

Cassytha pubescens:

Germination biology and interactions with native and introduced hosts

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Table of Contents

Table of Contents	ii
Abstract	v
Declaration	vii
Acknowledgements	viii
Chapter. 1 Introduction	1
1.1 General Introduction	1
1.2 Literature Review	3
1.2.1 Characteristics of parasitic control agents	3
1.2.2. Direct impacts on hosts	7
1.2.3. Indirect impacts on hosts	8
1.2.4. Summary	11
1.3 Research Aims	12
Chapter. 2 Study species	15
2.1 <i>Cassyltha pubescens</i>	15
2.2 <i>Cytisus scoparius</i>	15
2.3 <i>Ulex europaeus</i>	16
2.4 <i>Acacia myrtifolia</i>	16
2.5 <i>Leptospermum myrsinoides</i>	16
Chapter. 3 Dormancy and germination ecology of <i>Cassyltha pubescens</i>	17
3.1 Abstract	17
3.2 Introduction	18
3.3 Materials and Methods	20
3.3.1 Seed collection and seed viability assessment	20
3.3.2 Imbibition	20
3.3.3 Breaking Physical dormancy	21
3.3.4 Cold stratification experiment	22
3.3.5 Statistical Analyses	22
3.4 Results	23
3.4.1 Seed Viability and Imbibition	23
3.4.2 Breaking Physical Dormancy	23
3.4.3 Cold stratification experiment	23
3.5 Discussion	24
3.5.1 Dormancy	24
3.5.2 Germination ecology	25
3.5.3 Conclusion	27
Chapter. 4 Parasite, hosts and Rhizobium	32
4.1 Abstract	32
4.2 Introduction	33
4.3 Materials & Methods	35
4.3.1 Plant material and Experimental design	35

4.3.2 Nitrogen content.....	36
4.3.3 Host photosynthetic physiology.....	37
4.3.4 Growth and Biomass.....	38
4.3.5 Statistical analysis.....	38
4.4 Results.....	38
4.4.1 Infection with <i>C. pubescens</i>	38
4.4.2 Rhizobium nodulation.....	39
4.4.3 Plant nitrogen.....	39
4.4.4 Soil nitrogen.....	40
4.4.5 PSII efficiency and chlorophyll content.....	40
4.4.6 Growth and Biomass measurement.....	41
4.4.7 Effects of hosts condition on the parasite.....	43
4.5 Discussion.....	43
4.5.1 Symbiont specificity of <i>C. pubescens</i>	44
4.5.2 Effects of the parasite on the <i>Cytisus</i> -rhizobium system.....	45
4.5.3 Effects of host nodulation status on parasite.....	48
4.5.4 Effects on soil nitrogen.....	48
4.5.5 Conclusion.....	50
Chapter. 5 Host resistance to the parasite.....	68
5.1 Abstract.....	70
5.2 Introduction.....	71
5.3 Materials and Methods.....	72
5.3.1 Plant Materials and Growth Conditions.....	72
5.3.2 Experimental design.....	73
5.3.3 Statistical Analysis.....	74
5.4 Results.....	74
5.5 Discussion.....	74
5.5.1 Biotic resistance hypothesis.....	75
5.5.2 Resource availability hypothesis.....	76
5.5.3 Conclusion.....	77
Chapter. 6 Effects of <i>Cassitytha pubescens</i> on competition between native and invasive hosts.....	80
6.1 Abstract.....	80
6.2 Introduction.....	81
6.3 Materials & Methods.....	82
6.3.1 Experimental design.....	82
6.3.2 Harvest.....	83
6.3.3 Statistical analysis.....	84

6.4 Results.....	84
6.5 Discussion.....	84
6.5.1 Parasite-mediated competition.....	85
6.5.2 Effects of competition.....	86
6.5.3 Conclusion.....	87
Chapter. 7 General Discussion.....	94
7.1 Summary.....	94
7.1.1 Comparison between <i>Cassytha pubescens</i> and <i>Cuscuta</i> spp.....	94
7.1.2 Effectiveness as biological control agent.....	95
7.1.3 Effects of <i>Cytisus scoparius</i> on the environment.....	96
7.2 Future research.....	97
7.3 Conclusion.....	97
Appendix 1.....	99
References.....	100

Abstract

The native hemiparasitic vine *Cassytha pubescens* infects and often kills the invasive weeds *Cytisus scoparius* and *Ulex europaeus* in the Mount Lofty Ranges, South Australia. This leads to the consideration of whether this parasite is a suitable biological control agent for these weeds. The aims of this study were to investigate germination characteristics of the parasite, the direct effects of the parasite on both invasive and native hosts, and the indirect effects of the parasite on interactions between native and invasive hosts.

Seed dormancy and germination of *C. pubescens* were examined. Imbibition tests revealed that the seeds are enclosed in a water impermeable seed coat, which produces physical dormancy. Germination experiments showed that heat and scarification broke the physical dormancy, but the germination rate of heated seeds was over three times higher than that of scarified seeds. Thus this parasite may have evolved to share similar fire-related germination cues as some of its native hosts.

The direct impact of *C. pubescens* on growth of *Acacia myrtifolia* (a native legume) and *Cytisus scoparius* (an invasive legume) was investigated in a pot experiment. None of the parasites on infected *A. myrtifolia* survived, so none of the *A. myrtifolia* was successfully infected with the parasite. In contrast, *C. pubescens* successfully infected *C. scoparius*. Host biomass accumulation was reduced by 21%, relative to uninfected plants. Photosystem II efficiencies were reduced but only on the infected branches. The total nitrogen content of infected plants plus parasite was the same as that of uninfected plants, and there was no impact of infection on nodulation by *Rhizobium*. Thus, it is likely that the removal of nitrogen by the parasite, reduces the supply to the host, and this limits the biomass accumulation of *C. scoparius*.

The differences in resistance to the parasite by native and invasive hosts were studied. A pot experiment was conducted using ^{32}P to examine the uptake of nutrients by the parasite from either *C. scoparius* or *A. myrtifolia*. In this experiment, *C. pubescens*

was able to successfully attach to *A. myrtifolia* hosts. The parasite absorbed no ^{32}P when attached to the native host, but did take up ^{32}P from the invasive host. This suggests *A. myrtifolia* resists the formation of functional haustoria by the parasite, while the invasive host does not. It is likely that this resistance of the native host to the native parasite may have evolved through long-term coexistence, whereas the invasive host has had only a short-term association with the parasite.

To investigate if the differences in host resistance to *C. pubescens* lead to changes in competitive outcomes between hosts, plants of the invasive weed *Ulex europaeus* were grown together with either *A. myrtifolia*, or a native non-legume, *Leptospermum myrsinoides*, and either with or without *C. pubescens*. There was no effect on either biomass accumulation of hosts or on the intensity of competition between hosts. However, as these are perennial species, it is possible that the experiment was too short to detect any effects. Long-term experiments and field monitoring may be required to resolve these competitive interactions.

These results provide an important insight into the germination ecology of *C. pubescens*, and the nature of its impact on both native and invasive hosts. Unlike the morphologically similar holoparasites of the genus *Cuscuta*, *C. pubescens* does not seem to act as a carbon sink, thus had little effect on symbiotic nitrogen fixation. This suggests a different carbon-nitrogen economy model from the one proposed for the morphologically similar holoparasites, *Cuscuta* spp. The study also detected differences in resistance of hosts to the parasite; however, this appeared to have no effect on host competition in a short-term pot experiment.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Hong Tai (Steven), Tsang

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Chapter. 1 Introduction

1.1 General Introduction

Biological invasion of natural ecosystems arguably constitutes one of the worst threats to the environment. While biological invasions are a normal consequence of species dispersal, human transportation systems have enhanced the dispersal ability of many organisms. This has allowed numerous organisms to invade areas otherwise unreachable through natural dispersal mechanisms and has accelerated the spread of others. The introduction of exotic organisms affects the invaded habitat through different mechanisms, the impacts of these varying in different trophic levels and scales (Ehrenfeld 2003; Levine, Vila et al. 2003; Strayer, Eviner et al. 2006). While the management priority is usually given the prevention of introductions, once organisms are introduced their control and eradication becomes paramount, due to the significant negative effects of introduced species on invaded habitats. In Australia, at least \$116.4 million are spent by governments yearly in the control of invasive species in natural ecosystems (Sinden, Jones et al. 2004).

Among the methods used to control introduced populations, biological control is probably the most controversial one. The idea of biological control has arisen out of the enemy release hypothesis. This hypothesis states that absence of the specific predators and parasites of the introduced species in the invaded habitat allows the populations of many introduced species to increase rapidly, eventually leading to serious problems (Fagan, Lewis et al. 2002). Therefore, biological control programs aim to introduce some of the enemies of the introduced species from the original area into the invaded habitat. Many studies have shown that often the introductions of natural enemies successfully control the population of the introduced species, and hence restrict their impacts, however, there have also been many failures and unexpected negative effects.

Introduction of exotic biological control agents is not a risk-free solution for biological invasion. There are inherent risks, because they can negatively affect native

species as well as the target introduced species (Howarth 1991; McFadyen 1998; Pemberton 2000). Native species can be directly and indirectly affected by the introduction of a control agent (Louda, Kendall et al. 1997; Pearson and Callaway 2003; Pearson and Callaway 2005). The potential risks of introducing control agents have led to the search for a new source of biological control agents, namely native species that adopt the introduced species as a prey or host. For example, native parasitic plants could potentially be used as biological control agents for invasive weeds. Various detrimental impacts of parasitic plants on hosts have been well documented (Press, Graves et al. 1990; Stewart and Press 1990; Watling and Press 2001), however, to my knowledge, there are only two studies which have investigated the potential of parasitic plants as biological control agents. The results of these studies are promising, since the native parasitic plants induced severe harmful effects on the invasive hosts, at levels which could restrict population growth (Burch 1992; Hao, Ye et al. 2005; Prider, Watling et al. 2009). For instance, Burch (1992) firstly demonstrated the potential use of the parasitic plant *Cassythia filiformis*, as a biological control agent for the invasive weed, *Schinus terebinthifolius*.

Cytisus scoparius (broom) and *Ulex europaeus* L. (gorse), both leguminous shrubs originary from Western Europe, are highly problematic invasive weeds in many parts of southern Australia. It has been recently observed that, in the Mount Lofty Ranges in South Australia, patches of *C. scoparius* infected by the native parasitic plant, *Cassythia pubescens* suffered serious negative impacts, including senescence, inhibition of flowering and even plant death. In a preliminary study, Britton (2002) studied the impacts of *C. pubescens* on another invasive weed, *U. europaeus*, and reported that the photosynthetic efficiency and water potential in infected *U. europaeus* were lower than in uninfected plants. These results suggested that the energy acquisition of *U. europaeus* could be disrupted by infection by *C. pubescens*. A more recent study found that *C. scoparius*, suffers stronger negative effects from the parasite than a native host, *Leptospermum myrsinoides* (Prider et al. 2009). Infected hosts suffered significant reduction in foliage and fruit productions, which suggests that *C. pubescens* infection could limit the population growth of their invasive hosts. Based on the field observations and on the experimental results of this research, this study will further characterize the biology of *C. pubescens*, and investigate the direct and indirect impacts of *C. pubescens* on *C. scoparius*. The results of this study will

provide vital information for the possible implementation of *C. pubescens* as a biological control agent of *C. scoparius*.

1.2 Literature Review

1.2.1 Characteristics of parasitic control agents

Understanding the biological characteristics of potential control agents is essential to forecast the outcome of any biological control program. This includes interaction with the target species and native species in the invaded habitat. In the following sections I review some of the specific traits of parasitic plants that are of interest if they are to provide effective biological control of weeds.

Host range and host selectivity

Introductions of an exotic control agent can potentially threaten the native species in invaded habitats, especially if the agent is a generalist that can potentially affect a wide range of species. Therefore, prey specificity is an important concern in selecting a biological control agent for an introduced species. Host range of parasitic plants varies across species. In certain families of parasitic plants, the target hosts are limited to a particular plant family; and in extreme cases, only a few species may be infected. On the other hand, some parasitic plants have a wide host range, although it has been found that the pattern of hosts parasitized is seldom random (Kelly, Venable et al. 1988; Gibson and Watkinson 1989). The pattern of uneven host use in the system refers to host selectivity of the parasites, and this pattern is the outcome of two factors, the resistance of the host to the parasite infection (passive) (Cameron, Coats et al. 2006; Cameron and Seel 2007) or the ability of the parasite to locate and reach the preferred host (active) (Kelly et al. 1988; Runyon, Mescher et al. 2006).

For example, Gibson and Watkinson (1989) studied the host range and selectivity of *Rhinanthus minor L.* in a sand dune system. They found that while *R. minor* was a generalist using 34 different kinds of host in total. However, the infection frequencies of hosts differed, indicating that generalist parasitic plants still prefer certain species

in the host community rather than evenly infecting all the available hosts in the community. The two most common host families of *R. minor* were the *Poaceae* and *Fabaceae*, and because the members of *Gramineae* were the most common species in the community it was deduced that host availability could be one of the factors controlling host infection. However, while the availability of *Leguminosae* species was comparatively low, they were had much higher infection rates. The members of *Leguminosae* are capable of fixing nitrogen, and hence their nitrogen content is higher than that of other plants growing in nitrogen deficient soil. Thus, host preference of parasitic plants could also be affected by the nutrient value of the host.

However, the assumption that *R. minor* actively selects the preferred host have been proven to be incorrect, as recent studies on *R. minor* confirmed that this root parasite does not actively choose the host, instead its uneven distribution across hosts is actually the result of difference in host resistance to the infection (Cameron and Seel 2007). The performances (growth and photosynthetic capacity) of *R. minor* has been discovered to be closely related to the level of connection to the host vascular system, which is largely depends on the resistant response of the host toward the parasite (Cameron et al. 2006; Cameron and Seel 2007; Cameron, Geniez et al. 2008). Cameron et al. (2006) demonstrated that forb species have more effective defense mechanism against the invasion of the parasite than the grass and legume species, further Cameron and Seel (2007) confirmed that hosts with strong defense mechanism to the infection would supply less nutrient to the parasite. There is also a strong positive correlation between the amount of nutrient obtained from the host and the growth of the parasite. Therefore, these evidences firmly prove that the pattern of host preference of *R. minor* in the field is the result of difference in host resistance.

If must be noted however that there is only one genus of parasitic plants that has been sufficiently studied to support its active selecting ability. The stem holoparasitic, *Cuscuta* spp. have been suggested to selectively parasitize certain host in a salt marsh ecosystem in the studies conducted by Callaway and Pennings (1998) and Pennings and Callaway (1996). Afterward, this host selecting ability of *Cuscuta* spp. was demonstrated by the study of Runyon et al. (2006) on *Cuscuta pentagona*. This study found that the seedlings of *C. pentagona* grew towards its potential host, and this host recognition was proven to be related to detection of volatile chemical released by

favorable hosts. This shows that at least some parasitic plants can actively select the preferred host from the range of all available hosts in the ecosystem.

These studies have shown that even “generalist” parasitic plants are not strictly random in their host use, but this pattern of host range in the field are related to two equally important factors, the resistance of the host (passive selection) and the host recognition and selection of the parasite (active selection). Thus, the host range and host preference of parasitic plants have to be fully investigated to determine whether they preferentially parasitize the target invasive weeds. There is at present little knowledge of the host range or host preferences of the *Cassytha* genus, so detailed study of these features of *Cassytha* is needed.

Photosynthesis

Depending on their photosynthetic ability, parasitic plants can be divided into holoparasites, those which cannot undertake photosynthesis and therefore have to absorb organic carbon and all others resources from hosts, and hemiparasites, those which can photosynthesize, and are thus less or not dependent on the carbon supply from their hosts: they mainly absorb water and nutrients from them. Within hemiparasites, photosynthetic ability varies, and some hemiparasites acquire significant amounts of carbon from hosts (Marshall and Ehleringer 1990). The extent to which hemiparasites depend on host photosynthates could affect their impacts on the host and also on any relationship with other symbiotic partners, such as mycorrhizae and nitrogen fixing bacteria, that also depend on the carbon from hosts (see 1.2.3 Indirect impacts on hosts).

Understanding the photosynthetic characteristics of *C. pubescens* can provide vital information on the impact of *C. pubescens* on its hosts. However, only one study has investigated the photosynthetic ability of different species in the *Cassytha* genus (Close, Davidson et al. 2006). This study confirmed that the stem of *C. pubescens* contains chlorophyll, which indicates it is able to photosynthesize, but its chlorophyll concentration was quite low when compared with other species in the genus and two other hemiparasites in this study. In a more recent study, photosynthetic rates of *C.*

pubescens were shown to vary, its rate being higher on a nitrogen fixing host than a non-nitrogen fixing host (Prider et al. 2009). It is not known, however, whether *C. pubescens* represents a significant carbon sink on its hosts, and whether this could impact on the nitrogen-fixing capacity of leguminous hosts such as *C. scoparius*, *U. europaeus* and native nitrogen fixing hosts.

Germination

In biological control management, the control agents need to develop a self-sustaining population, which can control the target invasive species over time. Therefore, the lifecycle of a control agent is one of the concerns for selection of a suitable biological control agent. There is limited knowledge of the life cycles of many parasitic plants, including their pollination, seed dispersal and germination. The information available is restricted to certain genera of parasitic plants that are serious weeds crops (e.g. *Striga* and *Orobancha* (Parker and Riches 1993).

Most generalist parasitic plants have relatively large seeds, which allow the seedlings to have sufficient time to attach on a host (Press et al. 1990; Logan and Stewart 1992). However, very little is known of the dormancy mechanisms in these generalist parasitic plants, and in particular the *Cassutha* genus has received little attention. In *Cuscuta*, which is comprised of parasitic vines morphologically similar to *Cassutha*, it was found that seed germination of *Cuscuta campestris* was significantly increased by soaking in concentrated sulfuric acid for 10 minutes (Benvenuti, Dinelli et al. 2005). In contrast, Britton (2002) found no germination of *C. pubescens* after scarification with either a knife or with concentrated sulfuric acid. Therefore, the germination cues of *C. pubescens* have yet to be found. This requires a systematic investigation of dormancy mechanisms and possible means for breaking dormancy in the seeds using techniques such as those suggested by Baskin (2004) so that an effective method for achieving seed germination of *C. pubescens* can be developed.

1.2.2. Direct impacts on hosts

The effects of some parasitic plants on their hosts are well documented. Hosts can suffer different levels of negative effects from the infections, varying from negligible to lethal. However, most studies to date have focused on a limited range of parasitic species that have caused massive yield loss in commercially important crops, such as the members of the *Orobanche* and *Striga* genera (Watling and Press 2001). Comparatively, little attention has been given to parasitic plants that occur mainly in natural environments, and most notably, there is limited knowledge of the impacts of the genus *Cassytha* on its hosts.

The primary effect of parasitic plants is acting as an extra sink in the host system. The parasites take up resources including water and nutrients from the hosts, as well as photosynthates depending on the photosynthetic ability of the parasites. Holoparasites like *Orobanche* and *Cuscuta* spp. are largely dependent on the supply of photosynthates from their hosts, and they directly compete for photosynthates with other organs of the hosts, significantly reducing the biomass accumulation and reproductive output of the hosts. *Cuscuta reflexa* absorbed almost all carbon and nitrogen from *Lupinus albus*, and this loss of resources caused a 31% decrease in root biomass, and fruit production was completely inhibited (Jeschke, Baumel et al. 1994). With such a high demand of carbon, some studies have found sink-stimulated photosynthesis in some infected hosts as a mechanism that compensated the loss of carbon to holoparasites. Delay of leaf senescence was observed in tobacco plants infected with *Orobanche cernua*, which can increase photosynthesis by 20%. However, this increase in photosynthesis still could not balance out the loss of carbon as the biomass of the host was significantly reduced (Hibberd, Quick et al. 1999). As a result, the total biomass of the system (hosts plus parasites) would not change as this is only a redistribution of materials within the system.

Apart from acting as an extra sink and reducing resource availability for the hosts, materials within the system were only redistributed, instead of affecting the productivity of the system by the parasites. Some parasites has been documented to reduce the overall biomass of the system, and this has been suggested to be related to disruptions of host photosynthesis. Different mechanisms have been proposed to

cause this disruption, such as manipulation of plant growth regulator such as abscisic acid (ABA) by the parasite (Taylor, Martin et al. 1996), reduction in chlorophyll or Rubisco (Cameron et al. 2008) and reduction in the CO₂ concentrating ability by thinning the bundle-sheath cell wall (Watling and Press 2001). When *Striga hermonthica* significantly reduced the growth of its host *Zea mays*, three times higher concentration of ABA was detected in the leaves from infected hosts. This increase of ABA may be associated to the reduction of stomatal conductance, and hence can inhibit host photosynthesis (Taylor et al. 1996). Moreover, Cameron et al. (2008) found that the total chlorophyll concentration of *Phleum bertolonii* was halved by the infection of a hemiparasite, *Rhinanthus minor*, and that this reduction in chlorophyll concentration possibly further induced suppression on the quantum efficiency of PSII, hence limiting the growth of the host and the total productivity of the system.

1.2.3. Indirect impacts on hosts

Parasitic plants not only have direct effects on their hosts, but they also affect them through changes in the interactions with other organisms. In biological communities, species are immersed in a network of interactions with a variety of other species creating often complex indirect effects (Morin 1999). One potentially important indirect effect may arise between parasitic plants, host plant with symbiotic acquisition of nutrients. This could be particularly important when host species highly reliant on legume or mycorrhizal infection. In addition, differences in the resistance of hosts to the effects of parasitic plants and the host preferences of parasitic plants might alter interactions in the host community. These changes in the interactions of hosts could further negatively affect the performance of hosts.

Mycorrhizal relationships

In a mycorrhizal relationship, plants supply photosynthate to mycorrhizal fungi and this supports the nutrient exploration by mycorrhizal fungi (Smith and Read 2007). In the presence of a parasitic plant, the fungi must compete with the parasites for

photosynthate; hence the fungi may also be negatively affected by parasitism. Mycorrhizal infection of hosts' roots can be significantly reduced in hosts infected by parasitic plants (Gehring and Whitham 1992; Davies and Graves 1998). In a field study, Gehring and Whitham (1992) found that junipers infected with xylem-tapping mistletoes had lower levels of mycorrhizal infection, and proposed that one of the possible reasons for this was that the mistletoe competed for carbon with the mycorrhizal fungi.

Species richness, community composition and structure of mycorrhizal fungi can be altered by parasitic plant infection (Cullings, Raleigh et al. 2005; Mueller and Gehring 2006). Mueller and Gehring (2006) showed that the community structure of mycorrhizal fungi and the dominant species infecting pine roots changed with different intensities of dwarf mistletoe infections. Furthermore, Cullings (2005) revealed that dwarf mistletoe had similar effects on different *Pinus* species. The species richness was reduced and community composition was altered in the infected hosts, and in particular, Cullings (2005) suggested that mycorrhizal fungi shifted to a community with lower carbon demand.

Nitrogen-fixing relationship

Nitrogen fixation is another important mutualistic relationship between plants and soil microbes. This process is highly energy demanding, and in some relationships can take up to 10% of the energy fixed by the plants. Therefore, any reduction in photosynthesis of host plants produced by a parasitic angiosperms, is likely to affect the nitrogen fixation.

While the effects on nitrogen-fixing hosts on parasites have been well studied, there have only been a few studies that have quantified the effects of parasitic plants on the nitrogen fixing bacteria. The only two studies that included all three components (parasitic plant-host-nitrogen fixing bacteria) so far used a similar modelling technique to quantify the flows of carbon and nitrogen among the three components (Jeschke et al. 1994; Tennakoon, Pate et al. 1997). However, they obtained different results on the impacts of parasitism on nitrogen fixation. The first study was

conducted to investigate the impacts of a stem holoparasite, *Cuscuta reflexa*, on the nitrogen fixing host, *Lupinus albus* (Jeschke et al. 1994). Growth and fruit development of the host was suppressed by the parasite: infection also strongly suppressed the nitrogen fixation of the bacteria in *L. albus* nodules. The carbon flow model indicated that *C. reflexa* absorbed a significant amount of carbon from the host, and this reduced the carbon supply to the nodules. In addition, the nitrogen flow model proved that the reduction of carbon supply significantly restricted the nitrogen production of the bacteria in *L. albus* nodules. These models illustrated that the parasitic plant acted as a strong carbon sink in this tripartite interaction, and hence limited the nitrogen fixation of the bacteria. Moreover, it was suggested that the reduction of performances in *L. albus* could be explained by interruption in the nitrogen-fixing relationship. In contrast, the impact of parasitism on nitrogen fixation was less harmful, where a root hemiparasite, *Oxalis phyllanthii* was examined (Tennakoon et al. 1997). As *O. phyllanthii* is capable of fixing its own carbon, it would have been less likely to act as a strong competitor for host carbon with symbiotic nitrogen-fixing bacteria.

These studies suggest that the magnitude of the impact of parasitic plants on nitrogen fixation of hosts is correlated with the photosynthetic characteristics of the parasite. Since *Cassytha pubescens* is a hemiparasite, it should be less dependent on host photosynthates, and thus may not have a negative impact on nitrogen fixation in its hosts has been observed for holoparasites such as *Cuscuta* (Jeschke et al. 1994). However, whether the effect of this parasite on host nitrogen fixation is similar to other hemiparasites is still unknown.

Competition

Host selectivity and the difference in host susceptibility can lead to changes in the competition intensities between hosts in a community. Plants that suffer more from parasitism could be at a disadvantage even if they are competitively superior in the absence of the parasite. Therefore, parasitic plants might play an important role in influencing plant community structure. Cameron and Seel (2007) showed that different hosts have varied level of resistances to the infection of a generalist root

parasite, *Rhinanthus minor*. Cameron, White et al. (2009) further tested this effect in competitive interactions in an ecological modelling study. Based on the unevenness on the resistance of hosts to infection of *R. minor*, the community composition of grassland systems shifted from grasses dominated to forbs dominated (Cameron et al. 2009). This possible shift in community composition was supported by a meta-analysis study, which proved that the presence of *R. minor* generally suppressed grasses but increased on forbs abundance (Ameloot, Verheyen et al. 2005; Ameloot, Verheyen et al. 2006). The shift in community composition was driven by difference in hosts susceptibilities to the infection that acted on the competition between hosts.

Other studies conducted in salt marshes in California confirmed that *Cuscuta salina* actively preferred the dominant, and substantially suppressed the population of the dominant species, *Salicornia virginica*, and hence released neighbouring competitors from the competitive effect of *S. virginica* and promoted a system with higher diversity (Pennings and Callaway 1996; Callaway and Pennings 1998).

To my knowledge, nothing is known of the effect of *C. pubescens* on the host community where it naturally occurs. Indirect effects, such as those described above, could potentially impact on the effectiveness and suitability of *C. pubescens* as a biological control agent of introduced weeds in south-eastern Australia.

1.2.4. Summary

Limited studies on the impacts of native parasitic plants on invasive weeds have confirmed that these plants can harm invasive weeds. This review summarised several aspects of parasitic plant biology that need to be investigated to further our understanding of the interactions between plant parasites and their hosts, to increase our knowledge of the interactions between native and introduced species, and prior to the possible implementation of native parasitic plants as biological control agents. While the typical direct impacts of parasitic plants have been relatively well documented, most studies have focused on plant parasites that cause yield loss in crop production. In most cases these are annual species adapted to high resource

availability which ecological strategies and physiology are almost certainly widely different from those of perennial species. Therefore, there is not enough information to support the use of native parasitic plants as biological control agents in the natural environment. Especially, there are very limited numbers of studies on the genus *Cassytha*, so it is important to improve our knowledge of its biology and how it impacts hosts, both native and introduced.

In addition, the indirect effect of parasitic plants on hosts can be as important as direct effects. Both mycorrhizal and nitrogen-fixing relationships are vital to plants, and parasitism can disrupt these mutualistic associations. The effects of parasitic plants on the relationships between hosts and mycorrhizal fungi and nitrogen fixing rhizobia are poorly known. Furthermore, several studies have confirmed that parasitic plants can alter the competition between hosts, which in turn changes community structure. Hence, the indirect effect on competition in the host community is another aspect that has to be further investigated.

1.3 Research Aims

This project attempts to fill gaps in our knowledge of the stem hemiparasite, *C. pubescens*, and its relationship with introduced and native hosts. This knowledge is needed to determine whether *C. pubescens* can be deployed as a biological control agent of invasive weeds such as *C. scoparius* and *U. europaeus* in south eastern Australia. Here I report the results of experiments that investigated the germination biology of *C. pubescens*, its impact on the relationship of *C. scoparius* with nitrogen-fixing Rhizobium, nutrient transfer between the parasite and its hosts, and parasite impacts on competition between *U. europaeus* and native plant species. The major aim of this thesis was to improve understanding of the biology of the native hemiparasitic vine, *Cassytha pubescens*, in order to determine if it could be a suitable biological control agent for introduced weeds. The germination of *C. pubescens* and various interactions between the parasite and hosts are addressed in different chapters in this thesis.

Chapter 2

This chapter introduces all the plant species involved in this study, the native hemiparasite, *C. pubescens*, the two invasive hosts, *C. scoparius* and *U. europaeus* and the two native hosts, *Acacia myrtifolia* and *L. myrsinoides*.

Chapter 3

The dormancy and germination ecology of species in the genus *Cassyltha* have never been examined or classified according to the widely accepted classification system developed by Baskin and Baskin (2004). This chapter reviews all records on the seed structure and proposed germination cues of the native parasitic vine, *C. pubescens*, and present data on germination trials designed to examine the role of the seed coat in dormancy, and what cues are required to break seed dormancy in *C. pubescens*. The results are interpreted in the light of the life history strategies of the parasite and its hosts.

Chapter 4

This chapter examines the impact of *C. pubescens* on the nitrogen fixing relationship between *C. scoparius* and its symbiotic nitrogen-fixing rhizobia. The experiments was designed to investigate how the parasite affects the nitrogen budget of the host, and hence the impacts on the photosynthetic ability and growth of the hosts.

Chapter 5

From personal field observation and the results obtained in Chapter 4, the native legume *A. myrtifolia* shows stronger resistance to infection of *C. pubescens* than the introduced legume, *C. scoparius*. This chapter examines the nature of the difference in resistance between the native and introduced hosts. A radioactive tracer (^{32}P) was used to determine the effectiveness of the haustorial connection between host and parasite.

Chapter 6

Based on the finding of difference in resistance to the infection between native and introduced hosts, this chapter investigates the impacts of *C. pubescens* on the competition between *U. europaeus* and co-occurring native plants. The aims were to investigate any change in competitive intensity between native and invasive hosts, and to compare the difference of impacts on competition between *U. europaeus* with native nitrogen fixing and non-nitrogen fixing species.

Chapter. 2 Study species

2.1 *Cassytha pubescens*

Cassytha pubescens R.Br. (Lauraceae) is a perennial, stem-twining, hemi-parasitic vine native to Australia. Its leaves are reduced to scales, but the stem contains chlorophyll and is capable of photosynthesis (Close et al. 2006; Prider et al. 2009). *C. pubescens* is an obligate parasite, and has to attach to a host within around 6 weeks after the germination to survive (McLuckie 1924). It has a wide host range including many native Australian woody perennials and also non-native invasive perennial shrubs. Although morphologically similar to the well-studied parasitic vine *Cuscuta* (Convolvaceae), its life strategy is quite different. Whereas *Cuscuta* is a genus of annual holoparasites, in which the stem contains low concentrations of chlorophyll (Kuijt 1969; Press et al. 1990). *C. pubescens* is hemiparasite and perennial. *C. pubescens* spreads mostly through vegetative growth, moving across branches within a host and also moving from one host to another. There is very little information in the literature on sexual reproduction or germination ecology of *Cassytha* spp..

2.2 *Cytisus scoparius*

Cytisus scoparius (L.) Link (Leguminosae) is a tall, woody, perennial, shrub from the Mediterranean region of Europe, and is an invasive weed in Australia, the USA and New Zealand. This nitrogen fixing shrub has been reported to influence soil composition, especially soil nitrogen and phosphate content (Fogarty and Facelli 1999; Haubensak and Parker 2004; Caldwell 2006). Both its leaves and stems are photosynthetic, and in the summer it drops its leaves and relies on its stems for photosynthesis (Fogarty and Facelli 1999). *C. scoparius* accumulates a large, long-lived seedbank, which makes it difficult to control (Sheppard, Hodge et al. 2002). It has been declared as a noxious weed in more than one state in Australia.

2.3 *Ulex europaeus*

Ulex europaeus (L.) (Fabaceae) is a tall, spiny, evergreen perennial shrub native to Europe. At maturity the shrub is leafless and has photosynthetic stems. *Ulex europaeus* was introduced to Australia, New Zealand and many other countries at the time of European settlement as a hedging plant (Parsons and Cuthertson 1992). Like *C. scoparius*, it is also a nitrogen fixing plant, and has a high nitrogen fixation rate, and the ability to alter soil chemical composition (Augusto, Crampon et al. 2005; Leary, Hue et al. 2006). In Australia, the dense and spiny stands provide safe harbour for pests like pigs and rabbits (Muyt 2001). Because of its many negative impacts on the environment and a long lasting seedbank (Hill, Gurlay et al. 2001), it has been declared a Weeds of National Significance in Australia.

2.4 *Acacia myrtifolia*

Acacia myrtifolia (Sm.) Willd. (Leguminosae) is a woody, perennial shrub, to one metre tall, and is native to south-eastern Australia. In South Australia, it is distributed primarily around the southern Mount Lofty Ranges and the south-eastern part of the state. It is a native, nitrogen fixing plant, and it was chosen as the native host in this study as it co-occurs with the weeds mentioned above and has been observed supporting *C. pubescens*, although the parasite only occurs in low density on this native host.

2.5 *Leptospermum myrsinoides*

Leptospermum myrsinoides Schltdl. (Myrtaceae) is a perennial, native sclerophyllous shrub, to two metres tall that occurs throughout south-eastern Australia. It is one of the abundant understory species in the Mount Lofty Ranges and occurs in late successional stages (Cochrane 1963). This native shrub was chosen as a representative, non-nitrogen fixing species that is also parasitized by *C. pubescens*, at higher densities than *A. myrtifolia* (Prider et al. 2009).

Chapter. 3 Dormancy and germination ecology of *Cassyltha pubescens*

3.1 Abstract

Virtually nothing is known about the germination ecology of the Australian native parasitic angiosperm, *Cassyltha pubescens*. A number of experiments were conducted to determine what conditions are most likely to promote germination in *C. pubescens*. Imbibition tests were conducted to investigate water permeability of the seed coat. Scarified and non-scarified seeds were incubated at spring/autumn temperature for 4 days. The weight of scarified seeds increased by 90% and was significantly higher than non-scarified seeds. The impacts of both scarification and heat (boiling water) on germination were also tested. After 19 weeks incubation in spring/autumn temperatures, maximum germination rate of heat-treated seeds was 67%, whereas for the scarification treatment it was only 19%. In contrast, cold stratification negatively affected the germination of *C. pubescens*. When treated with heat after cold stratification, the germination rate was 69%, but it was only 14% when treatments were applied in the reverse order. However, the germination of scarified seeds was inhibited by cold stratification, independently of the sequence of the treatments. These experiments show that *C. pubescens* exhibits physical dormancy due to its water impermeable seed coat, and also shows some physiological dormancy that can be removed by heat, but is reversed by cold. The heat-stimulated germination suggests that bushfires may be the trigger for the germination of *C. pubescens* in the wild.

3.2 Introduction

Most parasitic plants rely on their hosts for their survival, either because they cannot photosynthesise and/or because they cannot obtain their own water and nutrients. Thus, they have often evolved to time their germination according to the availability of suitable hosts. The dormancy and germination ecology of parasitic plants is often closely related to the host range and lifespan of the parasites, and these have been studied thoroughly in certain genera of parasitic plants. In particular, root parasites in the genera *Orobanche* and *Striga* have been investigated in detail. These parasites can only parasitize a limited range of hosts, or in some cases only a single host (Matusova, van Mourik et al. 2004). Therefore, their seeds have evolved to detect root exudates that are specific to their hosts and these are used as to trigger germination; hence they ensure that germination only occurs with a host readily available for infection (Logan and Stewart 1992).

In contrast, annual generalist parasites with wider host ranges than the root parasites mentioned above, such as *Cuscuta* and *Rhinanthus* spp. have evolved to utilize the seasonal changes in abiotic factors used by their hosts (such as fluctuation in soil temperature) to indirectly indicate the presence of their hosts (Ter Borg 2005; Meulebrouck, Ameloot et al. 2008). The seeds of both these genera exhibit physiological dormancy and their germination are triggered by a period of cold stratification. Therefore, they germinate together with their annual hosts and maximize the chance of attaching to a suitable host. For example, physiological dormancy of *Cuscuta epithimum* acts to maximise the chances of seedlings encountering their hosts in the right season (Meulebrouck et al. 2008). In addition, the hard seed coat of *C. epithimum* may act to spread germination of seedlings over time, to reduce the risk of unsuccessful germination events, and intraspecific competition for hosts (Meulebrouck et al. 2008).

Cassytha, the only parasitic genus in the Lauraceae, includes hemiparasitic vines, morphologically similar to the well-studied parasitic genus, *Cuscuta*. However, unlike *Cuscuta*, *Cassytha* spp. are perennial generalists, and may differ from the former in their biology, including germination. Probably because it does not threaten crops, there are no studies on its dormancy and germination ecology, although the structure

of seeds and the embryo of *Cassytha* have been documented (Sastri 1962; Rangaswamy and Rangan 1971). The earliest report on the germination of *Cassytha* spp. was conducted by McLuckie (1924). Fresh fruits of *Cassytha paniculata*, *C. glabella* and *C. pubescens* were placed on top of a layer of pure sand. Flesh of the fruits decomposed and turned to a purple mass in few days, and the seeds germinated 4 weeks later. This was supported by another later study that proposed that the tough seed coat of *C. pubescens* could be softened by microbial action (Visser 1981). These studies suggested that it was relatively easy to germinate the seeds of *Cassytha*. However, neither of these records provided details of the experimental conditions used.

It has been suggested that the relatively large seed size of generalist relative to specialist parasites evolved to provide enough food reserves to sustain the seedlings for a few weeks until a host can be found (Logan and Stewart 1992). Furthermore, it is unlikely that parasites with wide host ranges, such as *Cassytha*, require stimulation by host exudates (Logan and Stewart 1992). According to a study on the embryology of several *Cassytha* species, the embryo is enclosed by a seed coat that has a layer of elongated cells filled with tannin (Sastri 1962). This layer of cells in the seed coat of *Cassytha* species has been suggested to make the seeds impermeable to water and thus to provide a mechanism of physical dormancy (Baskin and Baskin 1998). Weber (1981) proposed that the germination of *Cassytha* spp. may be stimulated by bushfire. Consequently, several attempts have been made to break this dormancy by heat treatment. Seeds of *C. pubescens* were found to still be viable after exposure to 80°C for 5 minutes, but died after 5 minutes in 150°C. No germination occurred after seeds were treated at 100°C for 15 minutes (French and Westoby 1996; Wills and Read 2002). Importantly, none of these studies successfully germinated the seeds of any *Cassytha* species. Moreover, other physical dormancy breaking methods, such as scarification, acid bathing and heat treatment have all been tried without success (Britton 2002).

Until now, there has been no comprehensive and systematic study of dormancy in any *Cassytha* species. In this study, the following questions were addressed: 1) is the seed coat of *C. pubescens* impermeable to water? 2) if yes, is there any other dormancy mechanism involved, except physical dormancy? 3) what is the impact of heat on the

germination of *C. pubescens*? and 4) what is the impact of cold stratification on the germination of *C. pubescens*?

3.3 Materials and Methods

3.3.1 Seed collection and seed viability assessment

Mature fruits of *C. pubescens* were collected from plants parasitizing a patch of *Cytisus scoparius* in the Mark Oliphant Conversation Park (35° 0'58.08"S and 138°45'58.45"E) in the Mt. Lofty Ranges, South Australia on December 2007. These fruits were stored in a dry-storage room with 15% RH in darkness until the seeds were examined for their viability in January 2008. The dried fruits were abraded with sandpaper to remove the dried flesh. Ten randomly selected seeds were dissected to examine the viability of the embryo and fullness of endosperm by visual inspection. To determine whether morphological dormancy occurs, the developmental stage of the embryo was investigated in five seeds by measuring the lengths of the seed and calculating embryo and the embryo to seed ratio (Baskin and Baskin 1998; Baskin and Baskin 2007).

3.3.2 Imbibition

The permeability of *C. pubescens* seeds to water was tested by measuring the rate of water uptake of scarified and non-scarified seeds. There were 3 replicates for each treatment, with 15 seeds in each replicate. Seeds were scarified by manually cutting the seed coat with a razor blade, producing small nicks exposing the whitish endosperm. In each replicate, 15 seeds were placed in petri dishes on a filter paper moistened with sterilized RO water, and then stored in a culture room with day/night temperatures of 15°C/22°C, and with 8 hours of light every day. The initial weight of the seeds was recorded, and then they were weighed at 1, 2, 3, 19, 24, 48, 72 and 96

hours. The change in weight was calculated by the formula of Hidayati, Baskin et al.(2001):

$$\%W_s = [(W_i - W_d) / W_d] \times 100$$

where W_s is the percentage increase in the mass of the seed, W_i is the seed mass at a given time and W_d is the initial mass of seeds.

3.3.3 Breaking Physical dormancy

Two methods were used to attempt to break the seed coat of *C. pubescens*: scarification and immersion in boiling water. Seeds were randomly allocated into three different treatments, control, mechanical scarification and boiling water. The method of mechanical scarification was the same as the one used in the imbibition experiment, but the point of scarification was carefully chosen to avoid damage to the embryo. In the boiling water treatment, the seeds were placed in a tea infuser and heated in boiling water (100°C) for one minute, and then placed on the bench at room temperature to cool for one minute. Treated seeds were then placed on top of a layer of sterilized sand in Petri dishes (20 per dish), and watered with 5mL of sterilized RO water: any excess water was removed a minute after watering. All dishes were incubated in an incubation room under the same conditions of temperature and light described above and 1 mL of sterilized water was added weekly to keep the sand moist. This regime simulated the spring and autumn conditions likely to be encountered in the field in South Australia. . There were 3 replicates for each treatment, and 20 seeds in each replicate. Seeds were inspected weekly and counted as germinated when the length of the radicle was half the length of the diameter of the seed. The experiment was terminated when there was no germination in any of the treatments for two consecutive weeks. Finally, all non-germinated seeds were dissected and inspected for fullness of the endosperm the viability of the seed was used to adjust the total initial number of viable seeds for calculation of the germination percentage.

3.3.4 Cold stratification experiment

To investigate whether cold released physiological dormancy is involved in the dormancy mechanism of *C. pubescens*, a cold stratification treatment was combined with the two treatments used in the previous experiment. There were 3 replicates for each treatment, and 20 seeds in each Petri dish (as described above). Seeds were allocated to 6 treatments (Fig. 3.1): warm control (T₁), boiling water prior to cold stratification (T₂), scarification prior to cold stratification (T₃), cold stratification control (T₄), cold stratification prior to boiling water (T₅) and cold stratification prior to scarification (T₆). The methods of scarification, the application of boiling water and the other experimental conditions were the same described above. Cold stratification was achieved by wrapping the Petri dishes with aluminium foil and incubated at 5°C in darkness for 6 weeks. Afterward, these five treatments were incubated in spring/autumn temperature without the cover of aluminium foil for the rest of experimental period. Weekly measurement of seed germination and seed examination at the end of the experiment, were as described above.

3.3.5 Statistical Analyses

The results of the imbibition experiment were analysed with MANOVA test for repeated measurement (JMPIN; version 4.0.3). The results of the two germination experiments were analysed with a Kaplan-Meier survival curve, germinated seed was treated as an event, 1, and the non-germinated seed at the end of the experiment was treated as a censored, 0, then these survival curves were compared in pairwise comparison by a Logrank test (GraphPad, PRISM; version 4.03).

3.4 Results

3.4.1 Seed Viability and Imbibition

All seeds tested were viable. The embryo of *C. pubescens* is linear with a mean embryo to seed length ratio of 0.58 ± 0.04 ($n=5$), hence it can be classified as linear fully developed (Baskin and Baskin 2007). In the imbibition experiment, mucilage formed on the surface of all seeds, independently of treatment, after water was added. The weight of scarified seeds increased by 95%, whereas the weight of non-scarified seeds only increased by 14% (Fig. 3.2) ($P < 0.0001$, MANOVA test for repeated measurement).

3.4.2 Breaking Physical Dormancy

Both treatments scarification and heat, stimulated germination of *C. pubescens* seeds (Fig.3.3). The germination rates of scarified seeds (Logrank test, $P = 0.0016$) and heated seeds (Logrank test, $P < 0.0001$) were significantly different from the rate of untreated control seeds (2% germination). Moreover, the boiling water treatment (68% germination) was more effective in stimulating the germination than the scarification treatment (19% germination) (Logrank test, $P < 0.0001$).

3.4.3 Cold stratification experiment

Germination of *C. pubescens* responded differently to the different combinations and sequence of treatments (Fig. 3.4). No germination occurred with cold stratification alone (T_1, T_4 ; Logrank test, $P = 0.9953$). The seeds germinated in the treatments which boiling water treatment combined with cold stratification (T_1, T_2 ; Logrank test, $P = 0.0305$ & T_1, T_5 ; Logrank test, $P < 0.0001$). Meanwhile, the effect of cold stratification on the boiling water treatment depended on the sequence of the

treatment (T₂, T₅; Logrank test, P<0.0001): seeds treated with cold stratification after the boiling water treatment had lower germination percentage than the seeds treated with boiling water after the cold stratification (Fig. 3.4a). In contrast, the combination of cold stratification and scarification had no effect on the germination of *C. pubescens* seeds (T₁, T₃; Logrank test, P=0.5538 & T₁, T₆; Logrank test, P=0.0976), nor did the sequence of the treatments had no effect (T₃, T₆; Logrank test, P=0.2588) (Fig. 3.4b).

3.5 Discussion

According to the seed dormancy classification system suggested by Baskin and Baskin (2007), if the length of embryo to the length of seed ratio is over 0.5, this indicates that the seed is mature and that there is no absence of morphological dormancy. The ratio of *C. pubescens* seeds was over 0.5, which indicated that the embryo of seeds in ripe fruits of *C. pubescens* are fully developed and mature, hence the seeds should be ready to germinate at the favourable condition and rules out the presence of morphological dormancy (seeds with undeveloped embryo). Imbibition test confirmed that the seed is enclosed in a water impermeable seed coat that imposes physical dormancy (Baskin and Baskin 2004; Baskin, Thompson et al. 2006). Nonetheless, the physical dormancy breaking experiment showed both scarification and heat treatment can trigger the germination of *C. pubescens* seeds, but differences in the germination rate between two treatments suggested that physical dormancy may not be the only dormancy mechanism present.

3.5.1 Dormancy

The imbibition test in this study revealed that the seed coat of *C. pubescens* was water impermeable and confirmed the presence of physical dormancy in the seeds of *C. pubescens*. Baskin et al. (2004) concluded that if germination is the same for heat and scarification treatments, then only physical dormancy is involved. In the study, seeds of *Dodonaea viscosa* were treated with heat and scarification, and the percentage of

germination in both treatments reached almost 100% within 2 weeks. Differences in the rate of the germination, between the two treatments were suggested to be a result of differences in the rate of water uptake generated by the two treatments. In contrast, different amounts of germination in the heat and scarification treatments were found in this experiment, indicating that physical dormancy may not be the only dormancy involved in *C. pubescens*. Bell and Williams (1998) found that species requiring fire as a germination cue, germinate in lower numbers when only their physical dormancy is broken by scarification. This is similar to present results for *C. pubescens*, and suggests combination dormancy, similar to that found for *Cuscuta epithymum* (Meulebrouck et al. 2008). In contrast to *C. epithymum*, however, heat rather than cold stratification stimulated germination of *C. pubescens*. This is consistent with germination of *C. pubescens* occurring in a post bushfire environment (Weber 1981).

It is possible, however, that the scarification method used in this experiments may have damaged the embryo and lowered the chance of germination, whereas heat may break physical dormancy without such damage (Baskin 2003). Li, Baskin et al. (1999) showed that physical dormancy of *Rhus glabra* could be broken by bathing 1 minute in boiling water, which opened the ‘water gap’ of the seed, referring to the first area on the seed coat that would open once the seeds are treated with the germination cue (Baskin, Baskin et al. 2000). Furthermore, the presence of a water gap has been confirmed in a species of the morphologically similar parasites, *Cuscuta* spp. Jayasuriya, Baskin et al. (2008) found that the heat treated seed of *Cuscuta australis* would imbibe water and germinate once the water gap of the seeds was opened. Therefore, a study of the water gap of *C. pubescens* and of the effect of heat on the water gap is needed to further clarify the dormancy mechanisms of this species.

3.5.2 Germination ecology

In both germination experiments, almost 70% of seeds germinated in heat treatments, suggesting that germination of *C. pubescens* may be triggered by fire in nature. The present results support the suggestion of bushfire as the germination trigger of *C. pubescens* (Weber 1981). Moreover, this suggestion of a close relationship between

bushfire and germination of *C. pubescens* is supported by various pieces of indirect evidence. Germination of a parasitic plant is often linked to that of its hosts and is also influenced by parasite lifecycle (i.e. annual or perennial), as seedlings of most parasites must attach rapidly to suitable hosts to obtain resources. *C. pubescens* infects a wide range of native understorey shrubs, including plants in the *Acacia*, *Hakea*, *Banksia* and *Leptospermum* genera (J. Prider pers. comm.). Because of this wide host range, *C. pubescens* may be similar to other generalist parasites that utilise abiotic factors as germination cues. The native hosts of *C. pubescens* are adapted to the Mediterranean climate found in the region of South Australia where they occur, and use fire related cues, including heat and smoke, to trigger germination (Clemens, Jones et al. 1977; Zammit and Westoby 1987; Whelan and York 1998; Wills and Read 2002). Some time after a fire many of these potential hosts are likely to be available at high density, either because germination or numerous stem sprouting from lignotubers, thus germination after this flush of new stems provides *C. pubescens* with a high chance of encountering the young shoots of these host species.

Unlike the two well studied annual generalist parasitic plants, *Cuscuta* and *Rhinanthus*, there is no record of how long *C. pubescens* can survive once attached to a host. It is reasonable to assume that vegetative growth is the most effective method for it to spread from host to host. Therefore, an annual population re-establishment may be unnecessary, and a strategy such as cold stratification, commonly found in annual species to ensure that germination occurs after winter, may not be as effective for the perennial *C. pubescens*. Meanwhile, physical dormancy allows seeds to persist in the soil seedbank until conditions are favourable, such as after the next fire event. Further tests on the longevity of *C. pubescens* are needed to confirm this. A possible scenario is that after annual flowering and fruit set, seed of *C. pubescens* may be dispersed by birds and/or other animals. The seed coat could protect the embryo from digestion in the guts of the seed dispersers and allow the seed to remain dormant in the soil until the next fire event (Kelly, Van Staden et al. 1992). Further evidence is needed, however, to support the role of animals in dispersal of *C. pubescens* (French and Westoby 1996).

Most of the potential hosts of *C. pubescens*, re-sprout or germinate as early as the first rains of early winter after a summer fire (personal observation). This results of this

experiment showed that *C. pubescens* seed took a relatively long time to germinate after heat treatment (reached max germination at 18 weeks, whereas the max germination of *C. epithymum* was at 50 days (Meulebrouck, 2008), and that cold stratification further postponed germination (reached max germination at 32 weeks). In either case, this delay in germination could be an advantage. Seedlings of *C. pubescens* can only survive 6 weeks without attaching to a host (McLuckie 1924), hence if they germinate too early after a fire, and the potential hosts are not available or still too young to sustain the parasite, *C. pubescens* could be at a disadvantage. Therefore, delays in germination following a fire, could ensure that suitable hosts are sufficiently well established when *C. pubescens* germinates.

3.5.3 Conclusion

This is the first study conducted to investigate the dormancy and germination ecology of *Cassyntha* species. The physical dormancy found in *C. pubescens* allows the seeds to persist in the soil seedbank and possibly re-establish in a post fire environment, also possibly providing protection for the embryo through the guts of dispersers, such as birds and other animals. Also, it suggests that *C. pubescens* has a somehow similar combination dormancy mechanisms as the morphologically similar parasitic species, *C. epithymum* (Meulebrouck et al. 2008). However, the different triggers required to break the physiological dormancy in each species, may be related to the contrasting life strategy and the host range of the parasite.

These germination characteristics of the seed of *C. pubescens* contribute to make it a suitable long term biological control agent for the target invasive species, *C. scoparius*. The seed of *C. scoparius* also has physical dormancy, and the germination of the seed can be triggered by scarification or heat treatment (Tarrega, Calvo et al. 1992). Furthermore, *C. scoparius* produces copious amounts of seed annually, and these physically dormant seeds persist in the soil seed bank. Therefore, a parasitic vine like *C. pubescens* with similar life history that can accumulate seeds in soil seedbank, and in which germination would be stimulated by fire would be an ideal agent to control the population of *C. scoparius* in the post-fire environment.

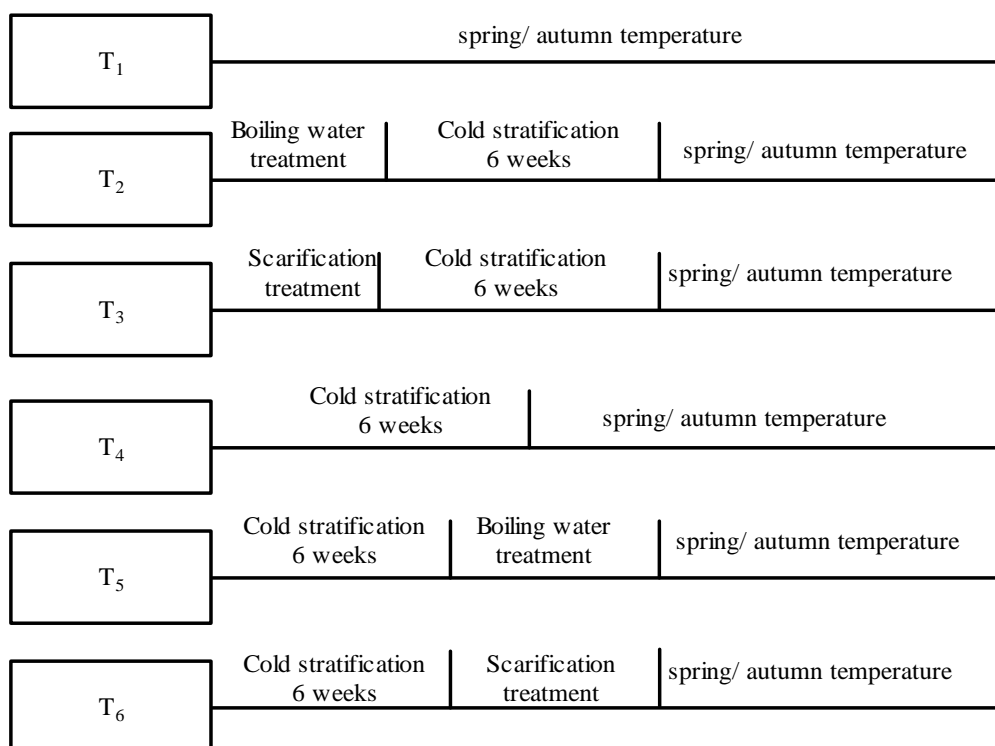


Figure 3.1. The experimental design of the 6 treatments in the cold stratification experiment. Cold stratification: seeds in Petri dish were wrapped with aluminium and cultured in 5°C and Spring/autumn temperature; seeds in Petri dish were cultured with temperature ranging from 15°C to 22°C with 8 hours of light daily.

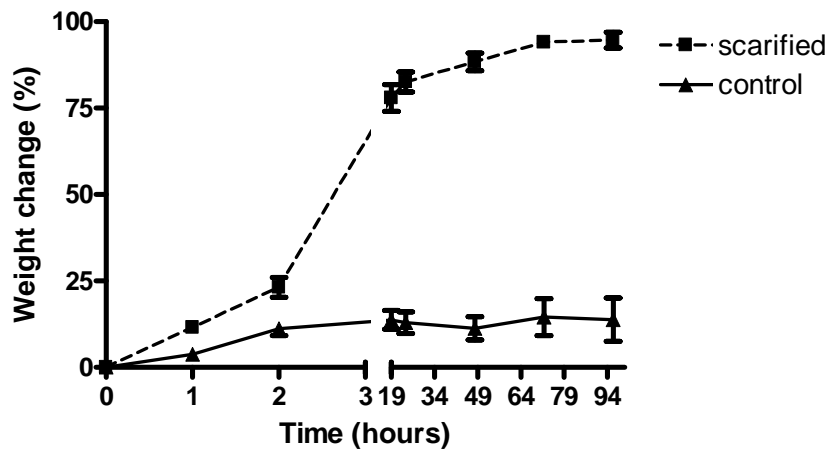


Figure 3.2. The water uptake of seeds of *Cassytha pubescens* in two treatments. square symbol: scarified treatment and triangular symbol: control treatment. Data points are means \pm s.e. (n=3).

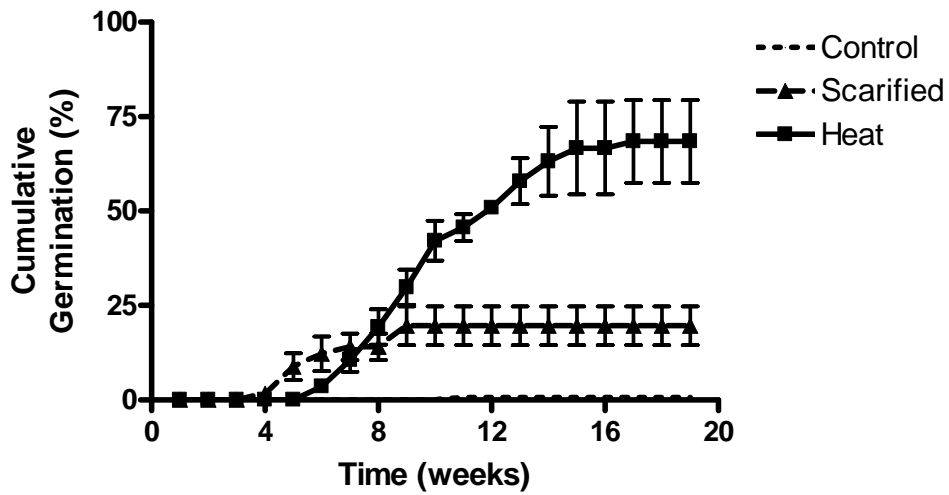
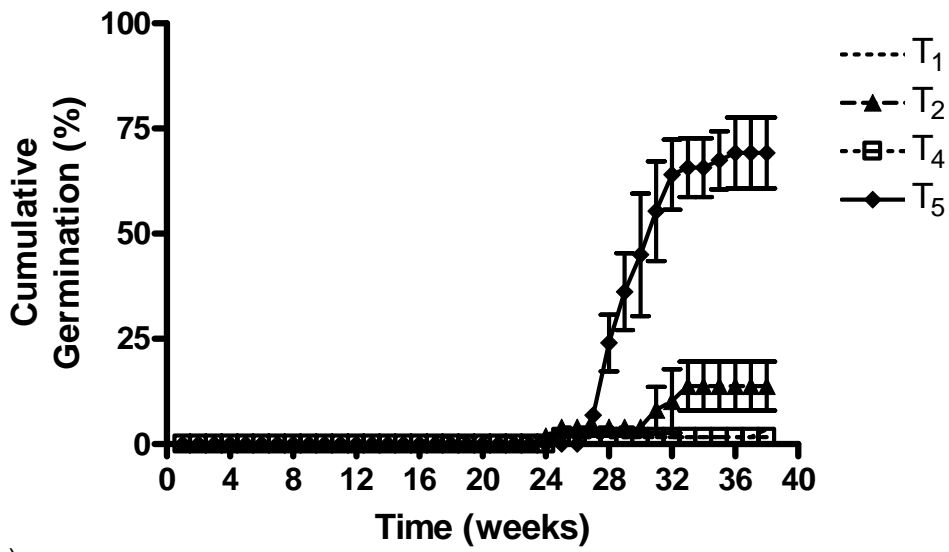


Figure 3.3. The cumulative germination of seeds of *Cassytha pubescens* in three treatments. No symbol: control, triangular symbol: scarification and square: heat. Data points are means \pm s.e. (n=3).

(a)



(b)

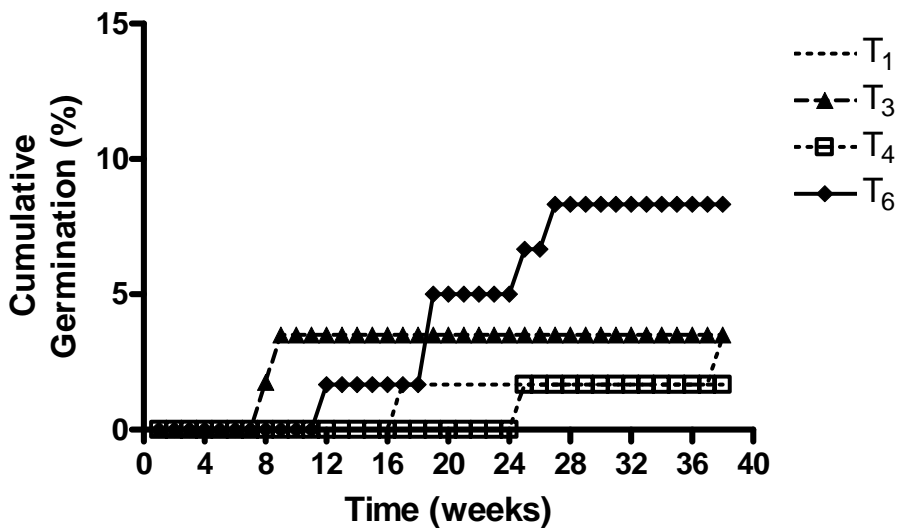


Figure 3.4. The cumulative germination of seeds of *Cassytha pubescens* in combinations of cold stratification and treatments. Treatments were according to the arrangement in Fig. 1. (a): cold stratification combined with heat and (b): cold stratification combined with scarification. Data points are means \pm s.e. (n=3). No error bar shown in (b) as large variance. Note different scales for both graphs.

Chapter. 4 Interactions between the parasite, hosts and Rhizobium

4.1 Abstract

The ability of invasive species to establish mutualistic symbiotic relationships with the soil biota of invaded areas can be critical for their success. However, such mutualistic relationship might be also affected by newly established antagonistic interactions with new enemies in the invaded habitat. This chapter reports the results of a glasshouse experiment designed to assess the effects of infection by a native plant parasite, *Cassytha pubescens*, on the nitrogen fixing relationship of an invasive and a native legume, *Cytisus scoparius* and *Acacia myrtifolia* respectively, growing with local soil biota. Nitrogen content, photosynthesis and growths of hosts and parasite were measured. *C. pubescens* did not survive on the native host, *A. myrtifolia*, thus only its impact on the invasive host, *C. scoparius*, was assessed. Parasite infection had no effect on the total nitrogen content of the parasite-*C. scoparius* system, but reduced the nitrogen availability to the host by 25%. Infected hosts accumulated 21% less biomass than the hosts without infection. Infection induced a negative effect on PSII efficiency of infected branches of the invasive host, but there was no effect on chlorophyll concentration of these same branches. Thus, the impact on PSII efficiency may have been a consequence of stomatal closure rather than nitrogen limitation. *C. pubescens* appears to have no effect on nitrogen fixation by symbiotic rhizobia, but acts as strong sink in this system and competes for nitrogen with the host. In conclusion, the uptake of nitrogen by the parasite limited nitrogen availability to the host, and reduced the photosynthetic surface area of leaves, leading to reduction of growth.

4.2 Introduction

The success of the establishment of an introduced species in a new habitat is determined by a combination of abiotic and biotic factors including interactions with native species in the invaded habitat (Simberloff and Von Holle 1999; Maron and Vila 2001). These interactions can be either mutualistic or antagonistic, and soil biotas in the new habitat have been suggested to play a critical part in the success of plant invasions. Direct mutualistic or antagonistic relationships between soil biota and invasive plants, or indirect effects to the relationship between native plants induced by the emerging direct effects, may have a dramatic effect on plant communities and ecosystem processes. The introduced species does not only interact with a single species in the new habitat, but may be subject concurrently to antagonistic interactions, such as predation and parasitism, and to mutualistic interactions, such as those with nitrogen-fixing bacteria. The outcome of these multiple opposing interactions can affect the success of the invasive plant in the community (Hawkes, Wren et al. 2005; Wolfe and Klironomos 2005; Mummey and Rillig 2006; Reinhart and Callaway 2006).

The two best understood mutualistic relationships between plants and soil biota are mycorrhizae and rhizobium-mediated nitrogen fixation. In both, the plants supply the soil symbionts with photosynthates as an energy source, and receive nutrients such as nitrogen and phosphorus in return. This trading between the plants and soil biota is controlled by various abiotic and biotic factors; any stress on either symbiotic partner can affect the interaction. In the case of parasitism, host plants are stressed through resource competition or through negative physiological effects, such as lowering of host photosynthesis by the parasite (Press et al. 1990; Watling and Press 2001). Reduction of available photosynthates produced may affect symbionts that rely on the host for their energy. Several studies have demonstrated the effects of parasitic angiosperms on either nitrogen fixation (Jeschke et al. 1994; Jeschke, Baig et al. 1997; Jeschke and Hilpert 1997; Tennakoon et al. 1997), or mycorrhizal associations of hosts (Gehring and Whitham 1992; Cullings et al. 2005; Mueller and Gehring 2006). In all these cases, the provision of resources from the host plants to the symbiotic partner was reduced by parasite infection, and the ability of the soil biota to provide the plants with nutrients was affected.

The performance of the parasites also depends on host condition. Parasitic plants totally or partly depend on the supply of water, nutrients, photosynthate and even secondary chemicals from their hosts (Press et al. 1990; Puustinen and Mutikainen 2001; Lehtonen, Helander et al. 2005; Schadler, Roeder et al. 2005). Obligate stem parasite like mistletoes, *Cuscuta* and *Cassytha* are heavily dependent on the supply of nutrients and water from their hosts. Therefore the nutrient and water status of the host can influence performance of such parasites (Wallace, Romney et al. 1978; Seel and Press 1994; Miller, Watling et al. 2003; Bickford and Kolb 2005). In contrast, some root parasites can supplement water and nutrients acquired from the hosts because they either retain a rudimentary root system, or are attached to multiple hosts and are thus less dependent on individual host nutrient and water status (Seel, Parsons et al. 1993; Loveys, Tyerman et al. 2002). The performance of the host can be correlated with the presence of mutualistic symbionts, such as mycorrhizae (Sanders, Koide et al. 1993; Seel, Cooper et al. 1993; Davies and Graves 1998; Salonen, Vestberg et al. 2001). Therefore, the performance of a parasite may indirectly depend on the mutualistic relationship between the hosts and its symbiotic partners, although, it has been reported that nitrogen-fixation may not provide a direct benefit to the root hemiparasite, *Rhinanthus minor* (Jiang, Jeschke et al. 2008).

Cytisus scoparius, a nitrogen fixing shrub native to Europe has been introduced to the United States, Australia and New Zealand. It is capable of rapid growth and has high nitrogen-fixation rates, which allows it to become invasive and to exclude many native species. It has been reported to threaten the native vegetation of the new habitat by altering the soil nitrogen content (Fogarty and Facelli 1999; Haubensak, D'Antonio et al. 2004). In this study the mechanism of the negative effects of the Australian native plant parasite *Cassytha pubescens* on the invasive legume *C. scoparius* was investigated. *Acacia myrtifolia*, a native nitrogen fixing shrub, was used to provide a comparison of the impacts of *C. pubescens* on a native legume. *A. myrtifolia* was selected because it has been observed to be infected with *C. pubescens* in the field, albeit at lower densities than on native non-legumes and invasive legumes. The native parasite may reduce the nitrogen-fixing ability of the invasive legume through its negative effects on host photosynthesis and/or competition for photosynthate with nitrogen-fixing bacteria. Thus, it could reduce the effect of *C. scoparius* on soil

nitrogen enrichment. Specifically the following questions were addressed: 1) does *C. pubescens* affect the photosynthetic physiology of the native and invasive nitrogen fixing species? 2) if so, does this influence host biomass accumulation? 3) does infection affect the nitrogen status of the host-parasite-rhizobium system? 4) can this effect reduce the soil enrichment by the invasive species? and 5) does the nitrogen status of the host influence the performance of the parasite?

4.3 Materials & Methods

4.3.1 Plant material and Experimental design

A three-way factorial experiment (infection by *C. pubescens*, the nodulation of the hosts with Rhizobium and the addition of nitrogen fertilizer) was conducted to investigate the impact of *C. pubescens* on the mutualistic relationship between rhizobium and a native or invasive nitrogen-fixing hosts, *A. myrtifolia* and *C. scoparius*, respectively. The soil used in this experiment was a mixture of soil collected from the field and commercial soil (a sandy loam soil from the Mt. Lofty area). Field soil was used to provide inoculum for the nodulation of the two nitrogen-fixing hosts with the rhizobium that naturally associated with these species in the Mount Lofty Ranges. Field soil was collected within a 30 cm radius of mature shrubs of the two host species, and was mixed in 1 to 4 ratios with the commercial soil. Depending on the treatments, the mixture of field and commercial soil was either sterilized in an autoclave (three hours at 120°C) or not. Seeds of *C. scoparius* and *A. myrtifolia* were surface-sterilized with bleach (4%) for 5 minutes. *C. scoparius* and *A. myrtifolia* were grown on either sterilized or unsterilized soil for 3 months, and then transplanted to 1.5 L pots containing either sterilized or unsterilized soil. A further three months after transplanting, half of the plants in each soil treatment were infected with *C. pubescens* by placing them next to an infected *C. scoparius* plant and directing the tendrils of the parasite to the stem of the target seedlings. *C. pubescens* was allowed to grow on the new hosts for 8 weeks before the connection between the donor and recipient host was severed. This experiment was conducted in a glasshouse from May 2008 to January 2009.

To test if the availability of nitrogen affects the host-parasite interaction, a subset of replicates without rhizobium (i.e. in sterilized soil) were supplied with full strength Hoagland's solution, while the rest of the replicates were supplied with nitrogen free Hoagland's solution. Later this setting was modified according to Fig. 4.1.1 and 4.1.2 when it was discovered that nodulation had occurred in sterilized *C. scoparius* (referred to novo-nodulated) and as all plants of *A. myrtifolia* failed to become infected by *C. pubescens* (see below). All the plants were watered three times weekly. The resulting final treatments were:

- 1) nodulated *C. scoparius* without parasite (n= 6)
- 2) nodulated *C. scoparius* with parasite (n= 6)
- 3) non-nodulated *C. scoparius* without parasite (n= 3)
- 4) non-nodulated *C. scoparius* with parasite (n= 2)
- 5) novo-nodulated *C. scoparius* without parasite with added nitrogen fertilizer (n= 4)
- 6) novo-nodulated *C. scoparius* with parasite with added nitrogen fertilizer (n= 3)
- 7) novo-nodulated *C. scoparius* without parasite without added nitrogen fertilizer(n=5)
- 8) novo-nodulated *C. scoparius* with parasite without added nitrogen fertilizer (n= 4)
- 9) nodulated *A. myrtifolia* with added nitrogen fertilizer (n= 6)
- 10) nodulated *A. myrtifolia* without added nitrogen fertilizer (n= 6)
- 11) non-nodulated *A. myrtifolia* with added nitrogen fertilizer (n= 9)
- 12) non-nodulated *A. myrtifolia* without added nitrogen fertilizer (n= 8)

4.3.2 Nitrogen content

Nitrogen content of tissue from nodulated *C. scoparius*, nodulated *C. scoparius* with *C. pubescens* and nodulated *A. myrtifolia* was measured using an Elementar Rapid N III Nitrogen Analyser (ELEMENTAR, Germany). Low numbers of replicates prevented measurement of tissues from the other treatments. Tissues for analysis were dried in an oven at 70°C for at least 3 days, and ground to a fine powder.

The effect of *C. pubescens* on soil nitrogen was evaluated by measuring nitrogen content of the soil in the pots before and after the experiment. The top 2 cm of soil was removed from the pot to avoid the influence the organic layer formed by the accumulation of dead leaves. Thus the effect of root activity was isolated. The collected soil was dried in an oven at 70°C for 5 days, and then sieved through a 1mm sieve three times to remove all rock and plant debris. The concentrations of nitrate and ammonia of the soil samples were measured.

4.3.3 Host photosynthetic physiology

Photosynthetic parameters of the hosts (chlorophyll content and PS II efficiency) were measured at the end of the experiment. For chlorophyll assays, the youngest fully mature leaves of each replicate were randomly collected and stored on ice for up to an hour until they were processed. The leaves were ground in a mortar and pestle with a pinch of sand and 3 ml of methanol, and the mortar and pestle was rinsed twice with 1 ml of methanol. The extract was then transferred into a centrifuge tube and topped up to 8 ml of methanol. The extracts were centrifuged at 2500 rpm for 15 minutes. The samples were measured by using a Spectronic Visible Spectrophotometer (Bausch & Lomb) at the wavelength of 652 and 665 nm, and the readings were used to calculate the concentration of chlorophyll a and b (Porra, Thompson et al. 1989).

The effect of *C. pubescens* on host PSII efficiency (yield) was measured at two hourly intervals from sunrise to sunset using a Mini-PAM Portable Chlorophyll Fluorometer fitted with a 2030-B leaf-clip holder (Heinz Walz GmbH, Effeltrich, Germany). Three youngest fully mature leaves of each replicate were randomly selected for the measurement; the recorded value was the average of the two closest measured values for each. The measurements were conducted on four sunny days with maximum PFDs around 1500 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Plants from all treatments were randomly allocated into these four measurement days.

4.3.4 Growth and Biomass

At the beginning of the experiment, four stems of the parasite and the hosts were marked with a permanent marker and their increase in length was recorded fortnightly. These measurements were used to assess their stem elongation rates. All the plant material of the hosts and parasite was harvested at the end of the experiment. The host material of all replicates was divided into four components: nodules, root, stem and leaf. Plant material was dried as described in 4.3.2 before weighing.

4.3.5 Statistical analysis

The data obtained from diurnal yield measurement were used to plot the relationship between photosynthetic yield and PFD. A single phase exponential decay function was used to fit this curve, and curves were compared using F-test in GraphPad Prism (version 4.03). All other measurements were analysed by ANOVA followed by a Tukey test to locate the significant difference in the comparison when appropriate (JMPIN, version: 4.0.3). Because of the unexpected nodulation of *C. scoparius* grown in sterilized soil, there were insufficient replicates of non-nodulated *C. scoparius* for statistical analysis.

4.4 Results

4.4.1 Infection with *C. pubescens*

C. pubescens readily infected *C. scoparius* but not *A. myrtifolia* in this experiment. After 8 weeks of infection, young stems of *C. pubescens* were firmly attached on all target hosts, however, after the connections between the source and target hosts were severed, all the stems of the parasite attached to *A. myrtifolia* wilted within a week. In contrast, young stems disconnected from the source hosts grew vigorously when

attached to *C. scoparius*. The lack of successful infection by *C. pubescens* on *A. myrtifolia* necessitated a change in the experimental design (Fig. 4.1.1 (b)).

4.4.2 Rhizobium nodulation

In the 3 months prior to transplanting, neither species grown in sterilized soil developed root nodules, whereas in non-sterilized soil both species developed obvious nodules. However, around 3 months after transplantation, it became apparent that the *C. scoparius* plants in the sterilized soil had become nodulated, as they turn greener and more vigorous within weeks (Fig. 4.2). Nodules were subsequently found on the roots of 16 plants of the *C. scoparius*. In contrast, only 4 *A. myrtifolia* growing in sterilized soil were found to be nodulated at the end of the experiment. Henceforth, the plants growing in sterilized soil that developed nodules were classified as “novo-nodulated”. The experimental design was consequently modified (Fig. 4.1.2 (b)). Due to the nodulation that occurred on *C. scoparius* in the sterilized soil, the nitrogen fertilizer treatment was only applied to the novo-nodulated plants of *C. scoparius*.

4.4.3 Plant nitrogen

The infection of *C. pubescens* on *C. scoparius* seemingly had no effect on the fixation of nitrogen by rhizobium, as there was no difference in the total nitrogen content of uninfected *C. scoparius* and the parasite-*C. scoparius* system (ANOVA, $f= 3.31$, d.f.= 1, $P=0.10$) (Fig. 4.3.1a). However, the nitrogen contents of parasite-infected *C. scoparius* were 25% lower than in uninfected *C. scoparius*, indicating that the parasite competed with the host for nitrogen (ANOVA, $f= 6.57$, d.f.= 1, $P=0.03$). Infection had no effect on the allocation of nitrogen to different organs of the host (Fig. 4.3.2a), except nitrogen content of the roots of infected hosts which was 6% lower than in the uninfected hosts (ANOVA, $f= 5.55$, d.f.= 1, $P=0.04$). Nitrogen content in the invasive legume, *C. scoparius* was 36% higher than in the native legume, *A. myrtifolia* (ANOVA, $f= 40.97$, d.f.= 1, $P<0.0001$) (Fig. 4.3.1b).

4.4.4 Soil nitrogen

The concentrations of nitrogen in the soil changed over the experimental period, and were related to the host identity growing in the pot but independent of the presence of *C. pubescens* (Fig. 4.4). The changes in the concentrations of nitrate ranged from no obvious change in both *C. scoparius* treatments (Fig. 4.4a) to significant 88% depletion in *A. myrtifolia* (Fig. 4.4b) (ANOVA, $f= 5.12$, $d.f.= 11$, $P=0.045$). Ammonia concentration rose substantially in all three treatments, however, there was no difference effect of *C. pubescens* on *C. scoparius* soils, also, none between native and invasive hosts. Although there was depletion of available nitrogen in soil from *A. myrtifolia*, this was not significant relative to the *C. scoparius* soil, and there was no effect of *C. pubescens*.

4.4.5 PSII efficiency and chlorophyll content

C. pubescens had no effect on the concentration of chlorophyll in *C. scoparius*, except the host in the non-nodulated treatments (Fig 4.5.1). In the non-nodulated plants, the total concentrations of chlorophyll in uninfected hosts was lower than the concentrations in both the infected and uninfected stems of infected hosts (ANOVA, $f= 15.10$, $d.f.= 1$, $P= 0.0116$). However, chlorophyll concentrations in the nodulated and novo-nodulated hosts were double those of non-nodulated hosts (ANOVA, $f= 60.49$, $d.f.= 1$, $P<0.0001$) (Figs 4.5.1 & 4.5.2). However, the chlorophyll a to b ratio in non-nodulated hosts was higher than for nodulated ones, and the addition of nitrogen fertilizer had no impact on this ratio in the novo-nodulated plants (Fig. 4.5.1 (d)). The presence of rhizobium had a similar effect on *A. myrtifolia*, the plants in nodulated treatments had higher concentrations of chlorophyll than the plants in non-nodulated treatments, except chlorophyll b. In the novo-nodulated treatment, addition of nitrogen fertilizer positively affected the concentration of chlorophyll; the concentrations were higher in the treatment with high nitrogen added than in to those without nitrogen supply (ANOVA, $f= 8.08$, $d.f.= 1$, $P= 0.008$). Furthermore, the addition of nitrogen fertilizer increased the concentration of total chlorophyll in *A. myrtifolia*, but this only occurred in the non-nodulated treatments (Fig. 4.5.3)

(ANOVA, $f= 4.63$, $d.f.= 1$, $P= 0.04$). That is, parasite infection had no effect on *C. scoparius* with rhizobium, whereas, the presence of rhizobium and addition of nitrogen fertilizer significantly influenced the chlorophyll content.

The parasite infection had only localised negative effect on PSII efficiency in the invasive host, *C. scoparius* regardless of the nodulation condition (Fig. 4.6.1). There was no difference in the photosynthetic yield response to light between the uninfected *C. scoparius* and the uninfected branches of infected *C. scoparius* (Fig. 4.6.4) ((a): $F=1.51$, $P=0.215$; (b): $F=1.40$, $P=0.257$; (c): $F=0.99$, $P=0.405$; (d): $F=2.20$, $P=0.099$). However, the infected branches suffered stronger reduction in yield with increasing PFD and at the highest PFD, the photosynthetic yield of infected branches were lower than the yield of uninfected branches ((a): $F=3.28$, $P=0.024$; (b): $F=3.55$, $P=0.026$; (c): $F=3.57$, $P=0.019$; (d): $F=3.01$, $P=0.039$).

Nodulation had a positive effect on the PSII efficiency of both native and invasive hosts, regardless of parasite infection or nitrogen fertilization in *C. scoparius* (Fig. 4.6.2 (a) and (b)). In *C. scoparius*, the light response of nodulated and novo-nodulated plants was the same with increasing light, but the photosynthetic yield of non-nodulated plants was lower than the other two treatments at any PFD ((a): $F=10.62$, $P<0.0001$). Meanwhile, in *A. myrtifolia*, the yield was lower in non-nodulated than nodulated plants with increasing light, but the yield was the same at the highest light ((b): $F=8.01$, $P<0.0001$). Furthermore, the addition of nitrogen fertilizer positively affected the photosynthetic yield of both hosts (Fig. 4.6.3 (a) and (b)). In novo-nodulated *C. scoparius*, the plants with addition of nitrogen fertilizer had higher yield with increasing light intensity than the plants without addition of nitrogen fertilizer ((a): $F=6.49$, $P=0.0003$). However, this increase in yield only occurred in the non-nodulated *A. myrtifolia* ((b): $F=18.08$, $P<0.0001$).

4.4.6 Growth and Biomass measurements

Stem elongation of *C. scoparius* was more strongly influenced by rhizobium than by the presence of parasite, with elongation rate higher in nodulated than in non-nodulated treatment (Fig. 4.7 (a)). In contrast, there was no difference in the

elongation rate between plants with or without *C. pubescens*. A similar result was found for the native legume, *A. myrtifolia*; where nodulated plants had higher elongation rates than plants in non-nodulated treatment. However, the low elongation rate of non-nodulated *A. myrtifolia* was increased by the addition of nitrogen fertilizer (Fig. 4.7 (c)).

Infection with *C. pubescens* reduced the biomass accumulation of nodulated *C. scoparius* (Fig. 4.8.1a). There was a trend for the biomass of each component of the infected host to be lower than the uninfected hosts, but only leaf biomass was significantly lower with infection (ANOVA, $f= 5.18$, $d.f.= 1$, $P= 0.04$). In consequence, the total biomass of the infected hosts (mean \pm s.e., 33.06 ± 2.75 g, $n=6$) was lower than the biomass of uninfected hosts (mean \pm s.e., 41.92 ± 2.75 g, $n=6$), but, this difference disappeared when the biomass of *C. pubescens* growing on these plants was included (ANOVA, $f= 1.48$, $d.f.= 1$, $P= 0.25$). This is, the reduction of biomass in *C. scoparius* seemed directly related to the biomass of *C. pubescens* growing on it.

In contrast, the parasite infection had no effect on the biomass of *C. scoparius* in the novo-nodulated treatments (ANOVA, $f= 0.32$, $d.f.= 1$, $P= 0.58$), nor did the addition of nitrogen fertilizer (ANOVA, $f= 0.25$, $d.f.= 1$, $P= 0.063$) (Fig. 4.8.1 (b)). In the non-nodulated *C. scoparius* treatments, no statistical analysis was conducted due to the low replication ($n=2$ and $n=3$). There was an obvious difference on the biomass of all components between nodulated and non-nodulated *C. scoparius*, which indicated that *C. scoparius* was highly dependent on the nitrogen provided by rhizobium (Fig. 4.8.1 (a) and (c)). Furthermore, the presence of rhizobium had positive effect on the biomass of *A. myrtifolia* (Fig 4.8.1 (d)). Its leaf and stem biomasses in nodulated treatments were higher than those in non-nodulated treatments, but the addition of nitrogen fertilizer increased the root biomass of the host in non-nodulated treatment. In terms of nodule formation, the nodules of *C. scoparius* were not affected by the infection of *C. pubescens* (Fig. 4.8.2 (a)) nor by addition of nitrogen fertilizer (Fig. 4.8.2 (b)). Furthermore, the addition of nitrogen fertilizer had no impact on the nodule formation of *A. myrtifolia* (Fig. 4.8.2 (c))

4.4.7 Effect of host condition on the parasite

The concentration of chlorophyll in *C. pubescens* was closely related to the relationship of the hosts with rhizobium (Fig. 4.5.4). In the treatment without nitrogen fertilizer, the concentrations of chlorophyll of the parasites growing on *C. scoparius* nodulated with and novo-nodulated with rhizobium were over 100% higher than those grown on the hosts without rhizobium. However, this positive effect of rhizobium on *C. pubescens* was only detected in the chlorophyll content, as there was no difference in photosynthetic yield (Fig. 4.6.5)($F=1.01$, $P=0.422$), stem elongation rate (Fig. 4.7(d)) or biomass (Fig. 4.8.3 (a)) of the parasites between the hosts in the various rhizobium treatments.

4.5 Discussion

C. pubescens had no effect on the nitrogen fixation of the invasive host, but competed with the host for nitrogen. This reduction in nitrogen availability to *C. scoparius* reduced the leaf biomass and hence lowered the photosynthetic surface area of the plant. There was also a negative effect on PSII efficiency on the infected branches of *C. scoparius*. The lower leaf area and PS II efficiency would have limited the photosynthates produced, but this leads to a decrease in the biomass accumulation of the host, rather than an impact on the symbiotic Rhizobium. The parasite performed differently on *C. scoparius* with different nodulation treatments. In addition, the two host species modified the soil nitrogen differently. Nodulation occurring in almost all of the sterilized treatment of *C. scoparius*, and this may indicate that *C. scoparius* has lower specificity in the mutualistic symbiosis with regard to *A. myrtifolia*. Further, this experiment revealed the high resistance of the native legume, *A. myrtifolia* to *C. pubescens*.

4.5.1 Symbiont specificity of *C. pubescens*

One of the setbacks in this experiment was the high rate of nodulation that occurred in the sterilized soil treatment of *C. scoparius*. This prevented an effective assessment of the importance of nitrogen fixation to *C. scoparius*, and how it may interact with the negative effect of parasite infection, as well as clarifies the effect of host nutrient status to the parasite. In contrast, only a minority of *A. myrtifolia* in the sterilized soil treatment were found to be nodulated at the end of the experiment. This difference in the nodulation in sterilized soil suggests that these two nitrogen fixing plants may have different degree of specialization with these microbial symbionts. This may contribute to the high invasiveness of *C. scoparius*.

Previous studies have found that the emerging mutualistic relationships between the invasive plants and the native soil biota can increase invasion success (Marler, Zabinski et al. 1999; Simberloff and Von Holle 1999; Rudgers, Mattingly et al. 2005; Wolfe and Klironomos 2005; Reinhart and Callaway 2006). For example, Marler, Zabinski *et al.*(1999) found that arbuscular mycorrhizae had a minor direct beneficial effect on growth of the invasive plant, *Centaruea maculosa*, but the soil biota significantly favoured the growth of the invasive plant when it was in competition with the native species, *Festuca idahoensis*. This showed that the emerging relationship between soil biota with invasive species can negatively affect native competitors. A correlation between competitive ability of a plant species and the degree of symbiotic specialization has been suggested by Wilkinson and Parker (1996), the generalists growing larger than those with high symbiotic specialization. Recently, van der Putten, Klironomos et al. (2007) proposed that the invasiveness of a plant species may depend on how it can interact with soil biota in the new habitat, especially nitrogen fixing species. Thus, species that can form mutualistic relationships with wider range of rhizobia (i.e. generalists) would be more invasive than those that can only form relationships with narrow range of rhizobia (i.e. specialists) (Sax, Stachowicz et al. 2007; van der Putten et al. 2007).

In my experiment, hosts growing in sterilized soil might have become nodulated by the bacteria surviving in the sterilized soil or by bacterial spores in the air. All hosts were cultured in the same glasshouse, hence all plants were subjected to same potential rate of contamination. However, the early timing and high level of nodulation in *C. scoparius* grown in sterilised soil may illustrate that this invasive species can form nitrogen-fixing relationships with a wider range of soil biota than that of the native species, *A. myrtifolia*. Alternatively, *C. scoparius* is more likely to form associations with strains of Rhizobium with higher dispersal ability. The first interpretation is consistent with studies showing that *C. scoparius* can form nitrogen-fixing relationships with a broad range of soil bacteria (Perez-Fernandez and Lamont 2003; Rodriguez-Echeverria, Perez-Fernandez et al. 2003; Lafay and Burdon 2006). Meanwhile, studies of the diversity of soil bacteria associated with *Acacia* species in Australia have shown that certain species of *Acacia* perform better when grown with soil that has previously supported the same species (Thrall, Burdon et al. 2000; Thrall, Slattery et al. 2007). In conclusion, the substantial novo-nodulation on *C. scoparius* in this experiment provides further evidence of the differences in degree of specialisation for soil bacteria between the invasive legume, *C. scoparius* and the native *A. myrtifolia*.

4.5.2 Effects of the parasite on the *C. scoparius*-rhizobium system

The lack of difference in total nitrogen content of the plant-parasite system and uninfected plants, and in nodule biomass in *C. scoparius* with or without the parasite, suggests that the nitrogen fixing capacity of rhizobium was unchanged by the presence of the parasite. This, in turn, suggests that the parasite probably did not reduce the resources provided by *C. scoparius* to the symbiont. Instead, the parasite constitutes a substantial drain of nitrogen from the host (Pate and Bell 2000). As a result, this suggests that *C. pubescens* acts as nitrogen sink and competes directly for nitrogen with the host.

The infected *C. scoparius* suffered reduction in photosynthetic yield, although this effect was only detected in the infected branches. This localised negative effect on the

infected branches has been observed in other related studies (Britton 2002; Prider, Facelli et al. 2009; Hao, Prider et al. 2010). Prider, Facelli *et al.* (2009) suggested that *C. scoparius* may reallocate resource from infected branches to other branches to compensate the reduction in photosynthesis. However, there was no difference in the chlorophyll content between infected and uninfected branches in infected hosts, suggesting that nitrogen is not the factor limiting photosynthesis in these branches. In this glasshouse experiment the decrease in the photosynthetic yield seemed much smaller than that reported by Britton (2002) in a similar infected invasive legume, *Ulex europaeus* and by Hao, Prider *et al.* (2010). This variation in the impact of the parasite on photosynthetic yield could relate to differences in the abiotic stress level produced by different growing condition. In this pot experiment, the host were subjected only to the biotic stress from the infection of *C. pubescens*. Since nutrient limitation was the focus of the study, the plants received ample water. In the other studies, particularly in the field, the hosts almost certainly experienced a whole range of abiotic stresses, such as water and nutrient limitations and high light intensity.

C. pubescens had no effect on the relative rate of the shoot elongation of *C. scoparius*, but reduced its final biomass. The biomass produced by the whole plant-parasite system, however, was the same as that of plants without parasite. The difference in effect on the biomass of whole system and the host indicates that the overall productivity of the whole system had not been affected; the lower biomass of the host may due to the loss of resources, such as water and nutrient (nitrogen mentioned above) from the host to the parasite (Hao et al. 2005). The impact of the hemiparasite on the invasive host in this study was unlike the effect of another hemiparasite, *Rhinanthus minor* that suppress the host photosynthesis (Cameron et al. 2008), instead, it was more similar to the resource sink interaction like between the holoparasite (Watling and Press 2001). The total chlorophyll content and total biomass of the system of infected host in this study were not affected by the parasite infection; hence the photosynthesis of the host was not disrupted by the parasite. Both stems and roots suffered only marginally negative effect by the infection, but the biomass of leaves was significantly reduced. This was most likely due to loss of leaves in infected plants. Considerable loss of leaves may be a strategy for the host to minimize water loss through the parasite (Prider et al. 2009). Parasitic plants usually have high transpiration rates, which allow them to withdraw minerals from the host, which

induces water stress on their hosts. Fogarty and Facelli (1999) reported that *C. scoparius* would drop its leaf at the onset of the summer drought of Australia thus reducing water loss. Similarly, Burch (1992) documented that the leaf biomass of *Schinus terebinthifolius* infected with *Cassytha filiformis* was halved. However, this may also relate to the reduction of nitrogen supply, since nitrogen deprivation may also trigger mortality of older leaves, when nitrogen is reallocated to newer organs.

By combining all the results regarding the effects of *C. pubescens* on *C. scoparius*, a tentative model can be proposed to describe the carbon-nitrogen relationship between the host, the rhizobium and the parasite. The parasite seemingly does not compete for host photosynthate with rhizobium, probably due to the relatively high photosynthetic capacity of the parasite (Prider et al. 2009), and hence there is no inhibition of nitrogen fixation. However, the parasite acts as an extra nitrogen sink, limiting host biomass accumulation, in particular lowering leaf biomass (photosynthetic area). The parasite also impairs host photosynthetic ability; although this is localized to infected branches, and may be the result of water stress rather than nitrogen limitation. Eventually, the amount of photosynthates produced by the host would be reduced by the lower photosynthetic surface area, and could further impact host biomass accumulation and may ultimately impact on the supply of carbon to rhizobium; although this may take more time than the length of this experiments.

The model suggested in this study is more similar to the one proposed for the root hemiparasite parasite *Oxalys phyllanthi*, than that proposed for the holoparasite *Cuscuta*. Like *C. pubescens* in my study, *O. phyllanthi* has a minor effect on nitrogen fixation of the host, but does affect the nitrogen allocation of host (Tennakoon et al. 1997). On the other hand, the morphologically similar stem holoparasite *Cuscuta reflexa* significantly inhibits nitrogen fixation and also affects the allocation of nitrogen to the host (Jeschke et al. 1994). The difference in the effect on nitrogen fixation is likely related to the photosynthetic capacity of the parasite, which has direct influence on the dependence of the parasite on host photosynthate. While *C. pubescens* and *C. reflexa* are both stem parasites, *C. pubescens* is a hemiparasite with a relatively high photosynthetic rate (Prider et al. 2009), while *C. reflexa* is a holoparasite, and mainly depends on its host for photosynthates, and thus competes with rhizobium for carbon.

4.5.3 Effects of host nodulation status on parasite

C. pubescens growing on non-nodulated *C. scoparius* had only half of the chlorophyll content of that growing on nodulated *C. scoparius*, although because of low replication in the non-nodulated treatment no statistical tests were made. However, photosynthetic yield, stem elongation rate and the biomass accumulation of *C. pubescens* did not differ between the hosts with/without nodulation. One possible explanation for this may be that *C. pubescens* absorbed at least some photosynthates from the non-nodulated *C. scoparius*, enabling it to achieve the same biomass as the parasites on the nodulated hosts. This would assume that this hemiparasite can vary their dependence on photosynthates produced by the host, which is consistent with the finding of xylem-tapping hemiparasite like mistletoes (Marshall and Ehleringer 1990). This study further suggests that the dependence on host photosynthate may vary with on the photosynthetic capacity of the parasite. To confirm this hypothesis, an experiment is required to measure the transpiration and measure the ^{13}C to ^{12}C isotope ratio of from the hosts and parasites. Following this line of thinking, suggests that there may be no clear-cut difference between holoparasite and hemiparasite, as the dependence of a hemiparasite on host photosynthate may depend on its photosynthetic capacity, which is controlled by a range of factors, such as nitrogen availability.

4.5.4 Effects on soil nitrogen

There were differences after 6 months in the soil nitrogen in the pots with the two host species, but the parasite had no effect on nitrogen in the soil with *C. scoparius* (no evidence was available for *A. myrtifolia* as it resisted the parasite). As suggested by the plant nitrogen data, the parasite seemingly did not reduce the carbon provided by the plant to the nitrogen-fixing bacteria; hence the amount of nitrogen fixed by the bacteria and incorporated into the soil may not have been different. However, the obvious differences in the soil nitrogen concentration detected in this pot experiment illustrate the ability of *C. scoparius* to alter soil nitrogen status. Watt, Clinton *et al.* (2003) found that at least 81% of the nitrogen in *C. scoparius* is acquired through nitrogen fixation. However, there is no such evidence for *A. myrtifolia*; and hence it is

hard to compare the nitrogen fixation capacity of these two species. Meanwhile, the total nitrogen content and concentration in *C. scoparius* were significantly higher than for *A. myrtifolia*, which may indirectly indicate that *C. scoparius* is a more effective fixer. In terms of the decomposition rate of plant material, the release of nutrient through fine root decomposition should also be counted as the high turnover rate of fine root (Usman, Singh et al. 2000; Chen, Harmon et al. 2002). The nitrogen content of the root of *C. scoparius* was higher than that of *A. myrtifolia*, therefore, the amount of nitrogen being released by fine root decomposition would likely be higher for *C. scoparius*.

Studies on the impact of *C. scoparius* invasions have all documented that this weed can alter the soil nutrient composition especially nitrogen content in the invaded area (Fogarty and Facelli 1999; Watt et al. 2003; Haubensak et al. 2004; Haubensak and Parker 2004; Prevosto, Dambrine et al. 2006). All these studies have found the soil nitrogen content in the invaded area with *C. scoparius* is higher than in adjacent areas without the weed. For example, a study on the effect of *C. scoparius* in the Mount Lofty Ranges documented that this invasive legume modifies soil nitrogen availability (Fogarty and Facelli 1999). However, most of these surveys were conducted in an invaded area without knowing how long the area had been invaded; therefore, the time scale of the change of soil nitrogen content by *C. scoparius* is still an unknown. This pot experiment provides evidence of high ability of *C. scoparius* to increase soil nitrogen in a brief period.

The increase in soil nitrogen could affect on the invasibility of the habitat in the future (Vitousek 1990; Ehrenfeld 2003; Levine et al. 2003). The ability of nitrogen fixing and decomposition rate of plant materials differ between species, hence the replacement of a new nitrogen fixing plant in the habitat could significantly affect the nitrogen availability in soil (Maron and Connors 1996; Evans, Rimer et al. 2001; Allison and Vitousek 2004; Hawkes et al. 2005; Laungani and Knops 2009). This increase in soil nutrient availability of soil may create new environmental conditions, making the system more easily invaded by other weeds (Davis, Grime et al. 2000; Carino and Daehler 2002; Bidwell, Attiwill et al. 2006). Consequently, the evidence provided by this study combined with the studies on the change in soil nitrogen

content in invaded areas by *C. scoparius* further proved the strong ecological engineer role of this weed in any new habitat.

4.5.5 Conclusion

This study suggests a model of the carbon-nitrogen relationship between the parasite and the host, which is different from the morphologically similar *Cuscuta* spp.. In this model, the parasite has a low dependence on host photosynthate due to the relatively high photosynthetic ability of the parasite and localised impact (branch level) on host photosynthetic performance; hence the parasite does not compete for carbon with rhizobium. Although it had no negative effect on nitrogen fixation, this parasite still acts as a strong nitrogen sink, and thus limits the nitrogen availability to the host. A consequence of this is lower host biomass and reduced photosynthetic surface area. Furthermore, the study provides a surprising result in the response of *C. pubescens* to *C. scoparius* with different nodulation treatments. The parasites on non-nodulated hosts had lower chlorophyll content than those on nodulated hosts, but there was no such negative effect on the growth of the parasite. This may suggest that *C. pubescens* shifts from being a hemiparasite to a holoparasite under the limited supply of nitrogen from the host.

In addition to the findings on the parasite-host interactions, this study provides clear evidence on the differences in the soil nitrogen modification between native and invasive legumes. Differences in soil nitrogen could increase the resource availability in the soil under *C. scoparius*, and possibly affect the invasibility of the system. Further, the invasiveness of *C. scoparius* may relate to the degree of specialization to mutualistic soil biota, this study suggests that it can interact with wider range of Rhizobium than the native *A. myrtifolia*; hence it has higher chance of establishing well in a new habitat and becoming invasive.

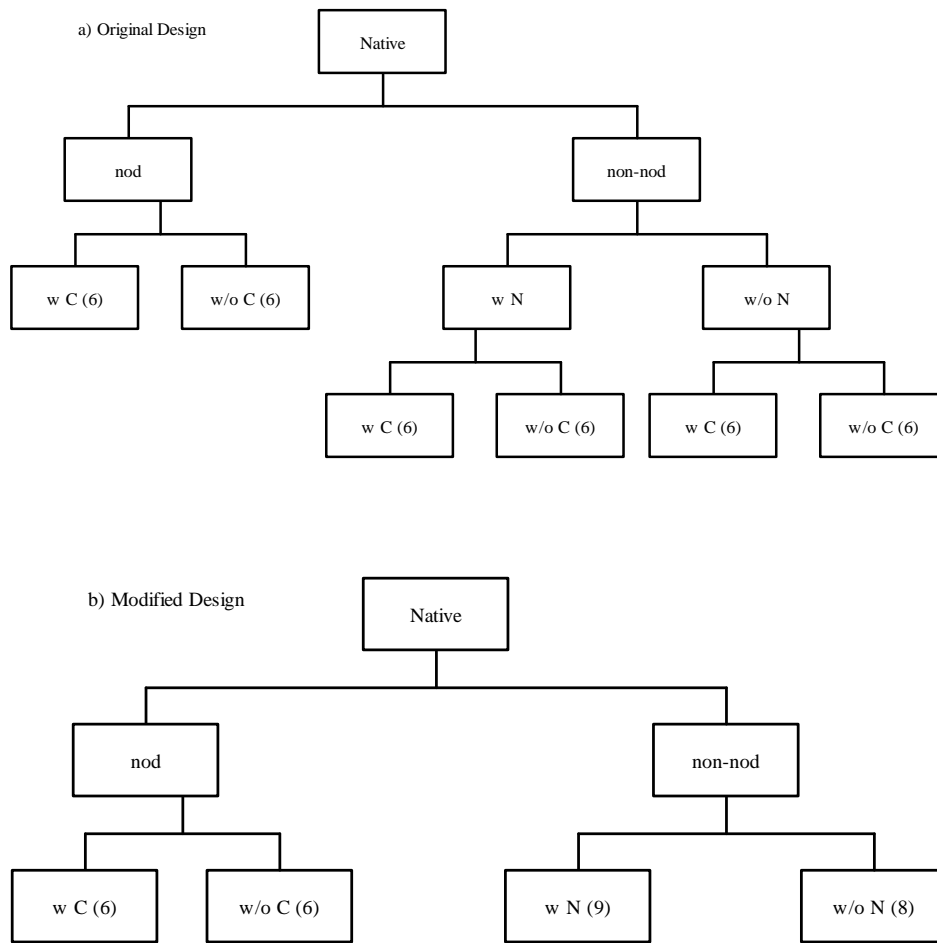


Figure 4.1.1. The experimental design for the native species, *Acacia myrtifolia*: a) original and b) modified designs. The number within bracket represents the number of replicate in the treatments.

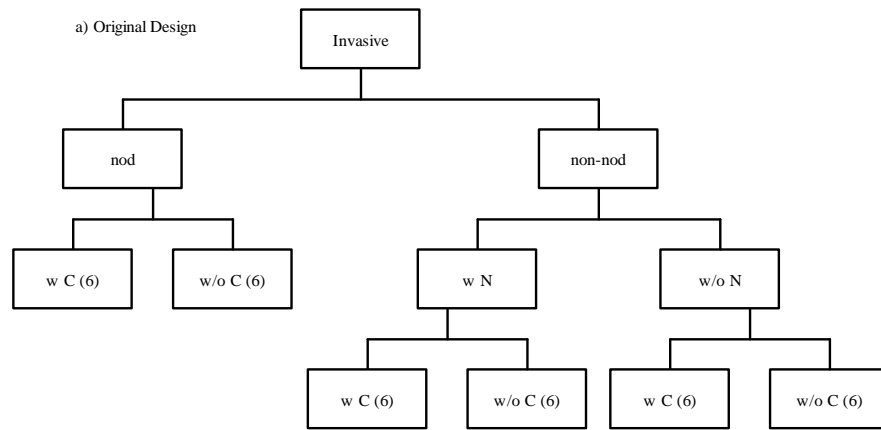


Figure 4.1.2. The experimental design for the invasive species, *Cytisus scoparius*: a) original and b) modified designs. The number within bracket represents the number of replicate in the treatments.

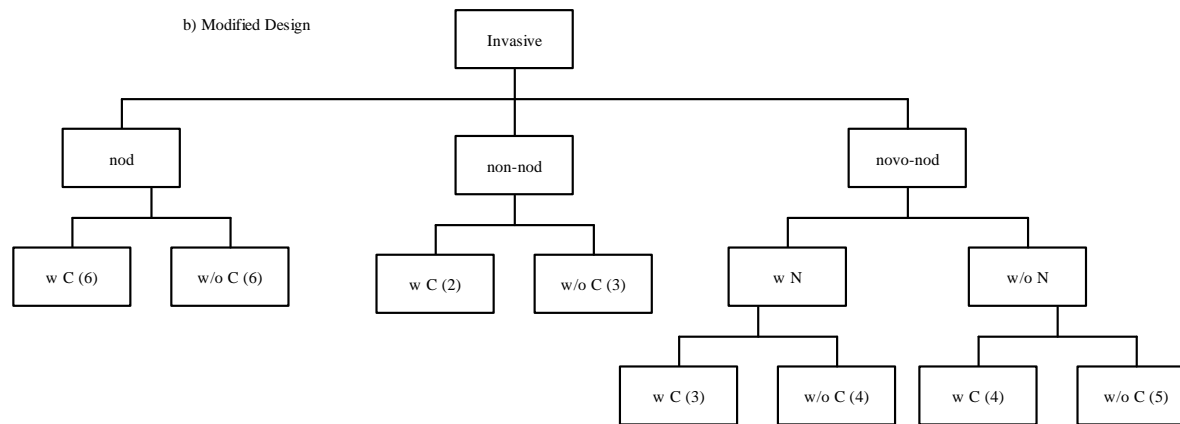
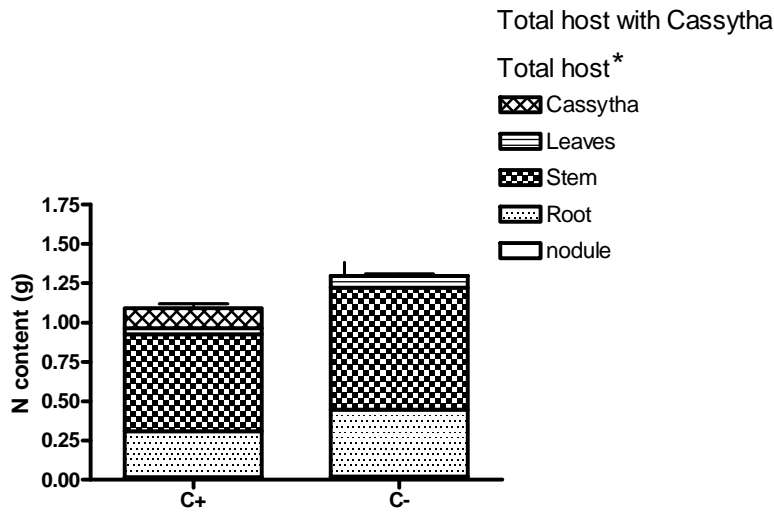




Figure 4.2: The difference in appearance of *Cytisus scoparius* between different nodulation treatments, non-nodulated, nodulated and novo-nodulated (from left to right).

(a)



(b)

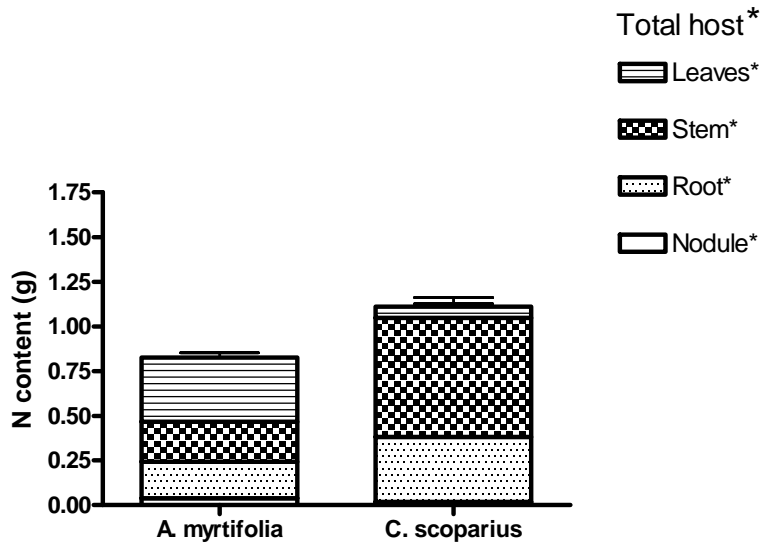


Figure 4.3.1: The total nitrogen content in different organs of hosts and parasite. (a): nodulated *Cytisus scoparius* in *Cassytha pubescens* treatments and (b): nodulated *Cytisus scoparius* and *Acacia myrtifolia*. C+: infected host and C-: uninfected host. Data points are means \pm s.e. (n=6). Asterisks indicate significant difference at $p < 0.05$.

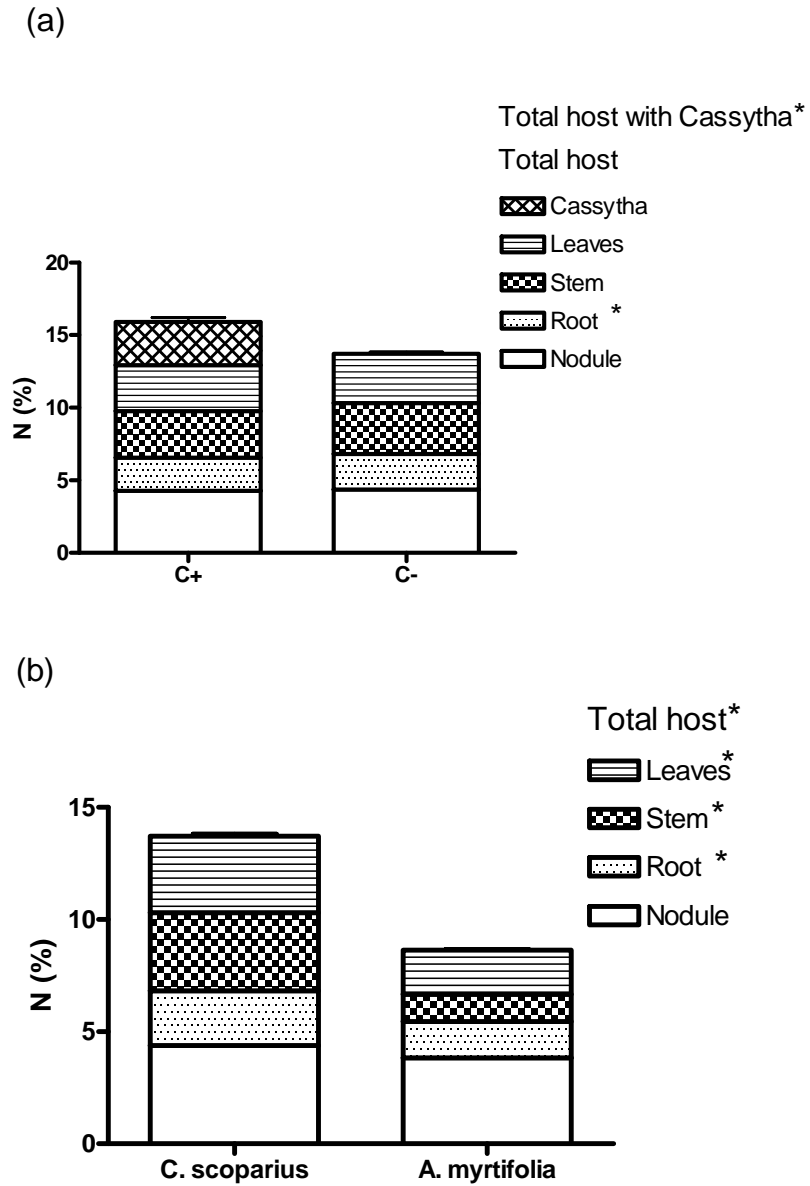


Figure 4.3.2: The concentration of nitrogen in different tissues of hosts and parasite, (a): nodulated *Cytisus scoparius* in *Cassytha pubescens* treatments and (b): nodulated *Cytisus scoparius* and *Acacia myrtifolia*. C+: infected host and C-: uninfected host. Data points are means \pm s.e. (n=6). Asterisks indicate significant difference at $p < 0.05$. Note different scales for both graphs.

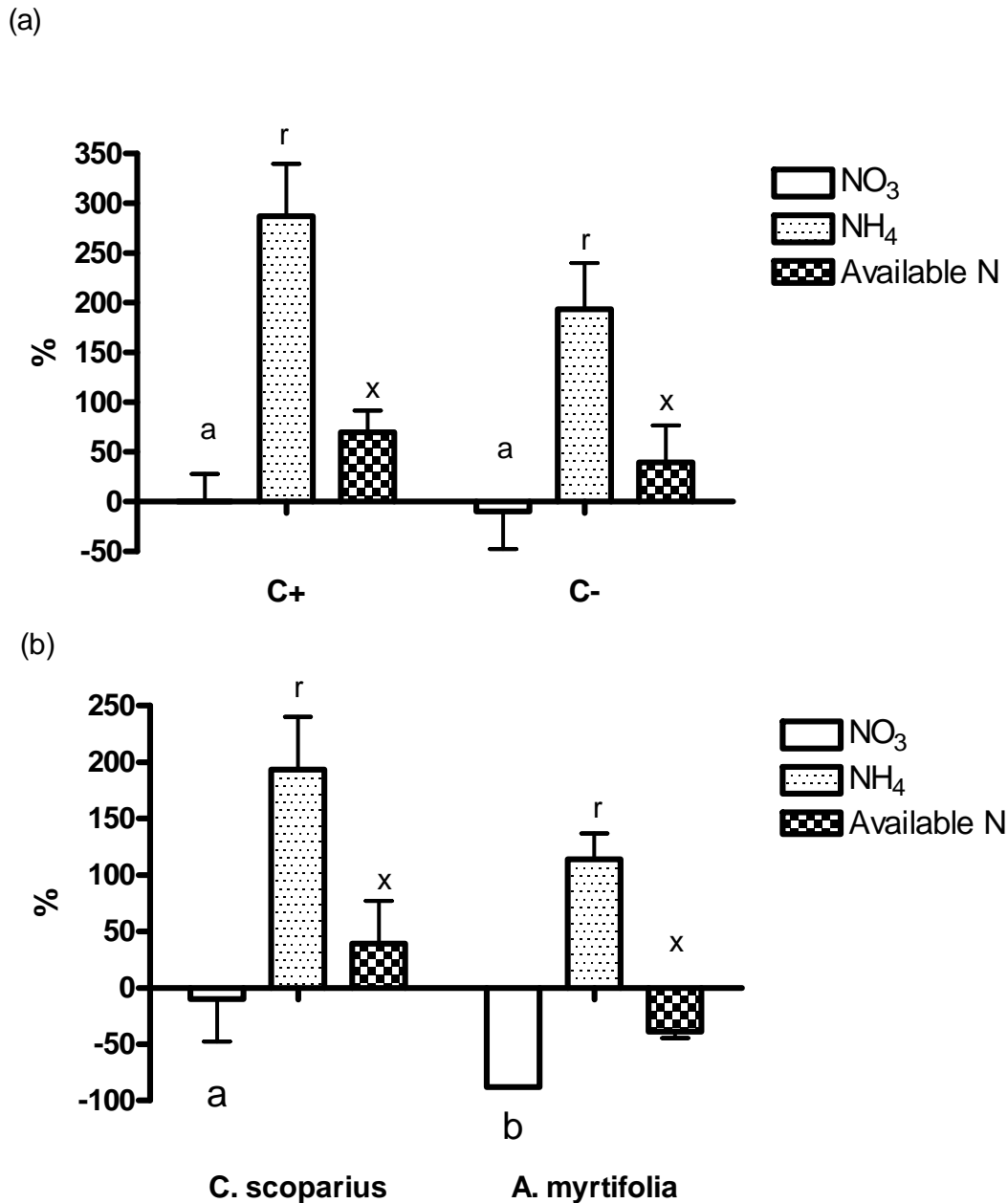


Figure 4.4.: The change in percentage on the concentrations of nitrogen compound, nitrate, ammonia and total available nitrogen in soil by hosts over the experimental period, (a): nodulated *Cytisus scoparius* in *Cassytha pubescens* treatments and (b): nodulated *Cytisus scoparius* and *Acacia myrtifolia*. C+: infected host and C-: uninfected host. Data points are means \pm s.e. (n=6). Different letters indicate significant difference at $p < 0.05$. Note different scales for both graphs.

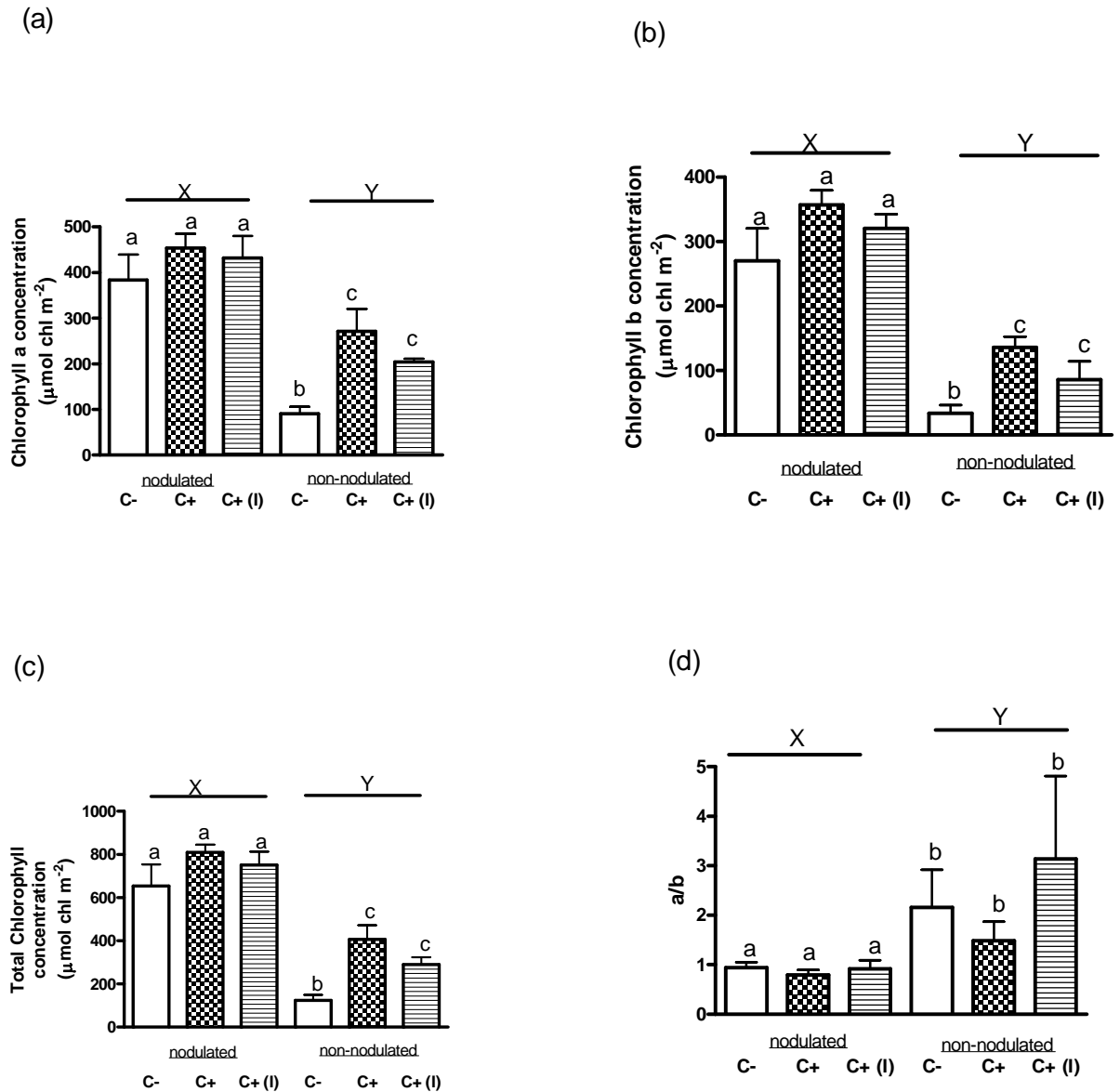


Figure 4.5.1: The concentration of chlorophyll of leaves of *Cytisus scoparius* in combination effect of nodulation and parasitism. C-: uninfected host, C+: uninfected branches of infected host and C+ (I): infected branches of infected host. (a): chlorophyll a, (b): chlorophyll b, (c): total chlorophyll and (d): chlorophyll a to b ratio. Data points are means \pm s.e. (n=2-6). Different letters indicate significant difference at $p < 0.05$, where upper case letters show comparison between nodulation treatment, and lower case letters show comparison parasite treatment.

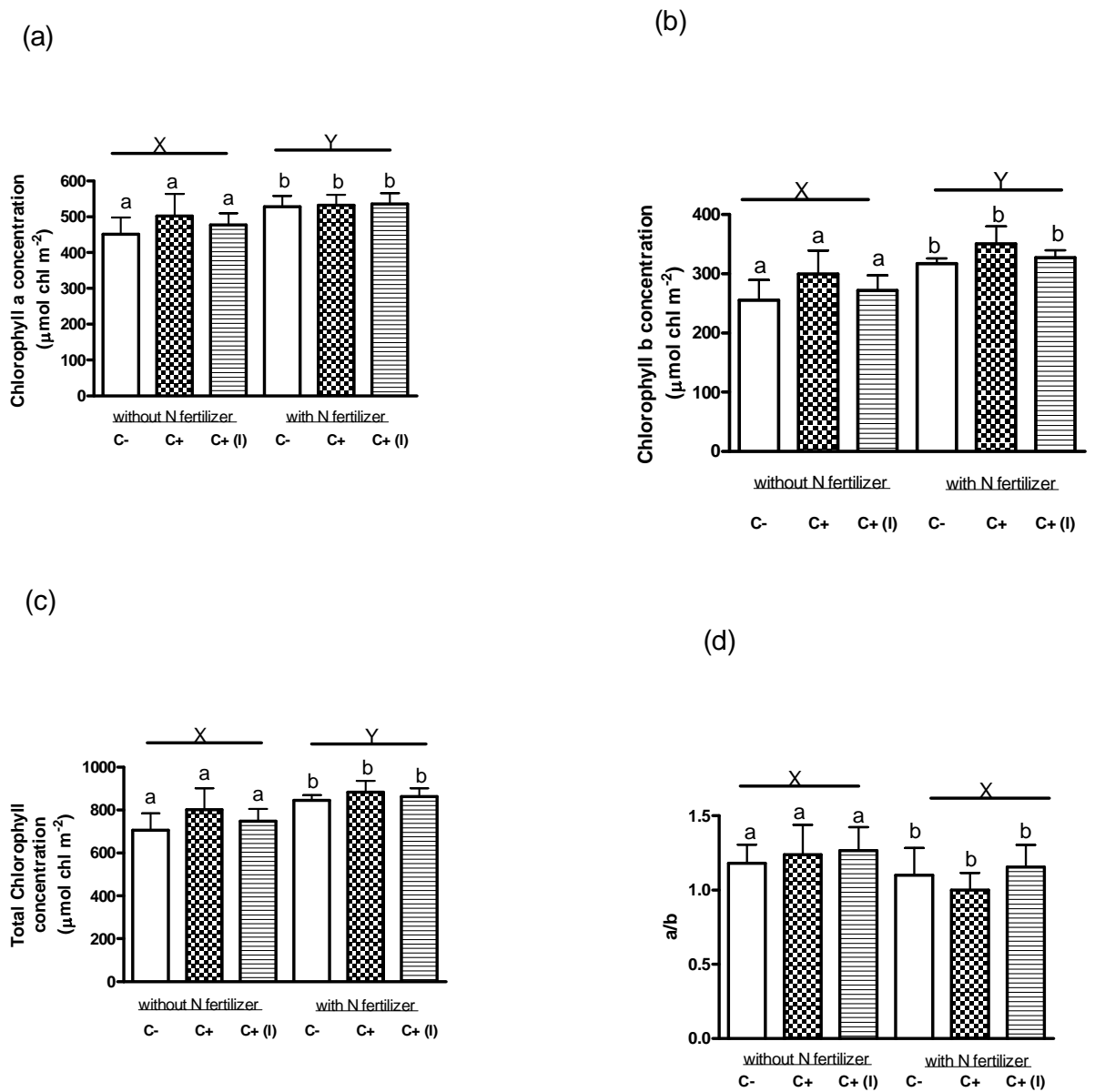


Figure 4.5.2: The concentration of chlorophyll of novo-nodulated *Cytisus scoparius* in combination effect of nitrogen supplies and *Cassitha pubescens*. C-: uninfected host, C+: uninfected branches of infected host and C+ (I): infected branches of infected host. (a): chlorophyll a, (b): chlorophyll b, (c): total chlorophyll and (d): chlorophyll a to b ratio. Data points are means \pm s.e. (n= 3-5). Different letters indicate significant difference at $p < 0.05$, where upper case letters show comparison between nitrogen fertilization treatment, and lower case letters show comparison parasite treatment.

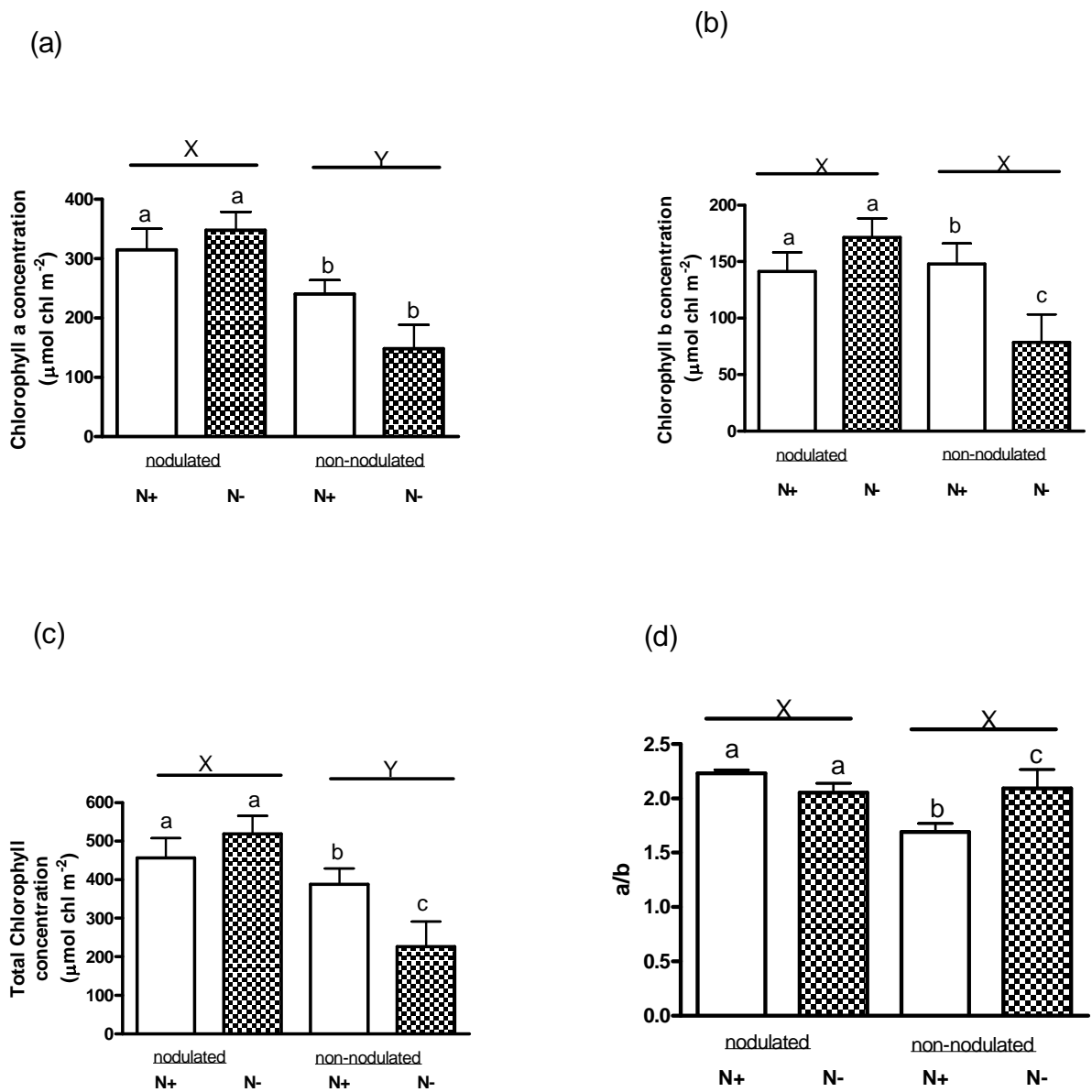


Figure 4.5.3: The concentration of chlorophyll of *Acacia myrtifolia* in combination effect of rhizobium and nitrogen supplies. N+: nitrogen fertilizer was added and N-: nitrogen free fertilizer was added (a): chlorophyll a, (b): chlorophyll b, (c): total chlorophyll and (d): chlorophyll a to b ratio. Data points are means \pm s.e. (n= 6-9). Different letters indicate significant difference at $p < 0.05$, where upper case letters show comparison between nodulation treatment, and lower case letters show comparison nitrogen fertilization treatment.

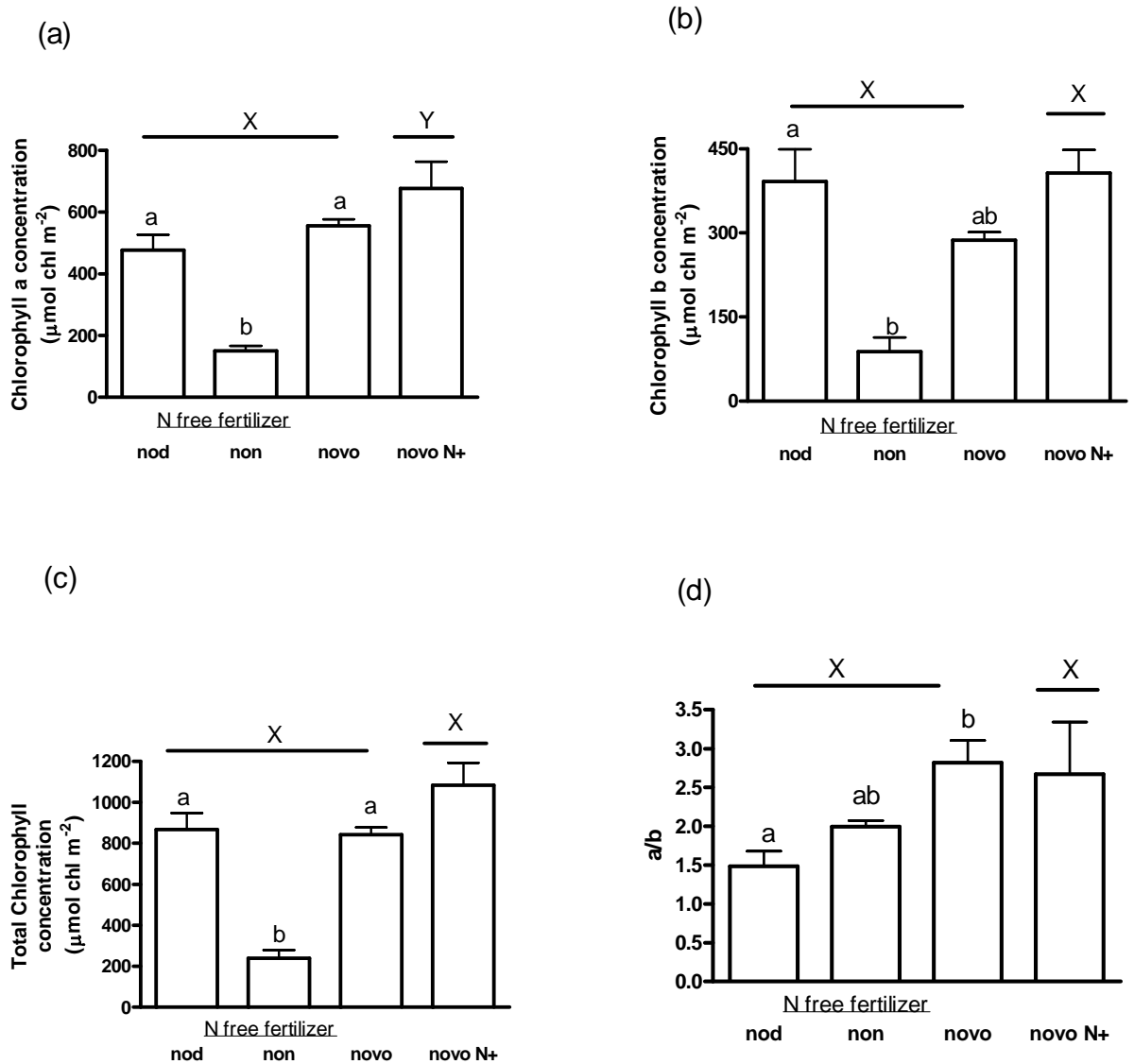


Figure 4.5.4: The concentration of chlorophyll of *Cassytha pubescens* on *Cytisus scoparius* in combination effect of rhizobium and nitrogen supplies. nod: nodulated, non: non-nodulated, novo: novo-nodulated and Novo N+: novo-nodulated with nitrogen fertilizer (a): chlorophyll a, (b): chlorophyll b, (c): total chlorophyll and (d): chlorophyll a to b ratio. Data points are means \pm s.e. (n=2-3). Different letters indicate significant difference at $p < 0.05$, where upper case letters show comparison between nodulation treatment, and lower case letters show comparison parasite treatment.

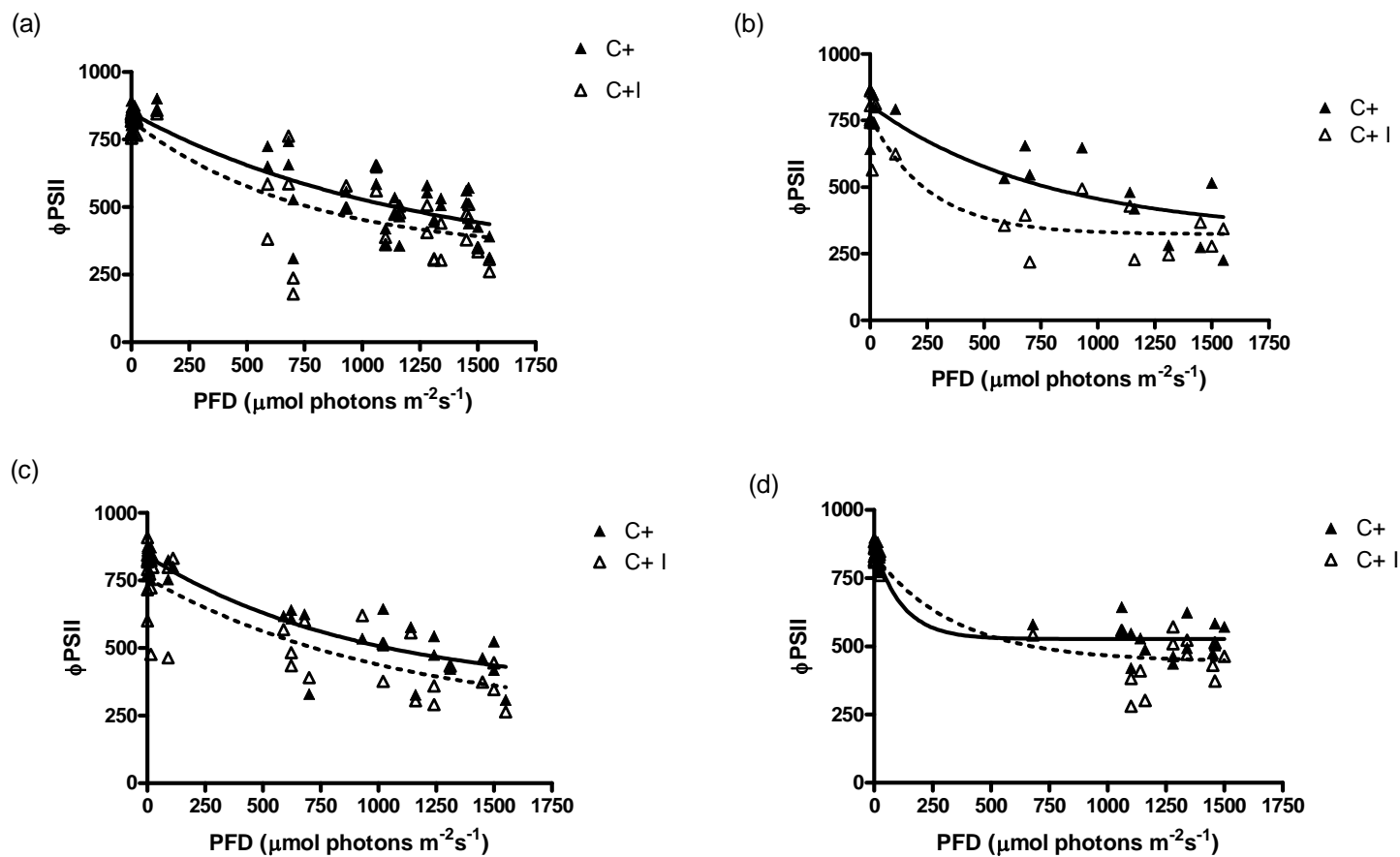
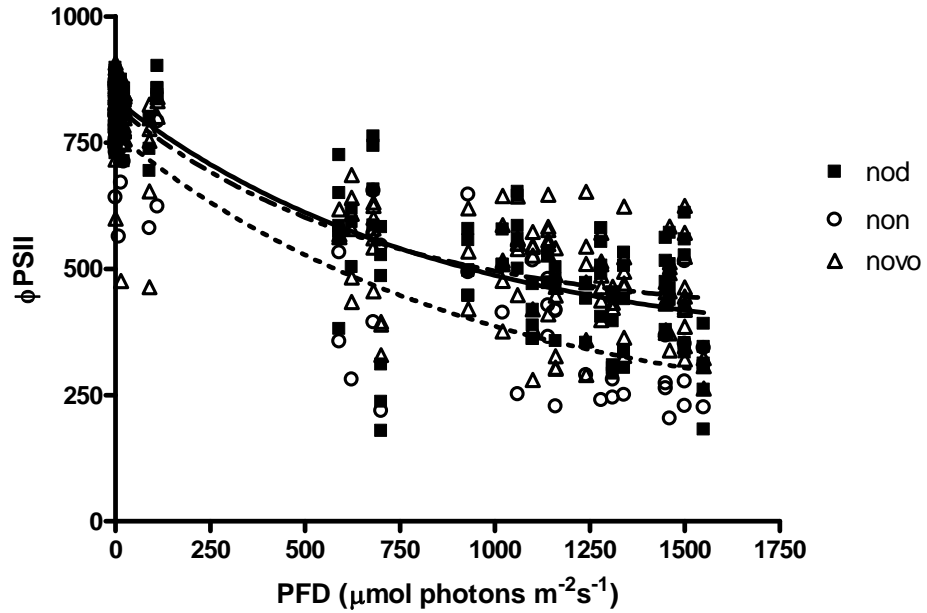


Figure 4.6.1: The response of PS II efficiency to light for leaves of infected *Cytisus scoparius* either uninfected branches (C+) or infected branches (C+ I). The infected *Cytisus scoparius* was in (a) nodulated, (b) non-nodulated, (c) novo-nodulated without nitrogen fertilizer and (d) novo-nodulated without nitrogen fertilizer treatment (n= 2-6).

(a)



(b)

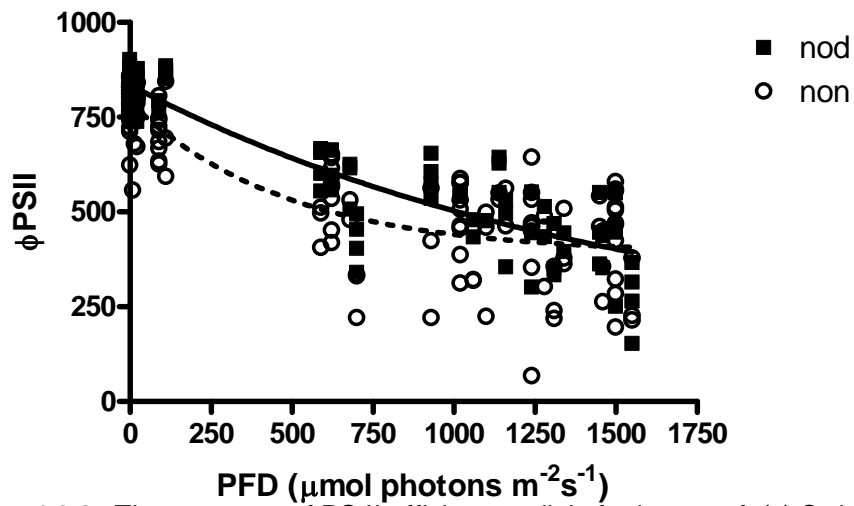


Figure 4.6.2: The response of PS II efficiency to light for leaves of (a) *Cytisus scoparius* and (b) *Acacia myrtifolia*, with different nodulation treatments, nod: nodulated, non: non-nodulation and novo: novo-nodulated (n= 2-9).

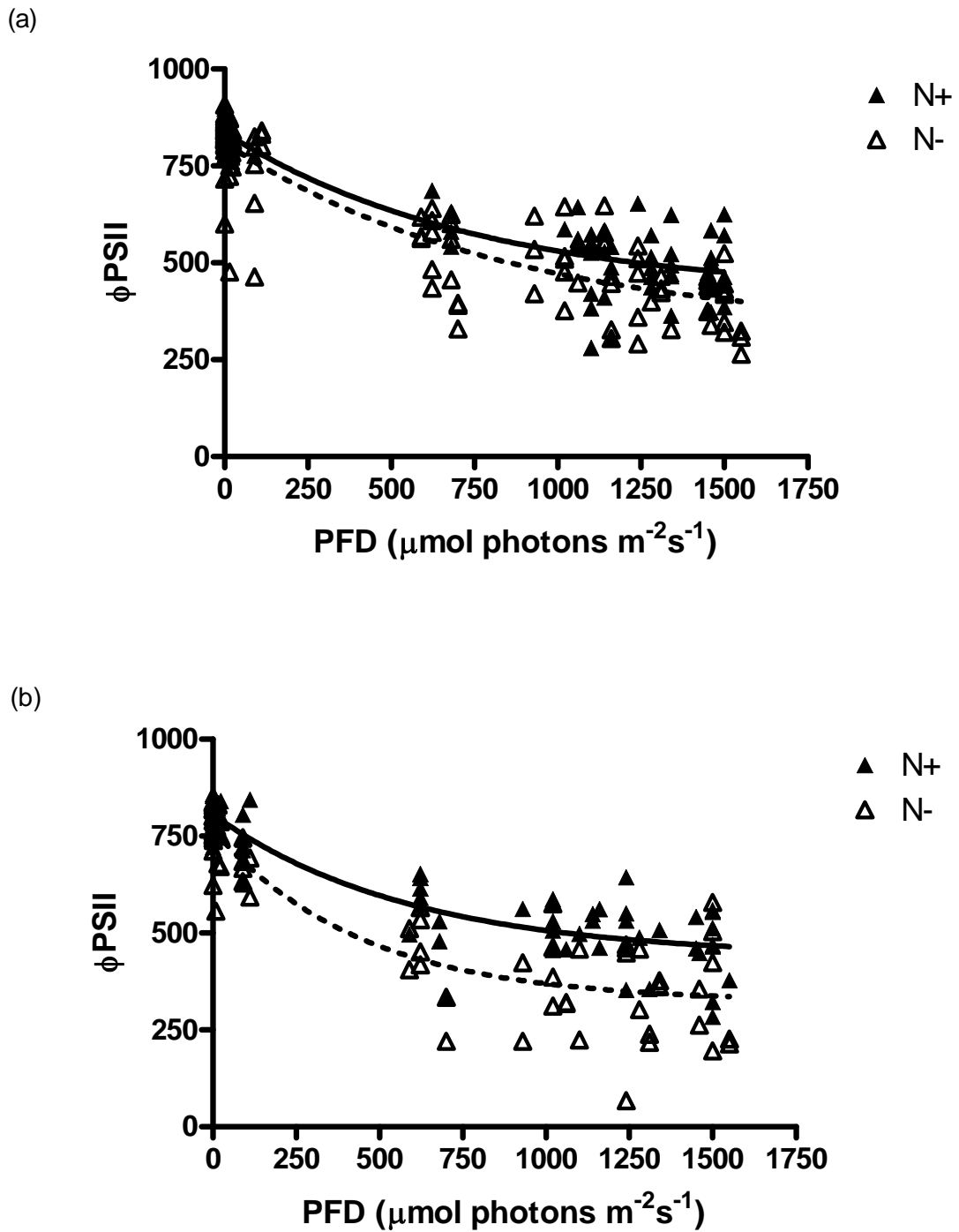
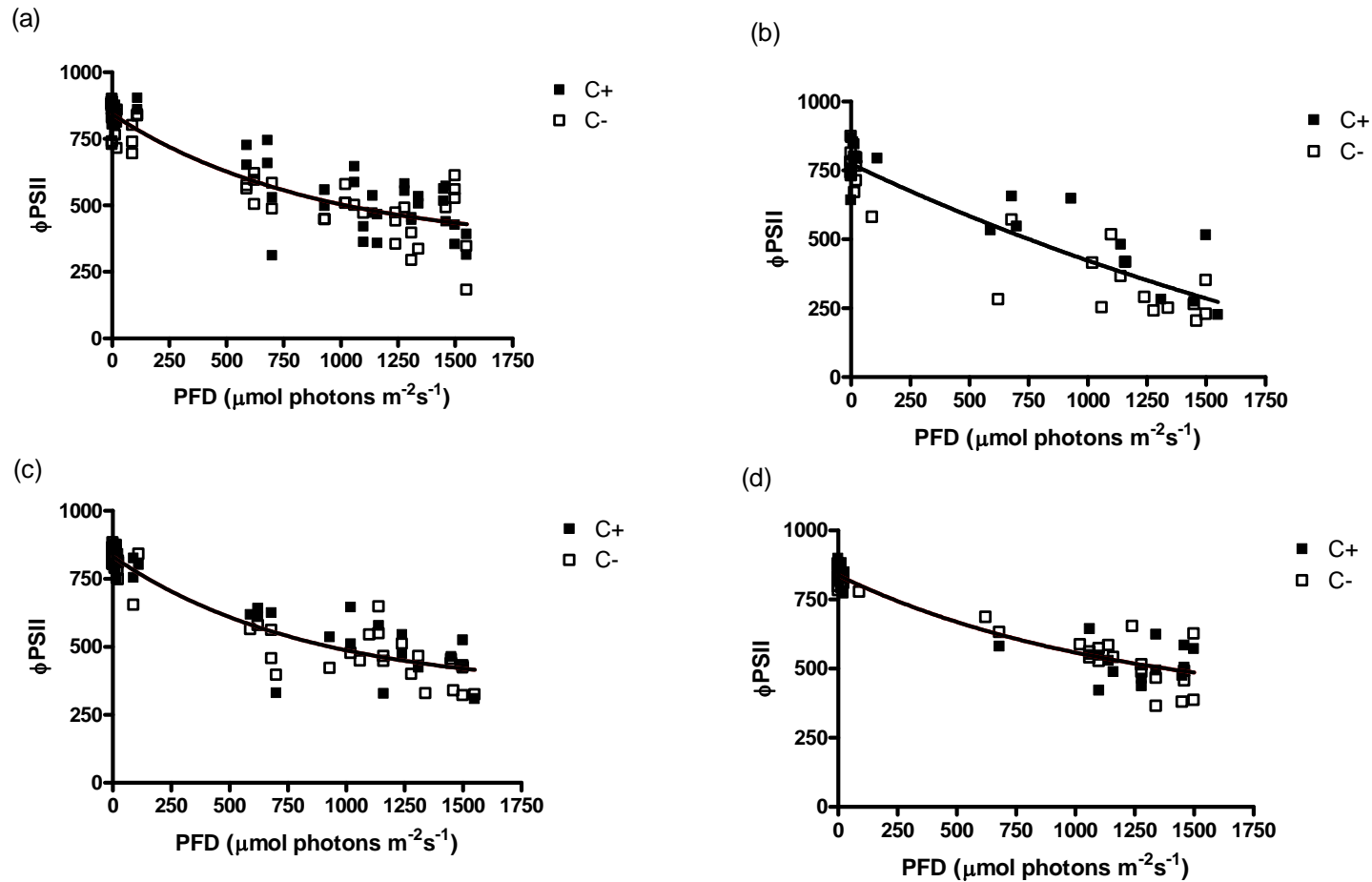


Figure 4.6.3: The response of PS II efficiency to light for leaves of (a) novo-nodulated *Cytisus scoparius* and (b) non-nodulated *Acacia myrtifolia* with different nitrogen fertilizer treatments, N+: with addition of nitrogen fertilizer and N-: without addition of nitrogen fertilizer (n= 3-9).



4.6.4: The response of PS II efficiency to light for leaves of uninfected *Cytisus scoparius* (C-) and uninfected branches of infected *Cytisus scoparius* (C+). The uninfected or infected *Cytisus scoparius* was in (a) nodulated, (b) non-nodulated, (c) novo-nodulated without nitrogen fertilizer and (d) novo-nodulated with nitrogen fertilizer (n= 2-6).

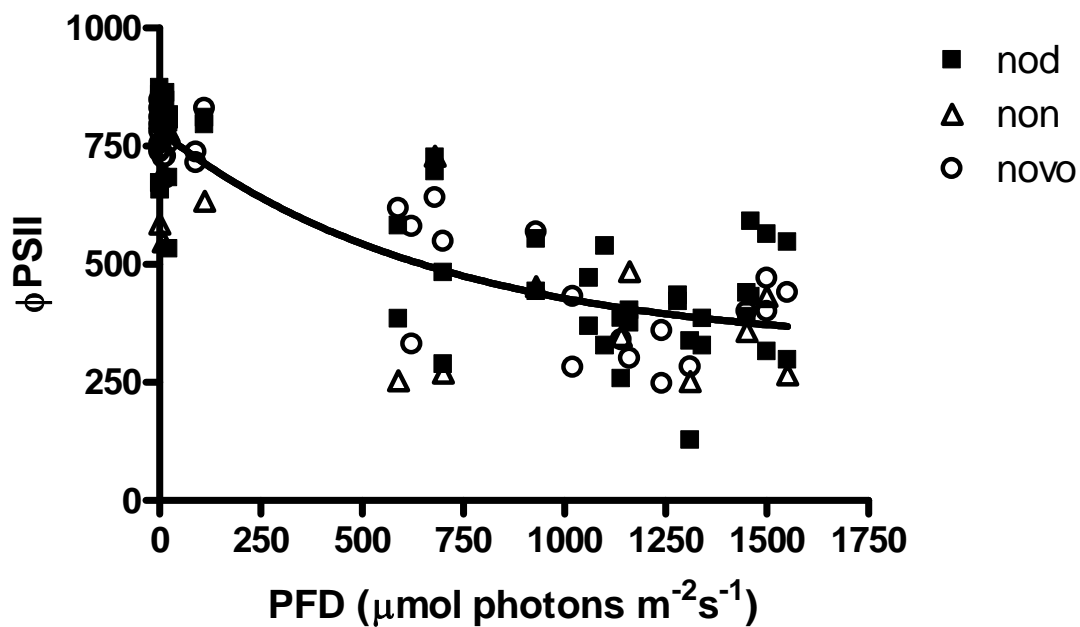


Figure 4.6.5: The response of PS II efficiency to light for stem of *Cassytha pubescens* on nodulated (nod), non-nodulated (non) and novo-nodulated (novo) *Cytisus scoparius* (n= 2-9).

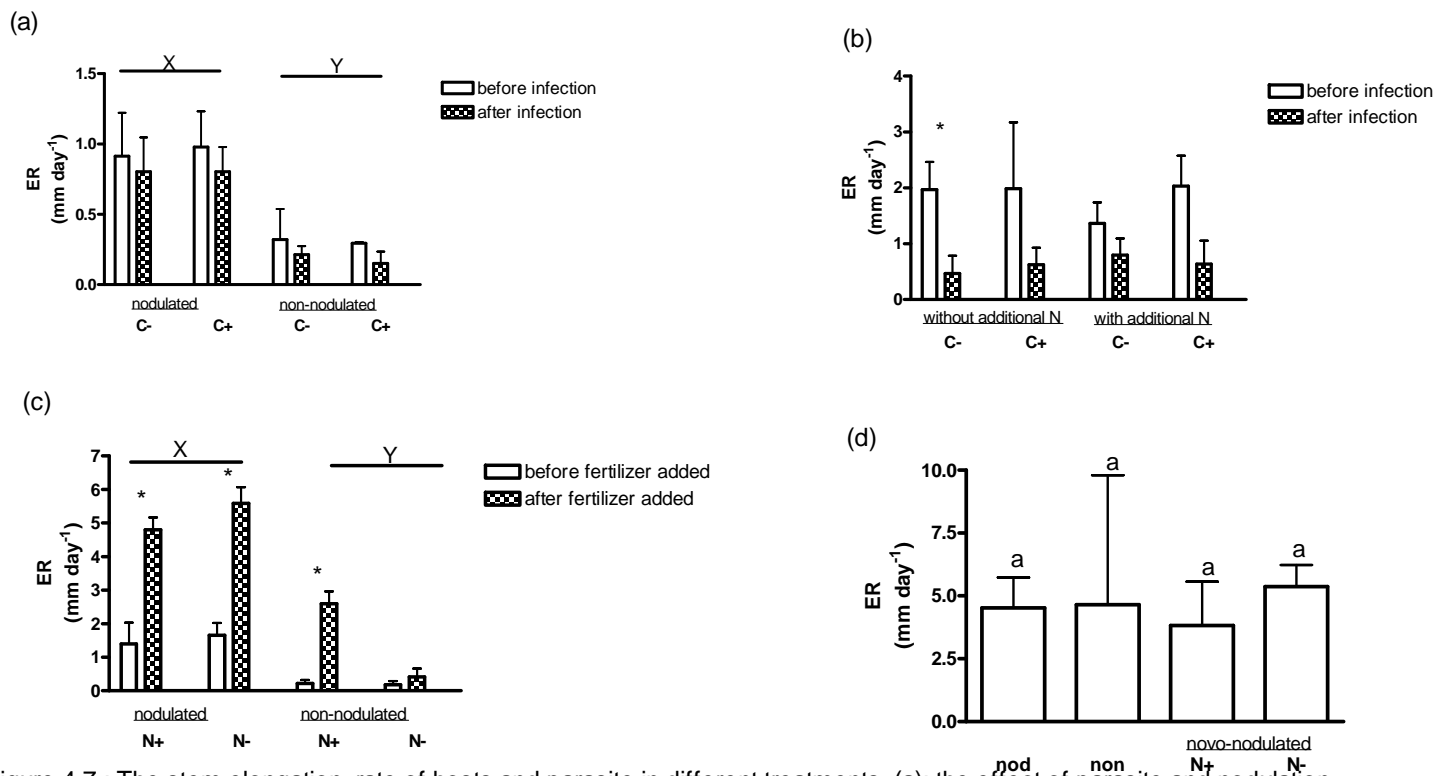


Figure 4.7.: The stem elongation rate of hosts and parasite in different treatments, (a): the effect of parasite and nodulation on *Cytisus scoparius*, (b) the effect of parasite and addition of nitrogen fertilizer on novo-nodulated *Cytisus scoparius*, (c) the effect of nodulation and addition of nitrogen fertilizer on *Acacia myrtifolia* and (d) the effect of nodulation and addition of nitrogen fertilizer on *Cassytha pubescens*. C-: uninfected host, C+: infected host, N+: nitrogen fertilizer added, N-: nitrogen free fertilizer added, nod: nodulated and non: non-nodulated. Data points are means \pm s.e. (n= 2-9). Different letters indicate significant difference at $p < 0.05$. Asterisks indicate significant difference at $p < 0.05$ between before/ after infection or N-fertilizer added. Note different scales for different graphs.

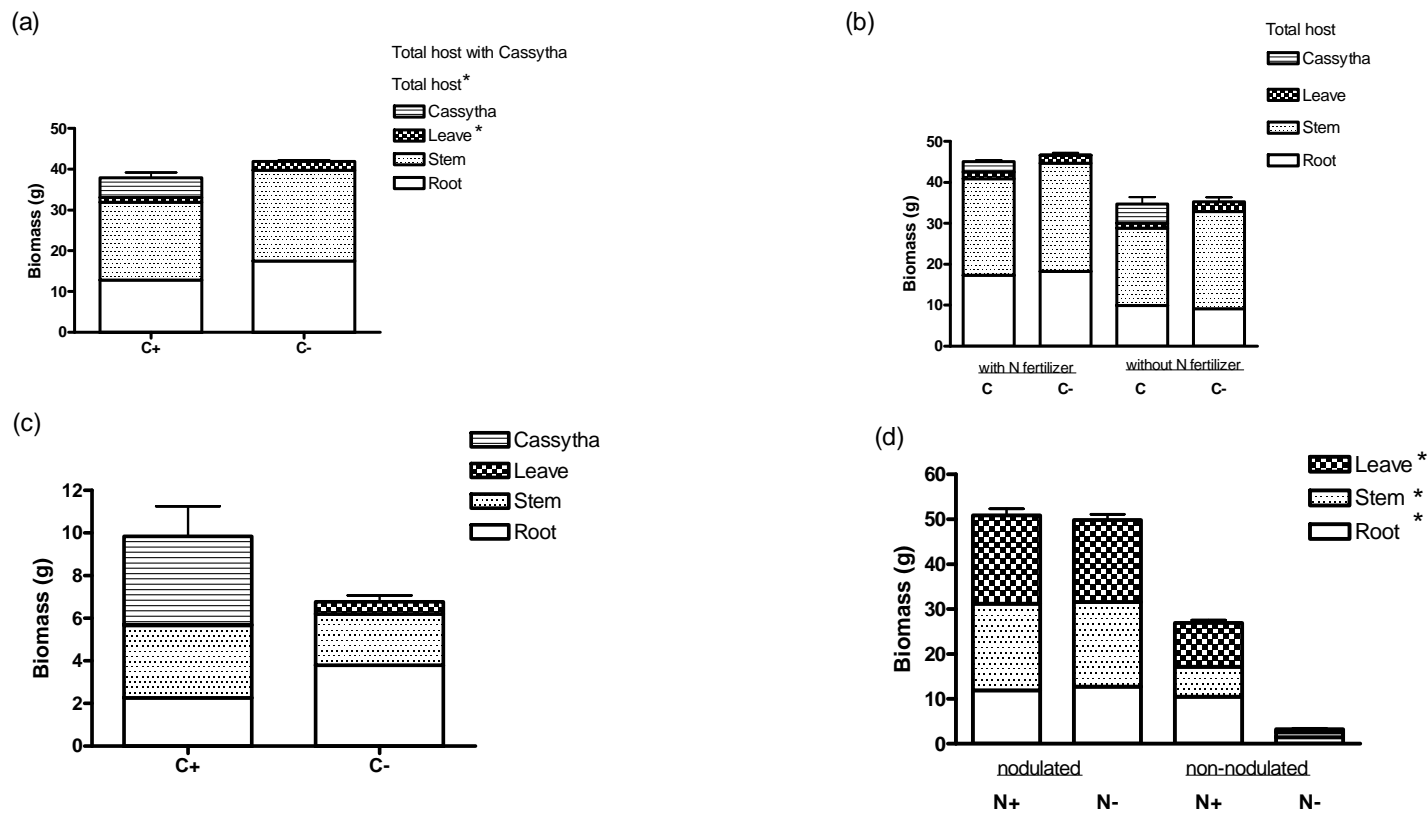


Figure 4.8.1: Biomass allocation (D.W.) of hosts in different combination of factors, (a): nodulated *Cytisus scoparius* in parasite treatments, (b): novo-nodulated *Cytisus scoparius* in combination parasite and nitrogen supply treatments, (c): non-nodulated *Cytisus scoparius* in parasite treatments and (d): *Acacia myrtifolia* in nodulation and nitrogen supply treatments. C-: uninfected host, C+: infected host, N+: nitrogen fertilizer added, N-: nitrogen free fertilizer added, Data points are means \pm s.e. (n= 2-9). Asterisks indicate significant difference at $p < 0.05$. Note different scales for different graphs.

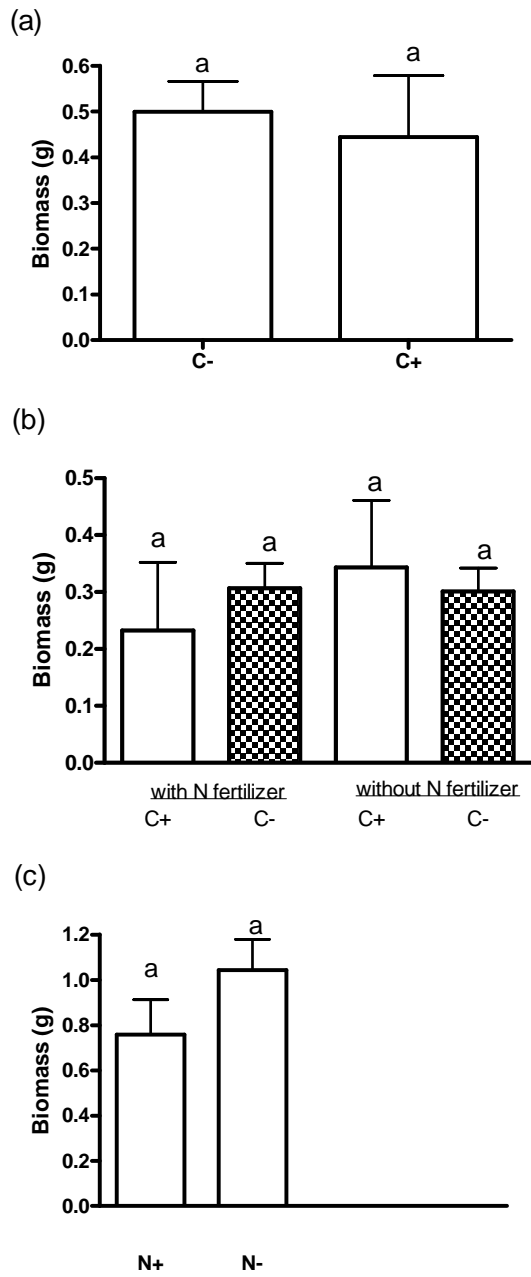


Figure 4.8.2: The biomass (D.W.) of nodule of hosts in different treatments, (a): nodulated *Cytisus scoparius* in parasite treatments, (b): novo-nodulated *Cytisus scoparius* in *Cassytha pubescens* and nitrogen supply treatments and (c): nodulated *Acacia myrtifolia* in nitrogen supply treatments. C-: uninfected host, C+: infected host, N+: nitrogen fertilizer added, N-: nitrogen free fertilizer added. Data points are means \pm s.e. (n= 3-6). Different letters indicate significant difference at $p < 0.05$. Note different scales for both graphs.

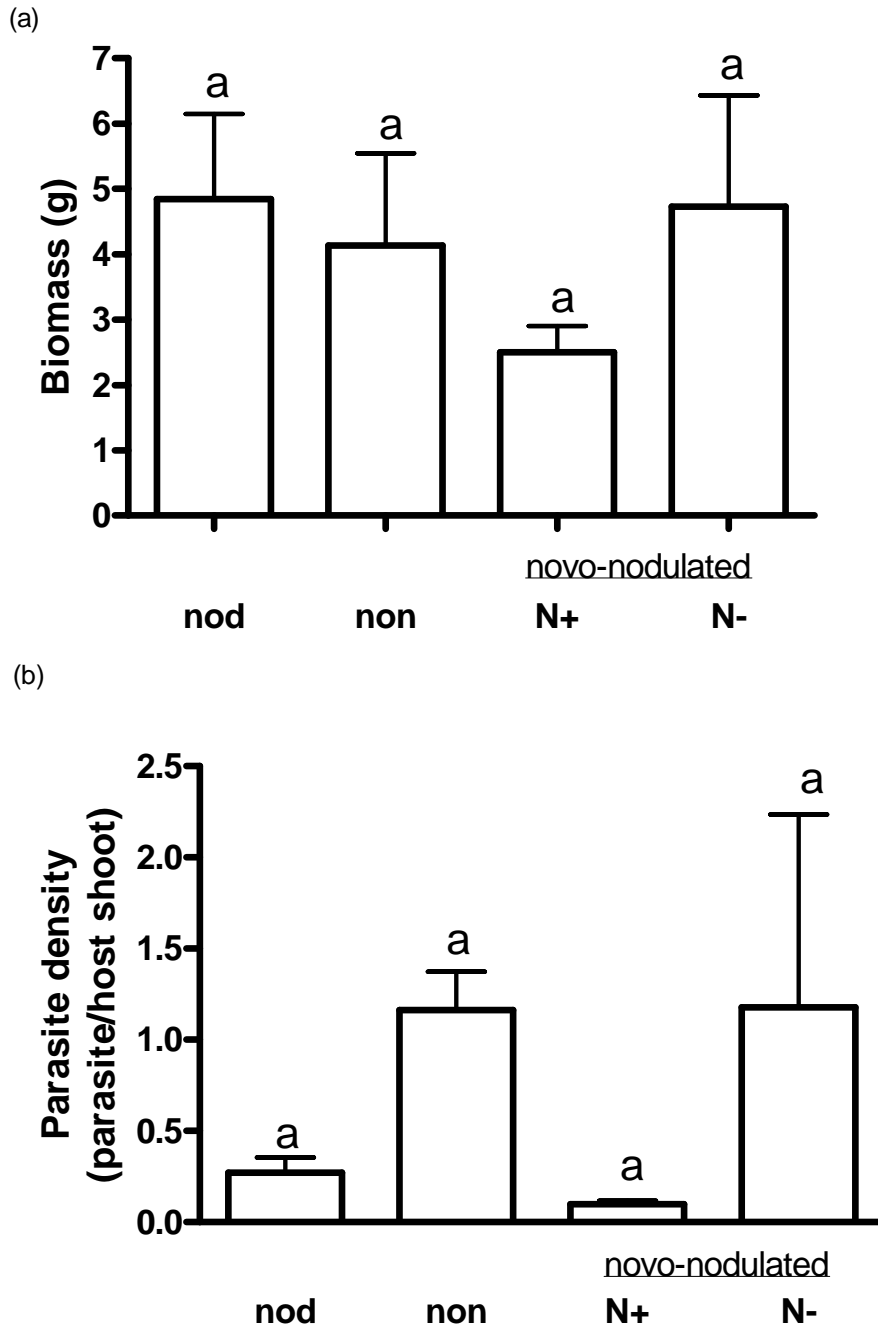


Figure 4.8.3: The biomass (D.W.) (a) and density (b) of *Cassytha pubescens* on *Cytisus scoparius* with different nodulation and fertilizer treatments. nod: nodulated host, non: non-nodulated host, N+: nitrogen fertilizer added and N-: nitrogen free fertilizer added. Data points are means \pm s.e. (n= 2-6). Different letters indicate significant difference at $p < 0.05$. Note different scales for both graphs.

Chapter. 5 Host resistance to the parasite

5.1 Abstract

The native parasitic vine *Cassytha pubescens* infects a wide range of perennial native and invasive species, although field surveys have shown that it is more abundant on invasive shrubs than on native ones. It also has stronger negative effects on the invasive species, *Ulex europaeus* and *Cytisus scoparius*, than on *Leptospermum myrsinoides* a native host. In addition, *Acacia myrtifolia*, is another native species which has been documented in the field supporting the parasite, but has been observed to show some tolerance or even resistance to *C. pubescens* (see Chapter 4). In this chapter the effectiveness of nutrient transfer from two hosts was compared, either *A. myrtifolia* or the invasive host *C. scoparius*, to the parasite using ^{32}P . Each host species was grown in a separate pot, but was connected to the other by the parasite. ^{32}P was then injected into the soil of either the pot with *C. scoparius* or *A. myrtifolia*. After introduction of ^{32}P into the soil, it was only found in the parasite when it has been applied to the pot containing *C. scoparius*. The concentration of ^{32}P in the parasite and the invasive host was the same, but no ^{32}P was detected in the parasite when applied to the pot with the native host. This suggests that while the vascular systems of the parasite and invasive host were functionally connected, the connection with the native host was not sufficiently effective to allow transfer of ^{32}P . This result supports the hypothesis that this native species has an effective resistance mechanism against the parasite possibly because it has co-evolved with it. If this pattern is found in other native species, this furthers the possibility of using *C. pubescens* as a biological control agent for *C. scoparius* and other invasive hosts.

5.2 Introduction

Much effort has been invested in the control of invasive species to minimize their negative effect in invaded habitats. Biological control is one of the many techniques used to manage invasive species, and it can either involve introduction of a known predator/pathogen from the original habitat of the invasive species, or by augmentation of new biological enemies that may arise in the invaded habitat. According to the enemy release hypothesis (Keane and Crawley 2002), the uncontrollable population growth of invasive species in a new habitat can be the result of the absence of its specific top-down regulators, such as predators, parasites or pathogens from its original habitat (Torchin, Lafferty et al. 2003). Therefore, the introduction of these enemies should reduce the performance of the invasive species in the new habitat. The introduction of a new species into a habitat, however, can be quite risky. The biotic resistance hypothesis suggests an alternative approach. This hypothesis focuses on the emerging interaction between the invader and generalist enemies in the new habitat, which could have negative impacts on the invasive species, limiting its establishment and spread (Maron and Vila 2001; DeRivera, Ruiz et al. 2005; Parker and Hay 2005). This hypothesis assumes that the native species in the invaded habitat have evolved resistance to their enemies through long term coevolution. In contrast, the newly arrived invaders have not been subjected to this selective pressure, so may succumb to these new enemies (Colautti, Ricciardi et al. 2004). Consequently, these 'new' enemies in the invaded habitat may feed preferentially on the invasive species making it possible to use a native generalist as a biological control agent for invasive species.

Variation in host preference of parasitic plants has been only well studied for a few species. Based on these studies, a range of host characteristics have been suggested that may influence host selection (active) of parasitic plants (Callaway and Pennings 1998) or the resistance (passive) of hosts (Cameron et al. 2009). The resistances of host plant toward the infections of parasitic plants have been investigated in various studies, and different defence mechanisms have been found to be involved. Host plants can secrete inhibitors to suppress the development of the haustoria of parasite, hence stop the connection between the host and parasite (Labrousse, Arnaud et al. 2001; Gurney, Grimanelli et al. 2003; Gurney, Slate et al. 2006). Also, the response of

some host plant is similar to the defence response of plant to bacteria or fungi infection, and the hypersensitive mechanism is triggered, blocking the connection between hosts and parasite (Cameron et al. 2006; Cameron and Seel 2007). All these studies have indicated that there is strong variation in the resistance to parasite infection between host species, which has been proposed as a possible factor that influences host preference (Cameron et al. 2006; Cameron and Seel 2007).

As mentioned in Chapter 1, the association between *Cassitytha pubescens*, its native hosts, and a number of invasive weedy hosts, may provide an example of the effect of long term co-existence on host resistance. This parasite has been found in significantly higher densities on the invasive weeds, *Ulex europaeus* and *Cytisus scoparius*, than on native hosts in the Mount Lofty Ranges of South Australia. Previous studies have confirmed that *C. scoparius* is preferred over native hosts, and it suffers stronger negative effects from the infection (Prider et al. 2009). Hence, evolution of biotic resistance in native species has been suggested to be the cause of this difference (Prider et al. 2009). *C. pubescens* may preferentially infect the invasive host, *C. scoparius*, because this host has lower resistance to infection than the native host, *Acacia myrtifolia*. The hypothesis was tested by quantifying the movement of ³²P from either the native or the invasive host to the parasite. This study aimed to answer the following questions: 1) can the parasite absorb nutrient from both native and invasive hosts, and 2) whether the parasite can establish functional haustoria on both hosts?

5.3 Materials and Methods

5.3.1 Plant Materials and Growth Conditions

Ten seedlings of *C. scoparius* were collected from the field site at the Mark Oliphant Conservation Park (35° 0'58.08"S and 138°45'58.45"E), South Australia. The seedlings were infected with *C. pubescens* by placing them next to an infected *C. scoparius* and directing the tendrils of the parasite to the stem of the target seedlings. Ten seedlings of *A. myrtifolia* were purchased from a local nursery and grown in 1.5L

pots in a glasshouse for six months. All seedlings were grown on the same commercial sandy loam soil used in the study reported in Chapter 4. Subsequently, infected *C. scoparius* seedlings were used to infect one seedling each of *A. myrtifolia*, using the method described above. The pots containing the *A. myrtifolia* seedlings were left for 10 weeks next to the infected *C. scoparius* plants to allow the haustoria of *C. pubescens* to develop. All plants were watered with 250 ml of RO water three times every week. Full strength Hoagland's solution was applied to all replicates during the 4th week. To ensure the uptake of ³²P at the 11th week, Hoagland's solution with only one fifth amount of phosphate was applied at the 8th week to create a phosphorous thirsty condition of the hosts.

5.3.2 Experimental design

At the 11th week, the 10 pairs of hosts, all having several haustoria of the parasite firmly attached to the two plants, were randomly assigned to two treatments. Radioactive phosphate (³²P) was injected either into the pot containing *C. scoparius* or into the pot with *A. myrtifolia* (Fig. 5.1(a) and (b)). Each injected pot received 6 MBq of radioactive phosphate dissolved in 125 ml of water, divided into 5 aliquots of 25 ml each. Each aliquot was injected using a syringe with a 10cm needle into 5 different locations in each pot to maximize the chance of it being absorbed by the host.

Two weeks after the injection of ³²P, each pair of plants and their parasite, were harvested and divided into the following components: 1) host shoot from the pot injected with ³²P, 2) *C. pubescens* on the radio-labelled host, 3) *C. pubescens* spanning the two hosts, 4) *C. pubescens* on the non-labelled host, 5) infected shoot of the non-labelled host and 6) uninfected shoot of the non-labelled host (Fig. 5.1 (a)).

Plant material was dried for 2 days at 70°C and then ground to a fine powder. For each replicate, 5 ml of nitric acid was added to 0.5g of ground plant material in a test tube, and heated in a hot water bath overnight. Following digestion, samples were diluted with RO water. *A. myrtifolia* digests were centrifugated at 2000 rpm for 10 minutes to remove a milky gelatinous residue. The radioactivity was determined using 2 ml aliquots of the digests in a liquid scintillation counter (Wallac 1215 RackBeta II)

by measuring the Cerenkov radiation produced by beta particles without any scintillation fluor cocktail and corrected for decay (Hanson 1950).

5.3.3 Statistical Analysis

The results of radioactivity measurements in each treatment were analysed using a One-way analysis of variance. Tukey tests were used for pairwise comparisons as required. There was no difference radioactivity between different sections within each plant species, so all sections in each species were averaged and treated as one sample (Appendix 1). All these analyses were done by JMPIN (version 4.0).

5.4 Results

There were differences in the radioactivity of shoot tissue between plants in the two treatments. When ^{32}P was injected into pots containing *C. scoparius*, the same level of radioactivity was detected in both *C. scoparius* and in *C. pubescens*, but only trace amounts were detected in *A. myrtifolia* (ANOVA, $F=12.17$, $d.f.=2$, $P=0.0013$; Fig. 5.2a). This contrasted with the distribution of ^{32}P when ^{32}P was injected into pots containing *A. myrtifolia*. In this case, the majority of radioactivity was found in *A. myrtifolia*, and only trace amounts were found in *C. pubescens* and *C. scoparius* (ANOVA, $F=10.07$, $d.f.=2$, $P=0.0027$; Fig. 5.2b).

5.5 Discussion

The results show clearly that *C. pubescens* was capable of absorbing ^{32}P from *C. scoparius*, but not from the native host, *A. myrtifolia*. This occurred despite the fact that the haustoria of *C. pubescens* were firmly attached to the shoots of both hosts, suggesting that *C. pubescens* connects functionally to the vascular system of *C. scoparius*, but not to that of *A. myrtifolia*. This failure of the connection between the native parasite and native host explains why *C. pubescens* attached to *A. myrtifolia*

died in a previous experiment, when the connection to the other host was severed (see Chapter. 4).

5.5.1 Biotic resistance hypothesis

In terms of nutrient transfer, *A. myrtifolia* had higher resistance to infection by *C. pubescens*, than the invasive host. This difference in their resistance is consistent with the prediction of the biotic resistance hypothesis. According to this interpretation the co-occurring native host has evolved in the presence of the parasite have coevolved and over time has developed suitable mechanisms to resist infection. In contrast, the invasive host, *C. scoparius*, which was introduced to Australia less than 200 years ago, has not evolved defence mechanisms capable of resisting infection by its new enemy, *C. pubescens*. By observation, *C. pubescens* will forms haustoria on any plant material (or even inanimate objects) that it encounters, but the results of the current study indicate that these haustoria are not always fully functional. This also indicates that the range of native species on which *C. pubescens* is found growing in the field does not represent the native host range of *C. pubescens*. Some of the native species like, *A. myrtifolia* may only provide physical support for the parasite, allowing it to reach other hosts, or harvest light for photosynthesis.

A preliminary survey of *C. pubescens* in the Mt Lofty Ranges found differences in the density of the parasite on various native hosts (Prider et al. 2009). Among all native hosts, a native non-nitrogen fixing shrub, *Leptospermum myrsinoides* was the most preferred host. Further, in other experiments of this project, *L. myrsinoides* could be easily infected with the parasite by the method mentioned above, surviving after the stem of *C. pubescens* had been severed from the donor hosts. This indicates that the resistance of *L. myrsinoides* is weaker than the resistance of *A. myrtifolia* to the native parasite, although it seems to still be greater than the resistance of invasive species to the parasite (Prider et al. 2009).

5.5.2 Resource availability hypothesis

Alternatively, this difference in the degree of plant defence system may relate to another driving force in the evolutionary history of the species. Coley et al. (1985) proposed that there is a relationship between the resource availability of the habitat and the level of plant defences. In low resource environments plants should evolve to invest more resources in defences to prevent the loss of valuable resources to their natural enemies, leaving less resource available for growth (Herms and Mattson 1992). In contrast, in high resource environments, plants should evolve to utilize more resources and achieve higher growth rates, thus allocating less resource toward defences. The hosts in this study have evolved in different geographical and climatic regions. The two invasive shrubs, *U. europaeus* and *C. scoparius* are originally from Western Europe where soils are rich in nutrients and water, while the two native species evolved under lower nutrient availability and unpredictable rainfall. Therefore, these plants may have evolved to have different degree of resistance to natural enemies based on the resource availability hypothesis.

However, this hypothesis does not explain the differences in resistance of native species to parasite infection. Differences in resistance to the parasite in native species may have evolved in species coexisting as hosts and parasites because of differences in susceptibility to the reduction in resource produced by the parasite. Species frugal in resource use (i.e. requiring less water or nutrient to saturate their growth potential) could suffer less from the same extraction of resources. This seems to be the case with *L. myrsinoides*, while *A. myrtifolia* seems to require more resources and hence it would suffer stronger negative effect from the parasite infection. This should result in stronger negative selective pressure on *A. myrtifolia*, eventually leading to the observed strong resistance of *A. myrtifolia* to the parasite infection in the present.

5.5.3 Conclusion

In summary, the native host in this study showed greater resistance to the native parasite than the invasive host and this suggests a possible relationship between the evolutionary histories of the hosts. This difference in host resistance may lead a reduction in the advantage of invasive hosts over native hosts, and promote co-existence of all host species (Levine, Adler et al. 2004). However, Prider, Watling *et al.* (2009) found that the photosynthesis of the native host *L. myrsinoides* suffered negative effects as a result of infection with *C. pubescens*, hence there is variation in the resistance to the parasite in various native hosts. Further research is needed to confirm whether this difference in the resistance between native and invasive hosts exists in other host species.

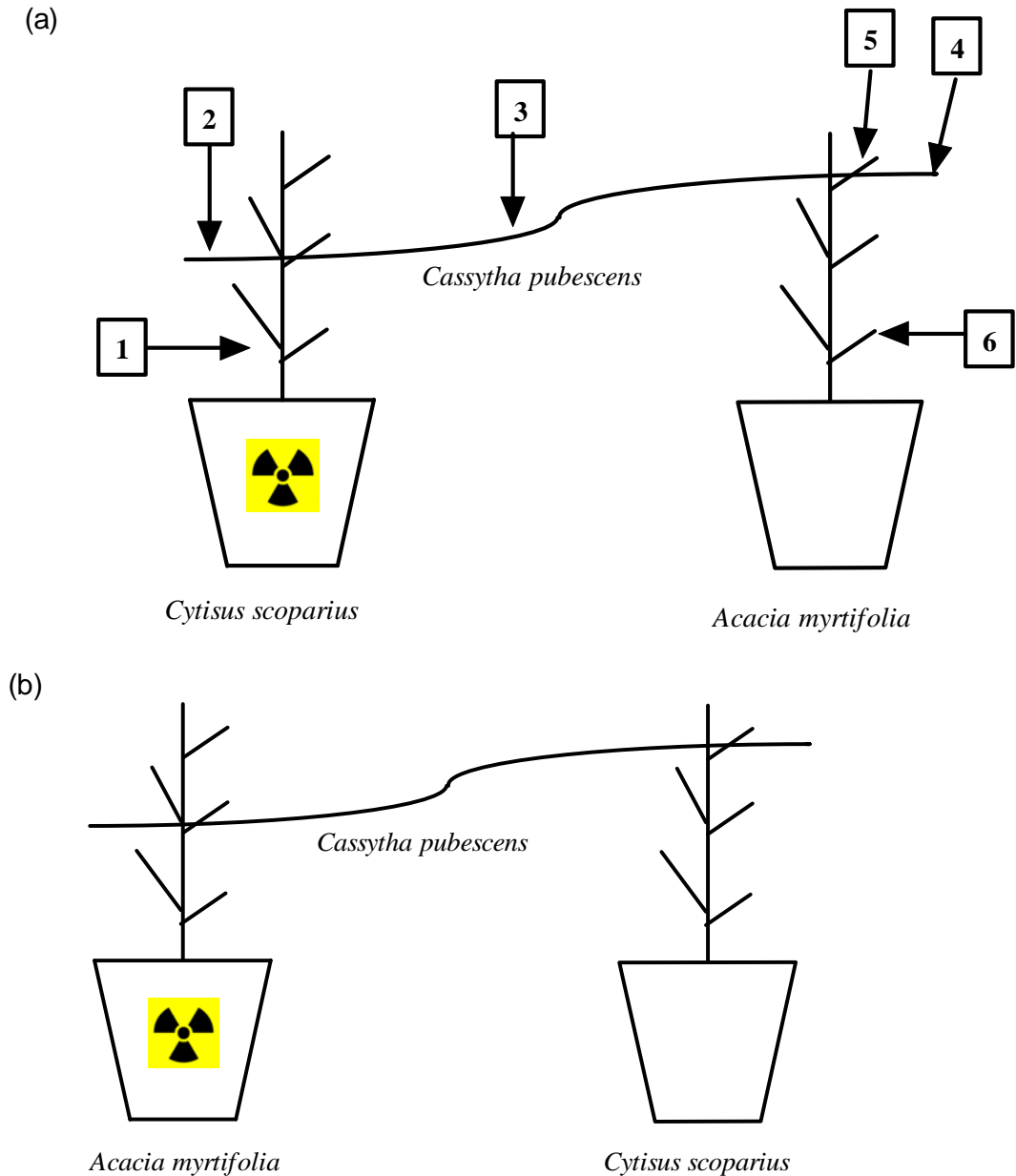
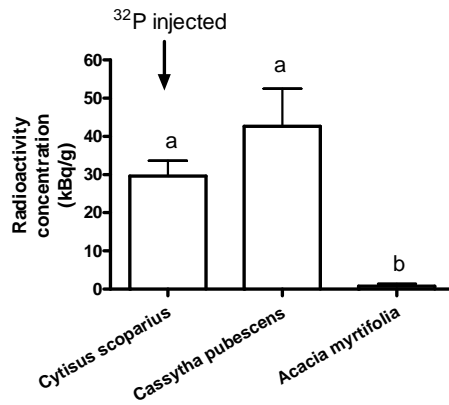


Figure 5.1. The experimental design of the two treatments in this experiment. The radiation symbol indicates the pot that was injected with ^{32}P in the treatment. (a): *Cytisus scoparius* injected with ^{32}P and (b): *Acacia myrtifolia* injected with ^{32}P . In (a), the six measured components of plants are indicated, (1) host shoot from the pot injected with ^{32}P , (2) *C. pubescens* on the radio-labelled host, (3) *C. pubescens* spanning the two hosts, (4) *C. pubescens* on the non-labelled host, (5) infected shoot of the non-labelled host and (6) uninfected shoot of the non-labelled host

(a)



(b)

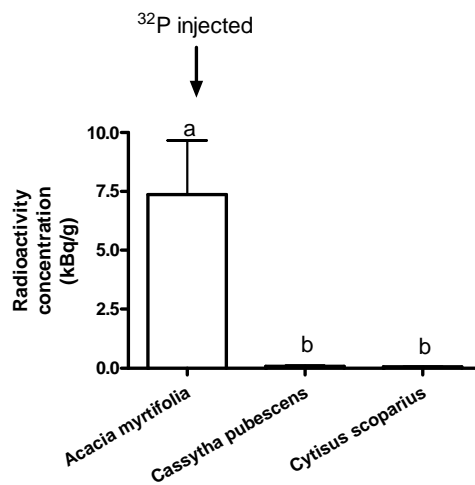


Figure 5.2. The radioactivity concentration in two treatments. (a): *Cytisus scoparius* injected with ^{32}P and (b): *Acacia myrtifolia* injected with ^{32}P . Data points are means \pm s.e. ($n=5$). Different letters indicate significant difference at $p < 0.05$. Note different scales for both graphs.

Chapter. 6 Effects of *Cassytha pubescens* on competition between native and invasive hosts

6.1 Abstract

Cassytha pubescens uses a wide range of hosts with different susceptibilities to the infection. Differences in impact between different hosts may modify competitive outcomes. A pot experiment was conducted to investigate whether the parasite influences competition between invasive and native hosts. Two native hosts, *Acacia myrtifolia* (legume) or *Leptospermum myrsinoides* (non-legume), were grown together in a pot with the invasive legume *Ulex europaeus*, and either with or without *C. pubescens*. After 6 months, infection had no effect on the biomass of the hosts, and there was no difference in the competitive effect of the invasive on the two native hosts. In contrast, biomass of *U. europaeus* was affected differently when grown with the two native species. The invasive host was almost 23% smaller with *A. myrtifolia* than when grown with *L. myrsinoides*. There was no effect of the parasite on the relative size of plants. However, the absence of the effect on host competition may relate to the species studied and the length of the experiment. They are all perennial species; hence the indirect effect between species may take longer to become apparent. A longer term study may be needed to confirm the lack of any impact of *C. pubescens* on competitive interactions. This study aimed to answer the following questions: 1) can the parasite absorb nutrient from both native and invasive hosts, and 2) can the parasite establish functionally haustoria on both hosts?

6.2 Introduction

Parasitism is a common interaction in all ecosystems, and can negatively impact the performance of hosts. Changes in the performance of host species can influence competition with other species, hence parasites can modify competitive outcomes (Price, Westoby et al. 1986; Price, Westoby et al. 1988; Hudson and Greenman 1998). Variation in negative effects on hosts induced by parasite may influence the competitive intensity of hosts over other species and hence the competition dynamic and community structure can be affected. Consequently, parasitism has been claimed as one of the determining factors in community structure (Minchella and Scott 1991; Bardgett, Smith et al. 2006; Hatcher, Dick et al. 2006; Hudson, Dobson et al. 2006; Wood, Byers et al. 2007).

Despite a long history of study of parasite-mediated competition, few studies have been made of the impact of parasitic angiosperms on host competition (Press and Phoenix 2005). Two systems that have received most attention are *Cuscuta salina* in coastal wetlands of California (Pennings and Callaway 1996; Callaway and Pennings 1998; Grewell 2008) and *Rhinanthus minor* in European grasslands (Gibson and Watkinson 1991; Gibson and Watkinson 1992; Joshi, Matthies et al. 2000; Bullock and Pywell 2005; Cameron, Hwangbo et al. 2005). In both cases, the parasites are annuals and have a wide host range. Although both species parasitize a wide range of hosts, certain host species suffer stronger negative effects from infection due to either active host preference (Callaway and Pennings 1998) or different host susceptibilities (Cameron et al. 2009), and hence this affects competitive outcome. This can eventually modify the community structure and dynamic. In studies of *C. salina*, the dominant shrub species, *Salicornia virginica* was heavily infected and suffered a stronger negative effect from infection than co-occurring hosts, leading to release of the sub-dominant species from competition (Grewell 2008). As a result, *C. salina* promotes diversity in this community. In contrast, in European grasslands the dominant grass species experience stronger suppression from infection with *R. minor* due to their weaker resistance compared to forbs species, hence the forbs become dominance in the presence of parasite (Cameron et al. 2009). In consequence, the effects of parasites on community structure depend on relative preferences for, and effects on, dominant or subdominant hosts. Due to the significant effect of parasitic

plants on the host community, it has even been suggested that parasitic plants can be used as a tool to restore diversity in disturbed ecosystems (Bullock and Pywell 2005; Grewell 2008).

Like the parasitic angiosperms mentioned above, *Cassytha pubescens* is a generalist parasite, parasitizing a wide range of co-occurring hosts. A previous field survey has found that there are differences in the effects of *C. pubescens* on native and invasive hosts (Prider et al. 2009). Both native and invasive hosts suffer negative effects from infection, but a stronger effect was found on invasive hosts. In addition, unsuccessful infection of *C. pubescens* on the native legume *Acacia myrtifolia* reported in Chapters 4 and 5, further supports evidence that at least some native hosts have greater resistance to the native parasite than the invasive species. As a result, *C. pubescens* may reduce the competitive advantage of the invasive species *Ulex europaeus* and *Cytisus scoparius* over native species, thus releasing the native hosts from the competitive effect of invasive hosts and benefit the growth of native species. Therefore, the aim of this experiment was to answer following questions: 1) does the impact on the growth of hosts affect competition between these hosts? Logistic constraints prevented also running controls without competition or with intraspecific competition. The focus, however, was on whether the presence of the parasite changed the competitive interactions.

6.3 Materials & Methods

6.3.1 Experimental design

An experiment was conducted using the invasive weed *U. europaeus* and two native hosts, *A. myrtifolia* (a nitrogen fixer) and *Leptospermum myrsinoides* (a non-nitrogen fixer) in a glasshouse from October 2008 to April 2009 (this experiment had been started before the harvest of experiment in Ch. 4 and 5). *C. scoparius* was originally intended to be used as the invasive host in this experiment, but the seedlings died due to an electrical fault in the automatic irrigation system of the glasshouse. Instead, 42 1-year old plants of *U. europaeus* that had been individually planted in 4L pots with

the sandy loam soil used in experiments reported in previous chapters, were used in this experiment. In half of these pots, one seedling of *A. myrtifolia* was planted, and the rest received one seedling of *L. myrsinoides*. Three months after setting up the competitive pairs, 14 *U. europaeus* from each of the *A. myrtifolia* and *L. myrsinoides* treatments were infected with *C. pubescens* as described in earlier chapters. *C. pubescens* was allowed to grow on the new hosts for 8 weeks before the connection between the donor and new host was severed. A month after separation, the young stems of *C. pubescens* that had grown on *U. europaeus* were used to infect the native competitor in half of the infected treatments.

The resulting treatments were then (Fig. 6.1):

- a) Uninfected *U. europaeus*, uninfected *A. myrtifolia*
- b) Uninfected *U. europaeus*, uninfected *L. myrsinoides*
- c) Infected *U. europaeus*, uninfected *A. myrtifolia*
- d) Infected *U. europaeus*, uninfected *L. myrsinoides*
- e) Infected *U. europaeus*, infected *A. myrtifolia*
- f) Infected *U. europaeus*, infected *L. myrsinoides*

No fertilizer was added over the experimental period, but all pots were watered three times weekly.

6.3.2 Harvest

All plants were harvested 6 months after infection of the native hosts. The aboveground component in each pot was separated into the following, depending on treatment: *U. europaeus*, *C. pubescens* growing on *U. europaeus*, the native hosts and *C. pubescens* growing on the native hosts. The root systems of the two plants were pooled for each pot, because they were too intertwined to separate. All the plant tissues were dried in an oven at 70°C for 3 days and then weighed.

6.3.3 Statistical analysis

Biomass data were analysed using one-way analysis of variance (ANOVA). The impact of *C. pubescens* on competition between the two hosts was determined by comparing the ratios of shoot biomass of each pair across treatments, and this ratio was compared using one-way analysis of variance. All the analyses were conducted using JMPIN (Version 4.0).

6.4 Results

Infection with *C. pubescens* had no effect on the aboveground or belowground biomass of any of the host species (Table. 6.1). In the *U. europaeus*-*A. myrtifolia* treatment, infection status had no impact on the aboveground biomass of either host (Fig. 6.2.1a), nor on the combined belowground biomass (Fig. 6.2.1b). Results of the *L. myrsinoides* treatment were similar. Parasite infection had no impact on aboveground biomass of *U. europaeus* or *L. myrsinoides* (Fig. 6.2.2a), nor on the combined belowground biomass (Fig. 6.2.2b). Moreover, infection had no impact on competition between *U. europaeus* and the two native species (Fig. 6.3). There was no infection effect on the aboveground biomass ratio of *U. europaeus* and *A. myrtifolia* (ANOVA, $F= 0.06$, d.f.= 2, $P= 0.94$; Fig. 6.3a), or *U. europaeus* and *L. myrsinoides* (ANOVA, $F= 0.34$, d.f.= 2, $P= 0.72$; Fig. 6.3b) in the different native plants treatments. Biomass of *U. europaeus* was 23% lower when grown with *A. myrtifolia* relative to plants grown with *L. myrsinoides* (t- test, $t= 0.33$, d.f.= 40, $P= 0.02$; Fig. 6.4).

6.5 Discussion

In this pot experiment the native parasite, *C. pubescens* induced no direct negative effect on the growth of either invasive or native hosts. Moreover, infection did not affect the competitive interaction between native and invasive hosts. Although this experiment did not detect any effect of the parasite on the competitive ability of the

hosts, it has revealed that *U. europaeus* suffers different intensities of competitive effect from two native species.

6.5.1 Parasite-mediated competition

The results of this experiment on the effect of the competition between hosts differed from the majority of the studies on parasitic angiosperm-mediated competition. Most studies so far have shown that the differences in the intensities of negative effect induced by parasites on different hosts alter the competitive outcome between hosts in the community (Gibson and Watkinson 1989; Gibson and Watkinson 1991; Gibson and Watkinson 1992; Matthies 1995; Pennings and Callaway 1996; Callaway and Pennings 1998; Marvier 1998; Joshi et al. 2000; Bardgett et al. 2006; Grewell 2008; Cameron et al. 2009). In these studies, a particular host within a wide range of available ones suffered stronger direct suppression relative to others due to either active host preference of the parasite or difference in susceptibilities to the parasite infection. This resulted in release of other hosts suffered less impact from the effect of competition, and the outcome of this modification on the communities depended on the dominance of the least resistant host.

The competitive effects of *C. scoparius* on native species in the Mount Lofty Ranges have been documented by Fogarty and Facelli (1999), who found that the invasive *C. scoparius* can out-compete native species with its high relative growth rate. The dominance of this invasive species can potentially lead to the displacement of the native species over time. Therefore, a native parasite that has strong negative effects on invasive species such as, *C. scoparius* and *U. europaeus* could be expected to suppress the dominance of the invasive species and release the native species from the negative competitive effect. However, this study found no effect of infection with *C. pubescens* on competition between the studied species. The absence of an effect on competition could be the result of a major factor: relatively weak direct effects of infection on hosts because of the relatively short experimental period in proportion to the life span of the species studied (Mitchell, Agrawal et al. 2006).

The intensity of direct negative effects that *C. pubescens* induced on both native and invasive hosts were weak compared to that produced by other parasitic plants on their hosts (see review of the impacts of the annuals (Cameron et al. 2005)). This may relate to the differences in life history strategy of the parasites used in other studies, which are annuals, and therefore only have one growing season to complete their life cycle. These parasites can obtain a large proportion of resources quickly from their hosts, and may even kill the host. Gibson and Watkinson (1991) documented changes in parasite mediated competition by an annual hemiparasite, *R. minor* within one growing season. In contrast, *C. pubescens* being a perennial species, may require the survival of the host over a longer period of time. As a result, it should optimise its negative effect on its hosts to maximise its reproductive output over time. This prediction is consistent with research by Press et al. (1988) who found that perennial hemiparasites regulate water potential to minimize the negative impact on hosts, whereas annual parasites do not. Therefore, detecting a negative effect strong enough to affect competitive outcomes with perennial parasites may require that the length of the experiment incorporates a larger portion of the life spans of both the host and the parasite. Due to time constraints, this study was restricted to for 6 months. It is possible that *C. pubescens* could induce a parasite-mediated competition effect on its hosts if a longer term pot experiment was conducted.

6.5.2 Effects of competition

The results also show that *U. europaeus* grew better with *L. myrsinoides* than with *A. myrtifolia*. This may relate to the relative growth rate and/or the initial size of the plants of the two native species. *U. europaeus* has a high growth rate and therefore easily out-competed *L. myrsinoides*, which has a slow growth rate. However, *U. europaeus* would seem to have less competitive effect on *A. myrtifolia* because of its possibly faster growth rate, according to the finding of Fogarty and Facelli (1999), who found that an invasive legume with high relative growth rate had a stronger competitive advantage on native species with low relative growth rate. However, this difference in the biomass accumulation of *U. europaeus* might also be explained by the initial biomass of the native species. There was a difference in the size of the

seedlings between the two native species that ordered from a local nursery, and the initial size of *A. myrtifolia* was significantly larger than that of *L. myrsinoides*; hence the demand for resources by seedlings of *A. myrtifolia* would have higher than that of *L. myrsinoides*, irrespective of any species difference in the demand for resources.

6.5.3 Conclusion

In conclusion, this pot experiment did not detect the predicated parasite-mediated changes in competition between the invasive and two native hosts. This result is different from other studies conducted on the effect of parasitic angiosperms on competition between hosts, and this possibly relates to the long life span of the hosts used in the current study. This study highlights a gap in knowledge of the indirect effects of perennial parasitic plants on perennial hosts, which may differ from the interactions between annual species. As a result, a longer-term pot experiment or field study will be needed to clarify this association.

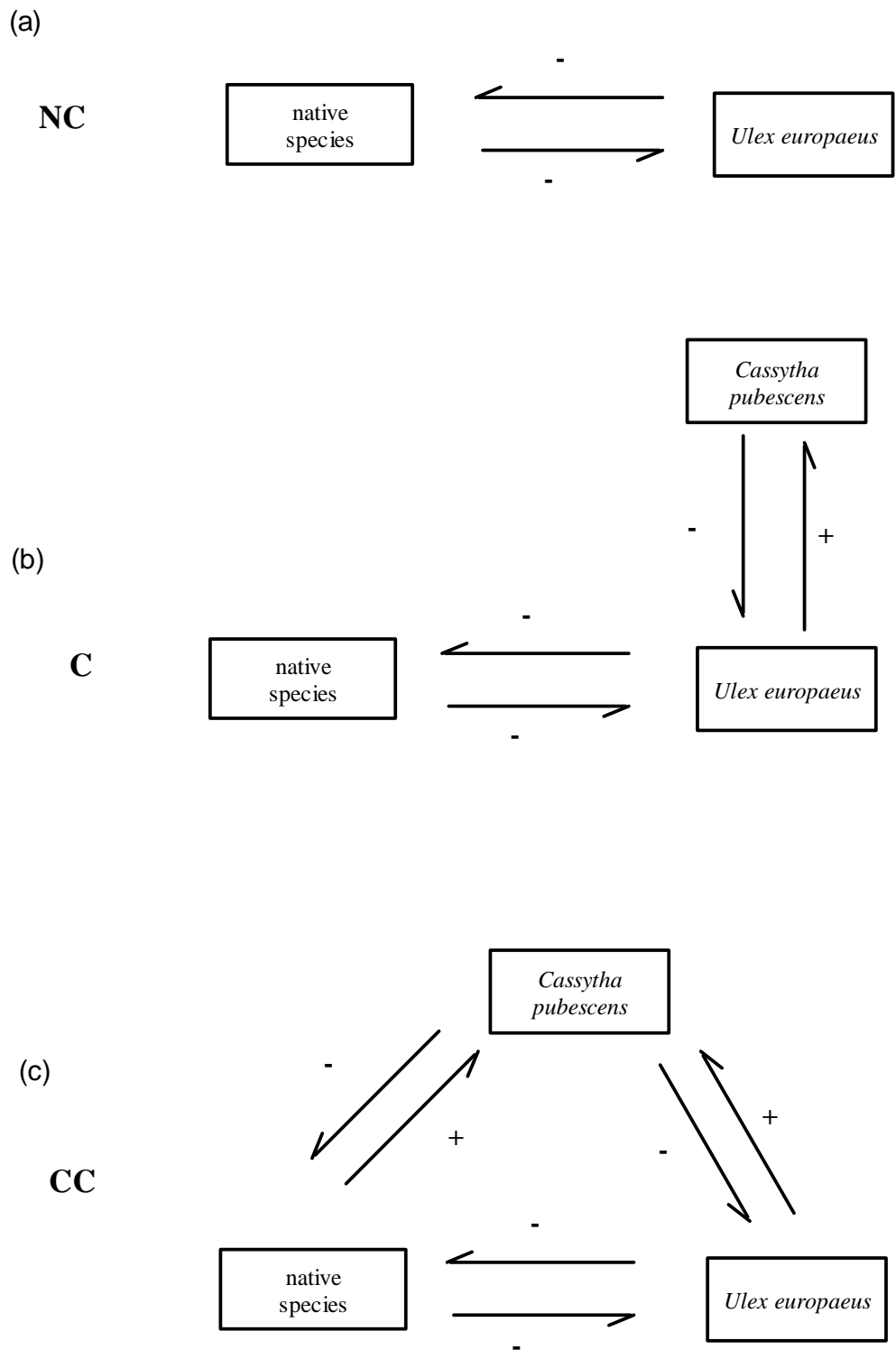
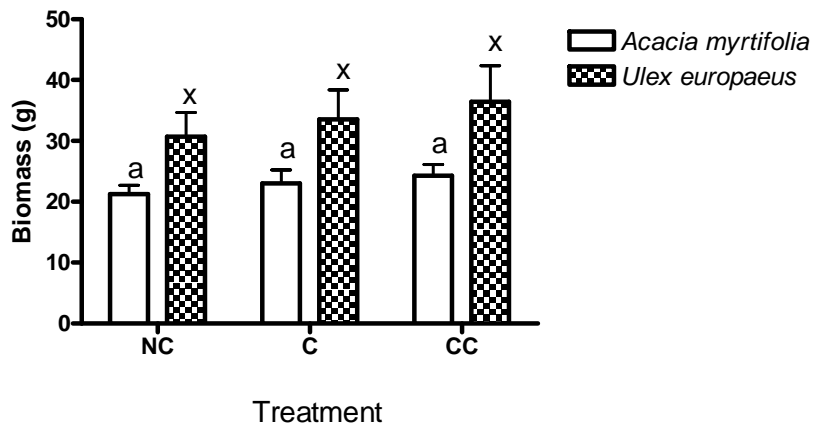


Figure. 6.1: The hypothetical interactions between plants in the three treatments, (a) NC: sole direct interaction between hosts, (b) C: infected *U. europaeus* interacted with native host and (c) CC: interaction between infected hosts. The arrows indicate the direction of the interactions and the signs on arrows represent the effect of the interactions, (+): positive effect and (-): negative effect.

(a)



(b)

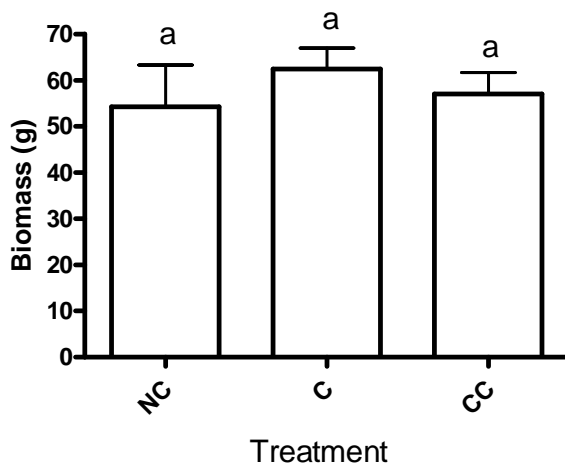
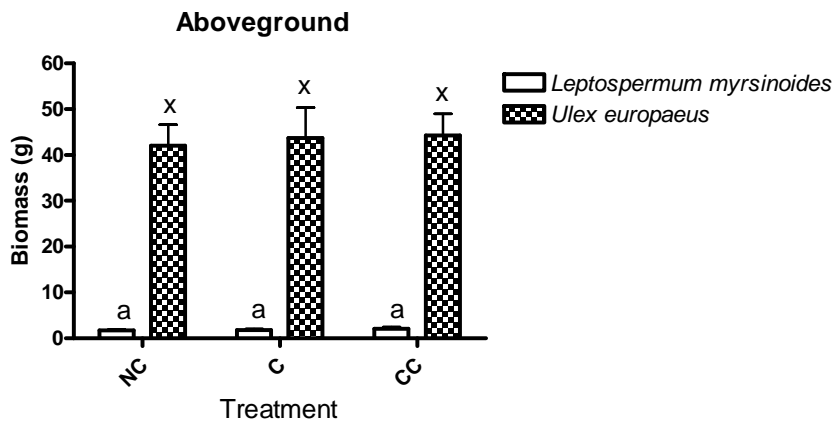


Figure 6.2.1: The dry weight of *Ulex europaeus* and *Acacia myrtifolia* in different *parasite* infection treatments. (a): aboveground and (b): belowground. Data points indicates means \pm s.e. (n=7). Different letters indicate significant difference at $p < 0.05$. The treatments in this figure is referring to the experimental design in Fig. 1.

(a)



(b)

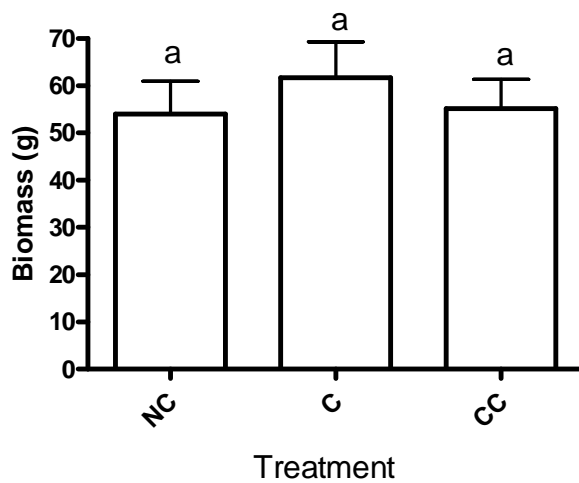
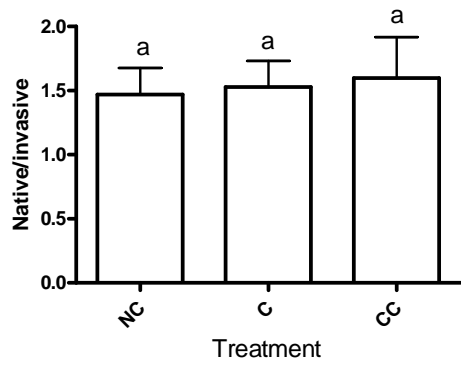


Figure 6.2.2: The dry weight of *Leptospermum myrsinoides* and *Ulex europaeus* in different parasite infection treatments. (a): aboveground and (b): belowground. Data points indicates means \pm s.e. (n=7). Different letters indicate significant difference at $p < 0.05$. The treatments in this figure is referring to the experimental design in Fig. 1.

		df	F	P
<i>Ulex-Acacia</i> interaction	<i>Ulex</i> shoot	2	0.33	0.72
	<i>Acacia</i> shoot	2	0.66	0.53
	total root biomass	2	0.41	0.67
<i>Ulex-Leptospermum</i> interaction	<i>Ulex</i> shoot	2	0.05	0.95
	<i>Leptospermum</i> shoot	2	0.46	0.64
	total root biomass	2	0.36	0.7

Table 6.1. ANOVA results for biomass (D.W.) of native and invasive hosts and the total belowground biomass between parasite infection treatments on the competition

(a)



(b)

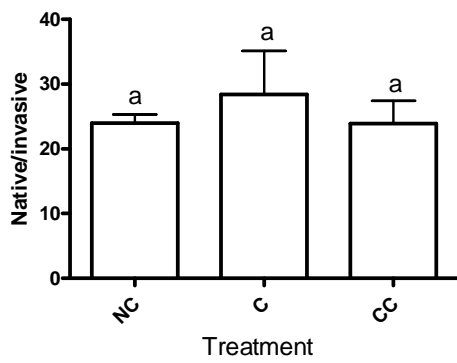


Figure 6.3: The ratio of aboveground dry weight between the native species and *Ulex europaeus* in different parasite infection treatments. (a): *Acacia myrtifolia* and (b): *Leptospermum myrsinoides*. Data points indicates means \pm s.e. (n=7). Different letters indicate significant difference at $p < 0.05$. Note different scales for both graphs. The treatments in this figure is referring to the experimental design in Fig. 1.

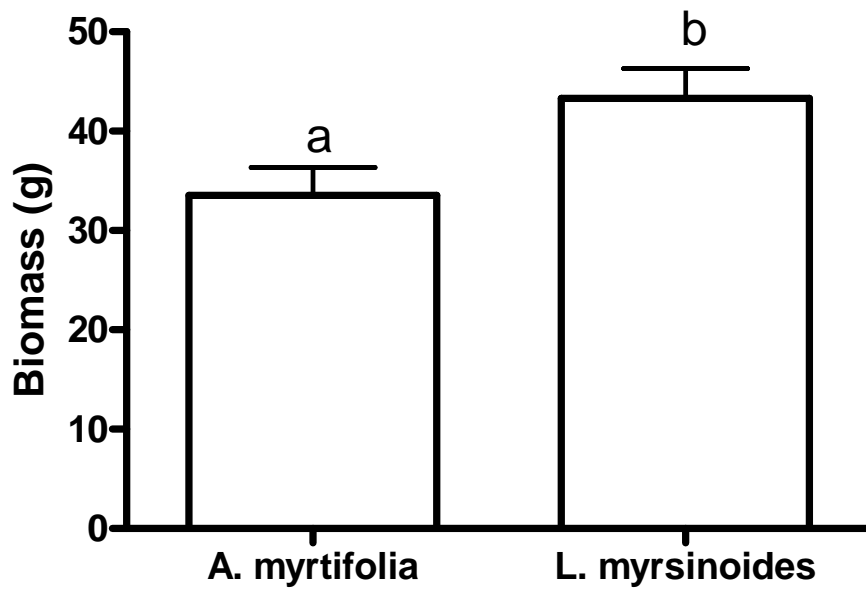


Figure. 6.4: The dry weight of *Ulex europaeus* grew with different native species. Data points indicates means \pm s.e. (n=21). Different letters indicate significant difference at $p < 0.05$

Chapter. 7 General Discussion

7.1 Summary

This project produced important new information on the biology of both *Cassytha pubescens* and the native and invasive hosts studied. The different ability of native and introduced legumes to resist infection, as well as fundamental aspects of the biology of *C. pubescens* contribute not only to the planning of the use of this species as a potential biological control agent, but also to our understanding of the interactions between plant parasites and their hosts. Important differences were also observed between *C. pubescens* and the well-studied genus *Cuscuta* which probably relate to differences in the life history strategies of these two parasites.

7.1.1 Comparison between *Cassytha pubescens* and *Cuscuta* spp.

Accumulation of seeds in the soil seedbank is an effective strategy for future generations of parasites to encounter their hosts. The seeds of both *C. pubescens* and *Cuscuta* spp. are enclosed in a water impermeable seed coat, hence they are physically dormant and can last in the soil for long periods of time until the right set of stimuli trigger germination. However, *C. pubescens* and *Cuscuta* spp. utilize different environmental cues as germination triggers, seemingly due to their different life history strategies and different habitats. As an annual species found in temperate climates, *Cuscuta* spp. have evolved to use the low temperatures in winter as a germination cue, timing germination to ensure they encounter newly germinated hosts in spring (Meulebrouck et al. 2008). In contrast, *C. pubescens* parasitizes mainly perennial shrubs in Mediterranean climate. Adult stems of these shrubs may be the only stems close to the ground for most of the time, and probably they are difficult to penetrate and at low density. The best opportunities for commencing parasitising new host for a seedling of *C. pubescens* is probably after a fire when there are abundant young stems close to the ground available for the parasite. Therefore, *C. pubescens* seemingly has evolved to germinate after bushfires trigger a germination or

production of new sprouts from lignotubers and rootstock of potential hosts. When vegetation is well established, probably it relies solely on clonal growth on the younger branches foraging from canopy to canopy until it latches on preferred hosts.

The difference in life history strategy between *C. pubescens* and *Cuscuta* spp. may explain the contrasting of strength of negative effects on their hosts. *Cuscuta* spp., as an annual parasite, need to complete their life cycle within a growing season, hence the competition for resources and relative negative effects on hosts are more intense than those observed for the perennial *C. pubescens*. On the other hand, being a perennial parasite, *C. pubescens* lives on its hosts for a long period of time, removing some resources from the host, but not necessarily producing its quick demise; at least for native hosts. Any lethal effects of perennial parasites on hosts would threaten the survival of the parasite as well (Press et al. 1988).

Further, differences in the photosynthetic ability of *C. pubescens* and *Cuscuta* may explain to the dissimilarity in their effect on nitrogen fixation. Being a holoparasite, *Cuscuta* spp. are highly dependent on the supply of photosynthate from its hosts, making it likely that they would directly compete for photosynthate with Rhizobium, and hence disrupt nitrogen fixation. On the other hand, as a hemiparasite, *C. pubescens* is less dependent on photosynthate from the hosts, although it may indirectly impact on the host's ability to fix carbon through reduction in water and nutrient availability. Despite these possible indirect effects, the results of this study, suggest that *C. pubescens* does not significantly disrupt host carbon supply to Rhizobium, thus having no negative effect on the nitrogen fixation of *Cytisus scoparius*.

7.1.2 Effectiveness as biological control agent

This research provides further insights into the suitability of this parasite as a biological control agent, particularly in relation to its life cycle and host specificity. The physical dormancy of its seeds may allow the parasite to persist in the soil for long period of time, making the parasite suitable to control invasive species with long

lasting seedbank like *C. scoparius*. Further, the germination of *C. pubescence* is seemingly triggered by fire, which also triggers the germination of the weeds under consideration. It is proposed that the delay in germination of the parasite could allow its seedlings to meet already established seedlings of *C. scoparius*, potentially allowing for controlling reinvasion after a fire.

Although *C. pubescens* is a generalist parasite, it may still be a suitable biological control agent, since it has stronger negative effects on invasive than native hosts. Some of this variation in its effect on its hosts could relate to resistance of the native hosts to *C. pubescens*. The radioactive labelling experiment revealed that *C. pubescens* could not acquire ^{32}P from *Acacia myrtifolia*, but did absorb a substantial amount of ^{32}P from *C. scoparius*. This suggests that native hosts might have evolved a degree of defence against infection by the parasite, whereas, *C. scoparius* behaves as a naïve host. Thus, observed differences in density of infection on native and introduced hosts in the field may be due to greater resistance of native hosts rather than *C. pubescens* actively selecting its hosts. However, it is important to consider how other native species that act as effective hosts (such as *L. myrsinoides*) respond to the infection (Hao et al. 2010).

7.1.3 Effects of *Cytisus scoparius* on the environment

C. scoparius seemingly has low degree of specificity for infection with legume-nodulating bacteria. This may contribute to the invasive ability of this weed, as this allows it to perform well with the soil biota in the new habitat. The results also further indicate the strong potential ecological engineer ability of *C. scoparius*. The soil nitrogen content in the pots was altered differently by different legumes in just 6 months. This reveals that the replacement of native legumes with invasive legumes would change the soil nitrogen dynamic and potentially alter the species composition in the community.

7.2 Future research

The difference in germination rate between the heat and scarification treatments in both germination experiments indicates that physical dormancy may not be the only mechanism involved. Therefore, studies are required to investigate the difference in the effect of these two treatments on *C. pubescens*. Another imbibition study should be conducted to compare the water absorption rate of seeds treated with heat and scarification. Afterward, to confirm whether a heat shock mechanism is involved, a molecular study would be required to detect the activation of heat shock proteins (Vierling 1991). Moreover, only laboratory experiments were conducted to test germination of *C. pubescens*. Field studies are required to confirm the role of bushfire in the germination of *C. pubescens*, for example, conducting surveys of areas where *C. pubescens* has been previously recorded and comparing the number of germinated seedlings in adjacent burnt and unburnt areas. Furthermore, the findings suggest that the seed coat of *C. pubescens* allows seeds to accumulate in the soil, but it is still unclear how long the soil seedbank lasts. Seed burial experiments followed by germination studies could resolve this question.

A wider range of both native and invasive hosts should be tested in an expanded radioactive labelling experiment, to obtain more information on the differences in resistance of various hosts to the parasite. Clearly, this information is critical to determine if this parasite could be used as a biological control agent without threatening the native flora. The uptake of ^{32}P by the parasite from *C. scoparius* could either be resource competition with the host, or may be due to stimulation of uptake by the host, making more nutrients available. This could be resolved by including a treatment of hosts without infection in the radioactive labelling experiment. The uptake of ^{32}P by uninfected and infected hosts could then be compared, allowing quantification of the effect of the parasite on nutrient uptake by the host.

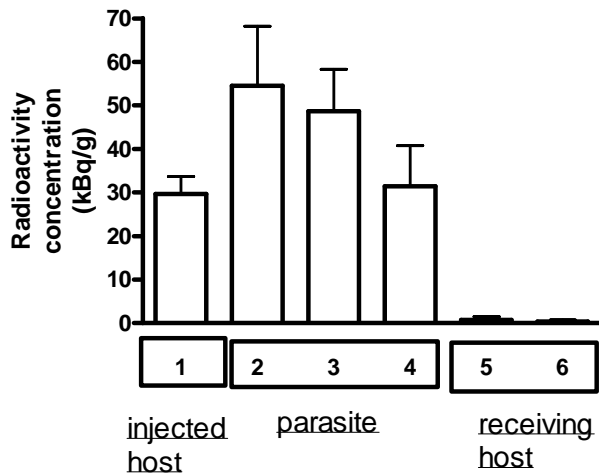
7.3 Conclusion

This study of *C. pubescens* suggest that the biology of this parasite and its interactions with hosts significantly differ from those of the better studied *Cuscuta* spp.. Species in

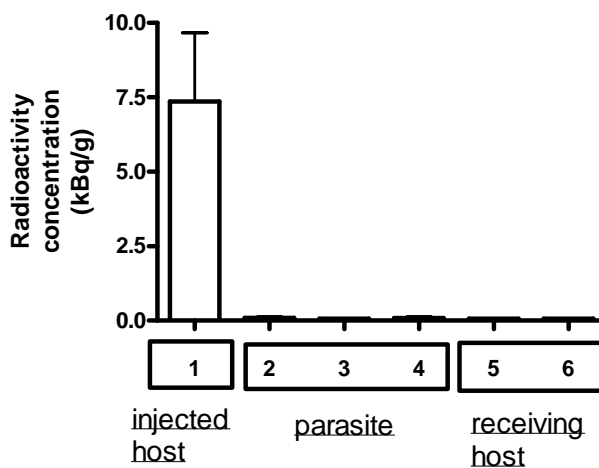
both these genera are obligate stem parasites, are morphologically similar, and have been cited as a classic example of convergent evolution (Kuijt 1969). However, my study shows that the two genera vary in germination strategy, the nature and the intensity of interactions with their hosts. These are most likely a result of different life history strategies and habitats. In all published studies *Cuscuta* species have strong negative effects on their hosts, which often strongly modify host interactions with other species. Only relatively minor or no negative effects (depending on the host species) were found in this study on the hosts of *C. pubescens*. This study highlights the need of better understanding the biology of generalist perennial parasitic angiosperms in natural system, and reveals that the information from species with similar morphology and seemingly same ecological niche could be irrelevant due to differences in life history strategy.

Appendix 1

(a)



(b)



Appendix 1. The concentration of radioactivity in different sections of each plant. (a): *Cytisus scoparius* injected with ^{32}P and (b): *Acacia myrtifolia* injected with ^{32}P . Data points are means \pm s.e. (n=5). (1): host shoot from the pot injected with ^{32}P , (2): *C. pubescens* on the radio-labelled host, (3): *C. pubescens* spanning the two hosts, (4): *C. pubescens* on the non-labelled host, (5): infected shoot of the non-labelled host and (6): uninfected shoot of the non-labelled host

References

- Allison S.D. and P.M. Vitousek (2004). "Rapid nutrient cycling in leaf litter from invasive plants in Hawaii." Oecologia **141**(4): 612-619.
- Ameloot E., K. Verheyen, J.P. Bakker, Y. De Vries and M. Hermy (2006). "Long-term dynamics of the hemiparasite *Rhinanthus angustifolius* and its relationship with vegetation structure." Journal of Vegetation Science **17**: 637-646.
- Ameloot E., K. Verheyen and M. Hermy (2005). "Meta-analysis of standing crop reduction by *Rhinanthus spp.* and its effect on vegetation structure." Folia Geobotanica **40**: 289-310.
- Augusto L., N. Crampon, E. Saur, M.R. Bakker, S. Pellerin, C. de Lavaissiere and P. Trichet (2005). "High rates of nitrogen fixation of *Ulex* species in the understory of maritime pine stands and the potential effect of phosphorous fertilization." Canadian Journal of Forest Research **35**: 1183-1192.
- Bardgett R.D., R.S. Smith, R.S. Shiel, S. Peacock, J.M. Simkin, H. Quirk and P.J. Hobbs (2006). "Parasitic plants indirectly regulate below-ground properties in grassland ecosystems." Nature **439**: 969-972.
- Baskin C.C. (2003). "Breaking physical dormancy in seeds: Focussing on the lens." New Phytologist **158**: 229-232.
- Baskin C.C. and J.M. Baskin (1998). Plants with Specialized Life Cycle and/or Habitats. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, Academic Press.
- Baskin C.C. and J.M. Baskin (1998). Seeds : ecology, biogeography, and evolution of dormancy and germination. San Diego, Academic Press.
- Baskin C.C. and J.M. Baskin (2007). "A revision of Martin's seed classification system, with particular reference to his dwarf-seed type." Seed Science Research **17**: 11-20.
- Baskin C.C., K. Thompson and J.M. Baskin (2006). "Mistakes in germination ecology and how to avoid them." Seed Science Research **16**: 165-168.
- Baskin J.M. and C.C. Baskin (2004). "A classification system for seed dormancy." Seed Science Research **14**: 1-16.
- Baskin J.M., C.C. Baskin and X. Li (2000). "Taxonom, anatomy and evolution of physical dormancy in seeds." Plant Species Biology **15**: 139-152.
- Baskin J.M., B.H. Davis, C.C. Baskin, S.M. Gleason and S. Cordell (2004). "Physical dormancy in seeds of *Dodonaea viscosa* (*Sapindales*, *Sapindaceae*) from

Hawaii." Seed Science Research **14**: 81-90.

Bell D.T. and D.S. Williams (1998). "Tolerance of thermal shock in seeds." Australian Journal of Botany **46**: 221-233.

Benvenuti S., G. Dinelli, A. Bonetti and P. Catizone (2005). "Germination ecology, emergence and host detection in *Cuscuta campestris*." Weed Research **45**(4): 270-278.

Bickford C.P. and T.E. Kolb (2005). "Host physiological condition regulates parasitic plant performance: *Arceuthobium vaginatum* subsp. *cryptopodium* on *Pinus ponderosa*." Oecologia **146**: 179-189.

Bidwell S., P.M. Attiwill and M.A. Adams (2006). "Nitrogen availability and weed invasion in a remnant native woodland in urban Melbourne." Austral Ecology **31**: 262-270.

Britton T. (2002). Impact of *Cassutha pubescens* R. Br. on the physiology and growth of gorse (*Ulex europaeus* L.) in South Australia. Department of Environmental Biology. Adelaide, Adelaide University: 27.

Bullock J.M. and R.F. Pywell (2005). "Rhinanthus: A tool for restoring diverse grassland?" Folia Geobotanica **40**: 273-288.

Burch J.N. (1992). "*Cassutha filiformis* and limits to growth and reproduction of *Schinus terebinthifolius* in Southern Florida." Florida Scientist **55**: 28-34.

Caldwell B.A. (2006). "Effects of invasive scotch broom on soil properties in a Pacific coastal prairie soil." Applied Soil Ecology **32**: 149-152.

Callaway R.M. and S.C. Pennings (1998). "Impact of a parasitic plant on the zonation of two salt marsh perennials." Oecologia **114**: 100-105.

Cameron D.D., A.M. Coats and W.E. Seel (2006). "Differential resistance among host and non-host species underlies the variable success of the hemi-parasitic plant *Rhinanthus minor*." Annals of Botany **98**: 1289-1299.

Cameron D.D., J.M. Geniez, W.E. Seel and L.J. Irving (2008). "Suppression of host photosynthesis by the parasitic plant *Rhinanthus minor*." Annals of Botany **101**: 573-578.

Cameron D.D., J.K. Hwangbo, A.M. Keith, J.M. Geniez, J. Rowntree and W.E. Seel (2005). "Interactions between the hemiparasitic angiosperm *Rhinanthus minor* and its hosts: from the cell to the ecosystem." Folia Geobotanica **40**: 217-229.

Cameron D.D. and W.E. Seel (2007). "Functional anatomy of haustoria formed by *Rhinanthus minor*: linkage evidence from histology and isotope tracing." New Phytologist **174**: 412-419.

Cameron D.D., A. White and J. Antonovics (2009). "Parasite-grass-forb interactions and rock-paper-scissor dynamics: predicting the effects of the parasitic plant

- Rhinanthus minor on host plant communities." Journal of Ecology **97**: 1311-1319.
- Carino D.A. and C.C. Daehler (2002). "Can inconspicuous legumes facilitate alien grass invasions? Partridge peas and fountain grass in Hawaii." ECOGRAPHY **25**: 33-41.
- Chen H., M.E. Harmon, J. Sexton and B. Fasth (2002). "Fine-root decomposition and N dynamics in coniferous forests of the Pacific Northwest, U.S.A." Canadian Journal of Forest Research **32**: 320-330.
- Clemens J., P.G. Jones and N.H. Gilbert (1977). "Effect of seed treatments on germination in *Acacia*." Australian Journal of Botany **25**: 269-276.
- Close D.C., N.J. Davidson and N.W. Davies (2006). "Seasonal fluctuations in pigment chemistry of co-occurring plant hemi-parasites of distinct form and function." Environmental and Experimental Botany **58**(1-3): 41-46.
- Cochrane G.R. (1963). "Vegetation studies in forest-fire areas of the Mount Lofty Ranges, South Australia." Ecology **44**: 41-52.
- Colautti R.I., A. Ricciardi, I.A. Grigorovich and H.J. MacIsaac (2004). "Is invasion success explained by the enemy release hypothesis?" Ecology Letters **7**: 721-733.
- Coley P.D., J.P. Bryant and F.S. Chapin (1985). "Resource availability and plant antiherbivore defense." Science **230**: 895-899.
- Cullings K., C. Raleigh and D.R. Vogler (2005). "Effects of severe dwarf mistletoe infection on the ectomycorrhizal community of a *Pinus contorta* stand in Yellowstone Park." Canadian Journal of Botany **83**: 1174-1180.
- Davies D.M. and J.D. Graves (1998). "Interactions between arbuscular mycorrhizal fungi and the hemiparasitic angiosperm *Rhinanthus minor* during co-infection of a host." New Phytologist **139**: 555-563.
- Davis M.A., J.P. Grime and K. Thompson (2000). "Fluctuating resources in plant communities: A general theory of invasibility." Journal of Ecology **88**: 528-534.
- DeRivera C.E., G.M. Ruiz, A.H. Hines and P. Jivoff (2005). "Biotic resistance to invasion: Native predator limits abundance and distribution of an introduced crab." Ecology **12**: 3364-3376.
- Ehrenfeld J.G. (2003). "Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes." Ecosystems **6**(6): 503-523.
- Evans R.D., R. Rimer, L. Sperry and J. Belnap (2001). "Exotic plant invasion alters nitrogen dynamics in an arid grassland." Ecological Applications **11**: 1301-1310.
- Fagan W.F., M.A. Lewis, M.G. Neubert and P. van den Driessche (2002). "Invasion theory and biological control." Ecology Letters **5**(1): 148-157.

Fogarty G. and J.M. Facelli (1999). "Growth and competition of *Cytisus scoparius*, an invasive shrub, and Australian native shrubs." Plant Ecology **144**: 27-35.

French K. and M. Westoby (1996). "Vertebrate-dispersed species in a fire-prone environment." Australian Journal of Ecology **21**: 379-385.

Gehring C.A. and T.G. Whitham (1992). "Reduced mycorrhizae on *Juniperus monosperma* with mistletoe: the influence of environmental stress and tree gender on a plant parasite and a plant-fungal mutualism." Oecologia **89**: 298-303.

Gibson C.C. and A.R. Watkinson (1989). "The host range and selectivity of a parasitic plant: *Rhinanthus minor* L." Oecologia **78**: 401-406.

Gibson C.C. and A.R. Watkinson (1991). "Host selectivity and the mediation of competition by the root hemiparasite *Rhinanthus minor*." Oecologia **86**: 81-87.

Gibson C.C. and A.R. Watkinson (1992). "The role of the hemiparasitic annual *Rhinanthus minor* in determining grassland community structure." Oecologia **89**: 62-68.

Grewell B.J. (2008). "Parasite facilitates plant species coexistence in a coastal wetland." Ecology **89**: 1481-1488.

Gurney A.L., D. Grimanelli, F. Kanampiu, D. Hoisington, J.D. Scholes and M.C. Press (2003). "Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides*, a wild relative of maize." New Phytologist **160**: 557-568.

Gurney A.L., J. Slate, M.C. Press and J.D. Scholes (2006). "A novel form of resistance in rice to the angiosperm parasite *Striga hermonthica*." New Phytologist **169**: 199-208.

Hanson W.C. (1950). "The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex." Journal of the Science of Food and Agriculture **1**: 172-173.

Hao S., J. Prider, J.M. Facelli and J.R. Watling (2010). "The influence of the hemiparasitic angiosperm *Cassytha pubescens* on photosynthesis of its host *Cytisus scoparius*." Functional Plant Biology **37**: 14-21.

Hao S., W. Ye, L. Hong, H. Cao and Z. Wang (2005). "Influence of the obligate parasite *Cuscuta campestris* on growth and biomass allocation of its host *Mikania micrantha*." Journal of Experimental Botany **56**: 1277-1284.

Hatcher M.J., J.T.A. Dick and A.M. Dunn (2006). "How parasites affect interactions between competitors and predators." Ecology Letters **9**: 1253-1271.

Haubensak K.A., C.M. D'Antonio and J. Alexander (2004). "Effects of nitrogen-fixing shrubs in Washington and Coastal California." Weed Technology **18**: 1475-1479.

Haubensak K.A. and I.M. Parker (2004). "Soil changes accompanying invasion of the

- exotic shrub *Cytisus scoparius* in glacial outwash prairies of western Washington [USA]." Plant Ecology **175**: 71-79.
- Hawkes C.V., I.F. Wren, D.J. Herman and M.K. Firestone (2005). "Plant invasion alters nitrogen cycling by modifying the soil nitrifying community." Ecology Letters **8**: 976-985.
- Herms D.A. and W.J. Mattson (1992). "The dilemma of plants: to grow or defend." The Quarterly Review of Biology **67**: 283-335.
- Hibberd J.M., W.P. Quick, M.C. Press, J.D. Scholes and W.D. Jeschke (1999). "Solute fluxes from tobacco to the parasitic angiosperm *Orobancha cernua* and the influence of infection on host carbon and nitrogen relations." Plant, Cell and Environment **22**: 937-947.
- Hidayati S.N., J.M. Baskin and C.C. Baskin (2001). "Dormancy-breaking and germination requirements for seeds of *Symphoricarpos orbiculatus* (*Caprifoliaceae*)." American Journal of Botany **88**(8): 1444-1451.
- Hill R.L., A.H. Gourlay and R.J. Barker (2001). "Survival of *Ulex europaeus* seeds in the soil at three sites in New Zealand." New Zealand Journal of Botany **39**: 235-244.
- Howarth F.G. (1991). "Environmental Impacts of Classical Biological Control." Annual Review of Entomology **36**(1): 485-509.
- Hudson J.P., A.P. Dobson and K.D. Lafferty (2006). "Is a healthy ecosystem one that is rich in parasites?" Trends in Ecology & Evolution **21**: 381-385.
- Hudson P. and J. Greenman (1998). "Competition mediated by parasites: biological and theoretical progress." TREE **13**: 387-390.
- Jayasuriya K.M.G.G., J.M. Baskin, R.L. Geneve, C.C. Baskin and C.T. Chien (2008). "Physical Dormancy in Seeds of the Holoparasitic Angiosperm *Cuscuta australis* (*Convolvulaceae, Cuscutaceae*): Dormancy-breaking Requirements, Anatomy of the Water Gap and Sensitivity Cycling." Annals of Botany **102**(1): 39-48.
- Jeschke W.D., A. Baig and A. Hilpert (1997). "Sink-stimulated photosynthesis, increased transpiration and increased demand-dependent stimulation of nitrate uptake: nitrogen and carbon relations in the parasitic association *Cuscuta reflexa*-*Coleus blumei*." Journal of Experimental Botany **48**: 915-925.
- Jeschke W.D., P. Baumel, N. Rath, F.C. Czygan and P. Proksch (1994). "Modelling of the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus Albus* L. II. Flows between host and parasite and within the parasitized host." Journal of Experimental Botany **45**: 801-812.
- Jeschke W.D. and A. Hilpert (1997). "Sink-stimulated photosynthesis and sink-dependent increase in nitrate uptake: nitrogen and carbon relations of the parasitic association *Cuscuta reflexa*-*Ricinus communis*." Plant, Cell and Environment **20**: 47-56.

Jiang F., W.D. Jeschke, W. Hartung and D.D. Cameron (2008). "Does legume nitrogen fixation underpin host quality for the hemiparasitic plant *Rhinanthus minor*?" Journal of Experimental Botany **59**: 917-925.

Joshi J., D. Matthies and B. Schmid (2000). "Root hemiparasites and plant diversity in experimental grassland communities." Journal of Ecology **88**: 634-644.

Keane R.M. and M.J. Crawley (2002). "Exotic plant invasions and the enemy release hypothesis." Trends in Ecology & Evolution **17**: 164-170.

Kelly C.K., D.L. Venable and K. Zimmerer (1988). "Host specialization in *Cuscuta Costaricensis*: An assessment of host use relative to host availability." Oikos **53**: 315-320.

Kelly K.M., J. Van Staden and W.E. Bell (1992). "Seed coat structure and dormancy." Plant Growth Regulation **11**: 201-209.

Kuijt J. (1969). The biology of parasitic flowering plants, University of California Press.

Labrousse P., M.C. Arnaud, A. Berville and P. Thalouarn (2001). "Several mechanisms are involved in resistance of *Helianthus* to *Orobanche cumana* Wallr." Annals of Botany **88**: 859-868.

Lafay B. and J.J. Burdon (2006). "Molecular diversity of rhizobia nodulating the invasive legume *Cytisus scoparius* in Australia." Journal of Applied Microbiology **100**: 1228-1238.

Laungani R. and J.M.H. Knops (2009). "Species-driven changes in nitrogen cycling can provide a mechanism for plant invasions." PNAS **106**: 12400-12405.

Leary J.K., N.V. Hue, P.W. Singleton and D. Borthakur (2006). "The major features of an infestation by the invasive weed legume gorse (*Ulex europaeus*) on volcanic soils in Hawaii." Biology and fertility of Soils **42**: 215-223.

Lehtonen P., M. Helander, M. Wink, F. Sporer and K. Saikkonen (2005). "Transfer of endophyte-origin defensive alkaloids from a grass to a hemiparasitic plant." Ecology Letters **8**: 1256-1263.

Levine J.M., L.S. Adler and S.G. Yelenik (2004). "A meta-analysis of biotic resistance to exotic plant invasions." Ecology Letters **7**: 975-989.

Levine J.M., M. Vila, C.M. D'Antonio, J.S. Dukes, K. Grigulis and S. Lavorel (2003). "Mechanisms underlying the impacts of exotic plant invasions." Proceedings- Royal Society of London. Biological sciences **270**: 775-781.

Li X., J.M. Baskin and C.C. Baskin (1999). "Anatomy of two mechanisms of breaking physical dormancy by experimental treatment in seeds of two North American *Rhus* species (*Anacardiaceae*)." American Journal of Botany **86**: 1505-1511.

- Logan D.C. and G.R. Stewart (1992). "Germination of the seeds of parasitic angiosperms." Seed Science Research **2**: 179-190.
- Louda S.M., D. Kendall, J. Connor and D. Simberloff (1997). "Ecological Effects of an Insect Introduced for the Biological Control of Weeds." Science **277**(5329): 1088-1090.
- Loveys B.R., S.D. Tyerman and B.R. Loveys (2002). "Effect of different host plants on the growth of the root hemiparasite *Santalum acuminatum* (quandong)." Australian Journal of Experimental Agriculture **42**: 97-102.
- Marler M.J., C.A. Zabinski and R.M. Callaway (1999). "Mycorrhizae indirectly enhance competitive effects of an invasive forb on a native bunchgrass." Ecology **80**: 1180-1186.
- Maron J.L. and P.G. Connors (1996). "A native nitrogen-fixing shrub facilitates weed invasion." Oecologia **105**: 302-312.
- Maron J.L. and M. Vila (2001). "When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses." Oikos **95**: 361-373.
- Marshall J.D. and J.R. Ehleringer (1990). "Are xylem-tapping mistletoes partially heterotrophic?" Oecologia **84**: 244-248.
- Marvier M.A. (1998). "Parasite impacts on host communities: Plant parasitism in a California coastal prairie." Ecology **79**: 2616-2623.
- Matthies D. (1995). "Parasitic and Competitive Interactions between the Hemiparasites *Rhinanthus Serotinus* and *Odontites Rubra* and their Host *Medicago Sativa*." The Journal of Ecology **83**(2): 245-251.
- Matusova R., T. van Mourik and H.J. Bouwmeester (2004). "Changes in the sensitivity of parasitic weed seeds to germination stimulants." Seed Science Research **14**: 335-344.
- McFadyen R.E.C. (1998). "Biological control of weeds." Annual Review of Entomology **43**: 369-393.
- McLuckie J. (1924). "Studies in parasitism I. A contribution to the physiology of the genus *Cassytha*." Proc. Linn. Soc. NSW **49**: 55-79.
- Meulebrouck K., E. Ameloot, J.A. Van Assche, K. Verheyen, M. Hermy and C.C. Baskin (2008). "Germination ecology of the holoparasite *Cuscuta epithimum*." Seed Science Research **18**: 25-34.
- Miller A.C., J.R. Watling, I.C. Overton and R. Sinclair (2003). "Does water status of *Eucalyptus largiflorens* (Myrtaceae) affect infection by the mistletoe *Amyema miquelii* (Loranthaceae)?" Functional Plant Biology **30**: 1239-1247.

Minchella D.J. and M.E. Scott (1991). "Parasitism: a cryptic determinant of animal community structure." TREE **6**: 250-254.

Mitchell C.E., A.A. Agrawal, J.D. Bever, G.S. Gilbert, R.A. Hufbauer, J.N. Klironomos, J.L. Maron, M. W.F., I.M. Parker, A.G. Power, E.W. Seabloom, M.E. Torchin and D.P. Vazquez (2006). "Biotic interactions and plant invasions." Ecology Letters **9**: 726-740.

Morin P.J. (1999). Community ecology, Blackwell Science.

Mueller R.C. and C.A. Gehring (2006). "Interactions between an above-ground plant parasite and below-ground ectomycorrhizal fungal communities on pinyon pine." Journal of Ecology **94**: 276-284.

Mummey D.L. and M.C. Rillig (2006). "The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field." Plant and Soil **288**: 81-90.

Muyt A. (2001). Bush invaders of South-East Australia : a guide to the identification and control of environmental weeds found in South-East Australia. Maryborough, Victoria, R.G. & F.J. Richardson.

Parker C. and C.R. Riches (1993). Parasitic weeds of the world: biology and control. Wallingford, CAB International.

Parker J.D. and M.E. Hay (2005). "Biotic resistance to plant invasions? Native herbivores prefer non-native plants." Ecology Letters **8**: 959-967.

Parsons W.T. and E.G. Cuthertson (1992). Noxious Weeds of Australia. Melbourne and Sydney, Australia, Inkata Press.

Pate J.S. and T.L. Bell (2000). "Host associations of the introduced annual root hemiparasite *Parentucellia viscosa* in agriculture and bushland settings in Western Australia." Annals of Botany **85**: 203-213.

Pearson D.E. and R.M. Callaway (2003). "Indirect effects of host-specific biological control agents." Trends in Ecology and Evolution **18**: 456-461.

Pearson D.E. and R.M. Callaway (2005). "Indirect nontarget effects of host-specific biological control agents: Implications for biological control." Biological Control **35**: 288-298.

Pemberton R.W. (2000). "Predictable risk to native plants in weed biological control." Oecologia **125**: 489-494.

Pennings S.C. and R.M. Callaway (1996). "Impact of a parasitic plant on the structure and dynamic of salt marsh vegetation." Ecology **77**: 1410-1419.

Perez-Fernandez M.A. and B.B. Lamont (2003). "Nodulation and performance of exotic and native legumes in Australian soils." Australian Journal of Botany **51**: 543-

553.

Porra R.J., W.A. Thompson and P.E. Kreidemann (1989). "Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy." Biochimica et Biophysica Acta **975**: 384-394.

Press M.C., J.D. Graves and G.R. Stewart (1988). "Transpiration and carbon acquisition in root hemiparasitic angiosperms." Journal of Experimental Botany **39**: 1009-1014.

Press M.C., J.D. Graves and G.R. Stewart (1990). "Physiology of the interaction of angiosperm parasites and their higher plant hosts." Plant, Cell and Environment **13**: 91-104.

Press M.C. and G.K. Phoenix (2005). "Impact of parasitic plants on natural communities." New Phytologist **166**: 737-751.

Prevosto B., E. Dambrine, P. Coquillard and A. Robert (2006). "Broom (*Cytisus scoparius*) colonization after grazing abandonment in the French Massif Central: impact on vegetation composition and resource availability." Acta Oecologica **30**: 258-268.

Price P.W., M. Westoby and B. Rice (1988). "Parasite-mediated competition: Some predictions and tests." The American Naturalist **131**: 544-555.

Price P.W., M. Westoby, B. Rice, P.R. Atsatt, R.S. Fritz, J.N. Thompson and K. Mobley (1986). "Parasite mediation in ecological interactions." Annual Review of Ecology and Systematics **17**: 487-505.

Prider J., J.M. Facelli and J.R. Watling (2009). Three is a crowd: Multispecies interactions among a plant parasite, a pollinator and a seed predator affect the reproductive output of an invasive plant, *Cytisus scoparius*: 23.

Prider J., J.R. Watling and J.M. Facelli (2009). "Impacts of a native parasitic plant on an introduced and a native host species: implications for the control of an invasive weed." Annals of Botany **103**: 107-115.

Puustinen S. and P. Mutikainen (2001). "Host-parasite-herbivore interactions: Implication of host cyanogenesis." Ecology **82**: 2059-2071.

Rangaswamy N.S. and T.S. Rangan (1971). "Morphogenic investigations on parasitic angiosperm. IV. Morphogenesis in decotylated embryos of *Cassytha filiformis* L. *Lauraceae*." Botanical Gazette **132**: 113-119.

Reinhart K.O. and R.M. Callaway (2006). "Soil biota and invasive plants." New Phytologist **170**: 445-457.

Rodriguez-Echeverria S., M.A. Perez-Fernandez, S. Vlaar and T. Finnan (2003).

- "Analysis of the legume-rhizobia symbiosis in shrubs from central western Spain." Journal of Applied Ecology **95**: 1367-1374.
- Rudgers J., W. Mattingly and J. Koslow (2005). "Mutualistic fungus promotes plant invasion into diverse communities." Oecologia **144**: 463-471.
- Runyon J.B., M.C. Mescher and C.M. De Moraes (2006). "Volatile chemical cues guide host location and host selection by parasitic plants." Science **313**: 1964-1967.
- Salonen V., M. Vestberg and M. Vauhkonen (2001). "The effect of host mycorrhizal status on host plant-parasitic plant interactions." Mycorrhiza **11**: 95-100.
- Sanders I.R., R.T. Koide and D.L. Shumway (1993). "Mycorrhizal stimulation of plant parasitism." Canadian Journal of Botany **71**: 1143-1146.
- Sastri R.L.N. (1962). "Studies in *Lauraceae*. III. Embryology of *Cassytha*." Botanical Gazette **123**(3): 197-206.
- Sax D.F., J.J. Stachowicz, J.H. Brown, B. J.F., M.N. Dawson, S.D. Gaines, R.K. Grosberg, A. Hastings, R.D. Holt, M.M. Mayfield, C. O, M.I. and W.R. Rice (2007). "Ecological and evolutionary insights from species invasions." Trends in Ecology & Evolution **22**: 465-471.
- Schadler M., M. Roeder, R. Brandl and D. Matthies (2005). "Is palatability of a root-hemiparasitic plant influenced by its host species?" Oecologia **146**: 227-233.
- Seel W.E., R.E. Cooper and M.C. Press (1993). "Growth, gas exchange and water use efficiency of the facultative hemiparasite *Rhinanthus minor* associated with hosts differing in foliar nitrogen concentration." Physiologia Plantarum **89**: 64-70.
- Seel W.E., A.N. Parsons and M.C. Press (1993). "Do inorganic solutes limit growth of the facultative hemiparasite *Rhinanthus minor* L. in the absence of a host?" New Phytologist **124**: 283-289.
- Seel W.E. and M.C. Press (1994). "Influence of the host on three sub-Arctic annual facultative root hemiparasites." New Phytologist **127**: 37-44.
- Sheppard A.W., P. Hodge, Q. Paynter and M. Rees (2002). "Factors affecting invasion and persistence of broom *Cytisus scoparius* in Australia." Journal of Applied Ecology **39**: 721-734.
- Simberloff D. and B. Von Holle (1999). "Positive interactions of nonindigenous species: invasional meltdown?" Biological Invasions **1**: 21-32.
- Sinden J., R. Jones, S. Hester, D. Odom, C. Kalisch, R. James and O. Cacho (2004). The economic impact of weeds in Australia: Summary. CRC for Australian Weed Management, Technical Series No 8: 4.
- Smith S.E. and D.L. Read (2007). Mycorrhizal symbiosis, Academic Press.

- Stewart G.R. and M.C. Press (1990). "The physiology and biochemistry of parasitic angiosperms." Annual Review of Plant Physiology and Plant Molecular Biology **41**: 127-151.
- Strayer D.L., V.T. Eviner, J.M. Jeschke and M.L. Pace (2006). "Understanding the long-term effects of species invasions." Trends in Ecology & Evolution **21**(11): 645-651.
- Tarrega R., L. Calvo and L. Trabaud (1992). "Effect of high temperature on seed germination of two woody Leguminosae." Vegetatio **102**: 139-147.
- Taylor A., J. Martin and W.E. Seel (1996). "Physiology of the parasitic association between maize and witchweed (*Striga hermonthica*): is ABA involved?" Journal of Experimental Botany **47**: 1057-1065.
- Tennakoon K.U., J.S. Pate and B.A. Fineran (1997). "Growth and partitioning of C and fixed N in the shrub legume *Acacia littorea* in the presence or absence of the root hemiparasite *Oxalys phyllanthi*." Journal of Experimental Botany **48**: 1047-1060.
- Ter Borg S.J. (2005). "Dormancy and germination of six *Rhinanthus* species in relation to climate." Folia Geobotanica **40**: 243-260.
- Thrall P.H., J.J. Burdon and M.J. Woods (2000). "Variation in the effectiveness of symbiotic associations between native rhizobia and temperate Australian legumes: interactions within and between genera." Journal of Applied Ecology **37**: 52-65.
- Thrall P.H., J.F. Slattery, L.M. Broadhurst and S. Bickford (2007). "Geographic patterns of symbiont abundance and adaptation in native Australian Acacia-rhizobia interactions." Journal of Ecology **95**: 1110-1122.
- Torchin M.E., K.D. Lafferty, A.P. Dobson, V.J. McKenzie and A.M. Kurlis (2003). "Introduced species and their missing parasites." Nature **421**: 628-630.
- Usman S., S.P. Singh, Y.S. Rawat and S.S. Bargali (2000). "Fine root decomposition and nitrogen mineralisation patterns in *Quercus leucotrichophora* and *Pinus roxburghii* forests in central Himalaya." Forest Ecology and Management **131**: 191-199.
- van der Putten W.H., J.N. Klironomos and D.A. Wardle (2007). "Microbial ecology of biological invasions." ISME Journal **1**: 28-37.
- Vierling E. (1991). "The roles of heat shock proteins in plants." Annual Review of Plant Physiology **42**: 579-620.
- Visser J. (1981). South African Parasitic Flowering Plants. Capetown, Juta & Co.
- Vitousek P.M. (1990). "Biological invasions and ecosystem processes: Toward an integration of population biology and ecosystem studies." Oikos **57**: 7-13.
- Wallace A., E.M. Romney and G.V. Alexander (1978). "Mineral composition of

Cuscuta nevadensis Johnston (dodder) in relationship to its hosts." Plant and Soil **50**: 227-231.

Watling J.R. and M.C. Press (2001). "Impacts of infection by parasitic angiosperms on host photosynthesis." Plant Biology **3**: 244-250.

Watt M.S., P.W. Clinton, D. Whitehead, B. Richardson, E.G. Mason and A.C. Leckie (2003). "Above-ground biomass accumulation and nitrogen fixation of broom (*Cytisus scoparius* L.) growing with juvenile *Pinus radiata* on a dryland site." Forest Ecology and Management **184**: 93-104.

Weber J.Z. (1981). "A taxonomic revision of *Cassytha* (*Lauraceae*) in Australia." Journal of Adelaide Botanic Gardens **3**: 187-262.

Whelan R.J. and J. York (1998). "Post-fire germination of *Hakea sericea* and *Petrophile sessilis* after spring burning." Australian Journal of Botany **46**: 367-376.

Wilkinson H.H. and M.A. Parker (1996). "Symbiotic specialization and the potential for genotypic coexistence in a plant-bacterial mutualism." Oecologia **108**: 361-367.

Wills T.J. and J. Read (2002). "Effects of heat and smoke on germination of soil-stored seed in a south-eastern Australian sand heathland." Australian Journal of Botany **50**: 197-206.

Wolfe B.E. and J.N. Klironomos (2005). "Breaking new ground: Soil communities and exotic plant invasion." BioScience **55**: 477-487.

Wood C.L., J.E. Byers, K.L. Cottingham, I. Altman, M.J. Donahue and A.M.H. Blakeslee (2007). "Parasites alter community structure." PNAS **104**: 9335-9339.

Zammit C. and M. Westoby (1987). "Seedling recruitment strategies in obligate-seeding and resprouting *Banksia* shrubs." Ecology **68**: 1984-1992.