competing pairs. We used sampling units to estimate each plants biomass (see Andrew *et al*. 1979). A small branch representative of the shape and density of each species was taken. For each calibration shrub, the number of equivalent 'units' contained within it is scored, then the number of unit-equivalents was converted to mass (g.DW^{-1}) via a calibration curve. Calibration shrubs were harvested and dry mass determined. To find the relationship between biomass and number of sampling units, regression was used to estimate the slope and use the formula below to predict the initial biomass of each shrub, from the number of sampling units.

$$\hat{F} = b \times N$$

Where $\hat{F}$ = calculated mass of shrub (g.DW^{-1}), N = number of unit-equivalents in a shrub, F = actual mass of shrub (g.DW^{-1}), b = conversion factor, obtained as the slope of the regression through the origin of F vs. N for the calibration shrubs (g.DW^{-1}).

Experiment 2: Initial biomass

We estimated the biomass of *A. paradoxa* and *U. europaeus* individuals prior to planting them together to mitigate effects due to the initial size disparity between species. We estimated the mass of each species using 10 replicates of each species. We estimated *A. paradoxa* dry weight to range between 5.5 (g) and 22.1 (g) ± 14% residual standard error (n = 10, Adj. R^2 = 0.73, res. std. deviation = 1.088 on 8 df) and *U. europaeus* ranged between 1.9 (g) and 21.1 (g) ± 25% residual standard error (n = 7, Adj. R^2 = 0.84, res. std. err. = 1.476 on 5 df)

Supplementary Figure 1: Linear model calibration curves for *Ulex europaeus* (left) and *Acacia paradoxa* (right) for the initial biomass of saplings using a sampling unit (see Andrews *et al*. 1979). From these models, we can estimate the initial biomass of plants using a sampling unit of known dry mass and how many of these units there are in each plant. Dashed red lines show the 95% CI around the mean estimate.

Supplementary Table 1: Linear model output for calibration curves, *Ulex europaeus*. Alpha = .05. Adj. R^2=0.84 for formula: estimated initial biomass (g) = -1.052 + 2.014 x (# SU). N = 7.

| Parameter	Estimate	Std. Error	T-value	P-value
Intercept	-1.051	1.278	-0.823	0.44 *NS*
Estimated mass	**2.014**	**0.341**	**5.901**	**0.00199**

Supplementary Table 2: Linear model output for calibration curves, *Acacia paradoxa*. Alpha = .05. Adj R^2 = 0.73 for formula: estimated initial biomass (g) = 1.3811 + 0.8299 x (# SU). N = 10.

Parameter	Estimate	Std. error	T-value	P-value
Intercept	1.381	1.260	1.096	0.305 *NS*
Estimated mass	**0.829**	**0.1633**	**5.081**	**0.000952**
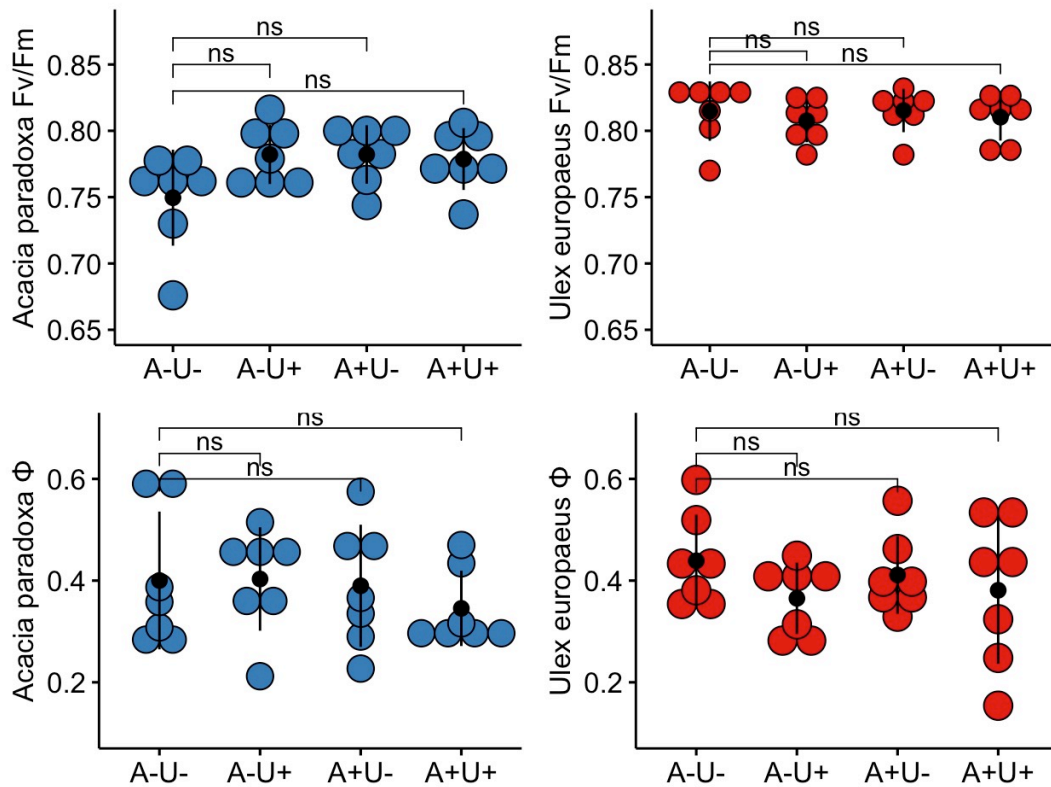
Supp. Figure 2: Quantum yields of *Acacia paradoxa* (blue) and *Ulex europaeus* (red), in pre-dawn (top panels) and midday (bottom panels), in each experimental treatment where neither species was uninfected (**A-U-**), only *U. europaeus* infected (**A-U+**), only *A. paradoxa* infected (**A+U-**), and both species infected with *Cassytha pubescens* (**A+U+**).
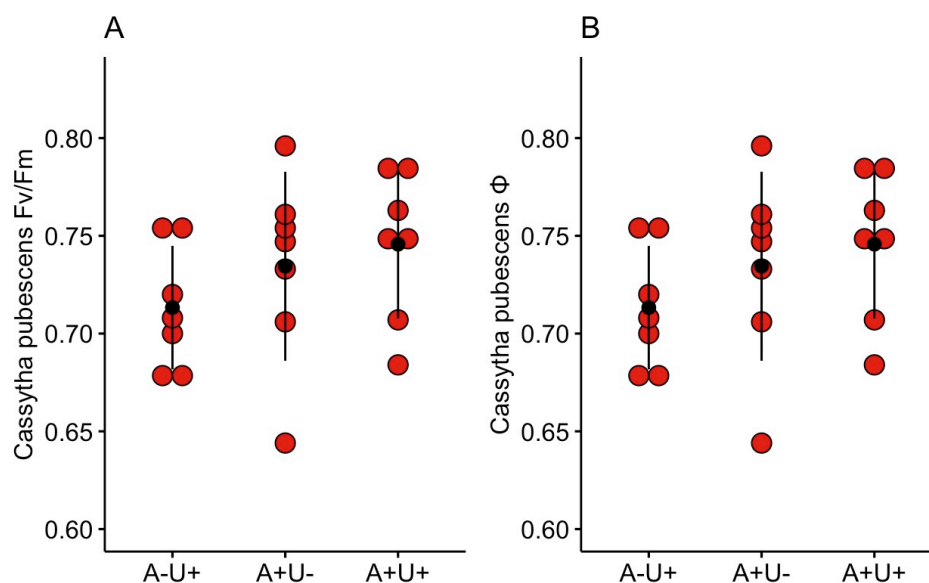
Supp. Figure 3: Quantum yields of *Cassytha pubescens* in pre-dawn (**A**) and midday (**B**), in each experimental treatment where neither species was uninfected (**A-U-**), only *Ulex europaeus* infected (**A-U+**), only *Acacia paradoxa* infected (**A+U-**), and both species infected with *C. pubescens* (**A+U+**). Black dots = mean, error bars = SD.

Supp. Table 3: Analysis of deviance table for pre-dawn QY of *Cassytha pubescens*. Alpha = 0.05, $R^2 = 0.38$. $n = 21$. From linear mixed model effect estimates;

	Chi sq.	df	P-value
Treatment	3.4482	2	0.1783 NS

Pre-dawn QY ~ treatment + block.

Supp. Table 4: Analysis of deviance table for midday *Cassytha pubescens* QY. Alpha = 0.05, $R^2 = 0.17$. $n = 21$. From linear mixed model effect estimates;

	Chi sq.	*Df*	*P*-value
Treatment	4.2884	2	0.1172 NS

Midday QY ~ treatment + block.

Supp. Table 5: Analysis of deviance table for dry mass of *Cassytha pubescens*. Alpha = 0.05, $R^2 = 0.42$. $n = $ 12 (parasite infecting single hosts only). From linear mixed model effect estimates;

	Chi sq.	*Df*	*P*-value
Host species	1.398	1	0.2371NS

Log(Cassytha dry mass) ~ species + block.

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Chapter 6: General Discussion

Ecological studies of plant parasites have largely focused on root hemiparasites (mainly Orobanchaceae), non-clonal stem hemiparasites (Loranthaceae, Santalaceae), and one stem-holoparasite genus (*Cuscuta* spp.). *Cassytha pubescens* (Lauraceae) is vastly different from these other parasitic plants: it attaches to stems, is leafless and rootless, can photosynthesise and attach to multiple hosts simultaneously. While these characters are found individually in the mentioned well studied parasites, none of them present this combination of characters. These traits combinations make *C. pubescens* a unique organism, with potential to help develop our understanding of how parasitic plants affect their biotic and abiotic environment. However, previous to this thesis no studies have investigated the influence of *C. pubescens,* or of any leafless stem-hemiparasite, on soil nutrients, litter dynamics, competitive interactions, arthropod communities, and co-occurring plants emergence and growth.

The main aim of this project was to understand how *C. pubescens* may indirectly affect some key ecological processes and whether these modifications would positively or negatively affect native or invasive plant species that commonly occur with the parasite. Overall, the project has provided further insight into how minor components of communities, such as parasites, can have strong and far-reaching effects on the ecology of the component species. Importantly, given that no serious negative effects of the parasites on ecological processes were detected, these studies largely support the use of this parasitic plant in its native range for suppressing invasive species, primarily *U. europaeus*, while having minimal impacts on native species.

Cassytha pubescens may have some indirect influence on vegetation, but its effects may be species specific. In communities invaded by *U. europaeus*, where *C. pubescens* infects both

native and invasive species, we can expect that *U. europaeus* will be negatively affected by the parasite more strongly than native species (Prider *et al*. 2011; Cirocco *et al*. 2017, 2018). The results reported in chapters 2 and 3 together demonstrate that increased soil resources (*i.e*. P, and K) under infected plants benefit the growth rate of invasive seedlings more than native species, probably because of their greater resource-use efficiency (Demey *et al*. 2013; Funk 2013) compared with native plants. However, the stronger negative effects of parasitism on invasive species may offset these growth benefits for invasive species particularly in smaller, early stages of plant development (Cirocco *et al*. 2020). Furthermore, by infecting *U. europaeus* or other weeds at early juvenile stages, the parasite may help reduce potential life-time fecundity, further reducing the spread of invasive weeds. It is widely acknowledged that parasitic plants can alter soil nutrients beneath their hosts, this is mainly due to the input of large volumes of high nutrient content leaf litter. Aside from chapter 2, only one other study has assessed whether a leafless (but holoparasitic in that case) vine can influence soil nutrients (Yu *et al*. 2009). This question had previously not been addressed. In this study I have demonstrated that *C. pubescens* can enhance the returns of some nutrients, despite not having a drastic effect on total litterfall under its hosts. These results also suggest the secondary metabolites present in *C. pubescens* may slow down its decomposition rate, possibly hindering its contributions to soil nutrient returns.

The results in chapter 3 indicate that *C. pubescens* litter can decrease seedling recruitment of *U. europaeus* by suppressing seedling emergence. Under the litter of *C. pubescens*, 27% fewer *U. europaeus* seedlings emerged than in treatments without litter. However, native seedlings were also negatively affected, as *Acacia pycnantha* and *Acacia myrtifolia* were also suppressed (29% and 36%, respectively). While it appears that *C. pubescens* litter had a greater negative impact on native seedlings, these have smaller germination proportions in comparison to *U. europaeus* – therefore there was a greater negative impact on *U. europaeus* emergence in terms

of total number of seedlings. Invasive species (*e.g.* *U. europaeus*) generally displace natives from a patch by recruiting large numbers of seedlings with high survival rates (Dewar *et al.* 2006; Udo *et al.* 2017; Paynter *et al.* 2018), *C. pubescens* may decrease flowering and seed output (*c.* -50%) of invasive plants due to a decreased C-budget (Prider *et al.* 2011; Cirocco *et al.* 2017). Therefore, *C. pubescens* may directly impact key processes that allow *U. europaeus* to displace native vegetation. I found that litter of *C. pubescens* had a negative effect on the emergence of seedlings of native species. Some common native hosts of *C. pubescens* (*e.g.* *A. pycnantha* and *B. spinosa*) may be "weedy" (*i.e.* respond to disturbances, particularly fire, establishing in large numbers) and may at times be relatively overabundant. Rarer native plant species may benefit from some suppression of these. Studies of the post-fire dynamics in situ with or without this parasite may be informative.

There were several unexpected events that reduced the amount of information collected for chapter 3. I conducted a study assessing how *A. pycnantha*, *Eucalyptus obliqua* and *U. europaeus* seedlings grew in soil collected from under infected or uninfected shrubs. The plants in this study died after a failure in the watering system during a time with restricted access to the glasshouse. This study had 10 replicates per treatment, 2 treatments, 3 species. Five seeds of each species were sown in small (5 x 5 cm wide, 15 cm tall) pots filled with soil from either infected or uninfected shrubs. After germination, seedlings were thinned out so that only one individual remained in each pot. This experiment would have been useful in understanding how native and invasive plants responded to soils affected by *C. pubescens*, without the influence of varying seedling density. I also conducted a third study as part of chapter 3, assessing whether leachates from soaked *C. pubescens* stems had an influence on germination of six species (same as chapter 3). This experiment was hindered by high amounts of fungal mould infection and high seed mortality because of it. The results from this study could not be

statistically analysed between treatments adding water and treatments adding *C. pubescens* stem leachates because of small sample sizes for *U. europaeus* and *A. pycnantha*.

Some parasitic plants may have bottom-up effects, by increasing the input of litter or by altering vegetation structure and composition, increasing the abundance and species richness of arthropod communities (Watson *et al.* 2011; Ndagurwa *et al.* 2014; Hartley *et al.* 2015; Mellado *et al.* 2019). It is not clear to what extent certain arthropod groups respond to resources in litter vs. the structure/microhabitats that litter provides. Previous studies have found large differences between infected and uninfected plants and the ground arthropods under their canopies (Ndagurwa *et al.* 2014; Hartley *et al.* 2015; Mellado *et al.* 2019). While root-hemiparasites boost arthropod abundance by altering vegetation structure (Hartley *et al.* 2015), stem-hemiparasites form dense layers of litter, providing resources and microhabitats for ground arthropods and increasing their abundance (Ndagurwa *et al.* 2014; Mellado *et al.* 2019). In contrast to those findings, the findings in chapter 4 suggests that both native and invasive host will have similar arthropod abundance, whether infected or not by *C. pubescens*. These results support the use of *C. pubescens* in the field to suppress invasive species, as *C. pubescens* does not appear to have a negative effect on arthropod communities. While I did not find any positive effects on arthropod abundance, it is quite likely that upon the death of the parasite or host, the large structure of dry *C. pubescens*, may have stronger effects similar to those under mistletoes. It is not surprising that *C. pubescens* may have little effect on arthropod communities since it contributes little to total litterfall under its hosts (see chapter 2), compared with mistletoes, or vegetation structure like rattles. However, it is surprising no effects were found on arthropod abundance and composition, given changes, albeit minor, in litter quality under infected shrubs. In chapter 4 I set up pitfall traps under shrubs to trap ground arthropods every season for one year. Unfortunately, during winter of 2020 and winter of 2021, very few

arthropods were captured and statistical analyses to compare abundance of total arthropods, or within-group differences could not be done. I suspect that the cold and heavy rains during these periods made trapping arthropods more difficult. Therefore, chapter 4 relied on comparing arthropod communities in summer and spring, between infected and uninfected shrubs.

Different parasitic plants may alter vegetation structure and other plants through different mechanisms. Mistletoes can enhance the growth of understory vegetation, by producing abundant of litter that rapidly decomposes and enhances soil nutrient returns (March and Watson 2007; Ndagurwa *et al*. 2016). Other parasitic plants, like *Rhinanthus* species, may alter vegetation structure by infecting several hosts simultaneously, decreasing grass biomass, increasing forbs abundance, and facilitating competitive release of less competitive species (Gibson and Watkinson 1992; Davies *et al*. 1997). *Cassytha pubescens* may be more similar to *Rhinanthus* spp. in its effects on co-occurring vegetation than to mistletoes. This is because *C. pubescens* contributed relatively little to soil fertility in comparison with mistletoes, and because *C. pubescens* can, like root-hemiparasites, infect several host species simultaneously like root-hemiparasites. The results from chapter 5 suggest that *C. pubescens* modifies competitive interactions between hosts, by indirectly having stronger negative effects on an invasive competitor than a native one. These results suggest that *C. pubescens* would facilitate competitive release onto native hosts, when in competition with invasive species that are vulnerable to *C. pubescens* infection (*e.g. Ulex europaeus, Cytisus scoparius, Rubus fruticosus* sp. aggregate).

While I had planned to assess whether or not *C. pubescens* could increase litterfall beneath infected hosts and then assess whether this litter increased the abundance and diversity of arthropods in the field, these studies were conducted simultaneously because of the time and

volunteer limitations imposed by the PhD structure and COVID-19 pandemic. Therefore I could not know that *C. pubescens* had a minimal impact on litterfall on hosts, prior to assessing whether litter input had impacts on arthropods. The order in which the studies were planned to be done could not have worked during the pandemic, forcing me to conduct studies simultaneously. Had it been possible to conduct chapter 2 (assessing litterfall) before I started chapter 4 (trapping arthropods), I would have used different methodologies to assess effects on arthropods (*e.g.* suction sampling upon host foliage).

Some parasitic plants have been used as habitat restoration tools because of their ability to suppress competitively dominant species and conferring competitive release onto inferior competitors (Těšitel *et al*. 2017, 2018). In chapter 5 I demonstrated that *C. pubescens* may modify the competitive balance between invasive *U. europaeus* and native *A. paradoxa*. When these plants were in direct competition, infected *U. europaeus* consistently grew smaller (*c.* 50% less biomass than uninfected *U. europaeus*) – regardless of the infection status of native competitor *A. paradoxa*. In the field, *C. pubescens* can infect several host species simultaneously. If *C. pubescens* is deployed to suppress invasive *U. europaeus*, the results in chapter 5 suggest that if *C. pubescens* co-infects a native and an invasive plant, while the native species may not gain competitive advantage by competing with an infected invasive species, the invasive species will not gain a competitive advantage by competing with an infected native species. Furthermore, even if both species are co-infected, the invasive will consistently have lower growth than the native.

Future Research

In this project I found that *C. pubescens* reduced the growth of an invasive host in direct competition with a native host that was unaffected in growth. Future studies should aim to

assess whether *C. pubescens* or other parasitic plants modify intraspecific competitive interactions of native and invasive plant species. This would help further our understanding of competition, as well as understanding the role of parasites in diversity maintenance. This is important to understand because parasites are not perceived as having conservation value, yet they may be 'keystone' organisms.

Future studies on *C. pubescens* and arthropods should quantify the abundance of arthropods on foliage of hosts and *C. pubescens* vines, through suction sampling – particularly of interest may be sap-suckers and gall formers. *Cassytha pubescens* may interact with sap-suckers and herbivores more strongly than ground arthropods because of its weak influence on litter, but potentially strong influence on host nutrient content. Of particular of interest would be assessing the potential additive effects of using the native parasite and introduced enemies. This has been looked at in one other invasive species (Prider *et al*. 2011), but not in *U. europaeus* and its many introduced specialist enemies.

Significance:

This work helps further our understanding of how parasitic plants that are different from mistletoes and rattles can influence ecological communities by altering basic ecological processes. This is important to understand, particularly if parasites will be used for the biocontrol of invasive species. By strongly modifying processes over relatively short timescales, my studies demonstrated the potential impact of a relatively rare plant species on ecological community structure and composition over its lifetime. My research also fills critical knowledge gaps in our understanding of the role of parasitic plants in ecological communities, by focusing on a unique group of parasitic plants that have biologically significant differences

from previously studied parasitic plants. Furthermore, these studies will have direct applications in assessing the benefits and drawbacks of using *C. pubescens* as a biocontrol agent. Given the potential of *C. pubescens* as a biocontrol agent against invasive plant species, and since trials in the field are ongoing, we need to understand the effects *C. pubescens* may have on co-occurring vegetation assemblages, their interactions, and arthropod communities.

General conclusion:

The impact of *C. pubescens* infection on native species is weak in comparison with effects on invasive species. While it is not practical to test the effect of *C. pubescens* on all potential host species in its range, common and abundant native species appear to be only mildly affected. Furthermore, considering that invasive species displace native species and profoundly disrupt ecosystem processes, negative effects on native plant species may be a small cost as native hosts may act as infection reservoirs, allowing *C. pubescens* to remain in the patch, ready to vegetatively spread onto potential invasive hosts. Also, the long-lasting seeds of *C. pubescens* do not germinate readily, and may be really important in fire events to prevent further expansion of invasive species. Stem-hemiparasites and their indirect effect on host-host competitive interactions have never been studied. While root-hemiparasites have been well studied in this regard, there are many differences in life form and even nutrient acquisition (access to soil nutrients) that may alter how different parasites affect indirect interactions between hosts.

By modifying certain ecological processes *C. pubescens* can increase soil fertility, change seedling recruitment, changes competitive interactions among plants, with no negative effects on ground arthropod fauna. Further to exerting strong indirect effects on these processes, invasive plant species are consistently more disadvantaged by *C. pubescens* than native species.

In time, modifications to these processes can lead to substantial changes in ecological communities and their components to the benefit of native biota. *Cassytha pubescens* is a viable biocontrol agent against Weeds of National Significance in its native range, with no substantial negative impacts on native biota found in these studies.

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