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Acacia longifolia and gall networks in Portugal: Understanding the impacts and the implications for biocontrol

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Sérgio Diogo Costa Ribeiro

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Grande árvore é o carvalho
Dá quatro castas de fruto:
Bugalhos e bugalhinhos,
Bolotas e maçãs de cuco.

Provérbio popular

</div>

Tal como o carvalho, grande à lei da sabedoria popular por dar quatro castas de "fruto", grandes foram as pessoas que me acompanharam ao longo desta jornada e que me deram bastante e às quais deixo aqui o meu agradecimento.

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Abstract

Trichilogaster acaciaelongifoliae is a gall forming wasp native to Australia that is being considered as a biocontrol agent for *Acacia longifolia*, an invasive species with a long record of severe ecological impacts, in Portugal. This wasp has been introduced in South Africa, more than 20 years ago, controlling *A. longifolia* with great success and proving to be a highly specific agent, with no direct non-target effects. In Portugal, direct non-target tests showed promising results. Nevertheless, indirect non-target impacts are also possible, even if seldom considered in biocontrol programs. In order to make such evaluation, a thorough assessment of all possible biotic interactions with the biocontrol agent in the introduced range is needed. In this study, ecological networks quantifying the interactions between plants, gall formers, their parasitoids and gall inquilines were constructed in order to: 1) build the reference situation before the introduction of the biocontrol agent *T. acaciaelongifoliae*; 2) predict indirect non-target effects before introduction of the agent and 3) evaluate the current impacts of *A. longifolia* on gall former communities. For that, five sites were sampled for galls monthly, during six months. In each visit, three 20x2m transects were selected along a gradient of *A. longifolia* (< 30%; 30-69%; > 69%). Galls were monitored in laboratory conditions for the emergence of insects which were identified up to order or family. Tri-trophic networks of plants, gall formers and inquilines/parasitoids were constructed for each levels of invasion, for each site and a general network comprising all information. Species level parameters (degree, species strength and d' - specialization) were calculated for the general network and network level parameters (links per species, connectance, web asymmetry, interaction strength asymmetry, interaction evenness, H_2'-specialization and robustness) were calculated for the networks of the three levels of invasion. Thirty seven different gall formers were found inducing gall development on leaves, flower and vegetative buds, inflorescences and stems, on 19 different plant species. Gall formers are highly specialized entities, and gall former sharing was only observed within plants of the same genus, namely *Quercus* spp., *Cytisus* spp. and *Rosa* spp. With 11 different gall formers, *Quercus coccifera* was the plant with higher gall former diversity. *Quercus suber* was the only species with a low d'-specialization since none of its gall formers was exclusively dependant on it. No gall formers were found on any exotic plant species, and on native species no galls similar to *T. acaciaelongifoliae*'s were observed, thus encouraging the prospects of a safe biological control program. Quiaios dunes is suggested as the best place for the first introduction of the biocontrol agent since dunes are the preferential habitat of *A. longifolia* and since it has the lowest number of gall former species and a community with lower probability of interacting with *T. acaciaelongifoliae*, likely facilitating the establishment of the biocontrol agent. *Acacia longifolia* invasion was associated with declines in the abundance of gall formers and its communities, and with higher web asymmetry and robustness. This study did not reveal any particular reason of concern regarding the introduction of *T. acaciaelongifoliae*, instead it suggests that the control could alleviate the significant negative impacts of the invasive *A. longifolia*. The detail of this study is seldom, if ever, achieved as an *a priori* planning tool representing a clear step forward in the safety of biological control programs. These results envisage the likely introduction of *T. acaciaelongifoliae* as safe and needed.

Key-words: invasive species, biocontrol, gall former communities, ecological networks.

Resumo

Trichilogaster acaciaelongifoliae é uma vespa formadora de galhas originária da Austrália cuja introdução como agente de controlo biológico para *Acacia longifolia*, uma espécie invasora com severos impactes ecológicos, em Portugal, está a ser considerada. O agente já foi introduzido com sucesso na África do Sul, há mais de 20 anos, controlando a mesma com grande sucesso e sendo bastante específico. Em Portugal, testes de efeitos diretos revelaram resultados promissores, no entanto, efeitos indiretos são uma possibilidade mesmo que raramente considerados em programas de controlo biológico. Para ser feita essa avaliação, uma análise das possíveis interações bióticas com o agente na zona introduzida é necessária. Redes ecológicas quantificando interações de comunidades associadas a galhas foram construídas com o objetivo de: 1) criar a situação de referência antes da introdução do agente; 2) prever efeitos indiretos não-alvo antes da mesma e 3) avaliar o impacto da *A. longifolia* em comunidades de galhas. Para tal, cinco locais foram usados para amostragem de galhas mensalmente durante 6 meses. Em cada visita, três transectos de 20x2m foram selecionados ao longo de um gradiente de *A. longifolia* (< 30%; 30-69%; > 69%). As galhas colhidas foram monitorizadas em laboratorio para aguardar pela emergência de insetos que foram identificados até à ordem ou família. Redes de três níveis de plantas, formadores de galhas e inquilinos/parasitoides foram construídas cada nível de invasão, para cada local e uma rede geral contendo toda a informação. Parâmetros de redes ao nível das espécies (degree, species strength e d' - specialization) foram calculados para a rede geral e parâmetros ao nível da rede (links per species, connectance, web asymmetry, interaction strength asymmetry, interaction evenness, H_2'-specialization e robustness) para as dos níveis de invasão. Trinta e sete formadores de galhas diferentes foram encontrados induzindo a formação de galhas em folhas, gemas florais e vegetativas, inflorescências e ramos, em 19 plantas diferentes. Os formadores de galhas são muito especializados e partilha de formadores foi somente observada em espécies pertencentes ao mesmo género (*Quercus* spp., *Cytisus* spp. e *Rosa* spp.). Com 11 formadores de galhas *Quercus coccifera* foi a planta mais diversidade. *Quercus suber* foi a única espécie com uma baixa d'-specialization por nenhuma dos seus formadores de galhas ser dependente dessa espécie. Não foram encontrados formadores de galhas em plantas exóticas; e em plantas nativas nenhuma galha semelhante à do *T. acaciaelongifoliae* encorajando as perspetivas de um controlo biológico seguro. As dunas de Quiaios são sugeridas como o melhor local para as primeiras introduções do agente uma vez que as dunas são o habitat preferencial da *A. longifolia* e por ter o menor número de formadores de galhas e uma comunidade com menor probabilidade de interação com o agente, facilitando o seu estabelecimento. A invasão de *A. longifolia* está indiretamente associada a declínios na abundância de formadores de galhas e suas comunidades, e a uma maior web asymmetry e robustness. Este estudo não revelou qualquer motivo de preocupação em relação à introdução do *T. acaciaelongifoliae*, em vez disso, sugere que o controlo poderia atenuar os impactes negativos da *A. longifolia*. O detalhe deste estudo é raramente alcançado o que representa um claro passo em frente na segurança de programas de controlo biológico. Estes resultados preveem uma provável introdução do *T. acaciaelongifoliae* como segura e necessária.

Palavras-chave: espécies invasoras, controlo biológico, comunidades de formadores de galhas, redes ecológicas.

Chapter 1 – Introduction

1.1.   Introduction to biological invasions

Species are not static in space. It is part of their nature to disperse themselves and colonize other areas. It is a natural process that has always occurred on the history of life on Earth and is conditioned by the availability of resources as well as geographical and climatic barriers (DiCastri 1989, Vitousek et al. 1997a). However, the reason why we have Australian acacias (*Acacia* spp.) in Portugal, European zebra mussel (*Dreissena polymorpha*) in North America, and short-tailed weasels (*Mustela ermine*) in New Zealand that are most commonly seen in Eurasia and North America, eludes this assumption. Humans, intentionally or not, play an important role moving species from their natural ranges into new areas where they can become severe invaders as the examples referred above (Vitousek et al. 1997b).Thus, to distinguish between natural expansion of species and the current problem of biological invasions worldwide, modern Ecology defines Biological Invasions as the spread of non-native species that become problematic in one way or another, in new geographical ranges after their transport and introduction by humans(Hochberg and Gotelli 2005).

The process of invasion by non-native species follows several stages (fig. 1), from transport to spread, that initiates with the overcoming of the geographical barrier and the consequent move outside its natural range (Richardson et al. 2000, Richardson and Pyšek 2012). By overcoming geographical barriers and reaching a new region the species is called exotic (syn. alien, introduced, non-indigenous, non-native), simply by being outside its native range. The majority of exotic species need human help to survive and reproduce like corn (*Zea mays* L.) and the potato plant (*Solanum tuberosum* L.), but a small fraction of these can establish beyond the area of introduction, reproducing persistently and forming populations that stay in equilibrium with the native ecosystem, without human help. These species are called naturalized and can stay in equilibrium for a variable period of time.

In a fraction of naturalized species, the equilibrium can be interrupted by any phenomenon that facilitates the quick growth of the population, which can happen shortly after introduction (Burlakova et al. 2006) or after a long lag period (Rilov et al. 2004). The stimulus that breaks this equilibrium can be a natural disturbance like the occurrence of a storm, climatic changes or fires, or an anthropogenic disturbance like changes in land use and even the control of another invasive species. Such species spread over a considerable area, often producing offspring in very large numbers, and are now considered invasive species. The approximate scales for the spread of a naturalized plant to be considered invasive are 100m in less than 50 years for taxa spreading by seeds and other propagules and 6m in 3 years for taxa spreading by roots rhizomes, stolons or creeping stems (Richardson et al. 2000).
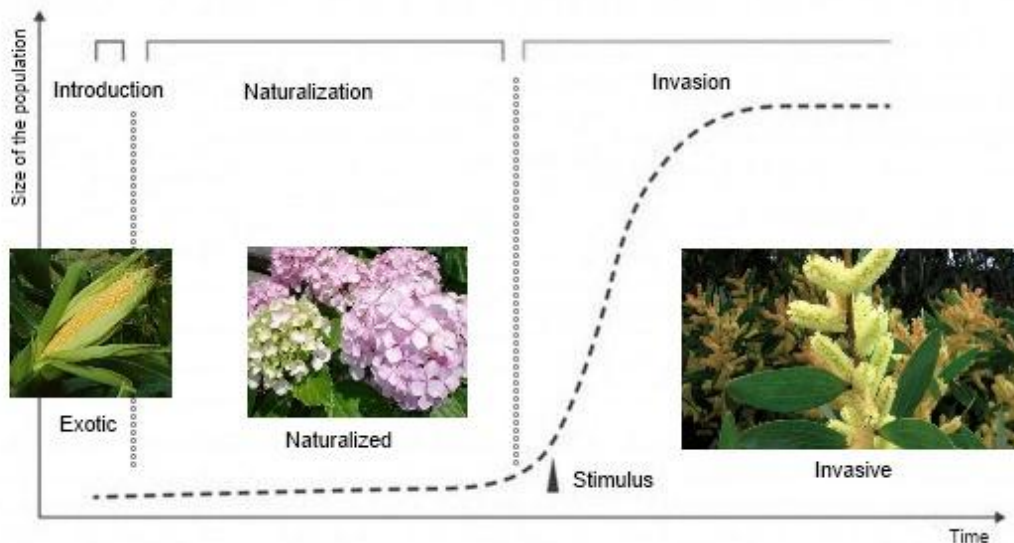
Figure 1. Main stages in the process of biological invasions (adapted from Marchante et al. 2014). Examples according to their status in Portugal: *Zea mays* L. (on the left, photo from: deeproot.co.uk), *Hydrangea macrophylla* (Thunb.) Ser. (on the centre, photo from: nossascozinhas.blogspot.pt), *Acacia longifolia* (Andrews) Willd. (on the right, photo from: invasoras.uc.pt)

The increase in the distributions of an invasive species depends, among other factors, on the characteristics of the invaded sites. Generally, well preserved habitats are more resistant to invasions but with global changes it is likely that disturbances facilitating biological invasions become more frequent, accelerating and aggravating those processes (Jeschke and Genovesi 2011).

Only a small fraction of new coming species are able to survive and become invasive. But those which succeed, that can reproduce without human assistance, are self sustaining and can disperse far away from the initial introduction point, they begin to interact with native species and frequently cause innumerous negative impacts (McNeely et al. 2001, Blackburn et al. 2014).

1.1.1. Causes of biological Invasions

There are several ways a species can reach a new area and numerous paths it can take, but when we talk about breaking the climatic and geographical barriers that naturally prevent the indiscriminate movement of species, there is a common denominator – humans (Vitousek et al. 1997a, Mack et al. 2000). This transfer of species from one region to another is undertaken since immemorial times, but since the huge growth of the human population, the intensification of trade and other activities, and more recently the advances in technology, it is definitely done in a faster pace and in greater quantities (Batabyal and Beladi 2006, Perrings et al. 2010).

With the great navigations and the European colonization of the New World, in the end of the 15th century, in what Alfred Crosby calls the "biological expansion of Europe", the first cases of

biological invasions appeared. He refers to this "expansion" as being responsible for excluding and even eliminating native fauna and flora from different regions of the world giving rise to "Neo-Europes", namely Australia, New Zealand and America (Crosby 1986). Recognized by many authors, The European Imperialism is a milestone in the history of species transfers, in which botanical institutions, zoos, naturalists, foresters and also anonymous carriers involved in the cultivation of plants and maintenance of the animals, played important roles in the introduction of species to new geographical ranges (DiCastri 1989, Baskin 2003, Wilson et al. 2009).

Particularly in the last half century, transportation systems have greatly improved, connecting even more the international markets, leading to distant regions being now more linked than ever (Hulme 2009). And as the volume of commercial movements increases, the same happens with species introduction (Costello et al. 2007). Non-indigenous species can be introduced intentional or unintentionally (Mack et al. 2000, Levine 2008). Most plants were deliberated introduced for agriculture and for ornamental purposes (Sanz-Elorza et al. 2009), while animals were introduced for farming, aquaculture, hunting/fishing and as pets, with some intentionally released to the wild and others escaping captivity or domestication (Nentwig 2007).

Unintentional introductions often occur when organisms, such as algae, plankton and larvae, are transported via ballast water and its sediments by ships (Carlton and Cohen 2003), or insects and pathogens that are carried with natural products (Nentwig 2007, Levine 2008). But even a simple tourist can be a major player in moving organisms from one region to another. Also, anthropogenic corridors, like roads and railways, play an important role on the spread of species (Hulme 2009, Wilson et al. 2009).

Other human actions can promote biological invasions: deforestation, urbanization and the creation of ruderal areas, together with habitat fragmentation (Vitousek et al. 1997a, McNeely 2006). Climate change through, for example, alteration of plant phenology or distribution of territorial and aquatic taxa, can also contribute to the invasion by exotic species (Parmesan 2006).

Nevertheless, the success of an invasive species cannot be explained only by human activities. Undoubtedly, it helps to understand 'why' we have biological invasions, but the 'how' is much more complex. The first studies of species with an invasive potential allowed defining a series of characteristics that are recurrent among invasive species along the globe. Many plant invaders share specific functional traits such as ability for clonal reproduction, high specific leaf area, rapid maturity or high production of seeds (Rejmánek et al. 2005). Nowadays, thanks to the increasing number of studies, new processes of invasion were discovered supporting the idea that biological invasions are idiosyncratic, that any successful invader is a success by its own reasons (Williamson and Fitter 1996) focusing either on the invasive ability of the species (invasiveness) or on the susceptibility of recipient communities to invasions (invasibility) (Lonsdale 1999).

1.1.2. Consequences of biological invasions

Biological invasions are recognized as one of the most important causes for ecosystem degradation and biodiversity loss worldwide (Vitousek et al. 1996, Mack et al. 2000). Invasive species can threaten biodiversity in many ways, by causing changes at every level of ecological organization, from genes to landscape (Van der Velde et al. 2006, Ehrenfeld 2010).

At the ecosystem level, impacts include alteration of pools and fluxes of nutrients and water (biogeochemical cycles), of energy flow through food webs and of availability of resources (Marchante et al. 2008a, Ehrenfeld 2010). These impacts can be driven by mechanisms of competition, disease (e.g. insects that act as vectors of pathogenic bacteria) and allelopathy (Van der Velde et al. 2006, Levine 2008). They can also be responsible by alterations in the fire regimes by changing fuel loads (Brooks et al. 2004), in species succession (Van der Putten et al. 2000), in the seed banks (Marchante et al. 2010) and in the relations between native plants and pollinators (Charles and Dukes 2007).

Ultimately, mechanisms of genetic changes as a consequence of hybridization between closely related invasive and native species or alteration of gene flow of native species can cause major evolutionary impacts by preventing allopatric speciation (Mack et al. 2000, Montesinos et al. 2012).

Biological invaders have also socio-economic consequences by afecting public health, provoking alergies, diseases and being vectors of plagues (Hatcher et al. 2012). Aditionaly, they cause massive economic losses by invading areas of agricultutal, florestal and aquacultual production and areas of conservation interest, implicating not only the production itself but by generating great costs in the application of management measures (Perrings et al. 2005, Binimelis et al. 2007).

Although some of these effects caused by invasive species occur by other means, like loss of biodiversity, they contribute severely for global changes and lead to a worldwide homogenization that cannot be overlooked (D'Antonio and Vitousek 1992, McKinney 2004).

1.2. *Acacia longifolia* invasion in Portugal

Acacia is a broad genus of trees and shrubs belonging to the family Fabaceae, sub-family Mimosoidae, with over 1300 species (Maslin et al. 2003). They spread around tropical and warm-temperate regions, thriving in different habitats from rainforests to deserts, from costal to subalpine regions, encompassing grasslands, forests and woodlands (Orchard and Wilson 2001). Over the last two centuries, acacias have been introduced in many parts of the globe becoming very well established in their new locations (Kull and Rangan 2008, Richardson et al. 2011). Among the close to 1000 acacia species that are native to Australia and have been introduced elsewhere, 71 species are naturalized or weedy and 23 are unequivocally invasive (Richardson et al. 2011).

From the late 19th century to mid-20th century, several acacia species were introduced, systematically planted and disseminated in Portugal by both governmental and private foresters mainly for reforestation and wood supply (Kull et al. 2011). This resulted in the 13 Australian species and 1 from South Africa nowadays present in the Portuguese flora (Almeida and Freitas 2006) with the majority being invasive with use and planting restricted by law (Ministério do Ambiente 1999)

Acacia longifolia (fig. 2 a), originated from southeast Australia, was largely planted in the Portuguese territory during the late 19th century and the beginning of the 20th. This species was introduced for ornamental purposes and in coastal dunes to curb sand movement and prevent erosion (Marchante et al. 2003, Marchante et al. 2008a, Kull et al. 2011). Since then, mostly because of the ability to germinate and colonize after fires, it has spread greatly in dune systems (fig. 2 c) being today the most dispersed invasive species in coastal areas of central-north of Portugal. It also occurs in the margins of roads and streams, as well as some mountain areas (Marchante et al. 2014).

Figure 2. a) *Acacia longifolia* close up showing the laminar phyllodes and the yellow flowers gathered in axillary spikes b) mature pods showing the seeds which are fire stimulated and can remain viable for more than 5 years c) dune systems are the preferential habitat of *A. longifolia* d) inside of an invaded area showing absence of other plant species. Photos from: invasoras.uc.pt.

Acacia longifolia has high growth rates, seed germination is stimulated by fire, produces a great amount of seeds that form seed banks with more than 1500 seeds/m^2 and that can remain viable for more than 5 years (fig. 2 b) (Marchante et al. 2010). It also produces a vast

amount of litter rich in nitrogen that induces changes in soil composition and microbiology (Marchante et al. 2008a). Moreover, it forms dense stands that outcompete native vegetation and hampers its recovery (fig. 2 d), reduce the flux of water lines and increase the fuel load causing changes in fire regimes (Marchante et al. 2011b). Adding to this, *A. longifolia* possesses a high resistance to water and salt stress, which in a region with Mediterranean climate with season drought periods is an incredible adaptive mechanism (Morais and Freitas 2012, Morais et al. 2012).

In an attempt to mitigate the numerous impacts of *A. longifolia* in Portuguese landscape, current control relies on mechanical methods, which consists in hand removal of seedlings and the cut of adult individuals (Marchante et al. 2005, Marchante et al. 2008b). These methods work if they have follow-up, essential to account for the new germination and/or burst of shoots, which can result in a rapid reinvasion of the controlled area (Le Maitre et al. 2011). Still, these methods have huge associated costs and workforce, often failing to reach a lasting control, mainly because of the abundant seed banks (Marchante et al. 2010). In this context, a biological control agent which reduces the seed bank may be a promising option for the control of this species in the near future (Dennill et al. 1993).

1.3. What is biological control?

Biological control, or biocontrol, is defined as the reduction of pest populations by natural enemies that involve an active human role (McFadyen 1998, Bale et al. 2008). In this definition it is important to underline the pest concept and the human aspect because all species are suppressed by naturally occurring organisms and environmental factors, with no human intervention, which is referred as natural control (Solomon 1949). In the case of invasive plants, which thrives in a new range that is free of its natural enemies, the objective of biocontrol is to reduce the vitality or reproductive potential of the plant ultimately reducing their abundance to values below the damage threshold (Hoddle 2004).

Biological control does not aim for, nor is it likely to achieve complete eradication of the target invasive plant. At best, a successful program will reduce the vigor and the abundance of the weed to a point in which reduces economic losses and impacts to environment (below the damage threshold; fig. 3) (Culliney 2005). Following introduction and establishment, populations of biological control agents build up to very high levels due to the abundance of their host plant. Over time, as the natural enemy population increases, a significant stress is generated, causing the invasive population to decrease. This, in turn, leads to a decline in the numbers of biological control agents until equilibrium is reached between the amount of damage caused by the agent and regeneration by the weed. In other words, there will always be a few invasive plants on a given site, but there will also be a few biocontrol agents to keep the equilibrium below the damage threshold the ecosystem can tolerate.
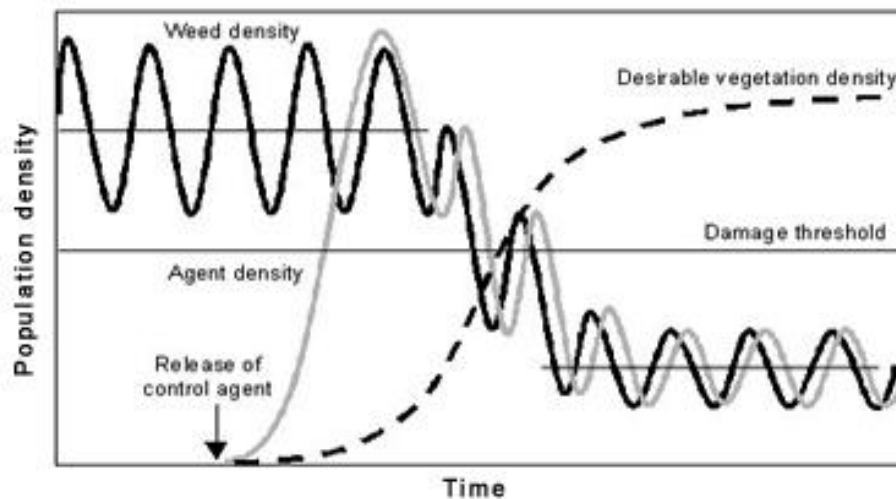
Figure 3. Best case scenario for the introduction of a biocontrol agent, considering the population densities of the introduced agent, the target invasive plant (weed) and other plant species. Photo from: csiro.au

The advantages and limitations of biological control are often expressed by comparison with pesticides (or chemical control) (Culliney 2005, Bale et al. 2008). These last ones are less specific, species often develop resistance to them (Manosa et al. 2001), and the effect are limited to the area where they were released, implying that frequent application may be required, increasing the risk of pollution and to human health (Culliney 2005). Biological control, on the other hand, uses natural enemies that actively seek out their prey and can increase the level of control over time, it is unlikely that resistance will develop to a biocontrol agent and in many cases the control can be self-perpetuating over long periods of time.

Cost–benefit analyses suggest that research on biological control is more cost effective than on chemical control (Tisdell 1990, Zimmermann et al. 2004). Additionally, when well succeeded, biological control is a very inexpensive method that allows a reduction of the intended populations and its maintenance at low levels that are less able to cause economic and conservations losses. In the case of control of invasive plants this is a method that does not disturb the soil nor create large clearing areas, as it does not kill the invasive population all at once. Instead, allows a gradual recovery of other species in the areas created by the individuals that perish (Hoddle 2004).

The main limitations of biological control is that is generally slower to suppress the target populations than most pesticides (because it may take some time to establish and for the first results to become visible), and the risk of selecting agents that interfere with the equilibrium of non-target species (McFadyen 1998, Culliney 2005). But in spite of the concerns for introductions of biocontrol agents being rightful, even some skeptics agree that in situations of biological invasions where an entire community is affected, biological control is the only viable alternative for management of some species (Thomas and Willis 1998, Marchante et al. 2011a). From a view of ecological costs to nature, particular invasions are so devastating that it is inconceivable that unintended consequences of a biological control introduction could be worse (Simberloff and Stiling 1996, Dean et al. 2012). The idea is that entire communities will be lost anyway if nothing is done. However, and despite some very bad examples in the past

(Phillips et al. 2006), nowadays all precautions are taken to prevent the introduction of biocontrol agents that can become disastrous.

Overall, when all precautions are taken biocontrol is long lasting, self-sustaining, environmentally friendly and low-priced. Other than the initial costs of collection, testing, importation and rearing, little expense is incurred. And for the particular case of the biocontrol for *A. longifolia,* where the introduction of an agent (*Trichilogaster acaciaelongifoliae*) is being considered (Marchante et al. 2011a) the costs and the whole process are simplified because it bypasses the first step of going to the native habitat in search of natural enemies suitable to be used as biocontrol agents. That selection was already performed by van den Berg in 1979.

1.3.1. Trichilogaster acaciaelongifoliae

All the known species of the pteromalid genus *Trichilogaster* (Hymenoptera: Chalcidoidea) are associated with galls on acacias. Different species induce galls on different acacias, and most species are native to Australia (Prinsloo and Neser 2007). *Trichilogaster acaciaelongifoliae* (Froggatt) (fig. 4 a) induces gall development in *A. longifolia*, deposit its eggs within immature flowering buds thereby inducing gall development, that ultimately prevent the formation of flowers and seeds (Dennill and Donnelly 1991, McGeoch and Wossler 2000).

Because of this characteristic *T. acaciaelongifoliae* was already introduced with success, in 1982, as a biocontrol agent in South Africa, where *A. longifolia* is also an invasive species (Dennill et al. 1993). Besides reducing the production of seeds by more than 75%, the galls of *T. acaciaelongifoliae* acted as nutrient sinks and, as a consequence, limit both the reproductive and vegetative development of *A. longifolia* causing the death of branches, and even entire plants when the environmental conditions are harsh. *Acacia longifolia* mortality was recorded to be around 35% in some sites (Dennill 1990).

Before introduction of this agent, *A. longifolia* was considered one of the worst invasive species in South Africa but nowadays is rarely mentioned as a problematic species, an accomplishment that is attributed to a successful biocontrol that by forming its galls reduces the reproductive potential of the plant and increases its stress, lowering its biomass and competitive ability (Veldtman et al. 2011).

The life cycle of *T. acaciaelongifoliae* is annual. The insect spends the great majority of its time inside the gall (fig. 4 b-d) (approximately 362 days of a 365 days life cycle), in the long-lasting pupal and larval stages. Almost in the end of the cycle, it will eat its way out of the gall and emerge as free-living adult, a phase that corresponds to only 2-3 days and if they do not find a host within that period of time they will die without laying their eggs (van den Berg 1979).

Adults are mostly females that reproduce by thelytokous parthenogenesis – only female offspring is produced (Noble 1940, Rabeling and Kronauer 2013) reducing the chances of hybridizing with native species. They are about 3 mm long, highly fecund organisms that produce an average of 409 eggs and are able to begin posture as soon as they exit the gall (Dennill 1988). Females are not active flyers and because of its short adult life, often lay their

eggs in the proximity of the galls they emerge from (van den Berg 1979, Dennill 1988). The potential loss of control of the introduced populations is limited because of this set of characteristics.

Figure 4. a) Adult *Trichilogaster acaciaelongifoliae* female (photo from: J. H. Hoffmann) b) mature *T. acaciaelongifoliae*'s galls on *A. longifolia* (photo from: wikipedia.org) c) *T. acaciaelongifoliae*'s galls after the emergence of insects (photo from: H. Marchante) d) cross section of *T. acaciaelongifoliae*'s gall (photo from: H. Marchante).

The safety of *T. acaciaelongifoliae* as a biocontrol agent is significantly assured by the degree of specialization and monospecificity to its host. Without the presence of *A. longifolia* it will not be able to produce galls, a vital step for its survival. The ability of this insect to produce galls in other species was already tested with gall formation only observed for *A. longifolia* (Neser 1982, Marchante et al. 2011a).

These findings and the fact that no negative effects were seen in South Africa after close to 30 years (Impson et al. 2011) suggest that the use of *T. acaciaelongifoliae* in Portugal could be a good choice to control *A. longifolia.* Presently, an official permit to introduce is pending on Portuguese authorities. Nevertheless, indirect non-target effects have not been studied up to now, so could a new gall former introduced in the Portuguese ecosystems potentially interact with native gall communities?

1.4. What is a gall?

Galls represent just one facet among thousands of ways plants and other organisms interact with each other. They are known for thousands of years since Classical Greece, where the first references come from the use of the Aleppo gall in medicine by Hippocrates (about 460-377 BC). But it was only with Marcello Malpighi (1628-1694), that galls were known to be caused by other organisms using the plant as a source of food, modifying its structure to accomplish its own needs (Redfern 2011). That is very much the current definition of gall: tumorlike growths produced by the host plant in response to a mechanic or chemical stimulus promoted by other organism (Russo 2006).

The gall is a structure of the plant modified in such a way to benefit the causing organism, and not just a healing response to the wound, although it is not always easy to distinguish between the two. All galls involve additional tissues, in the form of enlarged or additional cells or enhanced vascular tissues, which provide nutrients for the gall former (Redfern and Shirley 2002). Additionally it may provide refuge from harsh environments, including protection against enemies (Stone and Schönrogge 2003). In order to enable the formation of galls, cells must be omnipotent to be able to develop into any type of tissue intended by the gall former. Such cells are meristematic and can be found in leaves, flowering buds, cambium of young stems and roots. In some cases the gall former can induce older cells to de-differentiate and to revert to a more juvenile state, and then develop into new tissues. These cells, affected by the accelerated production of plant growth hormones, will either multiply in abnormally high numbers or develop into a greater size than normal (Redfern 2011, Malinowski et al. 2012). However, the exact mechanisms of gall formation vary greatly among different groups of gall formers.

Most of the advantages are to the gall former that receives food and shelter, while the host plant is the victim of parasitic interaction. Nevertheless, there are a few galls that benefit the host plant. Most fig wasps cause galls in the ovaries of the "fig fruit" and are essential for the fig tree survival (West et al. 1996, Redfern 2011). Another example of mutualism involves bacteria in root nodules, e.g. *Rhizobium* and their host legumes (Fabaceae), since both intervenient benefits from the interaction although neither is completely dependent on the other (Redfern and Shirley 2002, Redfern 2011).

Gall formers include insects, bacteria, viruses, fungi, nematodes, mites and even flowering plants like mistletoes (Viscaceae) and dodders (Cuscutaceae) (Redfern 2011). It is not well studied yet, but galls can also be caused by rotifera on algae (Wallace et al. 2001). As a result of different gall formers, hosts and affected organs, many different galls can be found in nature. They vary from simple dilated cells on algae to complex multicelular growths with the most distinct shapes and sizes; and from simple swellings, cavities, leaf rolls and folds to closed structures and highly organizes that involve several types of tissues. The most complex galls are induced by insects, particularly by gall midges (Diptera: Cecidomyiidae), gall flies (Diptera: Tephritidae) and gall wasps (Hymenoptera: Cynipidae) (Nyman et al. 2000, Russo 2006, Redfern 2011). Most of studies of galls involve insect galls only, particularly these last three groups.

From a plant perspective, gall formers are parasites, and as such evolution promotes resistance to the parasite. But even though the plant may produce defenses against gall causing organisms, generally, the overall vigor and health of the parasitized plant is not affected by the seasonal production of galls. However, localized damages can occur if a branch, group of leaves or even a single leave produces a large amount of galls (Russo 2006). Significant damages for the host plant depend on the:

1. Dimension of the plant – a younger plant may suffer more with the development of galls
2. Soil and environmental conditions in which the plant is growing – if a plant is already struggling with environmental conditions, if it is established in a poor soil and in a harsh climate, it will be more damaged by the production of galls.
3. Degree of infestation – the more galls are produced the more damages will be generated.
4. Type of the gall – some galls are more vicious than others.

Besides interacting with plants, there is often a whole community – a mini ecosystem – that develops within and around the gall.

1.4.1. The gall community

One of the reasons a gall former induces a gall is to escape predators. The gall former generally does not have a big life span outside the gall as it will spend its time mating and finding a spot to lay its eggs. Sometimes mating is not even a prerequisite as some species reproduce parthenogenically. When enclosed in the gall, the gall former has a very passive life style but it is the center of a community of specialists that depend on it. Besides interacting with the host plant, when it finds a spot where is able to deposit its eggs and they hatch, the larvae and the resulting gall can attract (Schonrogge et al. 1995, Hayward and Stone 2005):

1. Inquilines (herbivores) – are attracted by and feed on the succulent and nutritive tissues of the gall.
2. Parasitoids/Parasites – depending if they kill or not the host, respectively, they attack the larvae that produces the gall, and possibly inquilines.
3. Hyperparasitoids/Hyperparasites – attack the larvae of parasitoids/parasites.
4. Predators – e.g. birds or mammals that feed on any insect found in the galls despite these are not dependant on the galls unlike the others.

These communities can be so intricate that sometimes the organisms present polyphagous habits. One that feeds on the tissues of the gall can change its tactics and kill the gall forming larva, some parasitoids can also feed on the gall, and there are cases of parasitoids that besides affecting the gall former can also parasitize other parasitoids. These complex interactions networks have been explored (Kaartinen and Roslin 2011, Veldtman et al. 2011) but for few types of galls, almost exclusively caused by mites or insects, but little is still known about them.

1.5. Ecological networks

An ecological network is a representation of interactions in an ecosystem, in which species (nodes) are connected by pairwise interactions (links) (Pimm 2002, Montoya et al. 2006). In the last few years, many systems have been described as networks: food webs, protein networks, genetic networks, informatics networks, social networks, etc. (Bascompte 2007).

The most commonly described interaction in ecological networks is predation, through the creation of food webs (Woodward et al. 2005), but other types of interaction webs are increasingly being investigated, including a variety of trophic and non-trophic interactions such as pollination, seed dispersal, interference competition, habitat or shelter provisioning, and recruitment facilitation or inhibition (Menge 1995, Dicks et al. 2002, Bascompte et al. 2003, Allesina and Pascual 2008, Carvalheiro et al. 2008, Heleno et al. 2013a). These types of interactions can be divided in antagonistic (Pimm et al. 1991, Lafferty et al. 2008), involving predation, herbivory and pasasitism, or mutualistic (Olesen and Jordano 2002, Heleno et al. 2013b) such as pollination and seed dispersal.

Since the first food webs that appeared in scientific literature in the early 1900s (Lindeman 1942, Odum 1956, Borrett 2014), ecologist are discovering the rules by which ecological networks are organized, and how these rules help cherish biodiversity. By discovering early warning signs of networks in trouble, it is possible to predict eventual collapses and prevent them from occurring, something that would be impossible by looking only to individual paths (Memmott et al. 2007, Tylianakis et al. 2010). Being able to make predictions is a powerful asset intrinsic to ecological networks. How will an exotic species affect the structure of its new community? Which will be the first species to disappear? What will happen if we overexploit a species for food? By mapping the interactions of a certain community it is possible to study how threats (e.g. biological invasions, overfishing, etc.), mostly caused by human activities (Western 2001), are reorganizing these networks, and eventually putting them at risk of collapse.

Ecological networks are often described as complex since they represent objects (ecological communities) with complex dynamics (i.e., non-linear, sensitive to indirect interactions) (Pimm et al. 1991, Williams and Martinez 2000, Montoya et al. 2006). But complexity does not mean randomness, they are highly patterned (Pimm, 2002), illuminate the ecological mechanisms behind those patterns and allow a better understanding of the structure and function of ecosystems (Dunne et al. 2002). Although networks may miss important information, there is enough truth to allow the study of some of the features they represent (Pimm, 2002). But it was this complexity intrinsic to ecological networks, which as multi-faceted objects with a rich range of structure, that lead ecologists to look for emerging properties that can be easily measured and analyzed and that relate to ecological properties and processes.

Network thinking is not new to ecology (Bascompte 2007). There is increasing recognition that, in some cases, focusing on an sole species is not the only nor the best method to tackle ecological studies (Memmott et al. 2006). For example, the restoration of a given species will not be sustainable unless the ecological links with other species are also restored (Palmer et al. 1997). There is an increasing recognition that multispecies approaches are essential for

success, even when the conservation goals focus on protecting one single species (Schlapfer et al. 1999, Ehrenfeld 2000). By further exploring networks we are able to address how species networks, rather than pairs or single species, co-evolve (Bascompte 2007), a matter that marveled Darwin himself:

"*It is interesting to contemplate a tangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent upon each other in so complex a manner, have all been produced by laws acting around us. . .*" (Darwin 1859)

1.5.1. Ecological networks as a tool for predicting and evaluating the indirect effects of a biocontrol agent

Despite the current concerns to find highly specific biological control agents (McFadyen 1998, Hoddle 2004), the study of indirect effects of these agents is not common practice and is rarely considered (Carvalheiro et al. 2008). Indirect effects happen, for instance, when a biological control agent becomes a food source for natural enemies, like parasitoids, that include the new agent in their diets and become more abundant (Willis and Memmott 2005, Carvalheiro et al. 2008). Apparent competition, the competition due to shared natural enemies (Holt 1977), can then occur and have significant negative effects on the native biodiversity (Muller and Godfray 1997, Morris et al. 2005).

The ideal protocol to avoid/predict those negative effects is to make an assessment of what species are present in the target region and, based on information of the native range, predict what could interact with the biological control agent; this can be done using an ecological network approach (Carvalheiro et al. 2008). A number of difficulties about the non-existence of networks before the introduction of the agent have been reported, namely if any species has gone extinct in the process (Carvalheiro et al. 2008) or if species' abundance are significantly affected by the introduction (Dennill 1987). In such cases, those hypotheses can no longer be tested.

In the case of *T. acaciaelongifoliae*, considering that it is a gall former, the most likely scenario is to have species associated with galls interacting with it, namely parasitoids and inquilines. In South Africa *T. acaciaelongifoliae* formed new associations with native communities, but some of the parasitoids found had never been described which means that the original host was unknown as well as the effect of the introduction of an additional host on the populations dynamics (Manongi and Hoffmann 1995, McGeoch and Wossler 2000). Therefore in order to predict indirect effects is necessary to study gall communities from the introduced range. The resulting networks will be very useful to predict which species could interact with the biological control agent and its efficiency. Additionally, they will serve as a baseline to validate the predictions made before release and for future comparisons with the networks made after the release of the biocontrol agent.

More pre-release studies are needed in order to improve the predictive power of risk assessment tools, limit later concerns about unexpected non-target effects and to better manage the development of the biological control agent (McClay and Balciunas 2005, Veldtman et al. 2011). Network analysis, performed at a community scale, can be a valuable tools in biological control research, as they provide the tools to predict and assess negative impacts of introduced agents, by revealing how they will be integrated and affect the invaded community. However, is also important to realize that when invasive species have great and severe negative impacts eventual indirect effects can be outweighed by the positive effects of an effective biocontrol agent (Thomas and Willis 1998, Marchante et al. 2011a).

1.6. Thesis objectives

The main objective of this thesis is to establish a reference situation (t0) before the introduction of *T. acaciaelongifoliae* to control *A. longifolia* by identifying and quantifying the interactions among gall communities already present through the construction of ecological networks. With this information, predictions about the potential interactions between the gall former and its gall with parasitoids and inquilines will be made. It is expected that galls collected hold a community of gall formers, inquilines and parasitoids belonging to various orders and families. It is also anticipated the occurrence of a larger diversity of galls associated with *Quercus* spp. If galls morphologically similar to those formed by *T. acaciaelongifoliae*' are found, communities associated with them can be more prone to interact in some extension with *T. acaciaelongifoliae* after the release.

A second objective is to evaluate the impacts of *A. longifolia* in the native gall communities. *Acacia longifolia* has a long record of ecological impacts (Marchante et al. 2008a, Marchante et al. 2011b), drastically altering the invaded communities but the impacts on gall networks is yet unexplored. Since the introduction of a biocontrol agent that is a gall former is being evaluated this is a potentially important level to explore. Since *A. longifolia* has greatly altered invaded plant communities, it is expected that the galls and its communities are affected as well. In more invaded areas it is expected lower diversity of galls and consequently, communities with lower species richness (including parasitoids and inquilines) and smaller networks.

Besides the main objectives proposed for this study other contributions will arise from it:

1. Increased knowledge about native galls and associated communities, besides the ones that appear in *Quercus* spp. that are relatively known, particularly in Portugal.
2. The creation of the pre-release situation (native networks) for a posterior comparison with the situation after the release of *T. acaciaelongifoliae* to evaluate the impact of the agent and validate predictions, is a level of detail that is rarely attained to assess the safety of a biocontrol agent. In this context, this work will be a step forward in a better risk assessment of biocontrol agents and in a better monitoring of the evolution of the process.

Note: This thesis is included in the project INVADER-B (INVAsive plant species management in Portugal: from early DEtection to Remote sensing and Biocontrol of *Acacia longifolia*) that is planned for a whole year of samplings. The work presented in this thesis represents six months of samplings (August 2013 to January 2014) and for a portion of the sampling sites (5 of 11). The gall rearing and insect identification for this work ceased in February 2014, so emergences after that period are not presented. Therefore, this thesis is an important contribution for the outputs of the project, but the history to be told is still incomplete.

Chapter 2 – Materials and Methods

2.1. Experimental design – Sampling sites and methods

Four sites along the Portuguese coast were selected for gall collection: Pinhal do Rei in São Pedro de Moel, Quiaios Dunes, Mata Nacional das Dunas de Quiaios and Parque Florestal da Serra da Boa Viagem (fig. 5, table I). These sites were selected near the coast since this is where *A. longifolia* invades preferentially; selection was based on the presence of *A. longifolia* and of at least some species known to produce galls (e.g. oak forests), and relative proximity to the University of Coimbra. In addition, transects were run in an oak forest in Coimbra (fig. 5, table I; Escola Superior Agrária de Coimbra, ESAC) without *A. longifolia*; although currently this area does not include *A. longifolia*, its ecological conditions are suitable for the species; it was selected since this is an area with several *Quercus* species known to produce galls (Hayward and Stone 2005) and as such with higher probability for interactions with the biocontrol agent under study.

Legend
1 Pinhal do Rei – São Pedro de Moel
2 Quiaios Dunes
3 Mata Nacional das Dunas de Quiaios
4 Parque Florestal da Serra da Boa Viagem
5 Oak forest of Escola Superior Agrária de Coimbra
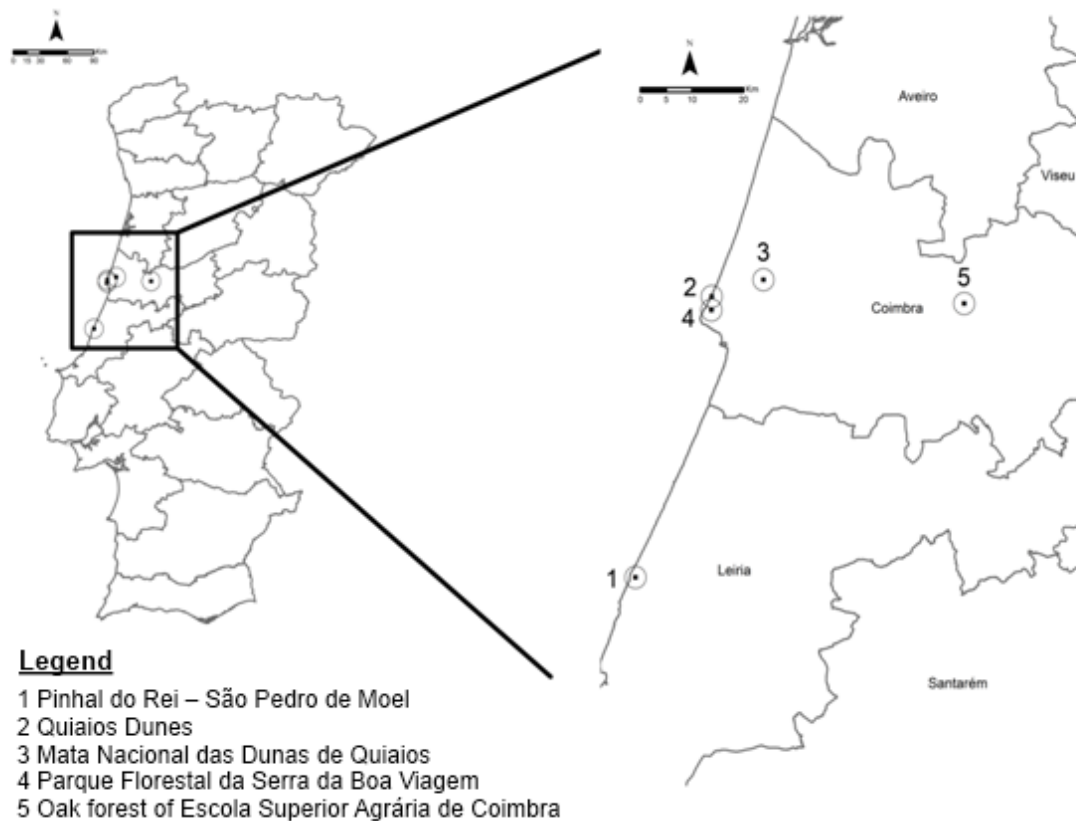
Figure 5. Study sites used for the collection of galls.

Table I. Location and general characterization of study sites.

Site	Code	Type of habitat/system	Dominant vegetation	Coordinates
Pinhal do Rei – São Pedro de Moel	SPM	Pine forest	*Pinus pinaster* **Acacia longifolia** *Cistus salvifolius* *Quercus coccifera* *Halimium calycinum* *Ulex* spp.	N 39° 45,612' W 009° 01,354'
Quiaios Dunes	QD	Primary dune	**Acacia longifolia** *Artemisia crithmifolia* *Corema album* *Carpobrotus edulis* *Crucianella maritima*	N 40° 13,402' W 008° 53,368'
Mata Nacional das Dunas de Quiaios	NFQ	Pine forest	**Acacia longifolia** *Pinus pinaster* *Corynephorus* sp. cf. *Acacia* melanoxylon *Ulex* spp.	N 40° 15,061' W 008° 47,955'
Parque Florestal da Serra da Boa Viagem	BV	Mediterranean shrubland	**Acacia longifolia** *Cistus* spp. *Pistacia lentiscus* *Ulex* spp. *Smilax aspera* *Olea europaea*	N 40° 12,059' W 008° 53,400'
Oak forest of Escola Superior Agrária de Coimbra	ESAC	Oak forest	*Quercus* spp. *Ulex* spp. *Arbutus unedo* *Pinus pinaster* *Ruscus aculeatus*	N 40° 12,707' W 008° 26,949'

In each site, three classes of invasion by *A. longifolia* were considered: low (0-29% of ground cover), medium (30-69%) and high invasion (70-100%). In each of these areas, one 20 x 2m transect was run each month between August 2013 and January 2014, the period when most galls are likely to form (Redfern 2011). As there are no *A. longifolia* invasion in the Coimbra oak forest, only the low invasion transects have been performed at this site. In total, 78 transects were run during the study period.

In each transect, all galls detected below 2m altitude (within reach) were collected and the position on the plant was registered. When the quantity of similar galls on the same host plant made collection impossible, these galls were counted and sub-sampled. Plant cover of all species was visually assessed in each transect and visit.

2.2. Gall storage and rearing

After collection, galls were maintained in plastic jars or bags, depending on the dimension and type of the gall (fig. 6 a-c). Galls were stored at room temperature and checked weekly for the emergence of insects. Galls were maintained and inspected for at least three months or until they rot or were severely infected by fungi. Collected insects were stored in individually labeled eppendorfs in 70% ethanol (fig. 6 d).

Figure 6. a, b & c) Different recipients used for gall storage d) Eppendorfs where emerged insects were stored with a solution of 70% ethyl alcohol.

2.3. Plant, gall formers and insect identifications

All plants were identified up to the species level following Bingre et al. 2007 for trees and shrubs, and Franco 1971, 1984 for other plants. Most gall formers were identified up to species or genus level using the galls, since each gall former induces a fairly singular and consistently similar gall that can be associated to the gall former (Redfern 2011, Redfern and Shirley 2002, Russo 2006). Due to this high correspondence between the gall type and species of gall former hereafter gall types are used as a proxy, and interchangeably referred to as gall former species.

As for insects, identification to the maximum resolution possible was performed, depending on the order and ease of identification (Goulet and Huber 1993, Barrientos 2004, Noyes 2014). Insect were sorted into morphospecies (individuals that have similar morphology and are probably members of the same species). Three guilds have been considered for the emerging insects: gall formers, parasitoids or inquilines. The guild of each species or morphospecies was identified based on the description of the family, since some families are recognized as including mostly a specific guild. For taxa with no available information, guild was attributed based on comparison with the closest genus with known feeding strategy.

2.4.   Construction of ecological networks

Tri-trophic, quantitative species-interaction networks were constructed for each site, for each level of invasion and for all pooled data. Each network comprised three levels: 1-Plants, quantified in terms of plant cover; 2- Gall formers, quantified by the number of galls observed; and 3-Parasitoids and Inquilines, quantified as the number of emergences from each gall. Networks were visualized with specific software written in Mathematica® 9.

To compare the networks from the three levels of invasion a variety of network structure descriptors were calculated, only for the first two levels (plants and gall formers) using the package bipartite 2.04 (Dormann 2008) for R (R Development Core Team 2014): links per species (mean number of links per species), connectance (realized proportion of all possible links), web asymmetry (balance between numbers in two levels where positive values indicate more lower-trophic species and negative values indicate more higher-trophic species), interaction strength asymmetry (quantifies the balance between the interaction strengths of species pairs), interaction evenness (Shannon's evenness of network interactions), H_2' (network specialization ranging from 0 (no specialization)) to 1 (complete specialization)) and robustness HL (rate of gall formers secondary extinctions after the primary extinction of plants).

Additionally, a series of species level descriptors have been computed to describe plant and gall former species interaction patterns, namely: species degree (number of interactions per species), species strength (sum of dependencies of each species, where dependencies are the proportion of interactions of each species to each of its partners) and d' - specialization (selectivity of the gall formers occurring on each plant species).

2.5.   Testing the impact of *A. longifolia*

The impact of *A. longifolia* invasion on species-interaction patterns was explored by using nested ANOVAS, with *level of invasion* nested within *site*, using the software SPSS 17.0.

Chapter 3 – Results

3.1. General gall network

A total of 6086 galls belonging to 37 different gall formers (fig. 7) were identified in the field from 19 plant species. Of these, 2183 galls were collected and their formers, parasites and inquilines reared in laboratorial conditions. Most galls were induced on leaves (16 species), followed by galls on stems (12 species), flower and vegetative buds (8 species), and inflorescences (1 species).

Overall, 130 plant species were identified along the transects. Of these, 7 species were exotic and invasive in Portugal. No galls were detected in any of the exotic species, including *A. longifolia* and other *Acacia* species, throughout the full duration of the study.

Of the species bearing galls, in average each plant species had 2,4 gall formers. *Quercus* species showed the highest number of gall formers varying from 3 to 11 depending on the species, only matched by *Ulex* spp. with 4 different galls (fig. 8, see degree). *Quercus coccifera* was not only the plant with more different galls found, but was also the host of the most abundant gall former (*Contarinia ilicis* cf.) (fig. 7 (13); fig. 9). In other plant species only one gall species, or occasionally two, were observed.

As can be seen by the high values of strength (importance of each plant species to the gall formers community) and d'-specialization (fig. 8) (which is maximum (=1) for many gall formers, as they interacted with a single plant species), the majority of gall formers induces gall development in just one plant species and only a few have a slightly wider spectrum of host species within the same genus. Only *Rosa*, *Cytisus*, and *Quercus* shared some gall formers, but always within species of the same genus. *Quercus suber* was the only species with a lower value of specialization, since it shares gall formers with several other species.

The 37 types of galls are created by 36 different gall former species, given that two of the currently presented types are induced by the same gall former, *Plagiotrochus quercusilicis,* in *Quercus coccifera* (fig. 7 (9 & 23)).These two interactions have been presented separately as they occur either in inflorescences or in leaves, inducing the formation of galls with different structures and that thus have different inquilines and parasitoids.
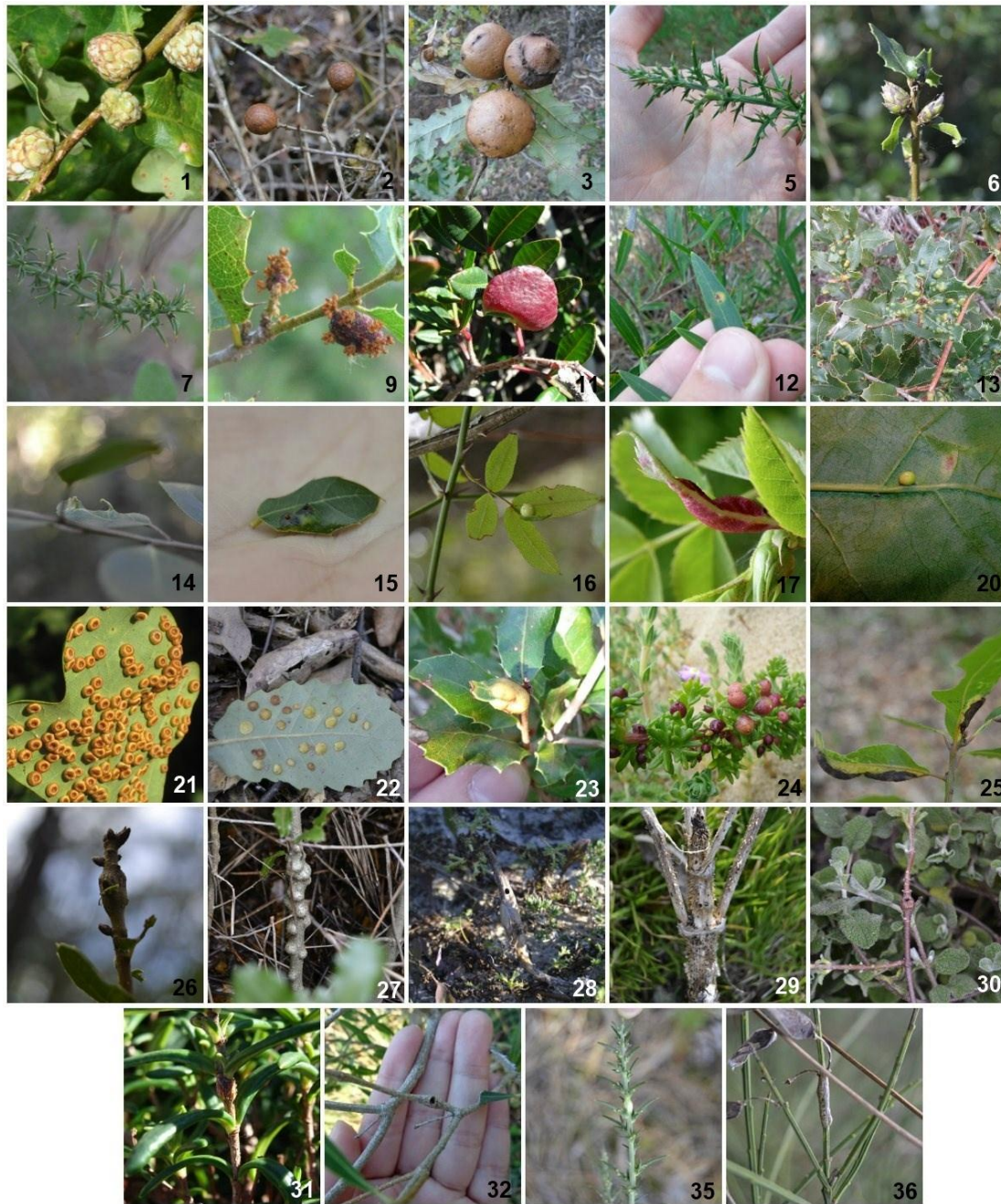
Figure 7. Some galls collected in the field. 1. *Andricus fecundator* on *Quercus faginea* 2. *Andricus kollari* on *Quercus robur* 3. *Andricus quercustozae* on *Quercus robur* 5. *Asphondylia ulicis* on *Ulex* sp. 6. *Phyllodiplosis cocciferae* cf. on *Quercus coccifera* 7. Undetermined on *Ulex* sp. 9. *Plagiotrochus quercuscilicis* fo. *fusifex* on *Quercus coccifera* 11. *Aploneura lentisci* on *Pistacia lentiscus* 12. *Braueriella phillyreae* cf. on *Phillyrea angustifolia* 13. *Contarinia ilicis* cf. on *Quercus coccifera* 14. Undetermined on *Rhamnus alaternus* 15. Undetermined on *Quercus coccifera* 16. *Diplolepis* sp. on *Rosa sempervirens* 17. *Dasineura rosae* cf. on *Rosa canina* 20. *Neuroterus anthracinus* on *Quercus robur* 21. *Neuroterus numismalis* on *Quercus* robur 22. *Neuroterus quercusbaccarum* on *Quercus* sp. 23. *Plagiotrochus quercuscilicis* fo. *quercuscilicis* on *Quercus coccifera* 24. *Rhopalomyia baccarum* on *Artemisia crithmifolia* 25. *Trioza alacris* on *Laurus nobilis* 26. *Andricus spectabilis* cf. on *Quercus coccifera* 27. Undetermined on *Quercus coccifera* 28. *Cochylimorpha hilarana* on *Artemisia crithmifolia* 29. Undetermined on *Dipsacus* sp. 30. Undetermined on *Cistus salvifolius* 31. Undetermined on *Halimium calycinum* 32. Undetermined on *Phillyrea angustifolia* 35. *Eutrichapion scutellare* on *Ulex* sp. 36. *Hexomyza sarothamni* cf. on *Cytisus* sp. Numeration of pictures is not continuous in order to correspond to the codes used for the galls on figures 9 and 10.
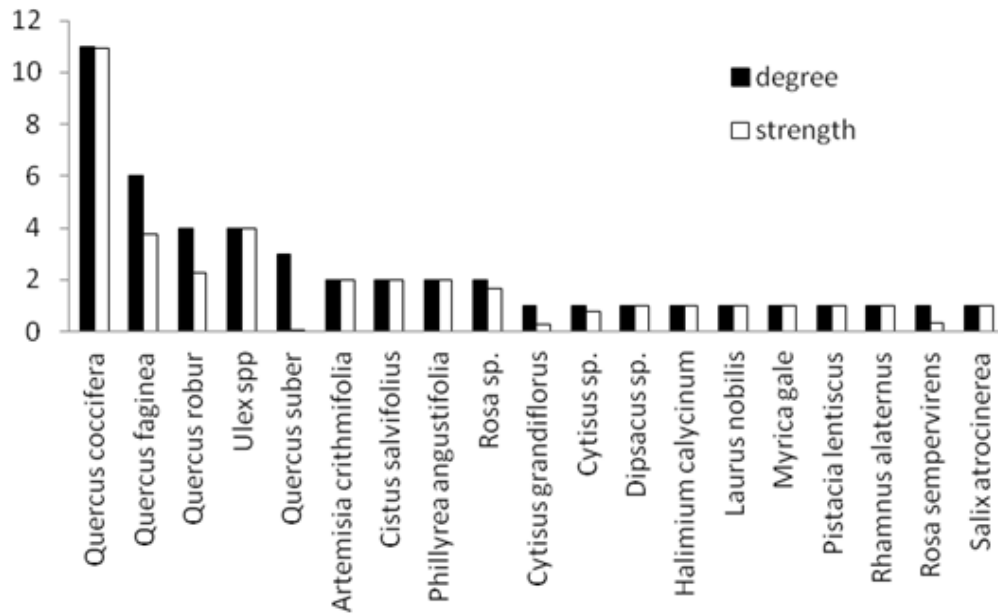
Figure 8. Degree, strength (top) and specialization (bottom) of plant species.
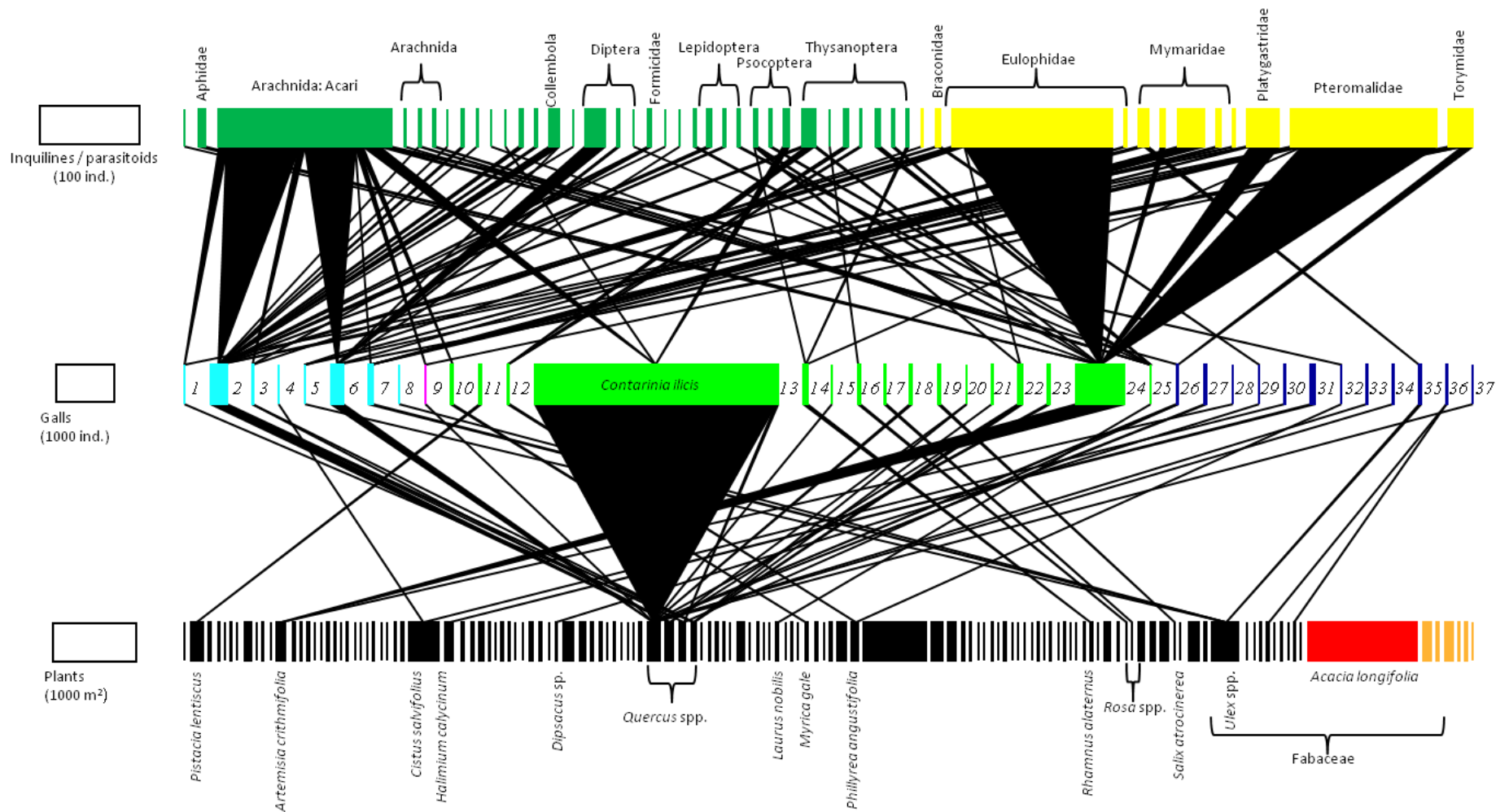
Figure 9. General quantitative network from 5 sites asserting the interactions among 3 levels of plant-gall networks: Plants, gall formers and inquilines and parasitoids. Each bar represents a species and its width represents relative abundance among all the individuals sampled. The area of the triangles and lines connecting each level represents the relative number of higher-level species interacting with lower-level species. Color codes: black - native plants; red - *Acacia longifolia*; orange – other invasive plants; light blue – bud galls; pink – inflorescence galls; light green – leaf galls; dark blue – stem galls; dark green – inquilines; yellow – parasitoids. The numbers associated with each gall former make correspondence to figures 7 and 10.

A total of 782 arthropods (individuals) emerged from the collected galls, namely, 227 gall formers, 250 parasitoids and 305 inquilines (fig. 9).

The emergences were recorded on 23 types of galls and the average number of emergences per type of gall was 21. *Rhopalomyia baccarum* on *Artemisia crithmifolia* (fig. 7 (24)) was the gall with most total emergences (278), followed by *Andricus kollari* on *Quercus* spp. (120; fig. 7 (24)), *Aploneura lentisci* on *Pistacia lentiscus* (106; fig. 7 (11)), *Phyllodiplosis cocciferae* on *Quercus coccifera* (84; fig. 7 (6)) and an undetermined bud gall on *Ulex* spp. (63; fig. 7 (7)). The remaining galls had less than 20 emergences (fig. 10).
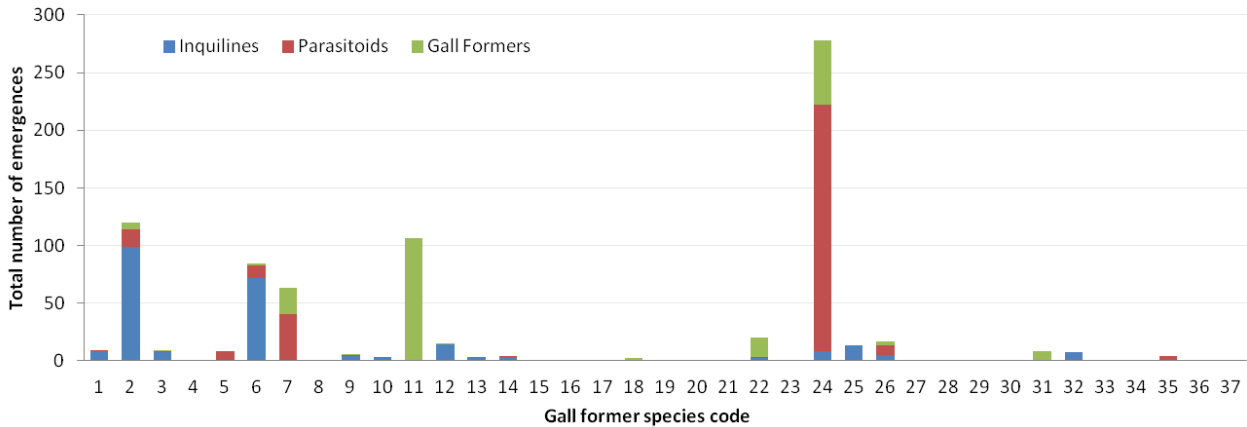
Figure 10. Total number of emergences divided by inquilines, parasitoids and gall formers for each gall type. Gall former species code makes correspondence to figures 7 and 9.

79.8% of the emerged arthopods were insects (624 individuals of 41 species), followed by Arachnida (154 individuals of 4 species), Chilopoda (3 individuals of 1 species), and Diplopoda (1 individual). These last three classes were only found to be gall inquilines. Other inquilines included: Arachnida: Acari, Coleoptera (families Anobiidae, Carabidae and Coccinelidae), Collembola, Diptera, Hemiptera (families Aphidae and Coccidae), Hymenoptera: Formicidae, Gryllidae, Lepidoptera, Psocoptera and Thysanoptera (fig. 11). On the other hand, all parasitoids (fig. 12) and gall formers (fig. 13) were insects. The first had representatives of the orders Hemiptera (Aphidae), Hymenoptera (Cynipidae), Diptera (Cecidomyiidae) and Coleoptera (Apionidae), while parasitoids were only Hymenoptera (families Pteromalidae, Torymidae, Platygastridae, Eulophidae, Mymaridae, Apoidea: Spheciformes and Braconidae).

Figure 11. Nine groups of inquilines reared from the collected galls (a. Class Arachnida b. Class Chilopoda c. Class Diplopoda d. Order Hemiptera: Coccidae cf. e. Order Coleoptera: Anobiidae cf. f. Order Hymeoptera: Formicidae g. Order Gryllidae h. Order Psocoptera i. Order Thysanoptera).
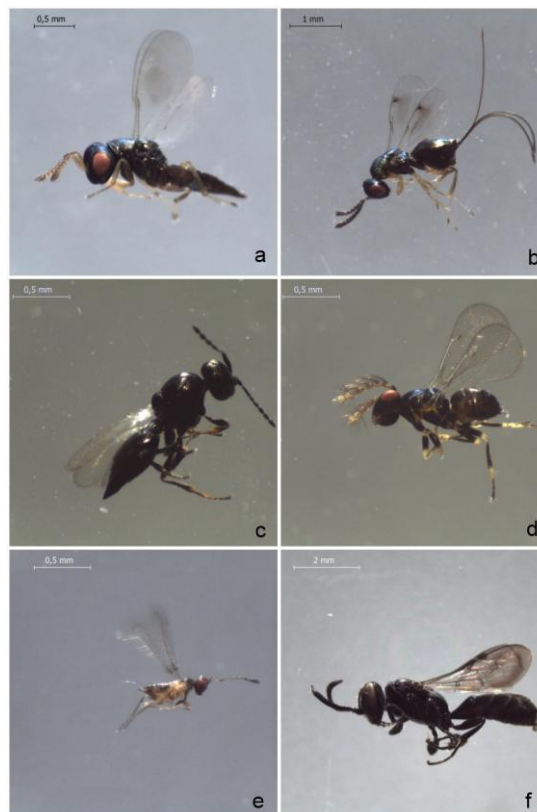
Figure 12. Six families of hymenopteran parasitoids reared from the galls (a. Pteromalidae b. Torymidae c. Platygastridae d. Eulophidae e. Mymaridae f. Apoidea: Spheciformes).
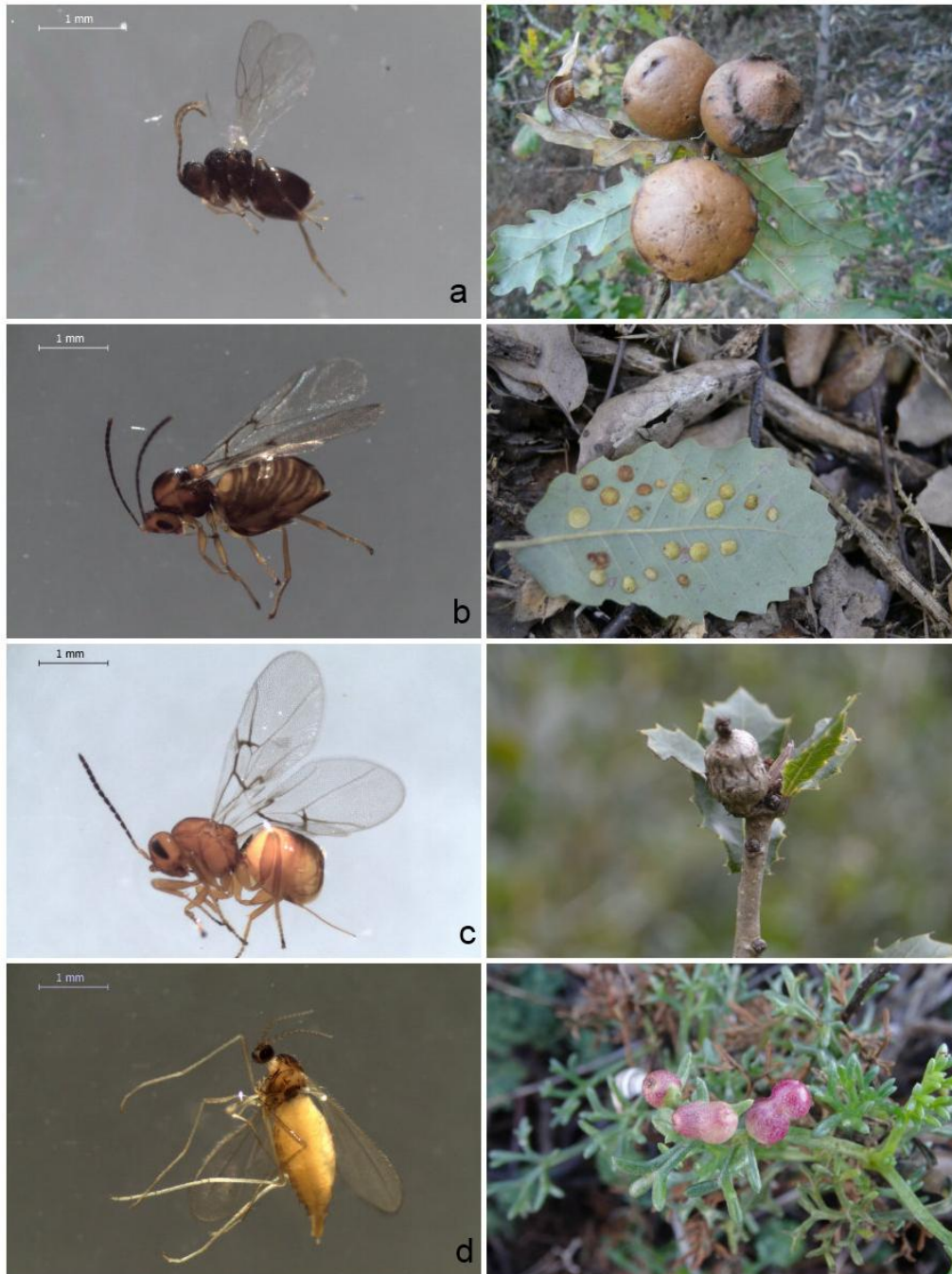
Figure 13. Four gall former species (on the left) and the respective galls (on the right); a. *Andricus quercustozae* (Hymenoptera: Cynipidae); b. *Neuroterus quercusbaccarum* (Hymenoptera: Cynipidae); c. *Andricus spectabilis* (Hymenoptera: Cynipidae); d. *Rhopalomyia baccarum* (Diptera: Cecidomyiidae).

3.2. Gall networks per site

Not every site has contributed equally for the general network. The individual networks for each site (fig. 14) show that São Pedro de Moel (SPM) was clearly the most represented, both in diversity (18 species) and abundance (4546 galls) of gall formers. In gall former richness was only followed by the oak forest of Escola Superior Agrária de Coimbra (ESAC; 12 species). These two sites were significantly influenced by the presence of *Quercus* species (11 of 18 gall former species for SP and 8 of 12 for ESAC) that greatly contributed for the overall gall richness. Serra da Boa Viagem (BV) was the only other site with *Quercus* species but its presence was vestigial.

In the third level of the network (emergences of inquilines and parasitoids) SPM was only surpassed by ESAC in diversity and by Quiaios Dunes (QD) in abundance. QD had one of the most abundant galls of the entire study (*Rhopalomyia baccarum* on *Artemisia crithmifolia*; fig 7 (24)), which was the second with higher emergences' richness.
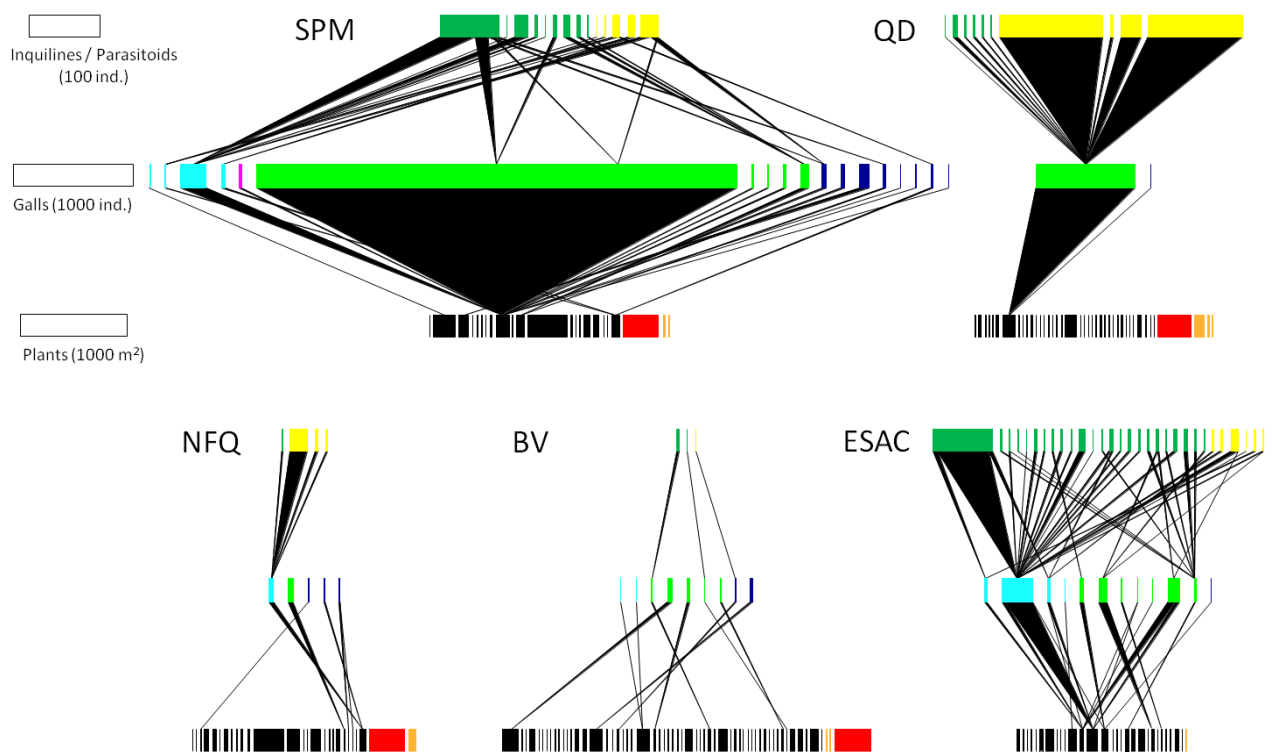
**Figure 14.** Quantitative networks of the 5 individual sites (SPM – Pinhal do Rei - São Pedro de Moel; QD – Quiaios Dunes; NFQ - Mata Nacional das Dunas de Quiaios; BV - Parque Florestal da Serra da Boa Viagem; ESAC - Oak forest of Escola Superior Agrária de Coimbra) asserting the interactions among 3 levels of gall networks: Plants, gall formers and inquilines/parasitoids. Symbol and color legend is as in figure 7.

3.3. Impact of *Acacia longifolia*

The increasing density of *A. longifolia* was associated with decreases in total abundance and diversity of plants and emergences from galls, and with a decrease in abundance of gall formers. The highest diversity of gall formers, however, was observed in the moderate level of invasion as well as the higher number of plant species bearing galls (table II). This resulted in smaller gall former networks as the level of invasion raised (fig. 15). Web asymmetry, interaction strength asymmetry and network robustness showed an increase with the increasing density of *A. longifolia* (table II).

On the other hand, other network parameters did not follow the same trend. For interaction evenness the network of moderate invasion showed the highest value while it had the lowest for connectance. H_2' – specialization was maximum for the 3 networks independently of the level of invasion (table II).

Table II. Comparison of network level properties of the first two levels of the networks (plants and galls) between the global network and the networks of 3 levels of invasion by *A. longifolia*: low (0 – 29%), moderate (30 – 69%) and high (70 – 100%).

	Global network	Low invasion	Moderate invasion	High invasion
Gall former richness	37	14	25	13
Plant richness	130	89	75	54
Plant species with galls	19	9	11	5
Links per species	0.821	0.609	0.722	0.722
Connectance	0.065	0.111	0.095	0.200
Web asymmetry	0.321	0.217	0.389	0.444
Interaction strength asymmetry	0.351	0.385	0.553	0.634
Interaction evenness	0.220	0.189	0.219	0.202
H_2' (Specialization)	0.948	1.000	1.000	1.000
Robustness to plant extinctions	0.623	0.545	0.612	0.659

A) Low *A. longifolia* invasion (0 – 29% coverage)
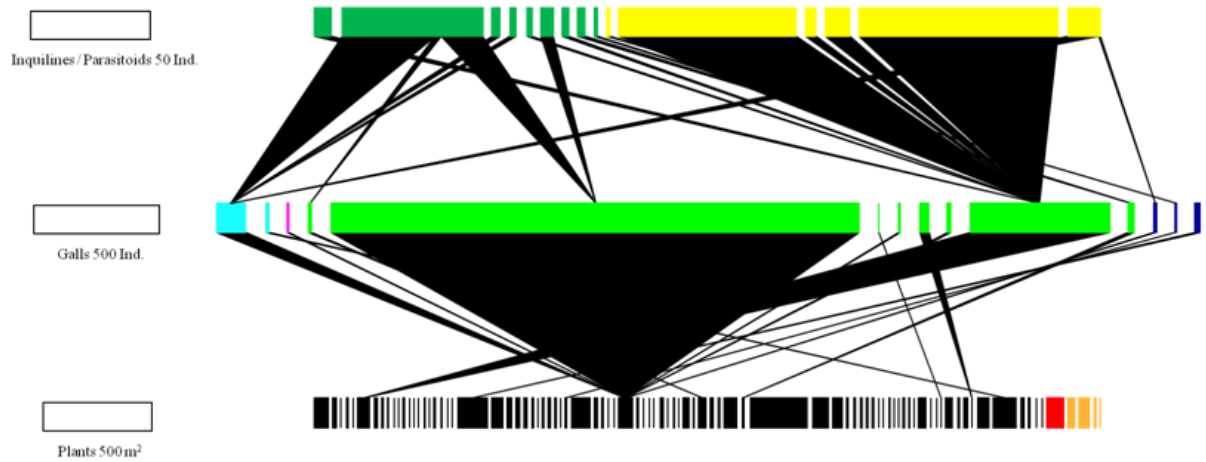
B) Moderate *A. longifolia* invasion (30 – 69% coverage)
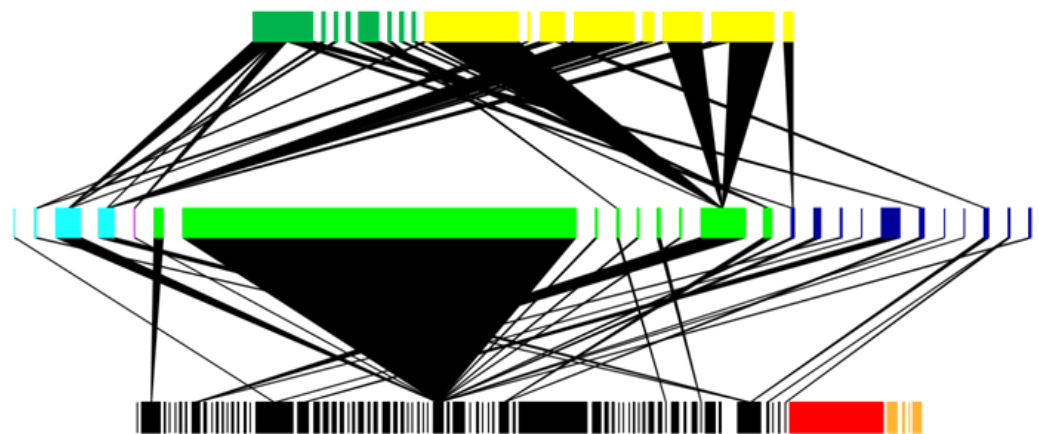
C) High *A. longifolia* invasion (70 – 100% coverage)
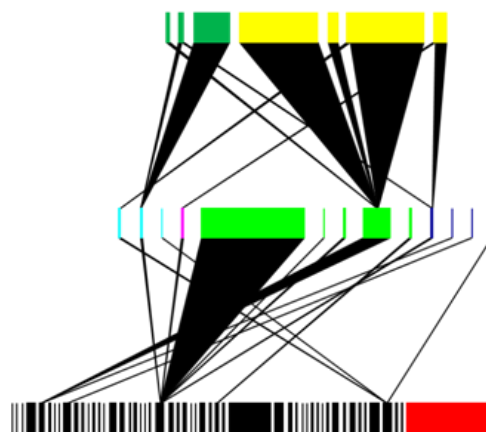
Figure 15. Quantitative networks from 4 sites representing the interactions between plants, galls and inquilines/parasitoids along a gradient of *A. longifolia* invasion. Sampling was performed via transects with variable importance of *A. longifolia* cover, namely: A) low invasion (0-29%), B) moderate invasion (30-69%) and C) high invasion (70-100%). Symbol and color legend is as in figure 7.
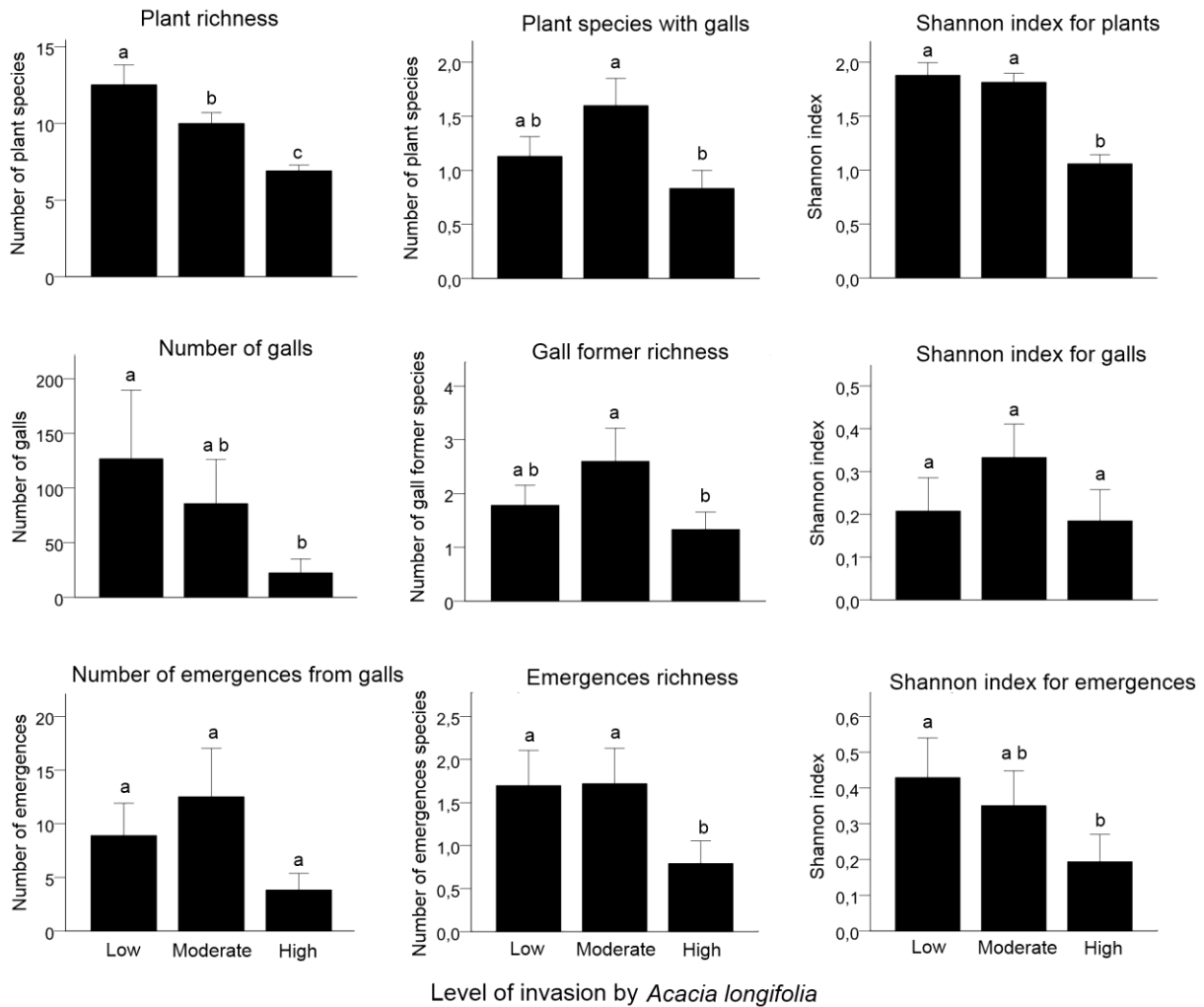
Figure 16. Values of nine variables (mean + SE) per level of invasion of *A. longifolia*. Different letters denote significant differences after nested ANOVAs to site and pairwise comparisons.

As seen on figure 14 not all sites contributed equally to the general networks, including the ones intended to show the effect of *A. longifolia* (fig. 15). Except for plant richness, most parameters analyzed showed no significant differences between low and moderately invaded communities (fig. 16). Highly invaded communities however had lower gall former diversity and significant differences when compared with low invasion in Shannon diversity for plants, emergences richness and Shannon diversity for emergences. Although highly invaded communities also had less emergences, these difference were not significant due to the high dispersal of the data (fig. 16)

Chapter 4 - Discussion

4.1. Gall communities in Portugal

To the best of my knowledge, the general network presented in this work constitutes the biggest gall former network ever constructed in the world and the first of this type in Portugal. Simultaneously, is the very first tri-trophic gall former network with a multi-species approach (other studies focused either on one species of plant or gall former, Kaartinen and Roslin 2011, Veldtman et al. 2011) and also the first with the intent of creating a reference situation before the introduction of a biocontrol agent.

With this work we were able to quantify interactions between plants, gall formers and inquilines/parasitoids, a poorly understood but highly diverse system. This information provides a detailed picture of the communities of organisms that can potentially interact with a biocontrol agent, in this case *T. acaciaelongifoliae,* before its release, what is seldom available in biocontrol processes (Carvalheiro et al. 2008). Having such a "time zero/reference" situation will allow a better planning of the introduction of *T. acaciaelongifoliae,* and a better monitoring of the evolution after the likely release of this agent. Furthermore, the tripartite plant-gall former-inquilines/parasitoids network allows a deeper understanding of the ecology of these interactions.

Overall, gall formers are highly specialized organisms interacting only with a closely related spectrum of hosts (Marchante et al. 2011a) (Redfern 2011). This specialization is reflected on the extremely high specialization of the overall network ($H_2' > 0.94$). It is interesting to note that specialization is much lower in pollination networks ($H_2' < 0.75$; Traveset et al. 2013) and seed dispersal networks ($H_2' < 0.40$; Schleuning et al. 2014). Gall sharing was only observed within the same genus, namely on *Quercus* spp., *Rosa* spp. and *Cytisus* spp. This specificity was not observed in the higher level of inquilines and parasitoids but this is most likely an artifact derived from the lower taxonomical resolution of parasitoids and inquilines attained during this study. Identification of parasitoids and inquilines up to genus or species level would likely change this picture, but was not possible in such a short study.

Importantly, no galls were found on any of the invasive species throughout this study, especially on species that are documented hosts of gall formers besides *A. longifolia*: *Acacia pychnantha* (Hoffmann et al. 2002)*, Acacia melanoxylon* (Morris et al. 2001)*, Arundo donax* (Moran and Goolsby 2014) and *Pittosporum undulatum* (Hering 1962). This is a striking result as it seems that no exotic gall former was introduced and no native gall former adapted to the introduced plants. It is one more piece of evidence of the specificity of gall formers even though in Portugal there are no native counterparts of the referred plants, which could constitute a greater risk. This helps to partly explain via the enemy release hypothesis (Keane and Crawley 2002) the success of these invasive species that thrives free of their natural enemies.

With 11 gall former species, *Q. coccifera* stands out as holding the higher diversity of galls and also by having highly specific gall formers (all exclusive, except for *Contarinia ilicis* cf. that appeared also in *Q. suber;* fig. 7 (10)), as shown by the high species strength (fig. 8). It does not seem to be like the other *Quercus* species that were found to share various gall formers. It is documented however that some of its galls appear on *Q. ilex* (fig. 7 (7, 10 & 18)) (Pujade-Villar

2000, Sánchez et al. 2012). *Quercus suber*, on the other hand, showed a low value of specialization (d'); despite being a species greatly recognized by its galls (Hayward and Stone 2005), apparently none of the gall formers depends solely on this species.

Not all galls were identified in this study so it is uncertain at this point if a new gall was discovered. Even if it is not the case, at least some new records for some species, for Portugal, are expected to arise and perhaps several new species of parasitoids and inquilines.

4.2. Predicting interactions with *T. acaciaelongifoliae*

The data of this study allows the formulation of predictions regarding potential indirect non-target interactions between *T. acaciaelongifoliae* (with plants and parasitoids) and its gall (with inquilines), which could affect its success as a biocontrol agent.

During this study no galls were found on *Acacia* species, which is very encouraging on the point of view of the non-target effects. Several species of this genus are hosts of *Trichilogaster* species, all forming very similar galls and were the prime candidates to hold species capable of interacting with *T. acaciaelongifoliae* (Prinsloo and Neser 2007). The closest relatives of *A. longifolia* that possessed galls were, within the same family Fabaceae, the genera *Cytisus* and *Ulex*. It is yet uncertain if galls on phylogenetically close plant species are more prone to share parasitoids or inquilines. Nonetheless, the galls found on *Citysus* spp. were located on stems and are structurally and morphologically very different from *T. acaciaelongifoliae*'s galls and the ones on *Ulex* spp. either followed the same trend or were considerably smaller (< 5mm).

The dimensions of a gall, position in the host plant and overall similarities with *T. acaciaelongifoliae*'s galls are important when talking about inquilines, which can increase in abundance after the introduction of the new tissue. One should focus then, on inquilines of galls morphologically similar to those formed by *T. acaciaelongifoliae*. Twenty eight out of the 37 gall formers' galls found in this study occur on leaves and stems and do not resemble in any way the gall of the intended biocontrol agent, whose galls occur on flower and vegetative buds. It is unlikely that inquilines will interact with clearly distinct gall formers (Sanver 2000). As for the 8 gall formers that induce galls on floral and vegetative buds, only those formed by *Andricus kollari* and *Andricus quercustozae* on *Quercus* spp. (fig 7 (3 & 4)) resemble *T. acaciaelongifoliae*'s in size and tissues (fig. 4). However, these galls were only found in ESAC, an area that does not include *A. longifolia*, decreasing the chances of interacting with this species. As for other bud galls they have either different tissues or are too small.

Regarding the emerged parasitoids, two families Torymidae and Pteromalidae have species known to parasite *T. acaciaelongifoliae* both in the home range and the introduced range in South Africa (McGeoch and Wossler 2000, Veldtman et al. 2011). Still, more resolution in the taxonomic identifications is desirable in order to have a full spectrum and to completely understand if any species could use *T. acaciaelongifoliae* as a resource.

Despite these considerations, it may be expected that the biocontrol agent and its gall can attract parasitoids and inquilines when it is introduced in the new range, like happened in South Africa (Manongi and Hoffmann 1995, Veldtman et al. 2011). It will be a new tissue introduced in the system and it is unlikely that it will stay isolated but at this stage significant interactions that could hamper the success of the biocontrol agent and cause unwanted effects in the introduced communities are improbable or unforeseen. This work though, is only a time window and there is more to be told since it spans only over six months.

It is important to state however, that even if the some negative impacts of the introduction of *T. acaciaelongifoliae* are predicted, it has to be considered that doing nothing it is a risk even bigger given that *A. longifolia* already has many associated and quantified negative impacts (see discussion bellow).

4.3.   Best sites for *T. acaciaelongifoliae* introduction

A carefully planned release strategy including the selection of best place(s) for the introductions is surmount to the success of the biocontrol, since the site is important to assure the successful establishment of viable populations of the agent. Analyzing the networks for each individual site, Quiaios Dunes seems the best option. Among all sites it has the lower number of plant species with galls (only 1), and just 2 gall formers. One of them (*Rhopalomyia baccarum*; fig. 7 (24)) has a wide array of inquilines and parasitoids, but its gall does not resemble neither in size nor in tissues the galls of *T. acaciaelongifoliae*. Furthermore, parasitoids often are very specific about what organisms they can use for hosts, so they target particular species (Sands 2002), and since it is a dipteran is less likely that its parasitoids could shift towards the biocontrol agent which is an hymenopteran). Additionally, dune systems are the most invaded habitats by *A. longifolia* and at the same time the most vulnerable so this is also the most adequate place to start the introductions of *T. acaciaelongifoliae*.

Nevertheless, one should bear in mind that after being released, and if the establishment is well succeeded, the biocontrol agent will spread further from the introduction places. This spread may be slow, since the species is not a very active flyer and it is mostly dispersed by wind (Dennill 1985), but it is expected to spread.

4.4.   Impact of *A. longifolia* invasion on gall communities

Acacia longifolia has well documented impacts in soil composition, microbiology (Marchante et al. 2008a), fire regimes, water fluxes (Marchante et al. 2011b), regeneration dynamics (Rascher et al. 2011) and nutrient fluxes (Rascher et al. 2012). The present work confirmed the impacts in plant abundance and added information on impacts in other unexplored level: gall former networks.

The impacts of invasive plants have been studied in some extent for pollination and seed dispersal networks for island systems. A recent study of pollination networks on the Galápagos

Islands suggests that alien species could stabilize disturbed island networks but in turn decreases diversity and disrupts native interactions (Traveset et al. 2013). In other study, the introduction of an exotic seed feeder insect was proven to be responsible for an increase in native parasitoids abundance which led to reductions of other seed feeders (Willis and Memmott 2005).

In this study in turn showed that the increasing abundance of the invasive *A. longifolia* was associated with declines in the abundance of other plants, gall formers, parasitoids and inquilines. These changes affected the gall community structure by increasing web asymmetry, interaction strength asymmetry and robustness (i.e. redundancy). Web asymmetry was also revealed to increase with the introduction of invasive plant species on island seed dispersal networks (Schleuning et al. 2014).These results were expected, as the presence of an invasive plant out-competing native vegetation, would naturally have indirect negative impacts on gall communities depending on plants. The increased proportion between lower trophic species (plants) and higher trophic species (gall formers) and increased robustness, results from the loss of plant diversity and the specialization of gall formers, which as a consequence of the disappearance of its hosts will disappear as well. Increased network robustness as good as it may sound it is not necessarily positive. Robustness increased after the disappearance of some plant species that served as hosts for gall formers, particularly plants with only one observed link, remaining the more generalist plant species (with more links), hence the higher values for the more invaded areas.

Other results, however, were not expected. Connectance increased slightly and unexpectedly, and interaction evenness decreased from low to moderately invaded communities. This is ultimately related to the gall former richness which in total was higher for moderately invaded communities. Despite of that, no significant differences were observed between low and moderate invasion besides plant richness.

These somewhat unexpected result could be explained by the intermediate disturbance hypothesis where small disturbances (in this case moderate invasion) could temporarily increase habitats heterogeneity (becoming the community a mosaic of fragmented habitats), rising biodiversity (Catford et al. 2012). And if disturbance becomes severe (as in advanced plant invasions) this increase will be followed by the reduction and eventual extinction of the most vulnerable species and a consequent biodiversity decline, as can be seen in the highly invaded areas in this study. Other factor contributing to these results was the type of implemented sampling. A limitation of sampling galls is that once a certain area is sampled it cannot be sampled again in the short period of time implemented in this study because the formation of new galls will likely take a year. This type of destructive sampling leads to the choosing of new transects within the same sampling site that had more differences than just the percentage cover of *A. longifolia* such as different plant species, proximity to roads, sun exposure (less covered areas as opposed to more covered by for example *Pinus* trees) or different arrangement of *A. longifolia* specimens (dense patches opposed to disperse trees). For this reason it was often impossible to select different areas where the only variable changing was the percentage cover of *A. longifolia*. Therefore, when data from several months were pulled together the diversity of plants across transects may have "buffered" further changes on some network descriptors..

Other possible explanation for the observed trends is that they are no more than artifacts that will disappear in the remaining months of sampling. The work on this thesis only covers half of the sampling period of the project (6 out of 12 months) and it is possible that these differences will be diluted over the remaining of the sampling, as suggested by the lack of significant differences.

Chapter 5 – Conclusions

The networks assembled in this work give the very first picture of gall former communities in Portugal, so far the largest gall's communities study in the world and the first multi-host tri-trophic: plant-gall former-parasitoid/inquiline network studies in any detail.

Despite the abundance and diversity of gall formers, not a single gall was found on exotic plants. The numbers of parasitoids and inquilines was also high and, in spite of the need for further taxonomical resolution it seems unlikely that any of these will explore either *T. acaciaelongifoliae* or its gall as a preferred resource. The diversity of plants with galls was high, showing gall communities as a highly diverse and ecologically important study system.

Quaios dunes was proposed as the best of the studied sites for the first introductions of *T. acaciaelongifoliae* since dunes are the preferential habitat for *A. longifolia* invasion and because this site possesses a gall community with lower probability of interacting with the agent, which gives more guaranties of a successful establishment.

Overall, this work encourages the prospects of a safe biological control program for *A. longifolia*. Up to this point there are no substantial reasons for not introducing *T. acaciaelongifoliae* as a biocontrol agent to counter the negative ecological impacts of *A. longifolia*, which had a significant impact in the diversity and abundance of plants which cascaded up the food chain to gall formers, inquilines and parasitoids. The reference situation before the introduction of the biocontrol agent was constructed which is a big step forward, and the first of his kind, in the safety of biological control programs and an example for more to come.

References

Allesina, S. and Pascual, M. (2008) Network structure, predator–prey modules, and stability in large food webs. Theoretical Ecology 1(1), 55-64.

Almeida, J.D. and Freitas, H. (2006) Exotic naturalized flora of continental Portugal - a reassessment. Botanica Complutensis 30, 117-130.

Bale, J.S., van Lenteren, J.C. and Bigler, F. (2008) Biological control and sustainable food production. Philosophical Transactions of the Royal Society B-Biological Sciences 363(1492), 761-776.

Barrientos, J. A. (2004) Curso práctico de entomología. Universidad Autonoma De Barcelona. 947 p.

Bascompte, J. (2007) Networks in ecology. Basic and Applied Ecology 8(6), 485-490.

Bascompte, J., Jordano, P., Melian, C.J. and Olesen, J.M. (2003) The nested assembly of plant-animal mutualistic networks. Proceedings of the National Academy of Sciences of the United States of America 100(16), 9383-9387.

Baskin, Y. (2003) A Plague of Rats and Rubbervines: The Growing Threat of Species Invasions, Shearwater Books.

Batabyal, A.A. and Beladi, H. (2006) International trade and biological invasions: A queuing theoretic analysis of the prevention problem. European Journal of Operational Research 170(3), 758-770.

Bingre, P., Aguiar, C., Espírito-Santo, D., Arsénio, P. and Monteiro-Henriques, T. (2007) - Guia de árvores e arbustos de Portugal Continental . [Lisboa]: Jornal Público, Fundação Luso-Americana para o Desenvolvimento, Liga para a Protecção da Natureza. (Árvores e Florestas de Portugal; IX)

Binimelis, R., Born, W., Monterroso, I. and Rodríguez-Labajos, B. (2007) Biological Invasions. W.Nentwig (ed), pp. 331-347, Springer-Verlag, Berlin Heidelberg.

Blackburn, T.M., Essl, F., Evans, T., Hulme, P.E., Jeschke, J.M., Kuhn, I., Kumschick, S., Markova, Z., Mrugala, A., Nentwig, W., Pergl, J., Pysek, P., Rabitsch, W., Ricciardi, A., Richardson, D.M., Sendek, A., Vila, M., Wilson, J.R.U., Winter, M., Genovesi, P. and Bacher, S. (2014) A Unified Classification of Alien Species Based on the Magnitude of their Environmental Impacts. Plos Biology 12(5).

Borrett, S. R., Moody J. and Edelmann A. (2014) The rise of Network Ecology: Maps of the topic diversity and scientific collaboration. Ecological Modelling

Brooks, M.L., D'Antonio, C.M., Richardson, D.M., Grace, J.B., Keeley, J.E., DiTomaso, J.M., Hobbs, R.J., Pellant, M. and Pyke, D. (2004) Effects of invasive alien plants on fire regimes. Bioscience 54(7), 677-688.

Burlakova, L.E., Karatayev, A.Y. and Padilla, D.K. (2006) Changes in the distribution and abundance of Dreissena polymorpha within lakes through time. Hydrobiologia 571, 133-146.

Carlton, J.T. and Cohen, A.N. (2003) Episodic global dispersal in shallow water marine organisms: the case history of the European shore crabs Carcinus maenas and C-aestuarii. Journal of Biogeography 30(12), 1809-1820.

Carvalheiro, L.G., Buckley, Y.M., Simon, R.V., Fowler, V. and Memmott, J. (2008) Apparent competition can compromise the safety of highly specific biocontrol agents. Ecology Letters 11(7), 690-700.

Catford, J.A., Daehler, C.C., Murphy, H.T., Sheppard, A.W., Hardesty, B.D., Westcott, D.A., Rejmanek, M., Bellingham, P.J., Pergl, J., Horvitz, C.C. and Hulme, P.E. (2012) The intermediate disturbance hypothesis and plant invasions: Implications for species richness and management. Perspectives in Plant Ecology Evolution and Systematics 14(3), 231-241.

Charles, H., and Dukes, J. S. (2007). Impacts of Invasive Species on Ecosystem Services. In W. Nentwig (Ed.), Biological Invasions - Ecological Studies (Vol. 193, pp. 217-237). Berlin, Germany: Springer-Verlag.

Costello, C., Springborn, M., McAusland, C. and Solow, A. (2007) Unintended biological invasions: Does risk vary by trading partner? Journal of Environmental Economics and Management 54(3), 262-276.

Crosby, A. W. (1986). Ecological Imperialism: The Biological Expansion of Europe, 900-1900. Cambridge, UK: Cambridge University Press.

Culliney, T.W. (2005) Benefits of classical biological control for managing invasive plants. Critical Reviews in Plant Sciences 24(2), 131-150.

Darwin, C. (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. Murray, London, 502 pp.

D'Antonio, C.M. and Vitousek, P.M. (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 23, 63-87.

Dean, K.M., Vandenberg, J.D., Griggs, M.H., Bauer, L.S. and Fierke, M.K. (2012) Susceptibility of two hymenopteran parasitoids of Agrilus planipennis (Coleoptera: Buprestidae) to the entomopathogenic fungus Beauveria bassiana (Ascomycota: Hypocreales). Journal of Invertebrate Pathology 109(3), 303-306.

Dennill, G.B. (1985) The effect of the gall wasp *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) on reproductive potential and vegetativa growth of the *Acacia longifolia*. Agriculture, Ecosystems & Environment 14, 53-61.

Dennill, G.B. (1987) ESTABLISHMENT OF THE GALL WASP TRICHILOGASTER-ACACIAELONGIFOLIAE (PTEROMALIDAE) FOR THE BIOLOGICAL-CONTROL OF ACACIA-LONGIFOLIA IN SOUTH-AFRICA. Agriculture Ecosystems & Environment 19(2), 155-168.

Dennill, G.B. (1988) Why a gall former can be a good biocontrol agent: the gall wasp <i>Trichilogaster acaciaelongifoliae</i> and the weed <i>Acacia longifolia</i>. Ecological Entomology 13(1), 1-9.

Dennill, G.B. (1990) The contribution of a successful biocontrol project to the theory of agent selection in weed biocontrol--the gall wasp Trichilogaster acaciaelongifoliae and the weed Acacia longifolia. Agriculture, Ecosystems & Environment 31(2), 147-154.

Dennill, G.B. and Donnelly, D. (1991) Biological control of *Acacia longifolia* and related weed species (*Fabaceae*) in South Africa. Agriculture, Ecosystems & Environment 37, 115-135.

Dennill, G.B., Donnelly, D. and Chown, S.L. (1993) Expansion of host-plant range of a biocontrol agent *Trichilogaster acaciaelongifoliae* (Pteromalidae) released against the weed *Acacia longifolia* in South Africa. Agriculture, Ecosystems & Environment 43(1), 1-10.

DiCastri, F. (1989). History of biological invasions with special emphasis on the Old World. In J. A. Drake, H. J. Mooney, & F. DiCastri (Edits.), Biological Invasions: a global perspective. Chichester, UK: Wiley.

Dicks, L.V., Corbet, S.A. and Pywell, R.F. (2002) Compartmentalization in plant-insect flower visitor webs. Journal of Animal Ecology 71(1), 32-43.

Dormann, C. F., Gruber B. and Fruend, J. (2008). Introducing the bipartite package: Analysing Ecological Networks. R news Vol 8/2, 8 – 11.

Dunne, J.A., Williams, R.J. and Martinez, N.D. (2002) Food-web structure and network theory: The role of connectance and size. Proceedings of the National Academy of Sciences of the United States of America 99(20), 12917-12922.

Ehrenfeld, J.G. (2000) Defining the limits of restoration: The need for realistic goals. Restoration Ecology 8(1), 2-9.

Ehrenfeld, J.G. (2010) Ecosystem Consequences of Biological Invasions. Annual Review of Ecology, Evolution, and Systematics 41(1), 59-80.

Franco, J. A. (1971) Nova Flora de Portugal Continental (Continente e Açores). Vol. I (Licopodiaceae - Umbelliferae). Lisboa

Franco, J. A. (1984) Nova Flora de Portugal Continental (Continente e Açores). Vol. II (Clethraceae - Compositae). Lisboa

Goulet, H. and Huber, J. T. (1993) Hymenoptera of the world: An identification guide to families. Ontario: Centre for Land and Biological Resources Research. Ottawa. 668 p.

Hatcher, M.J., Dick, J.T.A. and Dunn, A.M. (2012) Disease emergence and invasions. Functional Ecology 26(6), 1275-1287.

Hayward, A. and Stone, G.N. (2005) Oak gall wasp communities: Evolution and ecology. Basic and Applied Ecology 6(5), 435-443.

Heleno, R.H., Olesen, J.M., Nogales, M., Vargas, P. and Traveset, A. (2013a) Seed dispersal networks in the Galápagos and the consequences of alien plant invasions. Proceedings of the Royal Society B: Biological Sciences 280(1750).

Heleno, R.H., Ramos, J.A. and Memmott, J. (2013b) Integration of exotic seeds into an Azorean seed dispersal network. Biological Invasions 15(5), 1143-1154.

Hering, F. (1962) Galls of Agromyzidae (Dipt.) on Pittosporum undulatum Andr. Proc. Linn. Soc. N.S.W. Vol. 87 pp. 84-91

Hochberg, M.E. and Gotelli, N.J. (2005) An invasions special issue. Trends in Ecology & Evolution 20(5), 211-211.

Hoddle, M.S. (2004) Restoring balance: Using exotic species to control invasive exotic species. Conservation Biology 18(1), 38-49.

Hoffmann, J.H., Impson, F.A.C., Moran, V.C. and Donnelly, D. (2002) Biological control of invasive golden wattle trees (*Acacia pycnantha*) by a gall wasp, *Trichilogaster* sp. (*Hymenoptera: Pteromalidae*), in South Africa. Biological Control 25, 64-73.

Holt, R.D. (1977) PREDATION, APPARENT COMPETITION, AND STRUCTURE OF PREY COMMUNITIES. Theoretical population biology 12(2), 197-229.

Hulme, P.E. (2009) Trade, transport and trouble: managing invasive species pathways in an era of globalization. Journal of Applied Ecology 46(1), 10-18.

Impson, F.A.C., Kleinjan, C.A., Hoffmann, J.H., Post, J.A. and Wood, A.R. (2011) Biological control of Australian Acacia species and Paraserianthes lophantha (Willd.) Nielsen (Mimosaceae) in South Africa. African Entomology 19(2), 186-207.

Jeschke, J.M. and Genovesi, P. (2011) Do biodiversity and human impact influence the introduction or establishment of alien mammals? Oikos 120(1), 57-64.

Kaartinen, R. and Roslin, T. (2011) Shrinking by numbers: landscape context affects the species composition but not the quantitative structure of local food webs. Journal of Animal Ecology 80(3), 622-631.

Keane, R.M. and Crawley, M.J. (2002) Exotic plant invasions and the enemy release hypothesis. Trends in Ecology & Evolution 17(4), 164-170.

Kull, C.A. and Rangan, H. (2008) Acacia exchanges: Wattles, thorn trees, and the study of plant movements. Geoforum 39, 1258–1272.

Kull, C.A., Shackleton, C.M., Cunningham, P.J., Ducatillon, C., Dufour-Dror, J.-M., Esler, K.J., Friday, J.B., Gouveia, A.C., Griffin, A.R., Marchante, E., Midgley, S.J., Pauchard, A., Rangan, H., Richardson, D.M., Rinaudo, T., Tassin, J., Urgenson, L.S., von Maltitz, G.P., Zenni, R.D. and Zylstra, M.J. (2011) Adoption, use and perception of Australian acacias around the world. Diversity and Distributions 17(5), 822-836.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R. and Thieltges, D.W. (2008) Parasites in food webs: the ultimate missing links. Ecology Letters 11(6), 533-546.

Le Maitre, D.C., Gaertner, M., Marchante, E., Ens, E.-J., Holmes, P.M., Pauchard, A., O'Farrell, P.J., Rogers, A.M., Blanchard, R., Blignaut, J. and Richardson, D.M. (2011) Impacts of invasive Australian acacias: implications for management and restoration. Diversity and Distributions 17(5), 1015-1029.

Levine, J.M. (2008) Biological invasions. Current Biology 18(2), R57-R60.

Lindeman, R.L. (1942) The trophic-dynamic aspect of ecology. Ecology 23(4), 399-418.

Lonsdale, W.M. (1999) Global patterns of plant invasions and the concept of invasibility. Ecology 80(5), 1522-1536.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. and Bazzaz, F.A. (2000) Biotic invasions: Causes, epidemiology, global consequences, and control. Ecological Applications 10(3), 689-710.

Malinowski, R., Smith, J.A., Fleming, A.J., Scholes, J.D. and Rolfe, S.A. (2012) Gall formation in clubroot-infected Arabidopsis results from an increase in existing meristematic activities of the host but is not essential for the completion of the pathogen life cycle. Plant Journal 71(2), 226-238.

Manongi, F.S. and Hoffmann, J.H. (1995) The incidence of parasitism in *Trichilogaster acaciaelongifoliae* (Froggatt) (*Hymenoptera: Pteromalidae*), a gall-forming biological control agent of *Acacia longifolia* (Andr.) Willd. (*Fabaceae*) in South Africa. African Entomology 3, 147-115.

Manosa, S., Mateo, R. and Guitart, R. (2001) A review of the effects of agricultural and industrial contamination on the Ebro delta biota and wildlife. Environmental Monitoring and Assessment 71(2), 187-205.

Marchante, E., Kjøller, A., Struwe, S. and Freitas, H. (2008a) Short and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. Applied Soil Ecology 40, 210-217.

Marchante, E., Kjøller, A., Struwe, S. and Freitas, H. (2008b) Soil recovery after removal of the N_2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. Pyšek, P. and Pergl, J. (eds), p. 234, Institute of Botany Průhonice, Academy of Sciences, Prague (Czech Republic).

Marchante, H., Freitas, H. and Hoffmann, J.H. (2010) Seed ecology of an invasive alien species, *Acacia longifolia* (Fabaceae), in Portuguese dune ecosystems. American Journal of Botany 97(11), 1780-1790.

Marchante, H., Freitas, H. and Hoffmann, J.H. (2011a) Assessing the suitability and safety of a well-known bud-galling wasp, *Trichilogaster acaciaelongifolia*e, for biological control of *Acacia longifolia* in Portugal. Biological Control 56(2), 193-201.

Marchante, H., Freitas, H. and Hoffmann, J.H. (2011b) Post-clearing recovery of coastal dunes invaded by *Acacia longifolia*: is duration of invasion relevant for management success? Journal of Applied Ecology 48(5), 1295-1304.

Marchante, H., Marchante, E. and Freitas, H. (2003) Plant Invasion: Ecological Threats and Management Solutions. Child, L.E., Brock, J.H., Brundu, G., Prach, K., Pyšek, P., Wade, P.M. and Williamson, M. (eds), pp. 75-85, Backhuys Publishers, Leiden, The Netherlands.

Marchante, H., Marchante, E. and Freitas, H. (2005) Plantas Invasoras em Portugal - fichas para identificação e controlo, Ed. dos autores, Coimbra.

Marchante, H., Morais, M., Freitas, H. and Marchante, E. (2014) Guia prático para a identificação de Plantas Invasoras de Portugal, Imprensa da Universidade de Coimbra, Coimbra.

Maslin, B.R., Miller, J.T. and Seigler, D.S. (2003) Overview of the generic status of Acacia (Leguminosae : Mimosoideae). Australian Systematic Botany 16(1), 1-18.

McClay, A.S. and Balciunas, J.K. (2005) The role of pre-release efficacy assessment in selecting classical biological control agents for weeds - applying the Anna Karenina principle. Biological Control 35(3), 197-207.

McFadyen, R.E.C. (1998) Biological control of weeds. Annual Review of Entomology 43, 369-393.

McGeoch, M.A. and Wossler, T.C. (2000) Range expansion and success of the weed biocontrol agent Trichilogaster acaciaelongifoliae (Froggatt) (Hymenoptera : Pteromalidae) in South Africa. African Entomology 8(2), 273-280.

McKinney, M.L. (2004) Do exotics homogenize or differentiate communities? Roles of sampling and exotic species richness. Biological Invasions 6, 495-504.

McNeely, J.A. (2006) As the world gets smaller, the chances of invasion grow. Euphytica 148(1-2), 5-15.

McNeely, J.A., Mooney, H.A., Neville, L.E., Schei, P. and J.K.Waage (2001) A Global Strategy on Invasive Alien Species. , IUCN, Gland, Switzerland, and Cambridge, UK.

Memmott, J., Alonso, D., Berlow, E.L., Dobson, A., Dunne, J.A., Sole, R. and Weitz, J. (2006) Biodiversity loss and ecological network structure, Oxford Univ Press, New York.

Memmott, J., Craze, P.G., Waser, N.M. and Price, M.V. (2007) Global warming and the disruption of plant-pollinator interactions. Ecology Letters 10(8), 710-717.

Menge, B.A. (1995) INDIRECT EFFECTS IN MARINE ROCKY INTERTIDAL INTERACTION WEBS - PATTERNS AND IMPORTANCE. Ecological Monographs 65(1), 21-74.

Ministério do Ambiente (1999) Decreto-lei n.º 565/99 de 21 de Dezembro. In: Diário da República - I Série - A. 295: 9100-9114.

Montesinos, D., Santiago, G. and Callaway, R.M. (2012) Neo-Allopatry and Rapid Reproductive Isolation. The American Naturalist 180(4), 529-533.

Montoya, J.M., Pimm, S.L. and Sole, R.V. (2006) Ecological networks and their fragility. Nature 442(7100), 259-264.

Morais, M.C. and Freitas, H. (2012) The acclimation potential of *Acacia longifolia* to water stress: Implications for invasiveness. Plant Science 196(0), 77-84.

Morais, M.C., Panuccio, M.R., Muscolo, A. and Freitas, H. (2012) Does salt stress increase the ability of the exotic legume Acacia longifolia to compete with native legumes in sand dune ecosystems? Environmental and Experimental Botany 82(0), 74-79.

Moran, P.J. and Goolsby, J.A. (2014) Effect of nitrogen fertilization on growth of Arundo donax and on rearing of a biological control agent, the shoot gall-forming wasp Tetramesa romana. Biocontrol Science and Technology 24(5), 503-517.

Morris, D.C., Schwarz, M.P., Crespi, B.J. and Cooper, S.J.B. (2001) Phylogenetics of gall-inducing thrips on Australian Acacia. Biological Journal of the Linnean Society 74(1), 73-86.

Morris, R.J., Lewis, O.T. and Godfray, H.C.J. (2005) Apparent competition and insect community structure: towards a spatial perspective. Annales Zoologici Fennici 42(4), 449-462.

Muller, C.B. and Godfray, H.C.J. (1997) Apparent competition between two aphid species. Journal of Animal Ecology 66(1), 57-64.

Nentwig, W. (2007). Pathways in Animal Invasions. In: W. Nentwig (Ed.), Biological Invasions - Ecological Studies (Vol. 193). Berlin, Germany: Springer-Verlag.

Neser, S. (1982) Trichilogaster acaciaelongifoliae (Froggatt) (Hymenoptera: Pteromalidae) – a bud gall wasp of long-leaved acacia, Acacia longifolia (And.) Willd. Unpublished report of the Plant Protection Research Instititute. Pretoria

Noble, N.S. (1940) Trichilosgaster acaciae-longifoliae (Froggatt) (Hymenopt., Chalcidoidea), a wasp causing galling of the flower-buds of Acacia longifolia Willd., A. floribunda Sieber and A. sophorae R.Br. Transactions of the Royal Entomological Society of London 90, 13–37.

Noyes, J.S. (2014). Universal Chalcidoidea Database. World Wide Web electronic publication. http://www.nhm.ac.uk/chalcidoids

Nyman, T., Widmer, A. and Roininen, H. (2000) Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera : Tenthredinidae). Evolution 54(2), 526-533.

Odum, H.T. (1956) PRIMARY PRODUCTION IN FLOWING WATERS. Limnology and Oceanography 1(2), 102-117.

Olesen, J.M. and Jordano, P. (2002) Geographic patterns in plant-pollinator mutualistic networks. Ecology 83(9), 2416-2424.

Orchard, A. E., and Wilson, A. J. (2001) Flora of Australia, Mimosaceae, Acacia part 1 (Vol. 11 A). Melbourne, Australia: ABRS/CSIRO Publishing.

Palmer, M.A., Ambrose, R.F. and Poff, N.L. (1997) Ecological theory and community restoration ecology. Restoration Ecology 5(4), 291-300.

Parmesan, C. (2006) Annual Review of Ecology Evolution and Systematics, pp. 637-669, Annual Reviews, Palo Alto.

Perrings, C., Dalmazzone, S. and Williamson, M. (2005) Invasive Alien Species: A New Synthesis Mooney, H.A., Mack, R.N., McNeely, J.A., Neville, L.E., Schei, P.J. and Waage, J.K. (eds), pp. 16-35, Island Press, Washington, Covelo, London.

Perrings, C., Mooney, H. and Williamson, M. (2010) Bioinvasions and Globalization. Ecology, Economics, Management, and Policy, Oxford University Press, New York.

Phillips, B.L., Brown, G.P., Webb, J.K. and Shine, R. (2006) Invasion and the evolution of speed in toads. Nature 439(7078), 803-803.

Pimm, S.L., Lawton, J.H. and Cohen, J.E. (1991) FOOD WEB PATTERNS AND THEIR CONSEQUENCES. Nature 350(6320), 669-674.
Pimm, S. L. (2002). Food webs. Chicago: The University of Chicago Press.

Prinsloo, G.L. and Neser, O.C. (2007) Revision of the pteromalid wasp genus Trichilogaster Mayr (Hymenoptera : Chalcidoidea): gall-inducers on Australian acacias. African Entomology 15(1), 161-184.

Pujade-Villar, J., Villemant, C. and Andreï-Ruiz, M. C. (2000) Cynipidae associated with Quercus collected in Corsica with the description of a new Plagiotrochus species (Hymenoptera, Cynipoidea). Zoosystema Vol. 22 No. 4 pp. 835-846

R Development Core Team (2014) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Rabeling, C. and Kronauer, D.J.C. (2013) Thelytokous Parthenogenesis in Eusocial Hymenoptera. Annual Review of Entomology, Vol 58 58, 273-+.

Rascher, K., Große-Stoltenberg, A., Máguas, C., Meira-Neto, J. and Werner, C. (2011) *Acacia longifolia* invasion impacts vegetation structure and regeneration dynamics in open dunes and pine forests. Biological Invasions 13(5), 1099-1113.

Rascher, K.G., Hellmann, C., Máguas, C. and Werner, C. (2012) Community scale ^{15}N isoscapes: tracing the spatial impact of an exotic N_2-fixing invader. Ecology Letters 15(5), 484–491.

Redfern, M. (2011) Plant Galls. Collins (New Naturalist No. 117)

Redfern, M. and Shirley, P. (2002). British Plant Galls: Identification of galls on plants and fungi. Field Studies 10

Rejmánek, M., Richardson, D.M. and Pyšek, P. (2005) Vegetation ecology. E., V.d.M. (ed), pp. 332–355, Blackwell Science, Copyright (c) Blackwell Publishing Ltd Oxford.

Richardson, D.M., Carruthers, J., Hui, C., Impson, F.A.C., Miller, J.T., Robertson, M.P., Rouget, M., Le Roux, J.J. and Wilson, J.R.U. (2011) Human-mediated introductions of Australian acacias – a global experiment in biogeography. Diversity and Distributions 17(5), 771-787.

Richardson, D.M. and Pyšek, P. (2012) Naturalization of introduced plants: ecological drivers of biogeographical patterns. New Phytologist 196(2), 383-396.

Richardson, D.M., Pyšek, P., Rejmánek, M., Barbour, M.G., Panetta, F.D. and West, C.J. (2000) Naturalization and invasion of alien plants: concepts and definitions. Diversity and Distributions 6, 93-107.

Rilov, G., Benayahu, Y. and Gasith, A. (2004) Prolonged lag in population outbreak of an invasive mussel: a shifting-habitat model. Biological Invasions 6(3), 347-364.

Russo, R. (2006). Field guide to plant galls of California and other western states. Berkeley: University of California Press.

Sánchez, I., Skuhravá, M. and Skuhravý, V. (2012) Gall Midges (Diptera: Cecidomyiidae) Of Cádiz Province (South-Western Spain). Boletín de la Sociedad Entomológica Aragonesa (S.E.A.) nº 51

Sands, D., and Van Driesche, R. G. (2002). Host range testing techniques for parasitoids and predators. In: Proceedings of the First International Symposium on Biological Control of Arthropods", Honolulu, Hawaii (pp. 14-18).

Sanver, D. and Hawkins, B. A., (2000) Galls as habitats: The inquiline communities of insect galls. Basic and Applied Ecology 1(1): 3-11

Sanz-Elorza, M., Mateo, R.G. and Bernardo, F.G. (2009) The historical role of agriculture and gardening in the introduction of alien plants in the western Mediterranean. Plant Ecology 202, 247–256.

Schlapfer, F., Schmid, B. and Seidl, I. (1999) Expert estimates about effects of biodiversity on ecosystem processes and services. Oikos 84(2), 346-352.

Schleuning, M., Bohning-Gaese, K., Dehling, D.M. and Burns, K.C. (2014) At a loss for birds: insularity increases asymmetry in seed-dispersal networks. Global Ecology and Biogeography 23(4), 385-394.

Schonrogge, K., Stone, G.N. and Crawley, M.J. (1995) SPATIAL AND TEMPORAL VARIATION IN GUILD STRUCTURE - PARASITOIDS AND INQUILINES OF ANDRICUS-QUERCUSCALICIS (HYMENOPTERA, CYNIPIDAE) IN ITS NATIVE AND ALIEN RANGES. Oikos 72(1), 51-60.

Simberloff, D. and Stiling, P. (1996) Risks of species introduced for biological control. Biological Conservation 78, 185-192.

Solomon, M.E. (1949) THE NATURAL CONTROL OF ANIMAL POPULATIONS. Journal of Animal Ecology 18(1), 1-35.

Stone, G.N. and Schönrogge, K. (2003) The adaptive significance of insect gall morphology. Trends in ecology & evolution (Personal edition) 18(10), 512-522.

Thomas, M.B. and Willis, A.J. (1998) Biocontrol - risky but necessary? Trends in Ecology & Evolution 13(8), 325-329.

Tisdell, C.A. (1990) Economic impact of biological control of weeds and insects. In: Mackauer M, Ehler L.E, Roland J, editors. Critical issues in biological control. Intercept; Andover, UK. pp. 301–316.

Traveset, A., Heleno, R., Chamorro, S., Vargas, P., McMullen, C.K., Castro-Urgal, R., Nogales, M., Herrera, H.W. and Olesen, J.M. (2013) Invaders of pollination networks in the Galápagos Islands: emergence of novel communities. Proceedings of the Royal Society B: Biological Sciences 280(1758).

Tylianakis, J.M., Laliberte, E., Nielsen, A. and Bascompte, J. (2010) Conservation of species interaction networks. Biological Conservation 143(10), 2270-2279.

van den Berg, M.A. (1979) Trichilogaster acaciaelongifoliae (Froggatt) (Hymenoptera: Pteromalidae): a potential agent for the biological control of Acacia longifolia Willd. in South Africa. Proceedings of the Third National Weeds Conference of South Africa, pp.61-64

Van der Putten, W.H., Mortimer, S.R., Hedlund, K., Van Dijk, C., Brown, V.K., Leps, J., Rodriguez-Barrueco, C., Roy, J., Len, T.A.D., Gormsen, D., Korthals, G.W., Lavorel, S., Santa-Regina, I. and Smilauer, P. (2000) Plant species diversity as a driver of early succession in abandoned fields: a multi-site approach. Oecologia 124(1), 91-99.

Van der Velde, G., Rajagopal, S., Kuyper-Kollenaar, M., De Vaate, A.B., Thieltges, D.W. and MacIsaac, H.J. (2006) Wetlands: Functioning, Biodiversity Conservation, and Restoration. Bobbink, R., Beltman, B., Verhoeven, J.T.A. and Whigham, D.F. (eds), pp. 61-90, Springer-Verlag Berlin, Berlin.

Veldtman, R., Lado, T.F., Botes, A., Procheş, Ş., Timm, A.E., Geertsema, H. and Chown, S.L. (2011) Creating novel food webs on introduced Australian acacias: indirect effects of galling biological control agents. Diversity and Distributions 17(5), 958-967.

Vitousek, P., D'Antonio, C.M., Loope, L.L. and Westbrooks, R. (1996) Biological Invasions as global environmental change. American Scientist 84, 468-478.

Vitousek, P.M., DAntonio, C.M., Loope, L.L., Rejmanek, M. and Westbrooks, R. (1997a) Introduced species: A significant component of human-caused global change. New Zealand Journal of Ecology 21(1), 1-16.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. and Melillo, J.M. (1997b) Human domination of Earth's ecosystems. Science 277(5325), 494-499.

Wallace, R.L., Ott, D.W., Stiles, S.L. and Oldham-Ott, C.K. (2001) Bed and Breakfast: the parasitic life of Proales werneckii (Ploimida : Proalidae) within the alga Vaucheria (Xanthophyceae : Vaucheriales). Hydrobiologia 446, 129-137.

West, S.A., Herre, E.A., Windsor, D.M. and Green, P.R.S. (1996) The ecology and evolution of the New World non-pollinating fig wasp communities. Journal of Biogeography 23(4), 447-458.

Western, D. (2001) Human-modified ecosystems and future evolution. Proceedings of the National Academy of Sciences of the United States of America 98(10), 5458-5465.

Williams, R.J. and Martinez, N.D. (2000) Simple rules yield complex food webs. Nature 404(6774), 180-183.

Williamson, M. and Fitter, A. (1996) The varying success of invaders. Ecology 77, 1661–1666.

Willis, A.J. and Memmott, J. (2005) The potential for indirect effects between a weed, one of its biocontrol agents and native herbivores: A food web approach. Biological Control 35(3), 299-306.

Wilson, J.R.U., Dormontt, E.E., Prentis, P.J., Lowe, A.J. and Richardson, D.M. (2009) Something in the way you move: dispersal pathways affect invasion success. Trends in Ecology & Evolution 24(3), 136-144.

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J.M., Olesen, J.M., Valido, A. and Warren, P.H. (2005) Body size in ecological networks. Trends in Ecology & Evolution 20(7), 402-409.

Zimmermann, H.G., Moran, V.C. and Hoffmann, J.H. (2004) Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water programme. South African Journal of Science 100(1), 34-40.