Interactions between a gall–inducing wasp *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) and its host plant *Acacia longifolia* (Fabaceae)

by

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ABSTRACT

Sallow Wattle (*Acacia longifolia* subsp. *longifolia*) is a native Australian shrub which is an invasive weed in some parts of Australia, and internationally. A gall-forming wasp (*Trichilogaster acaciaelongifoliae*), also native to Australia, causes abnormal growth of tissues (galls) in Sallow Wattle. This wasp is used outside of Australia to control invasive populations of this plant species. However, in Australia, the wasp is not effective in managing the spread of Sallow Wattle. This study investigates various aspects of the relationship between the wasp and its host plant in Australian ecosystems to better understand the physiological and ecological processes involved. The study shows that this wasp is host-specific on Sallow Wattle. The feeding action of the larval wasps increase secondary plant compounds in gall tissue, which may assist the plant to defend itself chemically against other insects and microorganisms. The growth of the galls redirects resources which are otherwise used by the plant for growth and reproduction. A second insect species was found within the galls and was identified as *Megastigmus* sp. This second species is likely to be a parasitoid, killing the larvae of the gall-former and occupying the gall. The presence of *Megastigmus* sp. in Australian ecosystems may be a key factor affecting the ability of *T. acaciaelongifoliae* to control Sallow Wattle in its native range.

The structure of galls formed by each type of gall-inducing insects is unique and the process of gall induction also varies across species. The present study has specifically examined the initiation and development of galls formed by *T. acaciaelongifoliae* on *A. l. longifolia*. Unlike other hymenopteran groups, which induce galls during oviposition, *T. acaciaelongifoliae* appears to form galls on *A. l. longifolia* via the larval feeding process. Three major stages of gall development were identified and described: induction of gall, growth and maturation of gall, and shrinking and desiccation of gall. These findings have

significantly extended our current knowledge of gall induction and development by the hymenopteran group of insects.

Total antioxidant capacity **(**TAC), total phenol (TP), and total anthocyanin (TA) were measured in galls formed by *T. acaciaelongifoliae* at different growth stages of galls and in other plant tissue samples of *A. l. longifolia* to understand the effect of gall formation on plant phytochemistry. The results indicated differences in the amounts of phytochemicals in tissue samples from galls of different growth stages of galls and between gall tissue samples and other plant samples of *A. l. longifolia*. The highest amount of total antioxidant capacity, total phenols and total anthocyanin were recorded in samples of early stages galls, whereas the minimum amounts of phytochemicals were in stems of *A. l. longifolia*. Amounts of antioxidant capacity, phenols and anthocyanin gradually declined as galls developed and larvae became less active in their feeding activity prior to pupation. It is assumed that the active feeding action of the larvae results in increased amounts of these chemicals in the early growth stages of the galls.

The effect of galls formed by the wasp, *T. acaciaelongifoliae* on the growth and reproduction of *A. l. longifolia* was investigated in the native home range of both species, where the plant is invasive. Differences in the average number of phyllodes per sub-branch were found between galled and ungalled plants. Galls were also shown to affect the growth rate of branches. The number of galls correlated positively with twig mortality; and negatively with the number of seedpods per sub-branch. While galls formed by *T. acaciaelongifoliae* have impacts on the growth and reproduction of *A. l. longifolia* plants, the plant continues to invade Australian ecosystems.

An experiment was conducted to investigate the host plant preference of *T. acaciaelongifoliae*. Ten different native host plant species (co-occurring with *A. l. longifolia* in the study locations) were tested in two set of experiments; a 'free choice test' and 'no choice test'. The results showed that *T. acaciaelongifoliae* is highly host-specific on *A. l. longifolia* plants. Thus, it was concluded that the presence of other plant species does not explain the continued invasiveness of *A. l. longifolia* in Australia.

A second insect species was found in the galls developed by *T. acaciaelongifoliae* on *A. l. longifolia*. The insect species has been identified as another hymenopteran from the genus *Megastigmus*. Since no *T. acaciaelongifoliae* emerged from the galls occupied by *Megastigmus* sp, it is proposed that *Megastigmus* sp. may feed upon *T. acaciaelongifoliae* larvae and kill them inside the galls. This might be a key factor affecting the performance of the wasp, *T. acaciaelongifoliae* in controlling *A. l. longifolia* in its native distribution. Parasitism rates of *Megastigmus* sp. should be investigated in future experiments.

DECLARATION

This thesis, submitted as part of the requirements for the award of Doctor of Philosophy in the School of Science, Psychology and Sport (SciPS) at Federation University Australia, is wholly my own work unless otherwise referenced or acknowledged. This thesis describes the original work of the author, and has not been submitted previously for a degree or diploma from any university. It contains no material previously published or written by another person.

Signed: _____

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Candidate

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CHAPTER ONE

1. GENERAL INTRODUCTION

1.1. Background

1.1.1. Galls and their inducers

Galls (or cecidia; singular cecidium) are abnormal outgrowths of tissues formed in response to the presence and activity of an organism (M. Harris et al., 2003). The term is normally used to describe such outgrowths in plants, where they may be highly organized structures, which develop in response to the activities of various species of insects (predominantly Diptera (flies) and Hymenoptera (wasps) and mites. The gall-inducing insects are distributed in the Orders: Thysanoptera (thrips), Hemiptera (true bugs), Coleoptera (beetles), Diptera (flies), Lepidoptera (butterflies and moths), and Hymenoptera (bees, wasps and ants) (Fernandes et al. 2012). Invertebrate species which cause the formation of galls are generally described as gall inducers. The study of galls and gall inducers (known as cecidology) is a complex topic involving entomological and botanical approaches (Meyer & Maresquelle, 1983b).

The galls induced by some flies and wasps, form a closed environment made of a few layers of host-plant tissue, which protects the inducer's (eggs, larvae and pupae) from adverse ecological conditions (Askew, 1984; Bailey et al., 2009; Martel, 1995; Price, Fernandes, & Waring, 1987; Stone & Schönrogge, 2003). Protected within the gall, the eggs and larvae of the gall inducer can resist, for example extreme cold, extreme heat, desiccation and high salinity (Inbar, Wink, & Wool, 2004; Wool, Aloni, Ben-Zvi, & Wollberg, 1999). Galls are

specialised in their structure and the various species of gall inducers are closely associated with specific plants.

Gall-inducing insects may spend most of their lifespans within a gall, often emerging only for a short period of time as adults to reproduce before they die (J. D. Shorthouse & Rohfritsch, 1992). Many gall inducing insects can reproduce sexually and asexually (parthenogenetically) (Csoka, 1998). For example: Cynipid wasps reproduce parthenogenetically to complete their life cycle (Egan, Hood, Martinson, & Ott, 2018). Each species of gall inducer displays an intimate, and often specific, relationship with their host plant species. Specialised nutritional relationships between the insect and the host plant are common. For example, the insect may modify the vascular tissues of the plant in order to obtain nutrients and water (J. Meyer, 1987; Wool et al., 1999) that would otherwise be used for plant growth and reproduction (Fay, Hartnett, & Knapp, 1996; Kirst & Rapp, 1974; Larson & Whitham, 1991; McCrea, Abrahamson, & Weis, 1985). In such cases, the gall becomes a nutritional sink for the affected plant (Jankiewicz, Plich, & Antoszewski, 1970; Anantanarayanan Raman, 2003; Anantanarayanan Raman & Abrahamson, 1995), weakening the host plant metabolism (Fay et al., 1996; Kirst & Rapp, 1974; Larson & Whitham, 1991; McCrea et al., 1985).

Gall-inducing insects may feed actively within galls on flower buds (Lalonde & Shorthouse, 1984), stems (Gassmann & Shorthouse, 1990; A Raman & Dhileepan, 1999), leaves (R. West & Shorthouse, 1982) or roots (J. D. Shorthouse & Gassmann, 1994). Gall induction and development can alter the physiological processes of the host plant (Haiden, Hoffmann, & Cramer, 2012). Galls can also impair vegetative growth and the reproductive stages of the host

plant (N. Dorchin, M. D. Cramer, & J. H. Hoffmann, 2006; Hoffmann, Impson, Moran, & Donnelly, 2002b).

1.1.2. The process of gall induction

Insect-induced galls represent a unique interaction between the inducing insect and the susceptible plant (Weis, Walton, & Crego, 1988). In general, the response of the host plant to the stimulus of the gall inducing insect alters normal physiological processes within the plant, resulting in abnormal growth of plant tissues, and the formation of a gall (Haiden et al., 2012). This may occur in a number of ways. For example, flies of the family Cecidomyiidae and the hemipteroids (bugs, thrips and lice) induce galls via their feeding action and related salivary secretions (Miles, 1999). In this process, salivary secretions of the inducing insect inflict damage on the plant cells. As a consequence, plant metabolites, phenolic compound (detailed in chapter 5), in particular, the photoassimilates (energy storing monosaccharides), may change and alter tissue differentiation as well as growth promoters at the site of the damaged plant cells (Dorchin et al., 2009). As a result, abnormal growth responses are elicited which induce the gall to develop. Salivary secretions from these insects include phytohormone precursors which are able to regulate the development of the gall (Dorchin et al., 2009; Hori, 1974; Kloft, 1951; Miles, 1999). Another mechanism for gall induction is displayed by many Hymenoptera (wasps), including species of the families Cynipidae, Eulophidae and Pteromalidae, which are reported to induce galls via oviposition (detailed in chapter 4). Physical damage to the plant tissue caused during oviposition, and secretions associated with the egg-laying process, provide the stimulus for the growth of the gall (A. West & Shorthouse, 1989).

In hymenopteran insects, the female wasp inserts her ovipositor into plant tissues, about 5 mm below the tip of a vegetative shoot, creating an ovipositional channel into which she deposits one or several egg(s) (A. West & Shorthouse, 1989). After oviposition, the wasp then deliberately damages the apical tip of the plant parts by repeatedly stabbing the ovipositor into tissues above the oviposition sites. This wounds the plant tissue. Consequently, normal growth of that part of the plant stops (A. West & Shorthouse, 1989) and nutrients are redirected to the gall tissues that surround the eggs (J. Shorthouse, West, Landry, & Thibodeau, 1986). According to the nutrition hypothesis, which provides one possible explanation for the evolution of plant galls, changes in the plant tissue provide food resources for the gall inducing insect (Price et al., 1987). The inner layer of the gall induced by the hymenopteran and dipteran insects are typically transformed into nutritive tissue to supply food for the larvae of the gall inducer (W. Abrahamson & Weis, 1987; Mani, 1964). In addition, structural damage to the apical growing parts of the plant (caused during oviposition or feeding) can redirect nutrient flow towards the developing gall (J. Shorthouse et al., 1986).

1.1.3. Gall Inducing Insects

The richness of gall-inducing insect species is immense; there are 133,000 known species in a global context (Espírito-Santo & Fernandes, 2007; Redfern & Shirley, 2011). Most of them are host specific, and some are specific to individual plant organs within a particular plant species— monophagous (W. Abrahamson & Weis, 1987; G. Dennill, 1988). A small number of gall-inducers (for example: cynipid wasps) have more than one host plant species (Penzes et al., 2018), but in all such cases the alternative host plant species are closely related to their preferred (primary) host plant—narrowly oligophagous (Ronquist & Liljeblad, 2001; J. D.

Shorthouse, Wool, & Raman, 2005). Penzes et al. (2018) found gall inducing, cynipid wasps formed galls mainly on *Quercus*, but they can also cause galls on *Castanea, Castanopsis, Lithocarpus, Chrysolepis* and *Notholithocarpus*, all of which belong to the Fagaceae family.

1.1.4. *Trichilogaster acaciaelongifoliae* (Froggatt) (a wasp) and *Acacia longifolia* (Andrews) Will. subsp. *longifolia* (a shrub): a gall inducer-plant system.

Acacia longifolia (Andrews) Willd. subsp. *longifolia* (hereafter *A. l. longifolia*) is a member of the Fabaceae family. A shrub that can grow up to eight meters in height, *A. l. longifolia* has green phyllodes (modified leaf petioles) alternately arranged along the stem and yellow flowers densely arranged in elongated clusters about 20-50 mm long. The elongated seed pod of *A. l. longifolia* contains 4-10 black seeds. The plant typically reproduces via seeds; re-sprouting from the base of the plants also occurs when damaged, for example, by fire.

Acacia. l. longifolia is a shrub native to parts of south-eastern Australia, However, it has been naturalised outside of its native range including in many parts of Victoria (Correia, Montesinos, French, & Rodríguez-Echeverría, 2016; Costermans, 1981; Orchard, 2001). *Acacia l. longifolia* has also been introduced in many countries across the globe as a dune binder and to reduce soil erosion (G. Dennill & Donnelly, 1991; Hagemann & Rose, 1988; E. Marchante, Kjøller, Struwe, & Freitas, 2008a; Pieterse & Cairns, 1988; Stellatelli, Block, Vega, & Cruz, 2015). However, aggressive expansion of the species lead to invasive populations in many ecosystems around the world (Alberio & Comparatore, 2014; G. Dennill & Donnelly, 1991; H. Marchante, Marchante, & Freitas, 2003). The plant invades woodlands, grasslands, scrubs, swamps, watercourses, native bushland and roadsides (Morris, Esler, Barger, Jacobs, &

Cramer, 2011). Conversely, *Acacia l. longifolia* is used as an important landscape species in California and is a host for beneficial insects (Dreistadt & Hagen, 1994). The plant is also used in improving soil fertility (Brito, Reis, Mourão, & Coutinho, 2015; E. Marchante, Kjøller, Struwe, & Freitas, 2009; Weber, 2017).

Trichilogaster acaciaelongifoliae (Froggatt) (hereafter *T. acaciaelongifoliae*) is a wasp from the Pteromalidae family. It is native to Australia and co-occurs with *A. l. longifolia* (G. B. Dennill, Donnelly, Stewart, & Impson, 1999; S Neser, 1984; Noble, 1940). *T. acaciaelongifoliae* forms galls on *A. l. longifolia*, however, the relationship between *T. acaciaelongifoliae* and its host plant *A. l. longifolia* is not well understood, particularly in Australia. In particular, the mechanism of gall induction and development and the effects of gall formation on the growth and reproduction of the plant are unknown.

1.1.5. *Acacia l. longifolia*–an environmental weed

Although native to parts of south-eastern Australia, *A. l. longifolia* is regarded as a significant environmental weed in several Australian states and can alter native ecosystems by promoting a single-species monoculture and suppressing other species (De Wit, Crookes, & Van Wilgen, 2001; Gaertner, Den Breeyen, Hui, & Richardson, 2009; Hellmann et al., 2011; Le Maitre, Versfeld, & Chapman, 2000; E. Marchante et al., 2008a; E. Marchante, Kjøller, Struwe, & Freitas, 2008b; H. Marchante et al., 2003; H. S. D. C. Marchante, 2011; Rascher, Große-Stoltenberg, Máguas, Meira-Neto, & Werner, 2011).

Acacia l. longifolia has aggressive and rapid invasive characteristics (Gaertner et al., 2009; E. Marchante et al., 2008a; H. Marchante et al., 2003; Werner, Zumkier, Beyschlag, & Máguas, 2010; Yelenik, Stock, & Richardson, 2004). *Acacia l. longifolia* is a highly fecund species that can produce approximately 12,000 seeds per square metre per annum (H. Marchante et al., 2003). The seed can persist in the soil profile for decades and germination is enhanced by fire and other natural disturbances (Milton & Hall, 1981; Pieterse & Cairns, 1988). It outcompetes other native species (Chapin, 2003; Dietz & Steinlein, 2004), resulting in reduced biodiversity (Didham, Tylianakis, Gemmell, Rand, & Ewers, 2007; Levine et al., 2003; Pejchar & Mooney, 2009; D. Pimentel, 2001; Vilà et al., 2010; Vitousek, Mooney, Lubchenco, & Melillo, 1997). Dense populations of this plant can increase soil fertility by fixing nitrogen in the soil.

As an environmental weed, *A. l. longifolia*, is a major threat to native flora and fauna outside of its native range (Humphries, Groves, & Mitchell, 1991; Mack et al., 2000). Naturalised populations have established and spread rapidly; for example, in South Africa, Colombia, Portugal, Spain, Uruguay, Argentina and California, (Castro-Díez, Godoy, Saldaña, & Richardson, 2011; H. Marchante et al., 2003; Richardson et al., 2011; Richardson & Rejmánek, 2011). The negative impacts of invasive species biologically and ecologically on different ecosystems is now of significant concern to researchers and managers (Cronk & Fuller, 1995; Mack et al., 2000; David Pimentel, Lach, Zuniga, & Morrison, 2000; Vitousek, Antonio, Loope, & Westbrooks, 1996).

Invasive populations of *A. l. longifolia* in the Australian state of Victoria, within and outside of its natural range, create a significant threat in heathlands, woodlands and forest areas .

Currently, *Acacia l. longifolia* is considered a potentially threatening species in the Greater Grampians (GGr) Bioregion of Victoria, Australia (Milkins, 2017; Thomson, 2016), which is inside its natural range (AVH, 2014). In the Greater Grampians Bioregion, the Grampians National Park is the fourth largest, and one of the most iconic National Parks in the state of Victoria. It supports a wide range of plants, mammals, reptiles, amphibians, native fish, and invertebrate communities (Parks Victoria, 2006). *Acacia l. longifolia* threatens biodiversity in the park, and on nearby private property. It has, therefore, become an important issue to control *A. l. longifolia* in the region, in order to protect the environment and conserve biodiversity.

1.1.6. Consequences of environmental weeds on biodiversity and environment

Environmental weeds are a major threats to flora and fauna in Victoria (Thomson, 2016). Weeds which cause long term impacts on natural ecosystems and adversely affect the survival of native flora and fauna are known as environmental weeds (J. A. Williams & West, 2000). Where a weed spreads quickly, outcompeting other plants, it may also be termed 'invasive'. For instance, the invasive environmental weed, *Lantana camara* is a known threat to 275 native plants in Australia (Groves & Willis, 1999; Mack et al., 2000; Turner & Downey, 2010). *Acacia l. longifolia* is considered an invasive environmental weed (Hagemann & Rose, 1988; Thomson, 2016). A dense monoculture developed by the invasion of *A. l. longifolia* may decrease native plant species richness (Pieterse & Cairns, 1988; Weber, 2017) by suppressing ground flora species under the shade created by *A. l. longifolia* (Muyt, 2001).

Environmental weeds often establish themselves as monocultures by suppressing other native species. Weedy species grow rapidly and outcompete other native plant species for resources

such as nutrients and water, resulting in a loss of plant biodiversity. Consequently the habitats and food sources of native fauna are reduced by the actions of environmental weeds (Gurevitch & Padilla, 2004; Hejda, Pyšek, & Jarošík, 2009); leading to decreases in animal populations in the community (Bascompte & Jordano, 2007; Caujape-Castells et al., 2010).

Invasion by weeds has been recognised as a critical component in changing environments at a global scale (Vitousek, 1994). It changes land-use patterns and disturbance regimes resulting in loss of biodiversity worldwide (Soulé, 1991) and contributing to the homogenization of biological systems around the world (Mack, 1981; Mason & French, 2008). Such global changes include rising temperatures, increased frequency of bushfire, altered rainfall patterns, increasing carbon dioxide (CO_2) and nitrogen (N) accumulation (Dukes & Mooney, 1999; Thuiller, Richardson, & Midgley, 2008; Vilà, Corbin, Dukes, Pino, & Smith, 2007). Invasion by environmental weed species is considered to be a key factor in shifting the local climate and environment (Richardson et al., 2000). For example: invasion of *A. l. longifolia* can alter site conditions by adding nitrogen to the soil which benefits the weed and allows it to grow more densely in that area (E. Marchante et al., 2009). The dense population of the shrub (weed) can also influence fire frequency (D'Antonio, 2000), which may lead to local climate change. In order to develop appropriate management tools, it is important to understand the ecology of *A. l. longifolia,* including its relationship with the gall forming insect, *T. acaciaelongifoliae*.

1.1.7. Use of gall-inducing insects as biological control agents

Dube, Zachariades, Uyi, and Munyai (2019) reported that a gall inducing tephritid fly, *Polymorphomyia basilica* had been used as a biological control agent for the invasive

shrub *Chromolaena odorata* in southern Africa. Another gall inducing fly from the same family, *Cecidochares connexa* forms galls on the leaf buds of Siam weed, *C. odorata* resulting in reduced stem growth of the host plant (Horner, 2002). Siam weed is an invasive weed in tropical Asia, Africa, and the western Pacific. *C. connexa* is used to control the siam weed. *Prodiplosis longifila*, another dipteran insect from the family Cecidomyiidae, (Diptera), is used to control the invasion of bellyache bush, *Jatropha clavuligera.* It forms galls in the shoot-tips of the host plant (Dhileepan, Neser, & De Prins, 2014). The bellyache bush is a major weed of rangelands and riparian zones in northern Australia where its range is expanding (Bebawi et al., 2007). Likewise, skeletonweed, *Chondrilla juncea* is an important weed in Western Australia, which is being managed by the biological control agent *Aceria chondrillae* (Canestrini). *Aceria chondrillae* forms galls on leaves and flower buds resulting in reduced vegetative growth and seed set in the skeletonweed (Caresche & Wapshere, 1974). *Melaleuca quinquenervia* (Myrtaceae) is an invasive plant in wetland in southern Florida. The gall-forming biological control agent *Lophodiplosis trifida* (Diptera: Cecidomyiidae) can suppress the height of the sapling of *M. quinquenervia* and reduce the biomass of woody parts and roots of the plant via galls (Tipping, Martin, & Gettys, 2016). *T. acaciaelongifoliae* is a gall inducing insect, which has been successfully used to control *A. l. longifolia* in South Africa (G. B. Dennill et al., 1999). However *T. acaciaelongifoliae* does not appear to be able to control *A. l. longifolia* populations in Australia, since populations of *A. l. longifolia* remain invasive even in the presence of *T. acaciaelongifoliae* (Thomson, 2016). This observation underpins the investigations in this thesis, which are designed to fill the extensive knowledge gap about this host plant–wasp relationship; and to investigate why the wasp does not sufficiently control the invasiveness of the plant in Australian ecosystems.

1.1.8. *Trichilogaster acaciaelongifoliae* (Froggatt) as a biological control agent

This wasp species is currently used as a biological control agent in managing invasive *A. l. longifolia* populations in South Africa (Hoffmann, Impson, Moran, & Donnelly, 2002a). However, *Acacia l. longifolia* is still invasive in many parts of Victoria, despite the presence of *T. acaciaelongifoliae*. Therefore, one of the objectives of the research is to evaluate the effect of galls formed by *T. acaciaelongifoliae* on the vegetative growth and reproduction of *A. l. longifolia* in Australia. It is assumed that, like other gall-inducers, the wasp develops galls on *A. l. longifolia* by redirecting resources for the normal growth of the host plant's tissues at the point of attack. Several studies on other gall-inducing species have been undertaken to understand the developmental stages of gall induction and development, however it is still unclear how *T. acaciaelongifoliae* induces galls on its host plant (Giron, Huguet, Stone, & Body, 2016; Mani, 1964; Meyer & Maresquelle, 1983a; Myer, 1987; Stone & Schönrogge, 2003; A. West & Shorthouse, 1989). The mechanism of gall formation is an important factor to understand the relationship between *T. acaciaelongifoliae* and its host plant *A. l. longifolia.* Therefore, one of the aims of this study is to address how the *T. acaciaelongifoliae* develops galls on *A. l. longifolia.*

Gall-inducing insects are also currently being used as models for studying and interpreting the chemical and molecular ecology of insect-plant interactions (Gatjens-Boniche, 2019). The diversity of shapes, colours, unique structures and chemistry of the galls induced by insects could establish models to study biological systems of insect-plant interactions. However, insufficient studies have been made on the chemistry of galls induced by *T. acaciaelongifoliae*

in Australia, and thus the chemistry of galls *T. acaciaelongifoliae* on *A. l. longifolia* induced by this wasp in Australia is another objective to address in the study.

Although *T. acaciaelongifoliae* is widely used as a biological control agent to control *A. l. longifolia* in South Africa, the biology and ecology of this insect has not been well studied. As the various species of gall-inducing insects have remarkably different aspects to their life histories, it is important to closely study the biology and ecology of gall-inducing insects, especially those used as biological control agents. Several studies have been conducted on the biology and ecology of other gall-inducing insects (Ananthakrishnan, 1984; Csoka, 1998; La Salle, 2005; Mani, 1964; Whitham, 1992); however, limited knowledge is available on the detailed biology and ecology of *T. acaciaelongifoliae* in Australia. The host preference of *T. acaciaelongifoliae* and the presence or absence of other species within the gall community are also poorly understood in Australia. It is, therefore, essential to know the biology and ecology of *T. acaciaelongifoliae* in order to better understand the ecological relationships between the host plant and the gall forming wasp in the study area.

This thesis presents a series of studies intended to address the research gaps in the areas of i) mechanism of gall formation, ii) chemistry of galls, iii) effect of galls on plant, iv) host preference and v) gall community in galls on *A. l. longifolia* induced by *T. acaciaelongifoliae.* The cumulative effects of the study contribute to an understanding of the relationship between the gall-forming wasp *T. acaciaelongifoliae* and its host plant, *A. l. longifolia* in Australia.

1.2. Research questions

The overarching goal of this study is to gain a better understanding of the relationship between the gall–inducing wasp, *T. acaciaelongifoliae* and its host plant *A. l. longifolia* in an Australian ecosystem. To achieve this overarching research goal, the following research questions are explored:

Wasp–gall–plant relationships

I. What is the mechanism of gall formation by *T. acaciaelongifoliae* on *A. l. longifolia*?

II. What is the chemistry of galls formed on *A. l. longifolia* by *T. acaciaelongifoliae*?

III. What are the effects of galls formed by *T. acaciaelongifoliae* on the growth and reproduction of *A. l. longifolia* plants?

Community Interactions

IV. What is the host plant specificity of *T. acaciaelongifoliae*?

V. Are there any other inhabitants of galls induced by *T. acaciaelongifoliae* on *A. l. longifolia*?

1.3. Thesis structure

This thesis is presented in nine chapters (Figure 1.1). The present chapter provides a general introduction to the study including the background, significance, and goals of the study, also detailing the research questions. Chapter 1 continues with an outline of the thesis, identifying how the thesis will address research gaps and answer the research questions.

Chapter two presents a review of the relevant literature. The review is focused initially on the ecology of environmental weeds; in particular, the ecology, population biology and distribution of *A. l. longifolia*, a shrub species native to many parts of south-eastern Australia. The review progresses to describe the current state of knowledge regarding the ecology, biology and distribution of the gall-forming wasp, *T. acaciaelongifoliae*. This wasp species is a parasite of *A. l. longifolia.* Infestation results in the formation of galls or cecidia (abnormal outgrowths of plant tissue). The aim of the literature review is to present current knowledge about: environmental weeds including *A. l. longifolia*, gall–inducing insects including *T. acaciaelongifoliae* and the various mechanisms of gall induction by insects. The review identifies several knowledge gaps which are addressed in later chapters.

Chapter three describes the general approach and methods used to achieve the objectives of this PhD study. It includes details of study locations in the Greater Grampians Bioregion of Victoria, Australia, where populations of *A. l. longifolia* and *T. acaciaeloingifoliae* co-occur.

Chapters four to eight in this thesis describe discrete experiments or investigations, each of which contributes towards the overall objectives of the study. Chapters four, five, and six report the results of three separate investigations of the relationships between *A. l. longifolia* and *T. acaciaelongifoliae* (research questions I, II and III), and the galls formed on the former species by the latter. Chapter four then describes experiments and observations that revealed the mechanism of gall formation by the wasp (research question I), which has not been previously described. Chapter five describes the chemical characterization of gall tissues (research question II), filling another knowledge gap and Chapter six describes the effects of galls

formed by *T. acaciaelongifoliae* on the vegetative growth and reproductive capacity of *A. l. longifolia* (research question III). Chapters seven and eight consider interactions between the wasp, its host plant and other species within the ecological community (research questions IV and V). Chapter seven describes the specificity of *T. acaciaelongifoliae* for its host plant (research question IV). A previously unknown species that inhabits galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* is then described in chapter eight (research question V).

Chapter nine synthesises the findings of the previous chapters and draws general conclusions about the PhD research, including recommendations for future study.

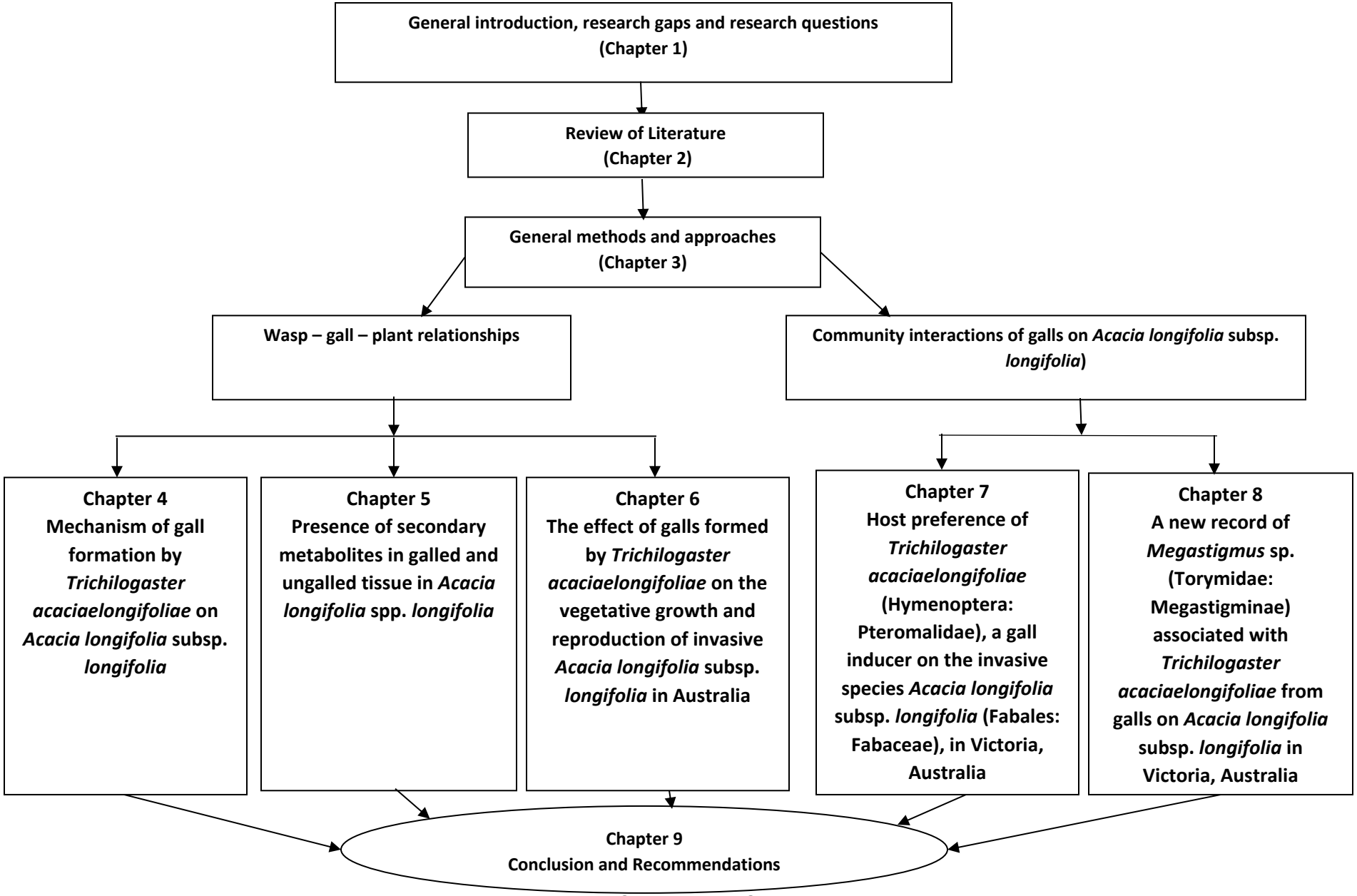

Figure 1. 1. Conceptual framework of thesis structure.

CHAPTER TWO

2. REVIEW OF LITERATURE

The aim of this chapter is to provide a clear understanding of the nature of galls on plants, including, the mechanisms by which galls typically develop, the nature and types of gall-causing agents, and their biology and interaction with their host plants. The use of galls in the control of environmental weeds is also considered; thus requiring an introduction to the concepts of 'weed' and 'environmental weed'. In addition, this chapter identifies research gaps related to the study of galls formed on *A. l. longifolia* by *T. acaciaelongifoliae*. The following information provides background to the research questions as presented in the previous chapter. Peer-reviewed journals, relevant books, conference papers and internet sources have been cited in this review, which is structured so as to inform the present study.

Each of the chapters 4-8 in this thesis has an additional introductory section, which provides further detail relevant to the investigation described. The present chapter is therefore a broad overview of the relevant literature.

2.1. The concept of weed

A weed can be defined as any plant species that is undesirable in an area or which interferes with the activities or welfare of humans (Benvenuti, 2007; Vencill, 2002). In other words, even plant species that are valuable or useful in some places, may be considered a weed where it occurs unexpectedly, where it is unwanted or where it decreases the value of agricultural products or environmental values (Zimdahl, 2013). The status of a plant as a weed is thus

dependent on where it is growing; a species which is a native in one region may be considered to be a major weed in another region (Pickering & Mount, 2010).

There are approximately 250,000 species of plants worldwide and about 8000 species have been recognised as weeds (PennState, 2017). In Australia, a weed can be an exotic species or a native species that colonises, persists and becomes naturalised in an ecosystem where it did not previously exist. Generally, the weed has established in a region outside of its natural range without human interference. Thus, an Australian native species can also be considered as a weed when it grows outside of its natural range. For instance, around 200 plant species have naturalised outside of their natural range in Victoria (SGA, 2014).

A weed can have adverse economic, environmental and/or societal effects. For example, the costs associated with weed management, reduced agricultural production and loss of stock, can adversely affect farm incomes (Cook, Sheppard, Liu, & Lonsdale, 2015; Sinden et al., 2005). In recognition of this, a weed is also understood to be any plant that requires some form of action to be taken to reduce its effect on the economy, the environment, human health and social amenities (Richardson et al., 2000).

Weeds are often characterised by a capacity to produce large numbers of seeds and an ability to survive in a wide range of environmental conditions. They are generally excellent colonisers of disturbed environments and are the first species to colonise and dominate in these conditions, outcompeting other species. Consequently, the presence of weedy species is often associated with losses of other species from the ecosystem. In general, weedy species have

the following characteristics: i) individuals and populations can become established quickly, ii) seeds are copious and have a long dormancy period, iii) plants show rapid vegetative growth; and iv) weeds commonly occupy sites disturbed by human activities.

2.2. Definition of an environmental weed

Weeds which invade native communities or ecosystems rather than agricultural areas are considered 'environmental weeds'. Such plants are unwanted from an ecological perspective, but not essentially an economic one. An environmental weed is any plant that causes potential threat to the environment and biodiversity. Serious environmental weeds are those that cause major modification to species richness, abundance or ecosystem function. Very serious environmental weeds are defined as those that can entirely and permanently destroy an ecosystem (Humphries et al., 1991).

An environmental weed can be an exotic plant which has established accidentally or following an intentional introduction to an area, or it can be an indigenous plant species that has spread outside of its normal range due to inappropriate management. An environmental weed has also been defined as a plant species that has established in native vegetation terrestrial or aquatic, outside their natural range by self-propagation (SM Csurhes, 1995).

Swarbrick and Skarratt (1994) defined environmental weeds as those plant species which invade, persist and multiply within an area, and causes of problems of maintenance, management of native vegetation, fauna or other natural or semi-natural environmental values.

2.3. Environmental weeds and their effects

An environmental weed can be considered invasive when it spreads rapidly and supresses other native flora. Invasive plants disrupt ecosystems and modify indigenous biodiversity resulting in a loss of native species (J. A. Williams & West, 2000). Environmental impacts of weeds include the degradation of natural ecosystems through reduction in biodiversity, an increase in fire risk, loss of habitat for native fauna, and reduced ecosystem amenities. For example a dense infestation of a weed, *Marrubium vulgare* (horehound) at Wyperfeld National Park, Victoria caused significant loss of biodiversity values (Adair & Groves, 1997). Bridal creeper is another invasive weed that poses a serious threat to natural ecosystems in southern Australia (Adair & Groves, 1997). Leigh and Briggs (1992) reported that 57 plant species are endangered nationally as a result of competition with weeds; 166 native plant taxa are threatened by environmental weeds in Victoria (Carr, Yugovic, & Robinson, 1992).

The term 'environmental weed' tends to be synonymous with several other terms including 'invasive plants', 'alien plants', 'weeds of conservation reserves', 'bushland weeds', 'exotic weeds' and 'nonindigenous—naturalised plants' in the literature (S Csurhes & Edwards, 1998).

2.4. The environmental weed *A. l. longifolia*

A total of 1059 species have been listed as environmental weeds in Australia (S Csurhes & Edwards, 1998). Among them, *A. l. longifolia* is a significant environmental weed causing ecological problems in the distribution of Victoria, especially in the Grampians National Park (GNP) and surrounding areas, where is has established populations within of its native range.

It is also problematic in other Australian states such as South Australia and Western Australia; and elsewhere in the world (Castro-Díez et al., 2011; H. Marchante et al., 2003; Richardson et al., 2011; Richardson & Rejmánek, 2011).

Acacia l. longifolia is a native weed species in Australia belonging to the family Fabaceae (Marchante et al. 2003). This species changes community composition and the ecosystem by suppressing other species and promoting a single-species monoculture; thus reducing species richness and biodiversity (Castro-Díez et al., 2011; De Wit et al., 2001; Gaertner et al., 2009; Hellmann et al., 2011; Le Maitre et al., 2000; E. Marchante et al., 2008a; H. Marchante et al., 2003; H. S. D. C. Marchante, 2011; Rascher et al., 2011).

Acacia l. longifolia can alter communities above and below ground, affecting: microclimates, soil moisture regimes and soil nutrient levels (Gaertner et al., 2009; E. Marchante et al., 2008a; H. Marchante et al., 2003; Werner et al., 2010; Yelenik et al., 2004).

Plate 2. 1. *A. l. longifolia* in the Grampians National Park, Victoria, Australia. The dense cover of *A. l. longifolia* does not allow other native plant species to grow study location PV5; Grampians National Park, Victoria.

Acacia l. longifolia is known to produce large numbers of seeds. It can produce nearly 12,000 seeds per square metre annually (Hélia Marchante, Freitas, & Hoffmann, 2010). The severity of invasions of *A. l. longifolia* is increasing (Richardson et al., 2011; Thomson, 2016). Currently, in the GNP, *A. l. longifolia* is expanding and establishing as a monospecific dense shrub community (Plate 1). This species has become a management priority within the Park (Thomson, 2016).

2.5. Management of *A. l. longifolia*

Sustainable control of *A. l. longifolia* is difficult because the seeds remain viable in the soil for decades (Milton & Hall, 1981; Pieterse & Cairns, 1988). Dormant seeds can germinate after above-ground efforts to control stands of this invasive species; or after some disturbance events (e.g. fire). Despite the popularity of biological control, it has been ineffective in some places. For example: the use of gall inducing flies *Urophora quadrifasciata* (Frfld.) and *U. affinis* (Meig.) were unsuccessful to control diffuse knapweed, *Centaurea diffusa* (Lam.), in North America after being established (P Harris, 1980; Myers, Risley, & Eng, 1988). Though, a gall forming wasp, *Trichilogaster acaciaelongifoliae* is used to control *A. l. longifolia* in South Africa, it has not been a successful biological control agent of the weed, *A. l. longifolia* in Australia. The goal of this research project is to understand the relationship between *T. acaciaelongifoliae* and *A. l. longifolia*, since the wasp is not successful in control of the weed in the native range in Australia.

T. acaciaelongifoliae is gall-forming wasp of the family Pteromalidae. Native to Australia, it is known to cause galls on *A. l. longifolia*. This wasp was introduced to South Africa in 1982 as a successful biological control agent to manage invasive populations of *A. l. longifolia* (G. Dennill & Donnelly, 1991). It has become a well-established and effective control agent for *A. l. longifolia* in South Africa (G. Dennill & Donnelly, 1991; G. B. Dennill et al., 1999; F. Impson, Kleinjan, Hoffmann, Post, & Wood, 2011).

Plate 2. 2. Galls on *A. l. longifolia*, formed by *T. acaciaelongifoliae* (Photo taken in the Greater Grampians Bioregion, location PP2; Private Property 2).

Although *T. acaciaelongifoliae* is widely used as a biological control agent to control *A. l. longifolia* in South Africa, the biology and ecology of this insect has not been well studied and the mechanisms of gall induction and formation are not well understood.

T. acaciaelongifoliae does not appear to control invasive populations of *A. l. longifolia* in Australia, since the plant continues to invade areas in Australia where the wasp species is co-located. Thus, it is essential to know the ecology and biology of *T. acaciaelongifoliae* with the host plant.

2.6. Galls formed by *T. acaciaelongifoliae*

Galls are novel plant structures resulting from changes in the normal pattern of growth and development of plant tissues or organs (Plate 2.2). Their formation may be induced by either insects or mites on a wide variety of host plants. There is often a highly specific relationship between a gall-forming insect and its plant host. Thus, a gall involves at least two participants: one is an arthropod that causes the gall and the other is the plant which determines the growth response (J. Shorthouse, 1982). Sometimes fungi can also be associated with or within a gall as a third component (S. A. Graham, 1995; Henrik & Biedermann, 2012). A series of complex interactions occur between the arthropod and the plant to express a gall (Colvin et al., 2006).

2.7. Types of galls

Galls vary in their structure and in their methods of induction and development (J. D. Shorthouse & Rohfritsch, 1992). Galls may be single-chambered or multi-chambered. Based on gall shape, galls are classified as described in Table 2.1.

Table 2. 1. Different types of plant galls and their causal agents.

Types of Galls	Definition and Examples (J. Meyer, 1987)
1. Blister and pit galls	Blister-like swellings of leaves, doming of one side of the leaf blade with a depression beneath; caused by gall midge, psyllids (sap sucking bugs) and scale insects.
2. Bud and rosette galls (Plate 2.2)	Deformities or enlargement of flower buds that vary from aborted buds to large swellings of bud structure; caused by eriophyoid mites, cecidomyiid gall midges , pteromalid wasps and adelgids (a family of insects closely related to aphids).
3. Filz galls	Usually occur on leaf blades; spherical with tufts of hairs; caused by eriophyoid mites.
4. Pouch galls	Simple, pouch-like deformities on the leaf surface; caused by eriophyoid mites, aphids, cecidomyiid gall midges, psyllids.
5. Roll and fold galls	A roll or fold of the leaf margin or leaf blade; caused by eriophyoid mites, aphids, cecidomyiid gall midges, psyllids, sawflies or thrips.
6. Covering galls	The gall inducer initially feeds on the surface of a leaf or stem and is then covered completely by plant tissue; caused by aphids, cecidomyiid gall midges, and scale insects.
7. Mark galls	The gall inducer deposits its eggs inside the plant tissue and the larvae are embedded in and feed on plant tissue; caused by cynipids (gall wasps), cecidomyiid gall midges, pteromalid wasps, tephritid flies (Plate 2.2).

2.8. Causes of plant galls

The mechanisms of gall induction by insects are thought to include mechanical injury to plant tissues and injection of chemical secretions, either during oviposition or feeding. Plant hormones such as auxin, amino acids and amides, and digestive enzymes (J. Shorthouse, 1982), may have roles in inducing galls.

2.9. Morphology of insect induced plant galls

Galls induced by different gall-inducing insects are morphologically distinctive (Ananthakrishnan, 1984; Metcalf & Kogan, 1987; Odette Rohfritsch, 1981). Coccoids (scale insects) can produce sexually dimorphic galls; that is, male and female insects produce different shaped galls (Gullan, 1984). Some gall-inducing insects (such as cynipid wasps, cecidomyiid flies) have an alternation of generations involving first sexual, and then asexual (parthenogenetic), reproductive forms. These alternative forms produce galls of different shapes on different host plants, or on even different parts of the same host plant species (Ananthakrishnan, 1984; Mani, 1964; Stone & Schönrogge, 2003).

2.10. Hypotheses of gall induction

There are three hypotheses describing the adaptive significance of gall formation for the gall-forming insect: the nutrition hypothesis; the microenvironment hypothesis; and the enemy hypothesis (Takei, Yoshida, Kawai, Hasegawa, & Suzuki, 2015).

2.10.1. The Nutrition hypothesis

The nutrition hypothesis suggests that galls provide nutrition to the gall inducer. Most gall-inducing insects (with the exception of fungus-feeding gall midges, which cultivate a fungal garden inside the gall (S. A. Graham, 1995; Henrik & Biedermann, 2012) feed on plant tissues or fluids. Galls form highly differentiated nutritive tissues around the larval gall inducer. These nutritive tissues are more nourishing and less well-defended (softer) than non-gall tissues on the same plant (Cornell, 1983; J. D. Shorthouse & Rohfritsch, 1992; Whitham, 1992).

2.10.2. The Microenvironment hypothesis

The microenvironment hypothesis suggests that gall tissues play an important role in protecting the gall-inducer (immature stage of insect, larva) from adverse environmental conditions, protecting especially against desiccation (K. Blanche, 2000; Cornell, 1983; Whitham, 1992). Gall-inducing insects live in enclosed structures within the boundary layers of moist plant cells. These act as a buffer against water stress for the insect body (J. D. Shorthouse & Rohfritsch, 1992). Generally, this hypothesis is broadly accepted as a selective advantage for the gall inducer (Cornell, 1983).

2.10.3. The Enemy hypothesis

The enemy hypothesis explains that inducers (larvae) shelter within galls where they are protected from predation. The structure of a gall also gives protection to the inducer from non-specialist predators and pathogens. However, the gall inducer can be attacked by other enemies within a gall such as fungi, predators, and parasitoids. These can cause high mortality for gall-inducers (W. G. Abrahamson & Weis, 1997; Cornell, 1983; Crespi & Abbot, 1999; J. D. Shorthouse & Rohfritsch, 1992; Waring & Price, 1989).

The relevance of these three hypotheses vary between gall forming insect species. There is little information about the mechanisms of gall induction and development by *T. acaciaelongifoliae,* nor is there information about which of the three hypotheses above are more likely to be important for this gall-forming wasp. This study aims to elucidate the host plant responses of *A. l. longifolia* to gall-induction by *T. acaciaelongifoliae*. This review provides a basis for the following chapters which address the research questions presented in Chapter One.

CHAPTER THREE

GENERAL METHODS

This chapter broadly describes the general approaches used to address the research questions outlined in chapter 1.2. It begins by providing information about the study areas for the research described in this thesis, including their physical condition, climate and vegetation. After that, the chapter describes methods and materials in two broad sections. First, the general methods used in investigating the relationships between the gall-forming wasp, *T. acaciaelongifoliae* and its host plant, *A. l. longifolia* are outlined. This section includes descriptions of the methods used to understand the nature of the galls formed on *A. l. longifolia* by *T. acaciaelongifoliae*, and the mechanisms by which they are formed (the results of these investigations are reported in chapters four, five and six of the thesis). Second, the methods and approaches used to investigate the role of other species in community interactions with *T. acaciaelongifoliae* and *A. l. longifolia* are described. The results of these investigations are reported in chapters seven and eight of the thesis.

More detailed descriptions of the methods used in each of the studies presented in chapters four to eight are included within each of those chapters.

3.1. Study area

The Greater Grampians Bioregion of Victoria was selected as the study area because of the prevalence of *A. l. longifolia* and concern about its effect (suppressing native plants) on the Grampians National Park (Milkins, 2017; Thomson, 2016) (see plate 2.1 and 3.1)

3.1.1. Physical description

For the research described in this thesis, field studies were conducted, and plant materials were sampled from a single study area in the Greater Grampians Bioregion of Victoria.

Specifically, there were six study locations comprising three locations within the Grampians National Park and three locations on private properties adjacent to the northern boundary of the National Park near the township of Laharum (Table 3.1) (Figure 3.1).

The Grampians National Park is the fourth largest, and one of the most well-known National Parks in the state of Victoria (Parks Victoria 1998). The park (36°55´S, 142°25´E) is situated south of the Western Highway, between the towns of Stawell and Horsham approximately 260 km to the north-west of Melbourne and 460 km to the south-east of Adelaide (Arrowsmith & Inbakaran, 2002). The park has a total area of 167,219 hectares (Parks Victoria, 2003) extending from Mount Zero in the north to Mount Abrupt and Mount Sturgeon in the south.

The six study locations were pseudo-randomly selected based on the presence of *A. l. longifolia*, evidence of wasp activity and accessibility. The minimum distance between any two study locations was at least 300 metres. The private properties adjoined with the northern side of the park boundary (Figure 3.1). The three study locations within the National Park supported only native vegetation (classified as Ecological Vegetation Class[1] (EVC): 285; Dry Creekline Woodland (Table 3.1). Two of the private properties (PP2 and PP3) also supported patches of Dry Creekline Woodland. Location PP1, a productive olive farm, was dominated by olive trees (approximately 28000, with spacing, 10 m x 10 m)) and pasture. A part of this farm supported remnant vegetation (classified as EVC: 48;

[1] The concept of Ecological Vegetation Class (EVCs) as mapping units for native vegetation was first introduced in Victoria in the 1990s. They are now the basic mapping units used in Victoria for biodiversity planning and conservation assessment at a landscape and regional scale. EVCs are based on criteria such as plant communities and forest types, including species and structural information; ecological information relevant to the species that comprise the communities, including life form and reproductive strategies; and information that describes important variations in the physical environment, including aspect, elevation, geology and soils, rainfall, and climate zones (DPI, 2008).

Heathy Woodland (DELWP (Department of Environment, 2004), nearly 100 m x 80 m of which was densely invaded by *A. l. longifolia*. Other native trees, such as *Eucalyptus* sp, *Acacia mearnsii* and *Acacia paradoxa* were commonly found in locations PP2, PP3, PV4, PV5 and PV6. All locations had stands of scattered, self-seeded *A. l. longifolia* trees of various heights. (Plate 3.1).

Plate 3. 1. Study location PP1 (private property 1), a part of the cultivated olive farm densely invaded by *A. l. longifolia* along with other native trees (A) and location PV5 (Park Victoria 5) densely covered by *A. l. longifolia* along with other native trees (B).

3.1.2. Climate

The Grampians region has a temperate climate with marked seasonal patterns. Summer and autumn are usually dry and hot whereas winter and spring are cool and wet (Day, McGregor, & Johnstone, 1984). Local microclimates across the park are further influenced by the abrupt topographic change in the landscape.

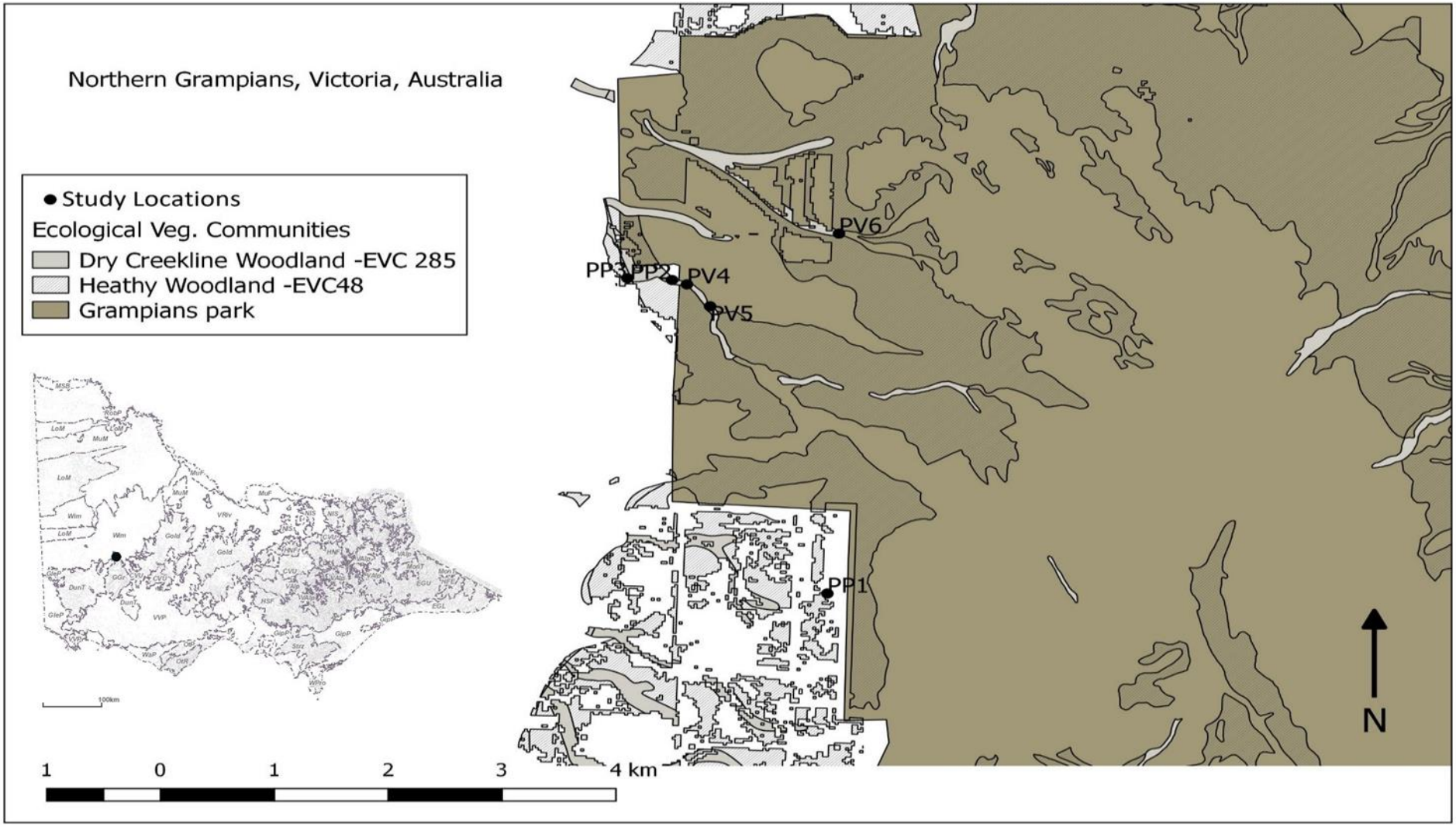

Figure 3. 1. Map of northern Grampians showing study locations (black dots) in two EVC classes; dark grey=EVC 285 (Dry Creekline Woodland) and light grey=EVC48 (Healthy Woodland) in Greater Grampians (GGr) Bioregion of Victoria, Australia.

Table 3. 1. Geographical information regarding the six study locations.

Location identification	Latitude / Longitude	Land tenure
PP1	36°56’07.34’’S, 142°22’22.15’’E	Private property
PP2	36°54’17.05’’S, 142°21’27.03’’E	Private property
PP3	36°54’16.44’’S, 142°21’11.20’’E	Private property
PV4	36°54’18.57’’S, 142°21’32.32’’E	Parks Victoria)GNP(*
PV5	36°54’26.29’’S, 142°21’40.53’’E	Parks Victoria)GNP(*
PV6	36°54’00.73’’S, 142°22’26.27’’E	Parks Victoria)GNP(*

*GNP=Grampians National Park, PP= Private Property, PV= Parks Victoria

The northern part of the park experiences higher rainfall than the southern part (Parks Victoria, 2006). An average annual rainfall of 1135.5 mm was recorded in the Grampians region for years 2005 to 2020. The months with heaviest rainfall are May to August each year (BOM, 2020).

The maximum average annual temparature recorded for the years 2005 to 2020 in the Grampians region was 12.8 degrees Celsius (BOM, 2020). January and February are the warmest months, with maximum average temperatures of 21.4 and 20.3 degrees Celsius respectively (BOM, 2020). Localised frosts are common throughout the winter months, particularly in sheltered areas where air can settle during night, such as valleys and on the downslopes of escarpments.

3.1.3. Vegetation

The Grampians National Park is recognised as a key botanical reserve for the state of Victoria (Parks Victoria, 2006). The diverse habitats throughout the park provide support for a wide range of plants. Around a third of the Victoria's vascular plants are found in the park with over 20 endemic species (Day et al., 1984; Parks Victoria, 2003). Among the plant communities, a significant proportion of plant species are considered to be threatened (J. A. Williams & West, 2000). Half of Victoria's endangered species are endemic to the Grampians National Park (DSE, 2003a).

3.2. Collection of mature galls from the field to raise adult *T. acaciaelongifoliae* in the laboratory

Acacia l. longifolia shoots and inflorescences bearing 10 galls were collected from each of the six study locations in the Greater Grampians bioregion. Small branchlets bearing galled shoots and inflorescences were collected from infested *A. l. longifolia* plants. Galls were collected from plant parts less than two meters from the ground to avoid the need for a ladder. To maximise the number of adults of *T. acaciaelongifoliae* obtained, samples were collected during field visits which occurred every 15 days throughout the 'mature gall season' across three years: September 2014 to February 2015, September 2015 to February 2016 and September 2016 to February 2017.

After collection from the field, whole branchlets bearing galled shoots and inflorescences were immediately transported to the laboratory at Federation University's Mount Helen Campus in Ballarat, Victoria. In the laboratory, branchlets were cut to lengths of approximately 15 cm.

The cut ends of the shoots were placed in Knop's solution in jars capped with sieve-lids, to facilitate proper aeration, until adults emerged from the galls. The Knop's solution provided moisture and nutrients to keep the plant material alive and so that the galls developed normally. These specimens were kept at ambient temperature (approximately 18°C) and light conditions and observed every three hours from 6 am to 12 pm to detect adult wasps emerging from the galls. On emergence, *T. acaciaelongifoliae* adults were immediately collected using an aspirator and used in the experiments described in chapters four and seven. Any insects which emerged from the galls which were not *T. acaciaelongifoliae* were kept in 70% ethyl alcohol for identification using microscopy (see chapters four, seven and eight).

3.3. Transfer of adults of *T. acaciaelongifoliae* to insect-proof cages containing plants in the glasshouse at Federation University Australia.

Immediately after emergence from galls collected in the field (usually 2-5 days after collection), adult *T. acaciaelongifoliae* were captured using an insect aspirator and placed in 120 cm x 80 cm x 90 cm insect-proof cages containing potted plants (see chapter four and seven). Each cage was made of an aluminium frame and muslin netting with a mesh size of 0.5mm. Each cage held adult wasps and contained a fiber filter dampened with water (Whatman 13 mms AA) with a drop of sugar solution to provide moisture and nutrition for the adult wasps. Observations of ovipositional behaviour of these wasps were made as described below in section 3.5. This is relevant to the research described in chapter four and seven.

3.4. Collection of adult *T. acaciaelongifoliae* from the field

Muslin bags were attached to plants growing in the study area, at each of the six study locations, so as to enclose newly developed galls. These bags facilitated the capture of adult insects as they emerged from the galls. This was repeated during the gall season across three years: September 2014 to February 2015, September 2015 to February 2016 and September 2016 to February 2017.

Galls enclosed in this way were observed as they developed in the field every 15 days from September to February of each year, while galls were green and growing. The frequency of observations was reduced to once a month from March–August of each year when galls were drying out or empty. Any bags which were noted to be damaged or torn during observations were immediately replaced. Adult *T. acaciaelongifoliae* wasps observed inside the muslin bags were immediately collected by aspirator and transferred into well-ventilated glass jars for transport to Federation University Australia's Mount Helen campus before being used for further experiments.

Wasps collected in this way were used to address the research questions examined in chapters four and seven. Insects observed in the muslin bags which were not *T. acaciaelongifoliae* were also collected by aspirator in the field and kept in 70% ethyl alcohol for research described in chapter eight.

3.5. Observation of ovipositional behaviour and gall development

Adult wasps, including those in glass jars containing plant materials in the laboratory and those in the insect-proof cages containing potted plants, were observed intensively (15-18 hours per day), using a magnifying glass where necessary, for 5 days following emergence to detect and observe ovipositional behaviour. The number and locations (i.e. on flower and shoot buds) of insertions of the female wasp's ovipositor into the plant tissue were of particular interest. A single ovipositional operation was defined as the time taken between the first stabbing action and the point at which the female moved away from that ovipositional site.

Following oviposition, the flower and shoot buds containing eggs deposited by *T. acaciaelongifoliae* were closely monitored. A marker pen was used to mark the points of oviposition; and observations continued at intervals of 24 hours to document gall formation and growth until emergence of the next generation of adults and desiccation of the galls Ovipositional behaviour and growth of gall were observed for the experiments described in chapters four and seven.

3.6. Microscopy

3.6.1. Light microscopy

The tissues of 90 galls (30 collected in each year and 10 of each growth stage comprising each batch of 30) were fixed in FAA (Formalin Aceto Alcohol)[2], followed by processing through an alcohol series (30, 50, 70, 80, 90, 100%, each change 12 hours), and histolene and paraffin-wax embedding at 65°C. The wax-embedded tissues were sectioned at 8 μm, deparaffinised in

[2] FAA was made up as follows: 10 ml formalin, 50 ml ethanol (95%), 5 ml glacial-acetic acid, 35 ml distilled water – making up to 100 ml

histolene, contrasted with 1% toluidine blue (in 1% aqueous-borax solution) and mounted in DPX (dibutyl-phthalate xylene) to observe under the compound light microscope. Insect samples were processed through graded ethyl alcohol series and mounted in glycerine on slide to observe under the light microscope. Samples were prepared for light microscopy for the experiments described in chapters four, seven and eight.

3.6.2. Scanning electron microscopy

The tissues of 90 galls (30 collected in each year and 10 of each growth stage comprising each batch of 30) were cut into small $2mm^3$ pieces for electron microscopy. Each cube of gall tissue was placed immediately into 10 ml 2.5% glutaraldehyde fixative and incubated for 48 hours with agitation. The glutaraldehyde was then discarded and the gall pieces were washed three times (the samples were left for 2 hours each time) in phosphate buffered saline (PBS)[3], (pH =7.4) followed by processing through an alcohol series (10, 30, 50, 70%, each change 6 hours). Prepared phosphate buffered saline was kept in the refrigerator below 4°C until further processing for scanning electron microscopy in the next week. For insect specimens, samples were processed through graded ethyl alcohol series as described earlier for dehydration followed by cleaning in a sonicator.

Further processing involved cleaning of the samples in a sonicator (Branson 2510, Danbury, Connecticut, USA) for 5 min, followed by drying in a critical-point drier (# 030, Bal-Tec AG, Schalksmühle, Germany). The samples were then coated with gold in a sputter coater (SC7620, Quorum Technologies Limited, Kent, UK) and viewed using a scanning electron microscope

[3] PBS was made up as follows: NaCl 8 g, KCl 0.2 g, Na_2HPO_4 1.44 g, KH_2PO_4 0.24 g – making up to 1000 ml volume

(SEM) microscope (S-4500, Hitachi Scientific Instruments, Tokyo, Japan). Micrographs were produced at 5 kV (Onagbola & Fadamiro, 2008; Saeung et al., 2014; L. X. Zheng, Wu, Liang, & Fu, 2014) Samples were prepared for electron microscopy for the experiments described in chapters four, seven and eight.

CHAPTER FOUR

Mechanism of gall formation by *Trichilogaster acaciaelongifoliae* on *Acacia longifolia* subspecies *longifolia*

4.1. Introduction:

Gall**s** induced by insects represent a distinctive interaction between the gall inducing insect and the host plant (Weis et al., 1988). Globally, there are 133,000 known gall forming insect species (Espírito-Santo & Fernandes, 2007; Redfern & Shirley, 2011) and each produces galls with unique characteristics in the host plant (A. Meyer, 1987).

Generally, insect-induced galls occur on plants because interactions between the inducing insect and the plant alter the normal physiological processes within the plant, causing the aberrant development of plant tissues and the formation of a gall (Haiden et al., 2012). The process of gall development varies between different insect species. For example, members of the orders Diptera (flies) and Hemiptera (bugs) form galls via their feeding action and chemicals related to salivary secretions (Miles, 1999). Galls form on flower buds (Lalonde & Shorthouse, 1984), stems (Gassmann & Shorthouse, 1990; A Raman & Dhileepan, 1999), leaves (R. West & Shorthouse, 1982) or roots (J. D. Shorthouse & Gassmann, 1994) in response to mechanical and chemical damage from the feeding action of these insects.

By contrast, some members of the Order Hymenoptera (including many gall inducing wasps) (A. West & Shorthouse, 1989) are reported to cause the formation of galls on plants via oviposition. In such cases, galls are induced by mechanical damage caused by repeated stabbing of plant tissues “and/or” by chemicals secreted during ovipositional behaviour (J. Shorthouse et al., 1986). The gall inducing hymenopterans in the superfamily Chalcidoidea

demonstrate this mechanism of gall formation (Stone, Schönrogge, Atkinson, Bellido, & Pujade-Villar, 2002). Chalcidoidea includes: the pteromalid, cynipid and eulophid wasp families such as *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) which form galls on lowbush blueberry in North America (Sliva & Shorthouse, 2006; A. West & Shorthouse, 1989).

Studies on the effects of galls induced by insects are often focussed on plants of horticultural importance such as galls formed by cynipids on chestnut trees (Kato & Hijii, 1997a). As a result, the mechanism by which galls are induced in many non-horticultural plants remains poorly understood (Mani, 1964; A. Meyer, 1987; Meyer & Maresquelle, 1983b; J. D. Shorthouse & Rohfritsch, 1992; M. Williams, 1994). The mechanism of gall induction on *A. l. longifolia* by *T. acaciaelongifoliae* is not known. This chapter aims to determine the mechanism of gall formation on *A. l. longifolia* by the wasp, *T. acaciaelongifoliae.* Understanding the mechanism of gall formation in *A. l. longifolia* may help us to understand the relationship between *T. acaciaelongifoliae* and *A. l. longifolia*. Since *T. acaciaelongifoliae* is a pteramalid hymenopteran**,** it was hypothesised that this species is likely to cause galls formation via ovipositional actions, like other members of the Pteromalidae family (Sliva & Shorthouse, 2006; A. West & Shorthouse, 1989).

4.2. Materials and Methods

To examine the mechanism by which *T. acaciaelongifoliae* forms galls on *A. l. longifolia*, the ovipositional and feeding behaviour of the wasps, and the development of galls on the plant, were both observed in the field under natural conditions, in the glasshouse and in the laboratory from September 2014 to December 2016.

4.2.1. Collection mature galls from the field

Acacia l. longifolia shoots and inflorescences bearing galls were collected during 54 visits (18 visits per gall season for three gall seasons; from September 2014 to February 2017 at each of six study locations in the Greater Grampians bioregion (detailed in chapter 3 section 3.2). Ten galls were collected from each location at each field visit.

A total of 3240 galls were collected. These galls were used for various purposes as indicated in Figure 4.1.

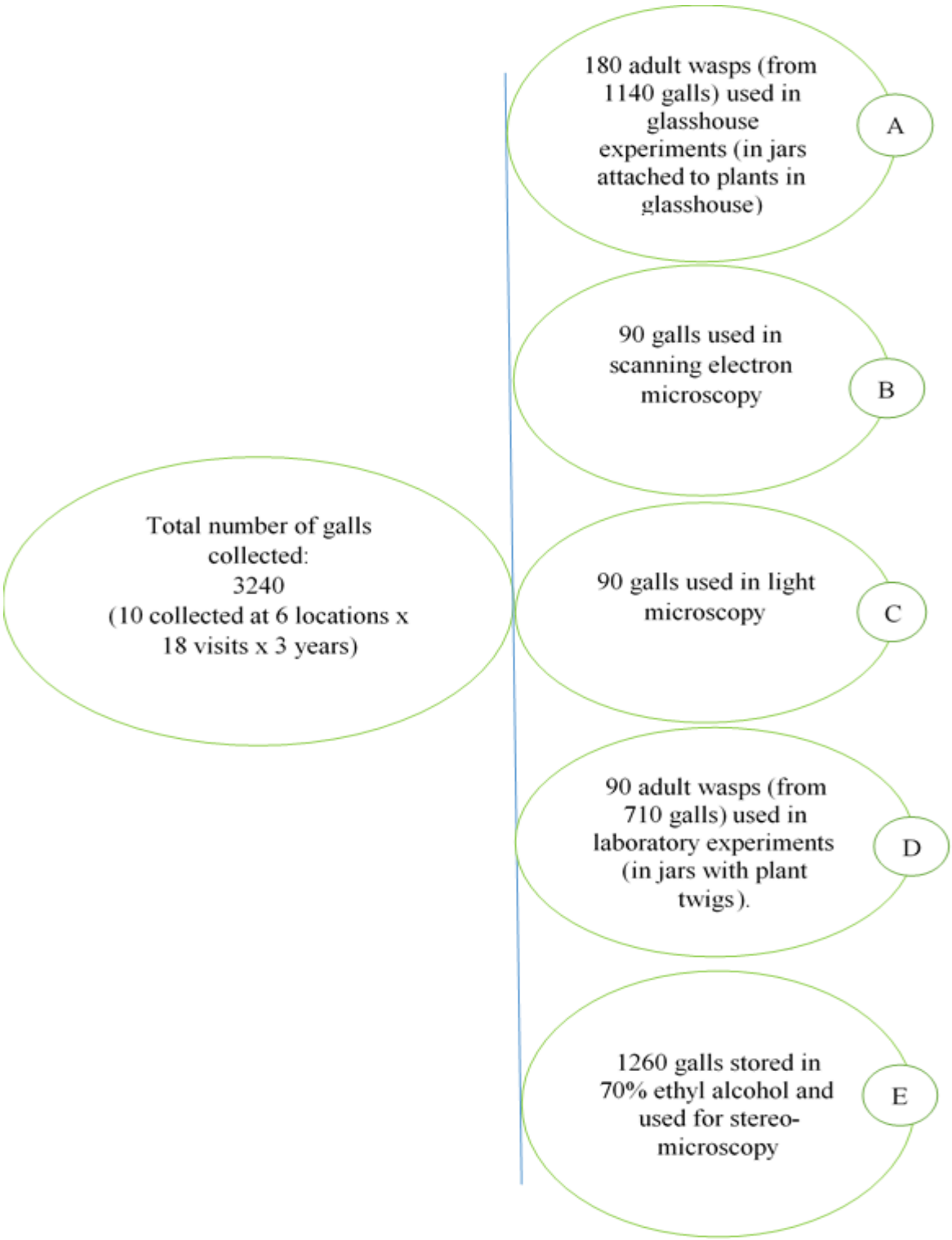

Figure 4. 1. Summary of numbers of galls collected from the Greater Grampians Bioregion during field visits between September 2014- February 2017 and how these were used in various experiments in the laboratory and glass house at Federation University Australia, Mt Helen campus. The letters A-E refer to separate experiments which are described below.

4.2.2. Transfer of adults of *T. acaciaelongifoliae* to the insect-proof cages containing *A. l. longifolia* in the glasshouse at Federation University Australia.

T. acaciaelongifoliae reproduces parthenogenetically, so that more than 90% wasps emerging from galls are females (McGeoch, 2000; Old, 2002). A total of 180 wasps emerging from 1140 field-collected galls (see circle A in figure 4.1) between November to December of 2014 (107 wasps), November to December of 2015 (46 wasps) and November to December of 2016 (27 wasps) were transferred to the foliage of potted *A. l. longifolia* plants in the glasshouse at Federation University's Mount Helen campus in Ballarat, Victoria for studies of ovipositional behaviour and gall growth (detailed methods of transferring of adults of *T. acaciaelongifoliae* to the insect-proof cages containing *A. l. longifolia* in the glasshouse are provided in chapter 3, section 3.3). These observational studies were each initially conducted in 2014 and then repeated in 2015 and 2016.

4.2.3. Preparation of galls for microscopy

A total of 90 galls collected from the field in September – December 2014, April – December 2015 and April – December 2016 (see circle B in Figure 4.1) were processed and used for scanning electron microscopy in December 2015 (60 samples collected in 2014 and 2015) and December 2016 (30 samples collected in 2016). A further 90 galls collected from the field in (September – December 2014, April– December 2015 and April – December 2016) (see circle C in Figure 4.1) were processed and used for light microscopy in December 2015 (60 samples collected in 2014 and 2015) and December 2016 (30 samples collected in 2016). These galls were of different sizes and were collected from the study locations at different stages of the gall season (as detailed in chapter 3, section 3.6). A stereo binocular microscope was used to isolate larvae from the galls. Galls were dissected along their median axis using a razor blade to expose the larvae. Larvae were then

extracted from the galls using a fine tipped camel hair brush. Larvae were immediately fixed in 70% ethanol for microscopy. Following removal of the insects, galls of each developmental stage were processed for microscopy as described in chapter 3, sections 3.7.1 (light microscopy) and 3.7.2 respectively (electron microscopy).

Of the 180 galls processed for light and electron microscopy, 60 samples were collected in 2014 (30 for light microscopy +30 for electron microscopy), 60 samples were collected in 2015 (30+30) and 60 samples were collected in 2016 (30+30). Each batch of 30 samples comprised 10 galls collected early in the season, 10 collected mid-season and 10 collected late in the season.

4.2.4. Observation of ovipositional behaviour and gall induction

Three studies of ovipositional behaviour of *T. acaciaelongifoliae* were undertaken in November to December of 2014, November to December of 2015 and November to December of 2016. Two studies on growth of galls were undertaken (April–December 2015 and April–December 2016). This part of the method is described in detail in chapter 3, section 3.5. These studies took place in the glasshouse (using a total of 180 adult wasps, circle A, Figure 4.1) and in the laboratory (using a total of 90 adult wasps, circle D, Figure 4.1) at Federation University.

4.2.5. Observation of eggs in the laboratory

To observe wasp's eggs, ten glass jars, each containing approximately 150 ml of Knop's solution were arranged in the laboratory between November to December in 2014, and again, at the same time of year, in 2015 and 2016. Three living *A. l. longifolia* branchlets approximately 15 cm long and bearing flower and shoot buds collected from the field were

placed inside the jars and enclosed with a well-ventilated insect proof lid. Adult *T. acaciaelongifoliae* wasps (three per jar, total *N*=90; see circle D in Figure 4.1) were transferred to the jars to allow egg deposition in between November to December in 2014, 2015 and 2016. After egg deposition, parts of the flower and shoot buds (*N*=30, total) presumed to contain eggs were excised and fixed in FAA for 24 hours. They were then treated in 10% aqueous sodium hydroxide for three to four days at 50°C to bleach and clear the bud to transparency (Johansen, 1940). After repeated rinses in distilled water, the bud portion that included the egg was contrasted with 1% toluidine blue solution, differentiated in acidic alcohol and mounted on a glass slide in 50% aqueous glycerine (A. West & Shorthouse, 1989). In addition, twenty one buds (flower and shoot buds) from the field presumed to contain eggs were prepared for light microscopy. These flower and shoot buds were collected January 2016.

4.2.6. Collection of galled plant material from the field to observe gall growth

To document the different stages of gall formation, additional galls were collected from *A. l. longifolia* plants growing in study locations in the Greater Grampians bioregion. Galls of different sizes, formed by *T. acaciaelongifoliae,* on *A. l. longifolia*, were collected once a month early in the gall season (April to August) and then twice a month late in the gall season (September to February in the following year) in 2014, 2015 and 2016. The diameter of all collected galls were measured using slide callipers. Galls collected in the field were kept in a cool box in the fridge until further processing in the laboratory which occurred on the day following collection. Observations of different stages of gall formation were conducted using light and scanning electron microscopy (see circles B, C and E in Figure 4.1). Photographs of key events of gall development were taken using a Canon EOS 80D DSLR camera attached to a stereo binocular microscope.

4.3. Results and discussions:

4.3.1. Gall formation by *T. acaciaelongifoliae* on *A. l. longifolia*

Chapter 4 has examined the initiation and development of galls formed by *T. acaciaelongifoliae* on *A. l. longifolia*.

Three main phases of gall development were observed: induction, growth and maturation, shrinking and desiccation. Emergence of the adult wasps occurred between the second and third of these stages. These observations were consistent for galls developing in the field, in the glasshouse and in the laboratory. Details are summarised in Table 4.1.

Table 4. 1. Developmental stages of galls observed in the field and laboratory during the study period by *T. acaciaelongifoliae* on *A. l. longifolia*.

Gall sizes (mm)	Growth stages of gall	Stage starts in the month of every year	Duration of each stage	Number of galls collected
1-2	induction	April/May	~5 days	260
3-26	growth and maturation	May/June	7-8 months	2080
≤26	shrinking and dessication	December	4-5 months	900

Gall induction

The first phase of gall formation is gall induction. Female adults of *T. acaciaelongifoliae* were observed to lay eggs in tissues of the young flower or shoot buds of *A. l. longifolia*, however it became clear during this study that the feeding activity of the larvae, rather than

the oviposition activity of the female, caused gall formation. This is supported by field and glasshouse experiments.

Adult wasps emerged during November to December in each year of the study period. After emerging from the mature gall, the adult female *T. acaciaelongifoliae* searched actively for suitable places for oviposition, usually moving from the base of young branches upward to the tips to find younger flower or shoot bud to lay eggs observed in the laboratory and in the glasshouse experiments. Once the female wasp found a younger flower or shoot bud, it appeared to check the suitability of the bud for oviposition with antennal movements.

Plate 4. 1. Adult *T. acaciaelongifoliae* ovipositing in the flower bud of the *A. l. longifolia* (X 3) (photograph taken by the author on 20 December 2014).

Once a bud was chosen as a suitable oviposition site, the wasp then moved forward and backward on the bud, presumably identifying a precise location for oviposition (Plate 4.1). Female wasps took an average of 10.94±1.25 (Mean±SD) minutes (10 minutes 56 seconds±1 minute 15 seconds) from emergence to find a suitable place for oviposition (Table 4.2). Only the youngest buds were used as oviposition sites. During oviposition, the wasp sat on the bud with her body at an angle of 45° with the head up and the abdomen down (Plate 4.1). Using her ovipositor, the wasp stabbed the plant tissue an average of 10.43±0.62 (Mean±SD) times before laying eggs. A single ovipositional operation was defined as the time taken between the first stabbing action and moving away from that ovipositional site. The average time taken for one ovipositional operation was 14.82±5.10 (Mean±SD) seconds. On average, each female wasp repeated this process 7.98±2.16 (Mean±SD) times before she dies (Table 4.2).

After completing one ovipositional operation (depositing 12-20 eggs), the wasp moves forward about 5-10 cm and immediately lays another batch of eggs in the same bud or on an adjacent bud.

Oviposition activity was very high during the first hour following emergence of the female wasp. Subsequent activity gradually decreased and ceased after 20 hours. On the second day following emergence, wasps were observed to sit on buds or stems, moving slowly. On average 85.59±5.59 (Mean±SD) per cent of observed adult wasps had died by the third day after emergence. The remainder lived until the fourth day following emergence, but did not actively move.

Table 4. 2. **Time spent by female *T. acaciaelongifoliae* wasps in various activities from the time of emergence to death**.

Year of emergence (November to December)	Number of adult wasps observed emerging in the laboratory	Average time period between emergence and start of ovipostion (minutes) (Mean±SD)	Average number of stabbing events before oviposition (Mean±SD)	Average time spent for one ovipositional operation (seconds) (Mean±SD)	Average number of ovipositional operations/ adult (Mean±SD)	Average period of time for all ovipositional operations (minutes) (Mean±SD)	Proportion of adults dead by third day after emergence (%)	Average time between oviposition and initiation of gall (days) (Mean±SD)
2014	151	11.05±1.99	9.81±1.40	20.71±5.33	8.33±1.57	9.67±2.52	79.34	139.33±14.97
2015	54	12.14±3.24	11.05±2.67	12.13±2.91	9.95±1.76	14.33±3.05	87.32	151.67±13.31
2016	65	9.64±2.50	10.43±2.04	11.63±2.07	5.67±1.08	11.67±2.08	90.12	142.33±11.01
Average (Mean±SD)		10.94±1.25	10.43±0.62	14.82±5.10	7.98±2.16	11.89±2.34	85.59±5.59	144.44±6.44

Plate 4. 2. An early stage gall formed by *T. acaciaelongifoliae* (X 3) on the flower bud of *A. l. longifolia* (photograph taken by the author on 21 June 2015).

Galls have a vast of diversity of structure, shape, size and mechanisms of development which are all dependant on the activities of the gall inducers (Csoka, 1998; Dreger-Jauffret, 1992; Stone et al., 2002). Regular observations of oviposition points in this study revealed that galls did not start to develop until April, or May. Examination of galls revealed that this coincided with the hatching of larvae from the egg and the initiation of larval feeding. Hence, although the adults of *T. acaciaelongifoliae* emerged from galls in November or December and oviposited within three days of their emergence, gall induction started at the point of oviposition much later (in the month of April or May in the subsequent year, when larvae began feeding). Eggs remain dormant and do not hatch until April/May. In April-May newly hatched larvae start feeding and as a result galls start to develop and larvae feed inside of the developing galls.

This study has clearly established that gall induction by *T. acaciaelongifoliae* on *A. l. longifolia* is due to the feeding activity of the larvae *T. acaciaelongifoliae* and not due to oviposition. This is uncommon in the hymenopteran group (Bronner, 1973; Miles, 1999; Rey, 1992; A. West & Shorthouse, 1989) and is an important novel finding. A majority of gall-inducing species of Hymenoptera develop galls on plants by their ovipositional activities (Rey, 1992). A. West and Shorthouse (1989) found that a hymenopteran gall–inducing insect, *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) develops galls on lowbush blueberry, *Vaccinium angustifolium* (Ericaceae) by their ovipositional actions. Sliva and Shorthouse (2006) also reported gall initiations, by *Aulacidea hieracii* and *Diplolepis spinose* on hawkweed (*Pilosella umbellatum*) and rose (*Rosa blanda*) respectively, began during oviposition. *A. hieracii* and *D. spinose*, are both Hymenopterans, of the Cynipidae family Hymenoptera. However, the results of the current study supports the relevance of the nutrition hypothesis (galls provide nutrition to the gall inducer) (Takei et al. (2015), which observed in dipteran and hemipteran insects (Miles, 1999). In this context, this is an advantage for the gall inducer getting food and protection from its own created gall in plant as larvae feed inside the gall. Since galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* do not form until larvae begin to feed, the eggs of *T. acaciaelongifoliae*, unlike those of other hymenopterans, which induce galls via oviposition, are not protected by gall tissue. Presumably, the stabbing action of the ovipositor ensures that eggs are laid safely beneath the epidermis of the plant tissue. Further studies are necessary to determine whether the eggs are protected by plant secretions.

The feeding activity and size of the larvae within the galls was observed by dissecting galls of different sizes collected from the study locations during the months of May to October of 2015, and 2016.

During this study, it was observed in the field and in the glasshouse that galls forming on *A. l. longifolia* by *T. acaciaelongifoliae* occur in two forms; rounded (one chambered) and elongated (multi-chambered) (see plate 4.3 and 4.4).

Plate 4. 3. Left: Elongated gall, Right: round gall on *A. l. longifolia* induced by *T. acaciaelongifoliae* (photographs taken by the author on 29 September 2014.

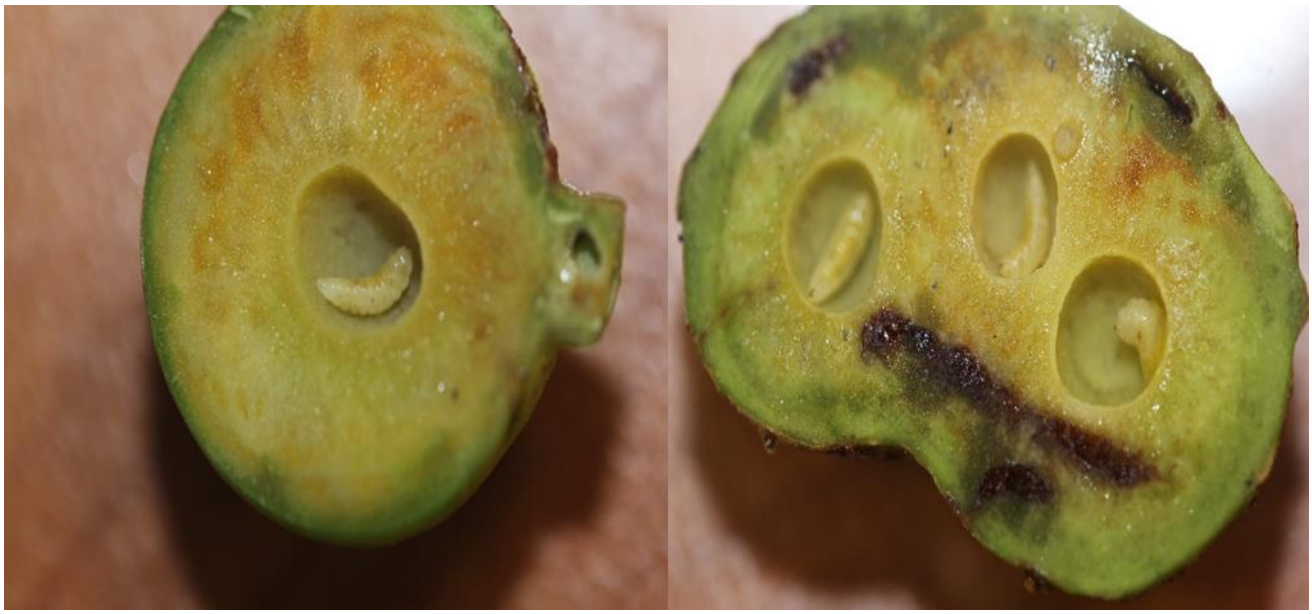

Plate 4. 4. Left: Mature gall on a flower bud of *A. l. longifolia* with full-grown *T. acaciaelongifoliae* larvae in a single-chamber (X 5). Right: multiple-chambered gall (X 3) (photographs taken by author on 01 October 2015).

It was found that rounded galls contained a single larva, while irregular and elongated galls contained multiple larvae (Plate 4.4). The average diameter of a full grown single chambered gall in the field was 16.72±1.20 mm (Mean±SD). Multiple chambered galls found in the field were double this size (Table 4.3).

Growth and maturation of galls observed in the field, glasshouse and laboratory

Galls formed following oviposition events observed in the glasshouse increased in size progressively from May to August, to an average gall size (for rounded, single-chambered galls) of 16.44±1.88 mm (Mean±SD) in diameter (in August). Growth rates remained steady and then ceased during September when average gall size was 16.72±1.20 mm (Mean±SD), presumably because larvae had ceased feeding activity in preparation for pupation (Figure 4.1).

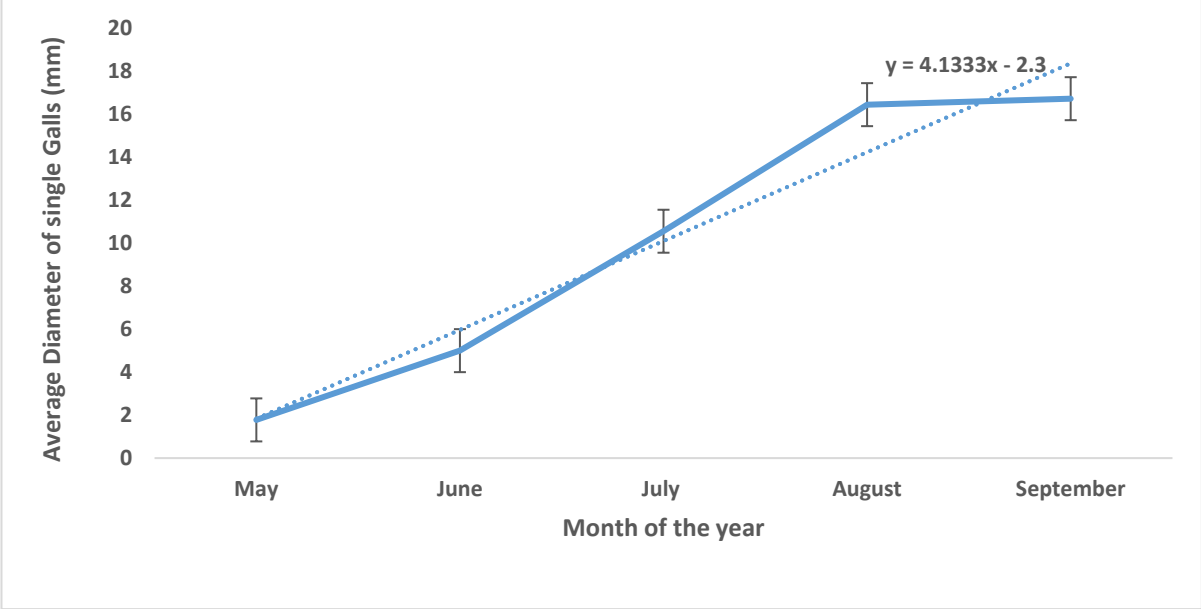

Figure 4. 1. Average growth of single chambered galls (round shaped) (n=36) formed on *A. l. longifolia* by *T. acaciaelongifoliae* observed in the field and glasshouse from May to September in 2015 and 2016.

During pupation, the pupa remained inside the gall and there was no hole visible from outside of the galls. Pupation inside the galls was confirmed by dissection of several galls at this stage (Plate 4. **5**).

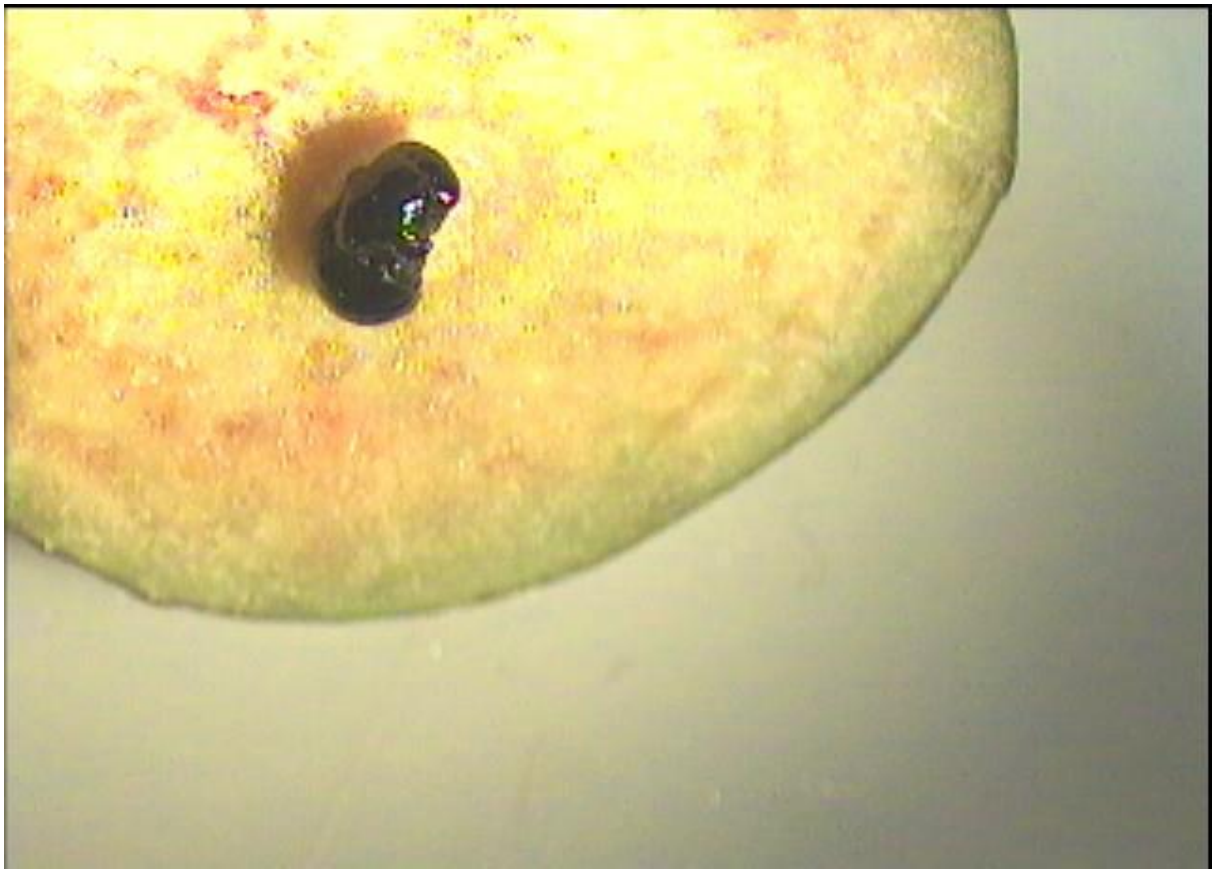

Plate 4. 5. Pupa of the *T. acaciaelongifoliae* inside the gall of *A. l. longifolia* (X 5) (photograph taken by author on 25 November 2015).

In the month of May 2015, six single galls began to develop on potted plants kept in the glass house. The development of these galls followed the ovipositional events observed during the ovipositional study in December 2014. The growth of the galls in glass house was slower than the growth of the galls in the field, however their overall shape and appearance were the same as the galls in the field. These grew to an average diameter of 14.33±1.2 (Mean±SD) mm when mature in September of the year of 2015.

Also in May 2015, 12 single galls which began to develop on plants growing in four of the six field locations were marked. These reached an average diameter of 16.72±1.2 (Mean±SD) mm when mature (in September 2015). Three multiple galls were tagged which began to develop on plants in the field from two locations in late of May 2015. These grew to an average diameter of 29.83±2.25 (Mean±SD) mm by September 2015 (Table 4.3). However, no multiple galls developed in the glass house over the study period.

Table 4. 3. Size (diameter) of the single and multiple galls in the glass house at Federation University Australia and at the study locations in 2015 and 2016.

	Single galls			**Multiple galls**		
	Number of galls observed	Average diameter (mm) (Mean±SD)		Number of galls observed	Average diameter (mm) (Mean±SD)	
		When mature	When shrunken		when mature	When shrunken
Observed in glass house	6	14.33±1.2	6.5±1.3	-	-	-
Observed in the field	12	16.72±1.2	9.67±3.05	3	29.83±2.25	18.66±1.53

Each gall grew as the larva within it developed, supporting the observation that *T. acaciaelongifoliae* induces gall formation by the feeding action of the larvae.

Gall dissection revealed that pupation occurred within the galls during October or November (Plate 4.5). No growth of the gall occurred during the pupation period. This further supports the contention that the feeding activity of the *T. acaciaelongifoliae* is the key factor causing the gall growth on *A. l. longifolia*. Brooks and Shorthouse (1998) found growth of galls on *Rosa blanda* happens during the period of feeding by larvae of the wasp,

Diplolepis nodulosa (Hymenoptera: Cynipidae). O Rohfritsch (1992) also reported the same for cynipid wasps. The growth of galls on oak trees (Fagaceae) is because of continuous larval feeding of cynipid wasps on gall tissues.

Shrinking and desiccation of gall

Following pupation inside the gall, the adult *T. acaciaelongifoliae* chews a channel through the wall of the gall in order to emerge. An exit hole remains in the empty gall structure. Emergence occurs in November or December (Plate 4.6).

Plate 4. 6. Adult of the *T. acaciaelongifoliae* emerging from the mature gall formed on *A. l. longifolia* (X 50) (photograph taken by author on 15 December 2015).

After the emergence of the wasp, the gall begins to shrink and dessicate, reducing in size by approximately 50% within a week (Plate 4. 7). The dried gall remained on the plant for

up to a year before dropping to the ground, see right photograph in Plate 4.7, which was taken March, 2016, the following year of gall development.

Plate 4. 7. A shrunken gall (left photograph) after one week of emergence of the wasp showing exit hole of *T. acaciaelongifoliae* (X 3) and dried galls (right photograph) of *A. l. longifolia* (actual size) (left photograph taken by author on 20 January 2016, right photograph on 15 March 2016).

A detailed understanding of the development of galls in plants is important to understand the gall inducers and their hosts (Rey, 1992; J. Shorthouse, 1993). The study described in this chapter provides information on the initiation of galls and their development which different from other hymenopteran gall-inducing insects. The mechanism of gall formation is known to be diverse among the gall-inducing insects even between species of the same family (A. West & Shorthouse, 1989). Nevertheless, the findings of this chapter contribute to a deeper understanding of the relationship between the wasp *T. acaciaelongifoliae* and its host, *A. l. longifolia.*

4.4. Conclusion

This study documents key events in the development of galls caused by *T. acaciaelongifoliae* on *A. l. longifolia.* It proved useful to consider three major stages of gall development: induction of gall, growth and maturation of gall, shrinking and desiccation of gall. *T. acaciaelongifoliae* induces galls on *A. l. longifolia* via the feeding activity of the larvae of the wasp. This is a novel finding and is unusual for the hymenopteran gall-forming insects, which typically induce galls via ovipositional behaviour. The growth of the gall by *T. acaciaelongifoliae* occurs from the month of May to August, when the larvae of the wasp actively feed inside the gall tissues. Growth of the gall ceases when the larva pupates. The gall starts to shrink when the adult wasp emerges from the gall (during December) and subsequently dessicates. It seems likley that the presence and feeding action of the larval wasp is required to maintain living plant tissue in the gall. It was also observed from the study that the wasp prefers younger flower or shoot buds for egg deposition. The information presented here significantly contributes to the understanding of galls formed by the pteromalid wasp *T. acaciaelongifoliae* on *A. l. longifolia*.

CHAPTER FIVE

Presence of secondary metabolites in galled and ungalled tissues of *Acacia longifolia* spp. *longifolia*

5.1. Introduction

Phytochemicals are chemicals produced by plants. Such chemicals may assist plants to thrive or grow, or have a role in defending plants against predators and pathogens (Lattanzio, Lattanzio, & Cardinali, 2006). Phytochemicals are often broadly classified depending on whether or not they have a direct role in the primary metabolism of the plant (i.e. contributing directly to growth and development). Compounds such as auxins, cytokinins and gibberellins are found in all plants and perform metabolic roles that are essential and evident (Davies, 2010). For example, auxins, a group of closely related hormones including indole-3-acetic acid (IAA), are synthesised in leaves, buds and growing shoots. They promote cell division and influence the carbohydrate mechanism. Auxins, acting in conjunction with other hormones, initiate root development and influence the elongation of roots and growth of the axillary shoot following germination (J. Li, Li, & Smith, 2017). Auxin also stimulates cell enlargement in plants (Davies, 2010; Leopold, 1964).

Plant chemicals with no known direct function in basic metabolism are known as secondary plant compounds, or secondary metabolites (Bell, 1981). The function of these chemicals in plants is still a topic of debate. Some have been proposed to act as growth regulators, or in the maintenance of ionic balance, or as nitrogen storage reservoirs (Wink, 2017). However, it is commonly understood that secondary plant compounds have an important role in interspecific interactions, such as in attracting pollinators and seed-dispersing

agents, as allelopathic agents to influence competition among plant species and as protection against herbivory and microbial infection (Metcalf & Kogan, 1987). Alkaloids, phenolics, antioxidants and anthocyanins are classes of secondary plant metabolites which are known to be important in insect-plant interactions. In some plants, alkaloids provide a chemical defence against herbivory. Certain plant families particularly rich in nitrogen-containing secondary metabolites which include caffeine, nicotine, morphine, strychnine and cocaine provide defence against herbivory (Howe & Jander, 2008).

Phenolic compounds are characterised by a chemical structure including an aromatic benzene ring and one or more hydroxyl groups. Some phenolics play important roles in plant development, or provide structural integrity to plants. Phenolic phytoalexins, secreted by wounded or stressed perturbed plants, repel or kill many microorganisms (Bhattacharya, Sood, & Citovsky, 2010). Phenolics have also been identified as important factors in protecting plants against insect attack (Cheeke, 1989; Hartley, 1999).

Antioxidants are compounds that defend cells or organisms from damage caused (Abdel-lateif, Eldeab, & Maghrabi, 2016) by free radicals produced through chemical reactions in the plant's system. For example, vitamins C and E, and carotenoids such as beta-carotene, lutein and lycopene, protect cells from damage caused by free radicals (Abdel-lateif et al., 2016).

Anthocyanins (a kind of antioxidants) are water-soluble pigments responsible for the blue, purple, red and orange colours of many fruits and vegetables (Miguel, 2011), with an important role in attracting and facilitating pollinators and seed dispersers (Karageorgou & Manetas, 2006). Anthocyanins are also thought to have a role in protecting plants from

tissue damage caused by extreme temperature (Zhang, Zhai, Shao, Lin, & Peng, 2019) as well as reducing insect herbivory (Karageorgou & Manetas, 2006).

Although several studies have described the morphology of galls formed on different plants by different insects, and the biology of the gall-inducing insects (M. Harris et al., 2003; Peter Harris & Shorthouse, 1996), very few studies have investigated the role of plant chemicals in gall formation, or the chemical characteristics of the gall tissue. Gall chemistry is likely to play an important role in the development of the gall as well as in the growth and nutrition of the gall inducing wasp. The production of antioxidants and phenolic compounds in plants has been observed to increase in response to insect herbivory (Liu, Norris, Hartwig, & Xu, 1992) and are recognised as important in gall formation. Maffei, Mithöfer, and Boland (2007) have suggested that phytochemicals (antioxidants and phenolic compounds) are released by plants (chrysanthemum) in response to the feeding activity of aphids. Salivary secretions from some gall inducing insects are known to include phytohormone precursors which regulate the development of the gall (Guerrieri & Digilio, 2008; Miles, 1999). Miles (1999) reported that salivary secretions from gall-inducing Aphidoidea regulate abnormal growth in the plant, poplar tree (*Populus nigra*). The insertion of aphid saliva by a gall inducing aphid species creates hormonal imbalance of the attacked plant resulting in formation of gall (Guerrieri & Digilio, 2008). Guerrieri and Digilio (2008) demonstrated that, gall inducing insect, *Rhopalosiphum insertum* accumulates extra amino acids (five-fold) in the gall tissue than in ungalled tissue of the plant, *Sorbus commixta*, which enhances higher performance of the gall inducer. Tjia and Houston (1975) demonstrated that, during gall formation, antioxidants and phenolic compounds induce the growth of layers of tissue that forms a barrier between the area infested by the eastern spruce gall aphid (*Adelges abietis*) and the rest of the plant (*Picea abies*). The extract from galled leaves of *Clusia lanceolata* by *Clusiamyia nitida* (Diptera,

Cecidomyiidae) showed a higher level of antioxidant activity, phenolics and proanthocyanidins, however the content of flavonoids was lower in leaves (Ferreira et al., 2014). It is likely that phytochemicals are involved in the development of other gall tissues. In addition to their important role in plant physiology, phytochemicals – particularly secondary plant metabolites are also of interest to scientists because of their actual and potential medicinal properties (Epstein, Diaz, Frei, Vita, & Keaney Jr, 1997; Vinson, Su, Zubik, & Bose, 2001; Wolfe, Wu, & Liu, 2003). Possible roles for phytochemicals and antioxidant substances in human and animal health has triggered intense research in the field of plant science.

Plant tissues are natural sources of various phytochemicals with pharmacological properties such as antioxidant viz. ascorbic acid, phenolics viz. tocopherols, tocotrienols, flavonoid acids, flavones, isoflavones, flavanones, anthocyanins, catechins and alkaloids viz. carotenoids, (Cao, Sofic, & Prior, 1996; Hertog, Hollman, & Van de Putte, 1993; Roberts & Gordon, 2003; Seeram, 2008; Speisky et al., 2005; Su & Chien, 2007; Y. Zheng, Wang, Wang, & Zheng, 2003).

Ferreira et al. (2014) found that the amount of antioxidant activity, phenolics and proanthocyanidins is higher in gall tissues than in ungalled tissue on balsam apple (*Clusia lanceolate*) by gall inducing dipteran insect, *Clusiamyia nitida* (Cecidomyiidae) which trigger the formation of gall. However, the amount of antioxidant activity, phenolics and anthocyanidins are still unknown in case of galls on *A. l. longifolia* by *T. acaciaelongifoliae.* Thus, this study investigates the secondary plant compounds present in gall tissue formed by *T. acaciaelongifoliae* on *A. l. longifolia* plants, which may trigger the formation of gall and defend the plant against insect. In particular, I measured antioxidant

capacity, and the amount of phenolics and anthocyanins in the gall tissue to understand *T. acaciaelongifoliae* and *A. l. longifolia* relationships. This study's results are also of interest with respect to potential sources of natural antioxidants. If levels of phytochemicals are particularly high in gall tissues, these may be a useful source of such compounds in pharmaceuticals (Hausenblas, Schoulda, & Smoliga, 2015).

The phytochemicals selected for investigation were total antioxidant capacity (TAC), total phenolic content (TP) and total anthocyanin content (TA). The phytochemicals produced in response to gall former have been shown to induce galls formed by dipteran insects (Ferreira et al., 2014; Hutangura, Mathesius, Jones, & Rolfe, 1999) and to facilitate the growth of galls (Tooker & Helms, 2014).

5.2. Materials and Methods

Total antioxidant capacity (TAC) was estimated using CUPRAC method described by Apak, Güçlü, Özyürek, and Karademir (2004), Total phenolic content (TP) was assayed using a slight modification of the Folin-Coicalteu method (Ahmed & Abozed, 2015; Cai, Luo, Sun, & Corke, 2004; Fu et al., 2011; W. Y. Huang, H. C. Zhang, W. X. Liu, & C. Y. Li, 2012) and total anthocyanin content (TA) was determined using pH-differential method described by Sellappan, Akoh, and Krewer (2002).

5.2.1. Plant materials

Gall tissues (of different sizes; see below) were collected from galled *A. l. longifolia* plants. Flower and leaf buds, stems and leaves were also collected so that levels of plant secondary compounds in galls could be compared with levels in other plant tissues. The plant samples (pooling samples) were from different parts of plant to minimize the heterogeneity of the

plant extracts. Samples were pooled from flower buds and leaf buds including leaves (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017) as it was aimed to compare the amount of phytochemicals in the ungalled buds and gall (developed on buds). Sampled plants were growing in *A. l. longifolia* populations at six locations in the Greater Grampians Bioregion (reported in chapter three).

Galls formed by *T. acaciaelongifoliae* were collected and categorised into four different size classes (1-5mm, 6-15 mm, 16-25 mm, & ≥26 mm gall diameter). Galls were collected from six different plants in each study location during the period from September–December 2014, September–December 2015 and September–December 2016 using secateurs. Buds (flower buds and leaf buds), stems (~2 mm diameter) and leaves (young and mature) were also collected at the same time at each location from three different ungalled *A. l. longifolia* plants. Sample collection was replicated three times in each year with thirty days interval between collection periods. Approximately 500 g of each type of fresh plant material (galls, flower buds and leaf buds, stems and leaves) was collected on each occasion. The plant materials or sample include several galls of four different sizes, buds (flowers buds and leaf buds), stems (~2 mm diameter) and leaves (young and mature), which are all pooled to form 500g of fresh tissue of each type of sample.

Plant materials (galls, buds, stems and leaves) of *A. l. longifolia* were then transported from the field to the laboratory at Federation University's Mt Helen Campus in Ballarat, Victoria, in a cool box and kept at 4°C temperature until sample preparation, which took place the day after sample collection. Galls were cut into quarters (i.e. four subsamples) to allow fine grinding. The fresh weight of all gall materials (subsamples) and plant materials were noted before being dried in a DT6000 Food Lab™ electronic dehydrator for 15 hours at 50°C,

then weighed again to calculate the moisture content in the materials (Table 5.1). The buds, stems and leaves were weighed to calculate the moisture content and dried immediately as whole samples using the same electronic dehydrator. The dried samples were labelled and kept at 4°C temperature for chemical analysis on the next day.

5.2.2. Chemicals and reagents used in the following experiments

The following chemicals were purchased from Sigma-Aldrich Australia: Folin-Ciocalteu reagent (Sodium 3, 4-dioxo-3, 4-dihydronaphthalene-1-sulfonate), neocuproine (2, 9-dimethyl-1, 10-phenanthroline), Trolox® (6-hydroxy-2, 5, 7, 8-tetramethylchroman- 2-carboxylic acid), gallic acid (3, 4, 5-Trihydroxybenzoic acid), hydrogen chloride (36%) and copper (II) chloride dihydrate. Sodium carbonate anhydrous (Na_2CO_3), sodium acetate trihydrate, ammonium acetate ($NH_4CH_3CO_2$), potassium chloride, methanol (CH_3OH), ethanol (CH_3CH_2OH), glacial acetic acid (CH_3COOH), reverse osmosis water and other reagents used in this study were of analytical grade and purchased from common sources.

Solutions of the following chemicals were also required for determination of total antioxidant capacity (TAC), total phenolic content (TP) and total anthocyanin content (TA): cupric chloride solution, ammonium acetate, neocuproine, Trolox stock solution.. Their preparation is described below.

5.2.3. Preparing plant sample extracts

A plant extract is an element of the plant representing desirable properties which is separated from the tissue of a plant by using a solvent for a particular purpose. The following procedure was used to obtain plant extracts from the tissues of galls, buds, stems

and leaves of *A. l. longifolia*. This method is based on that of by W.-y. Huang, H.-c. Zhang, W.-x. Liu, and C.-y. Li (2012).

Extracts were prepared from 63 fresh samples (500 g) of seven different tissue types: galls (of four different size classes), buds (pooling flower buds and leaf buds), stems (~2 mm diameter) and leaves (pooling young and mature), For each sample type, the procedure began with 500 g fresh sample which was dried and ground using a Kenwood True Compact Blender BL380. This resulted in approximately one third dried sample of the fresh sample, from which 1.00 g was subsampled and placed in 15ml of solvent (mixture of 95% CH_3OH and 5% CH_3COOH) in a shaking incubator (RATEK OM15 Large Orbital Shaking Incubator) at 24^oC for 20 minutes at 220 rpm in order to create a chemical extract. The remainder of ground samples were kept in the refrigerator (4°C) for back up usage. These plant tissue extracts were then centrifuged using a Hettich Universal centrifuge 30F. Samples were spun three times; each spin at 10,000 rpm for 10 minutes. After each spin, the supernatant was collected from each sample in a labelled conical flask, and wrapped in aluminium foil to exclude light. This solution was filtered using a medium speed filter under a vacuum at room temperature. The filtrate was made three times and collected in volumetric flasks up to 50 ml by adding solvent. These extracts were then stored in 100 ml aluminium-wrapped vials at -20^oC until the bioassay. 0.1 ml of each extract was used to determine the total amount of antioxidants and 0.4mL was used to determine the total phenolic content.

5.2.4. Determination of Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) is a measure of the antioxidant status of the samples *A. l. longifolia*, TAC contains the properties to protect the living cells from damage by oxidation process.

The protocol used to determine the TAC of each extracted sample is described below.

Step 1. Required solutions were prepared as follows.

Cupric chloride solution $CuCl_2.H_2O$ ($1.0x10^{-2}$ M).

Prepared by dissolving 0.4265g $CuCl_2.H_2O$ in MilliQ water and diluting to 250 ml (Apak et al., 2004; Gülçin, 2010).

Ammonium acetate ($NH_4CH_3CO_2$) buffer at pH 7.0.

Prepared by dissolving 19.273 g of $NH_4CH_3CO_2$ in MilliQ water and diluting to 250 ml.

Neocuproine (Nc) solution (2, 9-dimethyl-1, 10-phenanthroline), $7.5x10^{-3}$ M.

Prepared by dissolving 0.078 g Neocuproine solution in 50 ml 100% ethanol (C_2H_6O).

Trolox stock solution (6-hydroxy-2, 5, 7, 8-tetramethylchroman- 2-carboxylic acid), 1.0 X 10^{-3} M.

Prepared by dissolving 1.25 mg (0.125 g) of Trolox in 100 ml of 100% ethanol (C_2H_6O).

Step 2. The following solutions were mixed in a 10 ml test tube: 1 ml $CuCl_2.H_2O$ solution, 1ml NH_4Ac buffer, 1ml Neocuproine (Nc) solution, 100 µl of sample and 1 ml of MilliQ water to make the final volume 4.1 ml. The mixed solutions were placed into a water bath at 50°C for 30 minutes in the chemistry laboratory of Federation University Australia.

Step 3. Absorbances were measured at 450 nm against a reagent blank (Ak & Gulcin, 2008) using a SHIMADZU UV-1800 UV-Vis Spectrophotometer (Kyoto 604-8511, Japan). The absorbance readings were equated by using the following formula to measure the total antioxidant content in milligram per gram of sample-

Antioxidant content = absorbance + b/m x DF/1000 x 50/sample weight.

Where, b = Y axis intercept of your standard curve (obtained from standard assay with the different concentrations of Trolox) by using y = mx + b formula.

m = the slope of the standard curve of Trolox.

DF = the dilution factor of the sample.

5.2.5. Determination of Total phenolic content (TP)

Total phenolic content (TP) was determined using a modification of the Folin-Coicalteu method (Ahmed & Abozed, 2015; Cai et al., 2004; Fu et al., 2011; W. Y. Huang et al., 2012). 2 ml of Folin-Coicalteu reagent was diluted 1:10 with reverse osmosis water. The diluted reagent was added to 400 µl of sample extract that had been diluted as necessary with extraction solvent. All samples were diluted similarly. This was left at room temperature for 10 minutes before 2 ml of 7.5% (w/v) sodium carbonate aqueous solution was added. The test tubes were capped and vortexed briefly before incubation in darkness in a 40°C water bath for 30 minutes. Absorbance was read using a SHIMADZU UV-1800 UV-Vis Spectrophotometer (Kyoto 604-8511, Japan) at 760 nm and standardised using gallic acid. The total phenolic content of the sample extract was determined against the equivalent absorbance of gallic acid in the range 5 $mg.L^{-1}$ to 80 $mg.L^{-1}$ GAE (R^2=0.9941).

5.2.6. Determination of total anthocyanin content (TA)

The total anthocyanin content of the plant tissue samples was determined using the same Spectrophotometer (SHIMADZU UV-1800 UV-Vis Spectrophotometer, Kyoto 604-8511, Japan) and the pH-differential method for Monomeric Anthocyanin (Sellappan & Akoh, 2002; Sellappan et al., 2002). Two buffer solutions of approximately 500mL in volume were prepared. A pH 4.5 buffer was made by adding 54.4 g of sodium acetate to 480 ml of distilled water and the pH was fixed with HCL 10 M using a Pasteur pipette. A pH 1.0 buffer was made by mixing 32.8 g of potassium chloride (KCl) with 480 ml of distilled water and fixing the pH to 1.0 by the dropwise addition of HCL 10 M.

Briefly, 400 µl of the extract was mixed with 3.6 mL of each of the two buffers and the absorbance of each was read against a blank at 510 nm and 700 nm. Absorbance (A) was calculated as: $A = (A_{510\ nm}-A_{700\ nm})\ pH\ 1.0 - (A_{510\ nm}-A_{700\ nm})\ pH\ 4.5$. Monomeric anthocyanin pigment concentration in the extract was calculated as-

$$\text{Anthocyanin pigment (cyanidin-3-glucoside, mg/l)} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

Where,

$A = (A_{510\ nm}-A_{700\ nm})_{pH\ 1.0} - (A_{510\ nm}-A_{700\ nm})_{pH\ 4.5}$

MW = molar weight 449.38 g/mol for cyanidin-3-glucoside

1 = path length in cm

DF= dilution factor (30)

Ɛ (molar absorptivity) = 26,900 molar extinction coefficient.

The total anthocyanin content was expressed as cyanidin-3-glucoside (mg/100 g).

5.2.7. Statistical analyses

The data were compiled and tabulated for statistical analysis. Analysis of variance was done in MSTAT-C. The mean value of TA, TP and TA were separated by Duncan's Multiple Range Test (DMRT) at 1% level of significance (Gomez, Gomez, & Gomez, 1984). The mean value of percent moisture content were also separated by same analysis at 1% level of significance and lettering was done by using LSD (Least Significant Difference). Correlation between antioxidant capacity and phenolic compound, antioxidant capacity and anthocyanin, phenolic compound and anthocyanin were made by using linear correlation co-efficient.

5.3. Results and Discussions

5.3.1. Moisture content in the plant tissue samples

The moisture content of various plant tissue samples from *A. l. longifolia* are described in Table 5.1

The moisture content of galls of all size classes was similar and did not differ significantly from that of stems, leaves, or buds.

Table 5. 1. Moisture content in the plant tissue samples of *A. l. longifolia*.

Plant tissue sample type	Number of samples collected	Mean fresh weight (g)	Mean dry weight (g)	% moisture content	$LSD_{(0.01)}$	%CV
Galls (≥26mm)	63	66.16	24.47	63.02ab		
Galls (16-25mm)	63	40.77	13.50	66.90ab		
Galls (6-15mm)	63	55.05	15.76	71.37a		
Galls (1-5mm)	63	41.44	13.41	67.65ab	10.94	6.98
Buds	63	16.26	7.02	56.85b		
Stems	63	20.28	7.73	61.90ab		
Leaves	63	45.74	16.68	63.54ab		

Note: Values for mean % moisture content are annotated by the letter a and or b. Values with the same letter are not significantly different under Duncan's Multiple Range test (DMRT) at 1% level of significance, LSD=Least Significance Difference, CV=Coefficient of Variance.

Moisture content was the lowest in buds *A. l. longifolia* (56.85%), however, this was not significantly different from the moisture content in other plant materials except galls sized 6-15 mm. Gall inducers are typically more active in the early stages of gall development (Mendel, Protasov, Fisher, & La Salle, 2004); in later stages, the insect becomes less active in preparation for pupation stage. The increased moisture content may be related to the feeding activity of the insect and also related to age of the gall. However, the moisture content of all samples of *A. l. longifolia* was more than 50%, which usually provides to have more antioxidant and phenolic compounds in the samples (Datta, Sinha, Bhattacharjee, & Seal, 2019).

5.3.2. Total antioxidant capacity (TAC) in the plant tissue samples

The ANOVA results showed significant differences in the TAC across all plant tissue sample types (1% level of significance). All but the smallest galls had significantly higher TAC compared to other plant tissue types.

Table 5. 2. Total antioxidant capacity (TAC) in the plant tissue samples of *A. l. longifolia*.

Plant tissue sample types	Number of samples used in the experiment	Mean Mass (g) of samples*	Mean Abs	Average TAC (mg/g)	$LSD_{(0.01)}$	%CV
Galls (≥26mm)	63	1.009	0.745	725c		
Galls (16-25mm)	63	1.009	0.796	752b		
Galls (6-15mm)	63	1.001	0.782	823a		
Galls (1-5mm)	63	1.000	0.453	387e	4.861	0.40
Buds	63	1.000	0.215	217f		
Stems	63	1.004	0.14	155g		
Leaves	63	1.003	0.453	416d		

Note: Values for mean antioxidant capacity are annotated by the letter a to g. Annotations also indicate a ranking of highest (a) to lowest (g) value. Values with the same letter are not significantly different (otherwise, they are significantly different**)** under Duncan's Multiple Range test (DMRT) at 1% level of significance, Abs=Absorbance, LSD=Least Significance Difference, CV=Coefficient of Variance * used to make up the extract.

The highest TAC was found in the samples of early stage (1-5 mm) galls formed by *T. acaciaelongifoliae* (6-15 mm), which was 823 mg/g (Table 2). The lowest TAC was observed in samples from stems (155 mg/g) (Table 5.2).

In gall samples, TAC ranged between 387 mg/g to 823 mg/g (Table 5.2). TAC increased from early to mid-stages of development of gall (by *T. acaciaelongifoliae*) and the highest levels of TAC were observed (823 mg/g) in mid-stage galls by *T. acaciaelongifoliae*. These results are similar to those of Hartley (1998) who showed that galls induced by insects can accumulate more phytochemicals than other plant parts. In the current study, the gall inducer was more active in feeding during the early stages of gall development than the later stages of galls (personal observation while dissecting galls). The antioxidant in the plant galls may play an important role in defensive reactions of the plant against wounding and in regulating the response of plants to environmental stresses. Odette Rohfritsch (1981) reported that galls by spruce gall aphid (*Chermes abietis*) increased resistance in the plant (*Picea excelsa*) to other insects. Similarly, increased defensive properties by the galls formed by *T. acaciaelongifoliae* may improve resistance in the plant *A. l. longifolia* against other insects aiding the plants ability to become invasive.

5.3.3. Total phenolic compounds (TP) in the plant tissue samples

The TP of samples from all but the smallest size class (1-5 mm) of galls was significantly different from samples of other plant tissue types (buds, stems and leaves). The highest TP value was found in the gall sample of size class 6-15 mm, which was 12.13 mg/g (Table 5.3). The phenolic compounds in the plants may also play an important role in the defence mechanism of the plant against foreign organisms and herbivores (Lattanzio et al., 2006).

The amount of phenolic compounds in the gall tissue of *A. l. longifolia* appears to have increased as a result of the feeding action of *T. acaciaelongifoliae*. It is possible that the increased phenolic content may reduce further infestation of insects, perhaps when a certain threshold is reached. These results concur with those of Lattanzio et al. (2006) and Johnson

and Dowd (2004). Lattanzio et al. (2006) noted phenolic compounds in plant enhanced defence mechanisms of plants against insect herbivores and fungal pathogens. Johnson and Dowd (2004) found feeding of *Spodoptera frugiperda* on *Arabidopsis thalianais* negatively correlated with the concentration of anthocyanins. Similar notes were mentioned by Gutterman and Chauser-Volfson (2000) who recorded the amount of phenolic metabolites increased in the leaves of *Aloe arborescens*, considering this may prevent or reduce further consumption by insects.

Table 5. 3. Total phenolic compounds (TP) in the plant tissue samples of *A. l. longifolia*.

Plant tissue sample type	Number of samples used in the experiment	Mean Mass(g) of samples*	Mean abs	Average TP (mg/g)	LSD (0.01)	%CV
Galls (≥26mm)	27	1.009	0.920	11.68a	4.210	23.56
Galls (16-25mm)	27	1.009	0.802	11.90a		
Galls (6-15mm)	27	1.001	0.786	12.13a		
Galls (1-5mm)	27	1.000	0.376	5.28b		
Buds	27	1.000	0.162	2.61b		
Stems	27	1.004	0.109	1.89b		
Leaves	27	1.003	0.446	5.98b		

Note: Values for mean phenolic compounds are annotated by the letter a and b. Annotations also indicate a ranking of highest (a) and lowest (b) value. Values with the same letter are not significantly different under Duncan's Multiple Range test (DMRT) at 1% level of significance, Abs=Absorbance, LSD=Least Significance Difference, CV=Coefficient of Variance * used to make up the extract.

5.3.4. Total anthocyanin (TA) compounds in the plant tissue samples

Table 5. 4. Total anthocyanin (TA) compounds in the plant tissue samples of *A. l. longifolia*.

Plant tissue sample type	Number of samples used in the experiment	Mean Mass (g)*	Mean Abs				A	Anthocyanin pigment (cyanidin-3-glucoside, mg/L)
			P^H1		$P^H4.5$			
			520nm	700nm	520nm	700nm		
Galls (≥26mm)	27	1.001	0.065	0.062	0.063	0.062	0.002	1.002d
Galls (16-25mm)	27	1.001	0.066	0.061	0.061	0.060	0.004	1.854b
Galls (6-15mm)	27	1.001	0.079	0.071	0.072	0.071	0.007	3.508a
Galls (1-5mm)	27	1.000	0.066	0.062	0.060	0.060	0.004	2.005b
Buds	27	1.000	0.063	0.060	0.062	0.061	0.002	1.002d
Stems	27	0.999	0.063	0.061	0.061	0.060	0.001	0.501e
Leaves	27	1.002	0.066	0.062	0.061	0.060	0.003	1.504c

Note: $A=(A_{510\ nm}-A_{700\ nm})_{pH1.0} - (A_{510\ nm}-A_{700\ nm})_{pH4.5.}$ Values for mean anthocyanin compounds are annotated by the letter a to e. Annotations also indicate a ranking of highest (a) to lowest (e) value Values with the same letter are not significantly different under Duncan's Multiple Range test (DMRT) at 1% level of significance, Abs=Absorbance, LSD=Least Significance Difference, CV=Coefficient of Variance * used to make up the extract.

The amount of anthocyanin was highly variable among the various plant tissue types, however gall tissue samples of the two smallest size classes, had higher anthocyanin amounts than other plant tissues. The maximum amount of anthocyanin was detected in the gall sample (6-15 mm) (3.508 mg/l) and the minimum amount of anthocyanin was found in stems (0.501 mg/l). The amount of anthocyanin was statistically similar in buds (flower buds and leaf buds) of *A. l. longifolia* and in the largest size class of galls (≥26 mm) (Table 5.4).

Active feeding of the gall inducing insects in their early stages of life may have the potential to increase the amount of the anthocyanin in the early stages of galls on the host plants. The increased amount of anthocyanin may protect plants against other organisms. Johnson and Dowd (2004) showed the increased amount of anthocyanin in the leaves of *Arabidosis thaliana* reduces the infestation of the insect (*Spodptera frugiperda*).

Of the four stages of gall growth (≥26 mm, 16-25 mm, 6-15 mm and 1-5 mm) galls from the second early growth stage (6-15 mm) contain more antioxidant, phenolic compounds and anthocyanin compared to other plant tissue samples examined. *T. acaciaelongifoliae* larvae were more active in feeding in this stage (see chapter 4). Mature galls on *A. l. longifolia*, contain mature larvae of *T. acaciaelongifoliae*. These mature larvae cease feeding activity and enter a pupal period (see details in chapter 4). Mature galls showed comparatively lower amounts of antioxidant, phenolic compounds and anthocyanin (Table 5.2, 5.3 and 5.4). Thus there appears to be a relationship between the feeding activities of *T. acaciaelongifoliae* and the production or accumulation of antioxidant, phenolic compounds and anthocyanin in the galls of *A. l. longifolia.* Similarly, Ferreira et al. (2014) found that antioxidant activity, phenolics and proanthocyanidins increased in the galled leaves of *C. lanceolata* due to the feeding action of *Clusiamyia nitida* (Diptera, Cecidomyiidae). Similar results were also observed by Maffei et al. (2007) in the case of chrysanthemum plants and aphids. Hartley (1998) also reported that gall tissue often contains higher amounts of phenolic compounds than ungalled tissue. He found the amount of phenolic compounds was higher in galled tissue of *Salix alba*, especially in the early stages of gall formation by the gall inducing insect, *Pontania proxima* (Hymenoptera) and declined at the later stage of gall development as the gall matured.

The increased amount of phenolic compounds in the galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* might protect the plant from other herbivores and pathogens. This may result in the protection of *A. l. longifolia* from attack by phytophagous insects or fungal and bacterial pathogens, enabling the plant to become invasive. Akhtar and Malik (2000) reported that a number of phenolic compounds act as protective chemicals against some pathogens and insects. Dakora and Phillips (1996), and Ravn, Andary, Kovács, and Mølgaard (1989) also revealed that phenolics serve as defence against phytophagous, nematodes, fungal and bacterial pathogens.

The amount of phenolic compounds in the galls by *T. acaciaelongifoliae* on *A. l. longifolia* (max. 12.13 mg/g in gall 6-15 mm) is higher than some fruits such as apple (pomace; pulpy substances from a crushed fruit) 8.9 mg/g, pomegranate (pomace) 9.9 mg/g, red grapes (marcs; pressed grapes with skins) 10.4 mg/g (Agourram et al., 2013) and some medicinal plants such as *Baphicacanthus cusia* 1.15 mg/g, *Lycium chinense* 6.22 mg/g, *Rehmannia glutinosa* 4.83 mg/g, *Stellaria dichotoma* 5.99 mg/g (H.-B. Li, Wong, Cheng, & Chen, 2008). Future studies could investigate the use of these galls as a possible source of natural substitutes for artificial phenolics in food processing and pharmaceuticals.

5.3.5. Relationships between TAC and TP; TAC and TA and TP and TA of plant samples

There was a strong relationship among amounts of TAC, TP, and TA (Figure 5.1, Figure 5.2 and Figure 5.3) across different sized galls (by *T. acaciaelongifoliae*) samples and other plant tissue samples (flower buds, stems and leaves). It was evident from Figure 5.1 that the equation $y = 0.0166x - 0.8648$ gave a good fit to the data and correlation ($R^2 = 0.992$) showed that, the amount of TAC and TP had strong and positive correlation with the

samples of *A. l. longifolia* (Figure 5.1). Guerrero et al. (2010) also observed a positive correlation between TAC and TP.

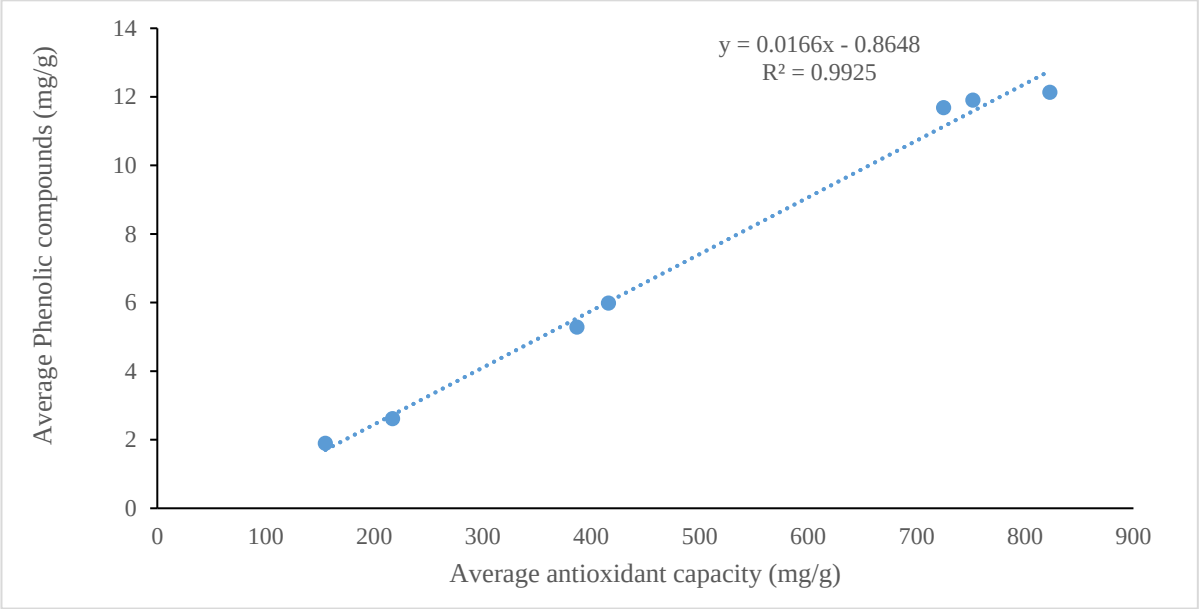

Figure 5. 1. Correlation between average antioxidant capacity and average phenolic compound in the plant tissue samples of *A. l. longifolia*.

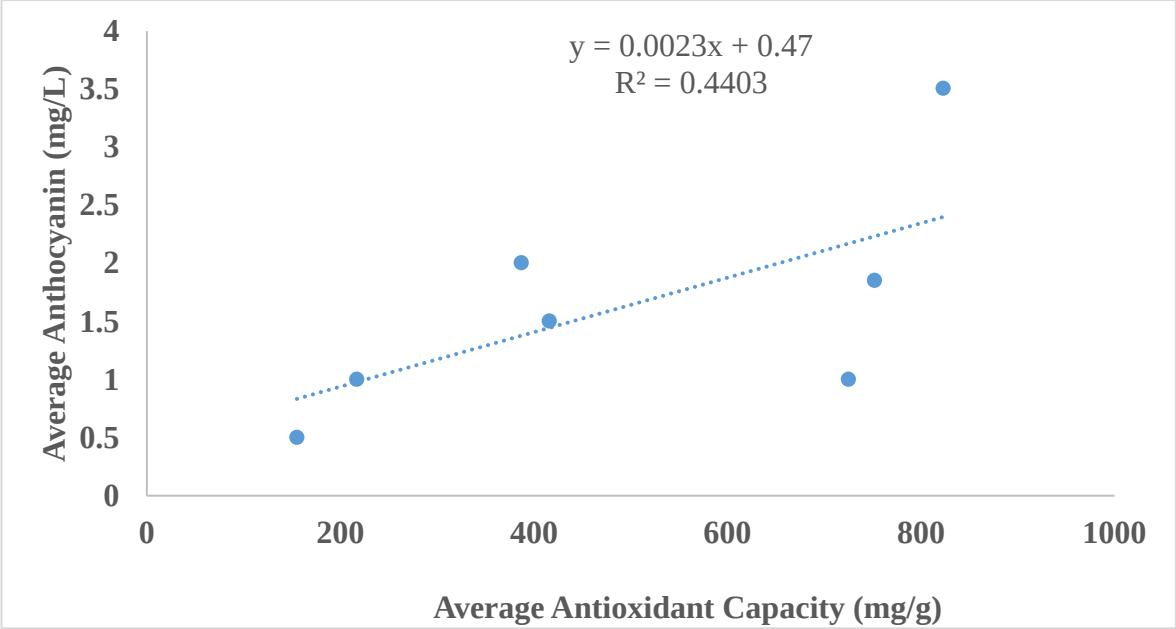

Figure 5. 2. Correlation between average antioxidant capacity and average anthocyanin in the plant tissue samples of *A. l. longifolia.*

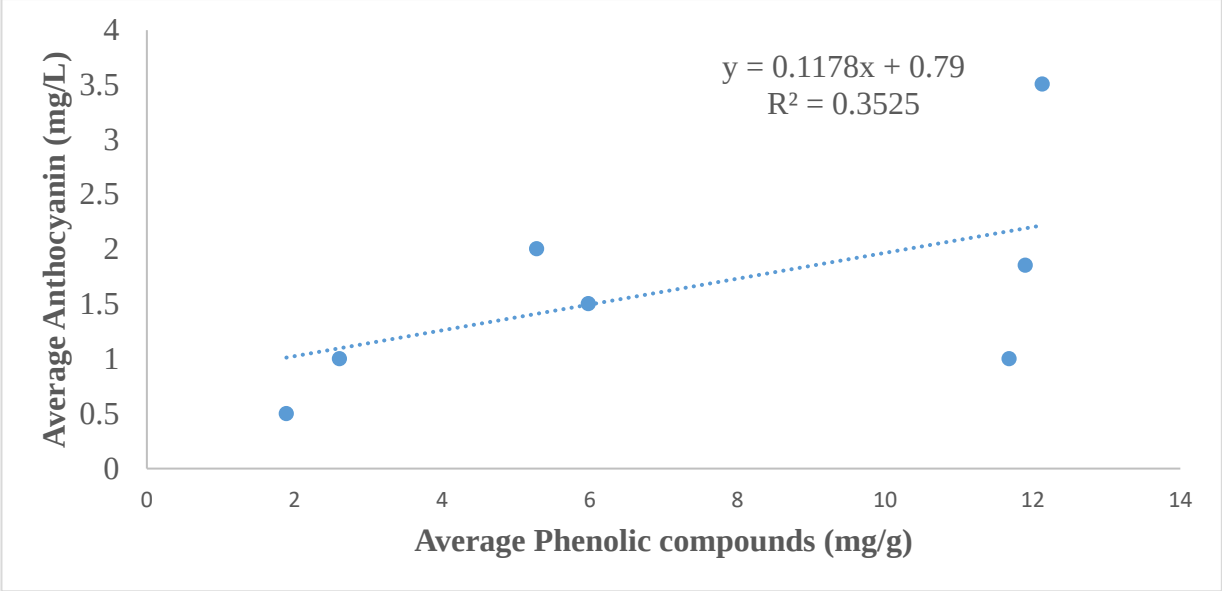

Figure 5. 3. Correlation between average phenols and average anthocyanin in the plant tissue samples of *A. l. longifolia*.

The relationships between TAC with TA and TP with TA, showed positive correlation between each pair of variables and the equation was y = 0.0023x + 0.47 and correlation (R^2 = 0.441) (Figure 5.2) for TAC with TA. In case of TP and TA, the equation was y = 0.1178x + 0.79 and correlation (R^2= 0.353) (Figure 5.3). A comparison of the correlation coefficients showed that neither relationship was as strong as in the case of TAC and TP. These results are also supported by Cai et al. (2004) who demonstrated that there is a strong relationship between TAC and TP. The galls on *A. l. longifolia* formed by *T. acaciaelongifoliae* contain a significant amount of phenolic compounds which contribute to antioxidant capacity of the plant and may contribute a defence mechanism against microorganisms. Moreover, the increased amount of the antioxidant capacity, phenols and anthocyanin may contribute to the invasiveness of the plant as these chemicals in plants play an important role in defence against microorganisms and insects (Johnson & Dowd, 2004; Lattanzio et al., 2006; Odette Rohfritsch, 1981).

5.4. Conclusion

Gall inducers and host plants have a remarkably close relationship and the chemical composition of galled tissue varies with the species of gall inducing insect. The nature of the gall chemicals is an important factor to understand in the relationship between the gall inducing agent and their host plant in terms of gall development and defence. Moreover, the defensive mechanism of the host plant is influenced by the chemical composition of galled tissue. For example: gall tissue with higher amounts of phenolic compounds may acts to defend the host plant against microorganisms and arthropods.

This chapter provides information about the chemistry of gall tissue of *A. l. longifolia* with respect to antioxidants, phenolic compounds and anthocyanins, which might affect the relationship of gall inducer and host plant and the defence mechanisms of the host plant. It has showed that the galls of *A. l. longifolia* have high antioxidant capacities. The highest amount of total antioxidant capacity, total phenols and total anthocyanins were found in early stage galls formed by *T. acaciaelongifoliae* (6-15 mm). The amount of the phytochemicals gradually decreased in the older stages of galls; however, it was still higher than in samples of other plant (buds, stems and leaves). Active feeding of *T. acaciaelongifoliae* larvae in the early stage of gall results in the formation of antioxidants, phenolic compound and anthocyanin in the gall tissue. The higher content of antioxidants, especially phenolic compounds, in the gall tissue might represent an attempt of the plant to protect itself from insects and microorganisms. However, the amount of the chemicals declines in later stage galls when larval feeding activity reduces and gradually ceases at the pupal stage of the wasp's development. Thus, feeding actions of the immature larvae of *T. acaciaelongifoliae* is correlated with the accumulation of antioxidant, phenolic compounds and anthocyanin in the gall tissue of *A. l. longifolia*. The maximum amount of total

antioxidant capacity, total phenols and total anthocyanin were detected in galls (6-15 mm) of *A. l. longifolia*, which were 823 mg/g, 12.13 mg/g and 3.50 1 mg/l respectively.

The results of this chapter showed positive correlations among TAC, TP, and TA in the plant tissue samples of *A. l. longifolia.* The amounts of antioxidant, phenolic compounds and anthocyanin in the gall tissue might be useful in a good source of natural antioxidants. Further research is needed to investigate their potential to be used as possible natural substitutes for artificial antioxidants currently used in food processing. The effect of the use of these natural antioxidants on food sensory properties (such as taste and odour) should also be considered.

CHAPTER SIX

The effect of galls formed by *Trichilogaster acaciaelongifoliae* on the vegetative growth and reproduction of invasive *Acacia longifolia* subspecies *longifolia* in Australia

6.1. Introduction

T. acaciaelongifoliae is a hymenopteran wasp, native to Australia and found in galls on *A. l. longifolia* (G. B. Dennill et al., 1999). Haiden et al. (2012) explains that galls develop because a stimulus from the insect alters normal physiological processes within the plant and chapter 4 of this thesis established that in the case of *T. acaciaelongifoliae* and *A. l. longifolia*, the stimulus resulting in gall formation is related to larval feeding activity. The redirection of resources associated with the formation of gall has previously been noted to impair vegetative growth and/or interrupt various reproductive stages of the host plant (Fernandes, Carneiro, & Isaias, 2012). For example, in a case study from South Africa, *Trichilogaster signiventris* causes the development of galls on vegetative or reproductive buds of *Acacia pycnantha*. Normal growth of the plant is hampered by the redirection of plant resources (Netta Dorchin, Michael D Cramer, & John H Hoffmann, 2006).

Among the numerous examples of negative effects on plant growth, or plant reproduction, due to the formation of galls is a study by Klöppel, Smith, and Syrett (2003), which showed that galls formed by the cynipid wasp, *Aulacidea subterminalis*, have a significant impact on growth of the grassland weed *Hieracium pilosella*. Klöppel et al. (2003) investigated the effect of the gall on the growth of potted plants kept in a shade house. Galled plants showed an average decrease in stolon length of 75% compared to ungalled plants (Klöppel et al., 2003). Another gall inducing cynipid wasp, *Phanacis taraxaci* (Ashmead) also

demonstrated that galls formed on the dandelion, *Taraxacum officinale* accumulate between 9 to 70% of the plants' resources and negatively affects the normal growth of the plant (Bagatto, Paquette, & Shorthouse, 1996). A stem galling moth, *Epiblema strenuana* was shown to reduce the number of immature capitula (36%), mature capitula (41%), and production of viable seeds (39%) in white top weed, *Parthenium hysterophorus* (Navie, Priest, McFadyen, & Adkins, 1998). The negative effects of galls on plant growth and reproduction have been successfully used to help control pest plants in some instances. The eurytomid wasp, *Eurytoma attiva* has been successfully used to control black sage, *Cordia curassavica* in Mauritius as it damages the reproductive parts of the plant and prevents reinvasion of the weed in areas that have proved difficult in managing the weed. This gall-forming wasp has also been used successfully in Malaysia and Sri Lanka to control the black sage (Cock, Bennett, Hughes, Simmonds, & Yaseen, 1985).

Fay et al. (1996) demonstrated that the gall inducing wasp, *Antistrophus silphii* (Cynipidae) has the potential to act as a successful biocontrol agent in Konza Prairie Research Natural Area, United States by reducing plant height, total leaf area, and inflorescence production of rosinweed, *Silphium integrifolium* (Asteraceae). *Silphium integrifolium* is a weed in dry areas of North America and in parts of Canada, and forms a large clump with many stems, which suppresses other native species.

Hartnett and Abrahamson (1979) showed that three stem gall inducing insects, *Gnorirnoshema gallaesolidaginis* (Gelechiidae), *Eurosta solidaginis* (Tephritidae), and *Rhopalomvia solidaginis* (Cecidomyiidae) can significantly reduce seed production and shoot height of goldenrods (*Solidago canadensis*). Goldenrod is a weed native to North

America that has now spread worldwide including coastal parts of Australia. The plant grows rapidly, especially after a disturbance such as bushfire, and outcompetes native flora. Biological control of weeds can be an effective strategy for long-term restoration of native ecosystems (Richardson & Kluge, 2008; Wilson et al., 2011). *T. acaciaelongifoliae* has been applied as a biological control agent to manage introduced, invasive *A. l. longifolia* populations in South Africa (Hoffmann et al., 2002a), where both the wasp and the plant are introduced. G. Dennill (1985) documented reduced vegetative growth and seed production in South African populations of *A. l. longifolia* as a result of gall formation by *T. acaciaelongifoliae*. It is the wasp alone which is responsible for reduced vegetative growth and seed production of *A. l. longifolia* by developing galls in South Africa.

Acacia l. longifolia is regarded as a significant environmental weed in its native distribution in several Australian states (Luke, Zed, & Craig, 2008; Milkins, 2017; Thomson, 2016), where it co-occurs naturally with *T. acaciaelongifoliae*. While it is apparent that the wasp is not acting to control *A. l. longiofolia* in these areas (since populations of the plant continue to spread) (Milkins, 2017), it is not understood whether this is because galls formed by the wasp do not negatively affect the plant in its native home range, or whether the plant is able to overcome the disadvantages posed by the presence of the wasp. Several aspects of the relationship between the wasp, *T. acaciaelongifoliae* and the plant, *A. l. longifolia*, remain poorly understood, especially in south eastern Australian environments; where both species co-occur naturally. The aim of this study is to test whether reduced vegetative growth and seed production in *A. l. longifolia* occurs due to galls formed by *T. acaciaelongifoliae* in the Australian context.

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6.2. Materials and Methods

6.2.1. Study area

Measurements of the growth of *A. l. longifolia* were made at six study locations in the Greater Grampians Bioregion of Victoria, Australia during a study period from September 2014–December 2016. The study location details are provided in chapter 3 section 3.1.

6.2.2. Data collection

The methods for data collection and analysis were derived by some modification (an increased number of sampling locations plus the addition of data on twig mortality and phyllodes) of methods used by F. A. Impson, Post, and Hoffmann (2013) and Hartnett and Abrahamson (1979). F. A. Impson et al. (2013) investigated the impact of a flower-galling midge, *Dasineura rubiformis* Kolesik, on the growth of its host plant, *Acacia mearnsii* De Wild, in South Africa and Hartnett and Abrahamson (1979) the effects of stem gall insects on the life history patterns of Canadian Goldenrod, *S. canadensis*.

The intensity of infestation of *A. l. longifolia* plants by *T. acaciaelongifoliae* was assessed once, at the beginning of the study period in September 2014. The intensity of infestation was calculated by positioning a 25 m^2 quadrat (considering the plant density and plant canopy of *A. l. longifolia* in the study locations) pseudo-randomly within each of the six study locations and assessing the number of *A. l. longifolia* plants present within the quadrat, noting how many plants bore galls. Infestation intensity was calculated as the number of *A. l. longifolia* plants bearing galls divided by the total number of *A. l. longifolia* plants present in each (5x5)=25 m^2 area; expressed as a percentage.

In order to understand the effects of the presence of galls on the growth and reproduction of *A. l. longifolia*, three galled and three un-galled mature *A. l. longifolia* plants were selected pseudo-randomly at the six study locations (*N*=36). One branch of each selected tree was tagged and several parameters were measured on these branches at monthly intervals throughout the study period (September 2014–August 2016) to provide proxy measures of growth and reproduction of *A. l. longifolia*. Each branch was considered to be comprised of many (usually 3-12) sub-branches and was chosen based on size (1.5 to 2.5 m long), and accessibility for monitoring without a ladder (R. Blanche, 2012). Initially, the length of galled and ungalled branches were roughly equal in size (Plate 6.1).

Plate 6. 1. Sub-branches of *A. l. longifolia* with galls (inset photo of gall at a month of age) formed by *T. acaciaelongifoliae* (white colour) and without galls (red colour) in the study location in the Greater Grampians Bioregion, Victoria, Australia.

For several parameters, sub-branches were used instead of branches as this was more practical and provided a larger data set for analysis.

Plant growth parameters measured were:

- Branch and sub-branch length: measured every month for the first year (September 2014–August 2015) but only every second month for the second year of the study (September 2015–August 2016) due to consistent trends of the data.
- Number of phyllodes per sub-branch: measured every month throughout the first year of the study (September 2014–August 2015).
- Twig mortality: measured once during each year of the entire study period by comparing the proportion of dead compared to live twigs per branch and sub-branch. Comparisons were made at the end of each gall season (between December 2014 to January 2015, December 2015 to January 2016 and December 2016 to January 2017).

Reproductive growth was measured by:

- Number of seedpods per sub-branch: measured once every year when mature seedpods were present on *A. l. longifolia* plants at the study locations (between December 2014 to January 2015, December 2015 to January 2016 and December 2016 to January 2017) by counting the number of seedpods formed per branch and sub-branch of *A. l. longifolia*.

The effect of galls on the vegetative and reproductive growth of *A. l. longifolia* was assessed by comparing the parameters described above for galled and ungalled trees. The effects of galls formed by *T. acaciaelongifoliae* on the twig mortality of *A. l. longifolia* were determined by correlating the percentage of twig mortality against the number of galls on

54 galled branches of *A. l. longifolia* plants. Three branches from three different plants at each of six locations were assessed at the end of each of three years (*N*=54 branches). The effects of galls formed by *T. acaciaelongifoliae* on the reproductive capacity of *A. l. longifolia* were examined by correlating the number of seedpods on 54 sub-branches of galled *A. l. longifolia* plants against the number of galls on those sub-branches. Three sub-branches from three different plants at each of six locations were assessed at the end of each of three years (*N*=54 sub-branches).

6.2.3. Statistical analyses

All data analysis was conducted using SPSS Statistics Version 22, with reference to Allen, Bennett, and Heritage (2014).

Infestation intensity

A chi-squared test was performed to assess whether infestation intensity differed significantly across locations. Assumptions of independence and expected frequencies were checked and found not to have been violated (Allen et al., 2014).

The effect of galls on sub-branch length

The effect of galls on the length of galled and ungalled sub-branches of *A. l. longifolia* was compared using a mixed model analysis of variance (ANOVA) at 5% level of significance. The Shapiro-Wilk and Levene's test statistics were used to check the assumptions of normality and homogeneity of variance. Assumptions of normality and homogeneity of variance for the mixed model ANOVA were not violated (Allen et al., 2014). Differences between the mean lengths of galled and ungalled sub-branches were compared using the Least Significant Difference (LSD) at 5% level of significance (Allen et al., 2014).

A mixed model within subjects ANOVA was conducted to compare the effect of time on the length of galled and ungalled sub-branches of *A. l. longifolia*; in other words, to examine the effect of galls on the rate of growth of the sub-branches throughout the study period. Here, the variable: time represents the 24 months of the study period from September 2014–August 2016.

The effect of galls on number of phyllodes

The effect of galls on the number of phyllodes on *A. l. longifolia* sub-branches was examined using a repeated measure ANOVA. The skewness (Z_s) and kurtosis (Z_k) values of the data were within ±1.96. Inspection of a boxplot and the Shapiro-Wilk and Levene's test statistics suggested the data were normally distributed and had equal variance (Allen et al., 2014). The assumptions of normality and homogeneity of variance for the repeated measure ANOVA were not violated. The mean data were separated using the LSD test to compare the number of phyllodes of galled and ungalled sub-branches of *A. l. longifolia* at 5% level of significance (Allen et al., 2014). A mixed model within subjects ANOVA was conducted to compare the effect of time on the number of phyllodes on sub-branches of galled and ungalled *A. l. longifolia*. Here, time is the 12 months of the study period from September 2014–August 2015.

The effect of galls on twig mortality and seed formation

The relationships between the number of galls per branch formed by *T. acaciaelongifoliae* and the proportion of twig mortality per branch, and the number of seedpods per sub-branch were explored using separate simple linear regressions. The assumptions of normality, linearity, and homoscedasticity were assessed and considered to be within the necessary limits (Allen et al., 2014). The Shapiro-Wilk test and normal Q-Q and detrended Q-Q plots

were considered for normality tests. The mahalanobis distance (2.32) did not exceed the critical chi-square (χ^2)=10.83 for *df*=1 at α=.001, suggesting that the outliers were not of concern (Allen et al., 2014). A visual inspection of a scatterplot for linearity and homoscedasticity of the data confirmed that the relationship between these variables was linear and homoscedastic (Allen et al., 2014).

6.3. Results and Discussion

6.3.1. Infestation intensity

Infestation of *A. l. longifolia* by *T. acaciaelongifoliae* was observed across all study locations at the beginning of the study period (Table 6.1).

Table 6. 1. Percent infestation of *A. l. longifolia* by *T. acaciaelogifoliae* in 25m^2 quadrats assessed at six study locations in the Greater Grampians Bioregion, Victoria, Australia in September 2014.

Locations	Total number of *A. l. longifolia* plants in 25 m^2	Number of *A. l. longifolia* plants infested by *T. acaciaelongifoliae* in 25 m^2	Percent infestation)%(of *A. l. longifolia* plants by *T. acaciaelongifoliae* in 25 m^2
PP1	18	15	83.3
PP2	14	6	42.9
PP3	9	5	55.6
PV4	15	9	60.0
PV5	12	9	75.0
PV6	11	6	54.5

The highest proportion of infested trees (83.3%) was found at location PP1 (Table 6.1), a private property densely covered by *A. l. longifolia* plants known to be more than three years of age (G. Hopkins, personal communication, 2014). The lowest proportion of infested trees (42.9%) was found at location PP2 (Table 6.1). The proportion of trees

affected by *T. acaciaelongifoliae* was more than 50% at all four other study locations; and the chi-squared test indicated no significant differences in infestation intensity between the locations (p=1.00). With the exception of PP1, all locations were covered by a mix of *A. l. longifolia* and other native vegetation; locations PV4 and PV5 had been recently invaded by *A. l. longifolia* plants, following a fire in 2014 (Milkins, 2017) enhanced germination of the *A. l. longifolia* seeds. Its spread has impeded the germination and growth of other native plants in these locations (personal observation and G. Hopkins, personal communication, 2014). *Acacia l. longifolia* trees with more branches and more flower buds may provide greater opportunities for *T. acaciaelongifoliae* infestation (PP1) since these structures are thought to be preferred by the wasp for egg deposition (see details in chapter 4) (G. Dennill & Donnelly, 1991). Chapter 4 of this thesis documented that *T. acaciaelongifoliae* shows a preference for flower or twig buds on younger branches of *A. l. longifolia* plants for egg deposition. Such branches are more common at locations PV4 and PV5 (Plate 6.2).

Plate 6. 2. Abundance of younger branches of *A. l. longifolia* in the study locations PV4 (A) and PV5 (B).

6.3.2. The effects of galls on vegetative growth

The impact of galls formed by *T. acaciaelongifoliae* on the vegetative growth of *A. l. longifolia* was evaluated by measuring sub-branch length (growth rate), twig mortality per branch and numbers of phyllodes per sub-branch at six different locations throughout the study period and comparing these measures for galled and ungalled branches.

The effects of galls on the length of sub-branches

Initially, the average length of galled and ungalled branches of *A. l. longifolia* were roughly equal in size (Plate 6.1), however, the average length of ungalled branches increased over the time compared to galled branches. The average length of galled sub-branches (mean length, M=94.98 cm; standard deviation, SD=13.63 cm) were significantly shorter than ungalled sub-branches (mean length, M=117.37 cm; SD=25.9 cm) of *A. l. longifolia* at the end of the study $F(1, 24)=47.39$, $p<.005$, partial $\eta^2=.66$. The partial η^2, indicates that the presence of galls has a large effect on the length of galled sub-branches (Cohen, 1988) (Figure 6.1). There were no significant effects of location on the length of sub-branches $F(5, 24)=1.87$, $p=.137$, partial $\eta^2=.281$ and no significant interaction between treatments (galled/ungalled) and locations $F(5, 24)=0.364$, $p=.868$, partial $\eta^2=.071$.

Not surprisingly, time (the months of the study period) explains a great deal of the variation in the lengths of both galled and ungalled sub-branches (galled and ungalled) of *A. l. longifolia* (Cohen, 1988). All sub-branches increased in length over time ($F(17, 408)=2202.55$, $p<.001$, partial $\eta^2=.99$), however galled branches increased at a slower rate than ungalled branches ($F(1, 24)=47.39$, $p<.005$, partial $\eta^2=.66$). A slower growth rate in galled sub-branches is implied by a significant combined effect of time and the presence

of galls on the length of sub-branches $F(17, 408)=563.01$, $p<.001$, partial $\eta^2=.96$. The effect size of changes in the length of the sub-branches over time was almost five times higher ($\eta^2=.77$) in ungalled branches than galled branches ($\eta^2=.15$) of *A. l. longifolia* (Figure 6.1).

Seasonal differences in growth were also evident; and were also influenced by the presence of galls. Mean lengths of galled sub-branches did not differ significantly from their initial lengths (measured in September 2014) until after January 2015 when season growth began (Post hoc comparisons using the LSD test with $\alpha=.05$; M=90.95 cm, SD=11.31 cm).

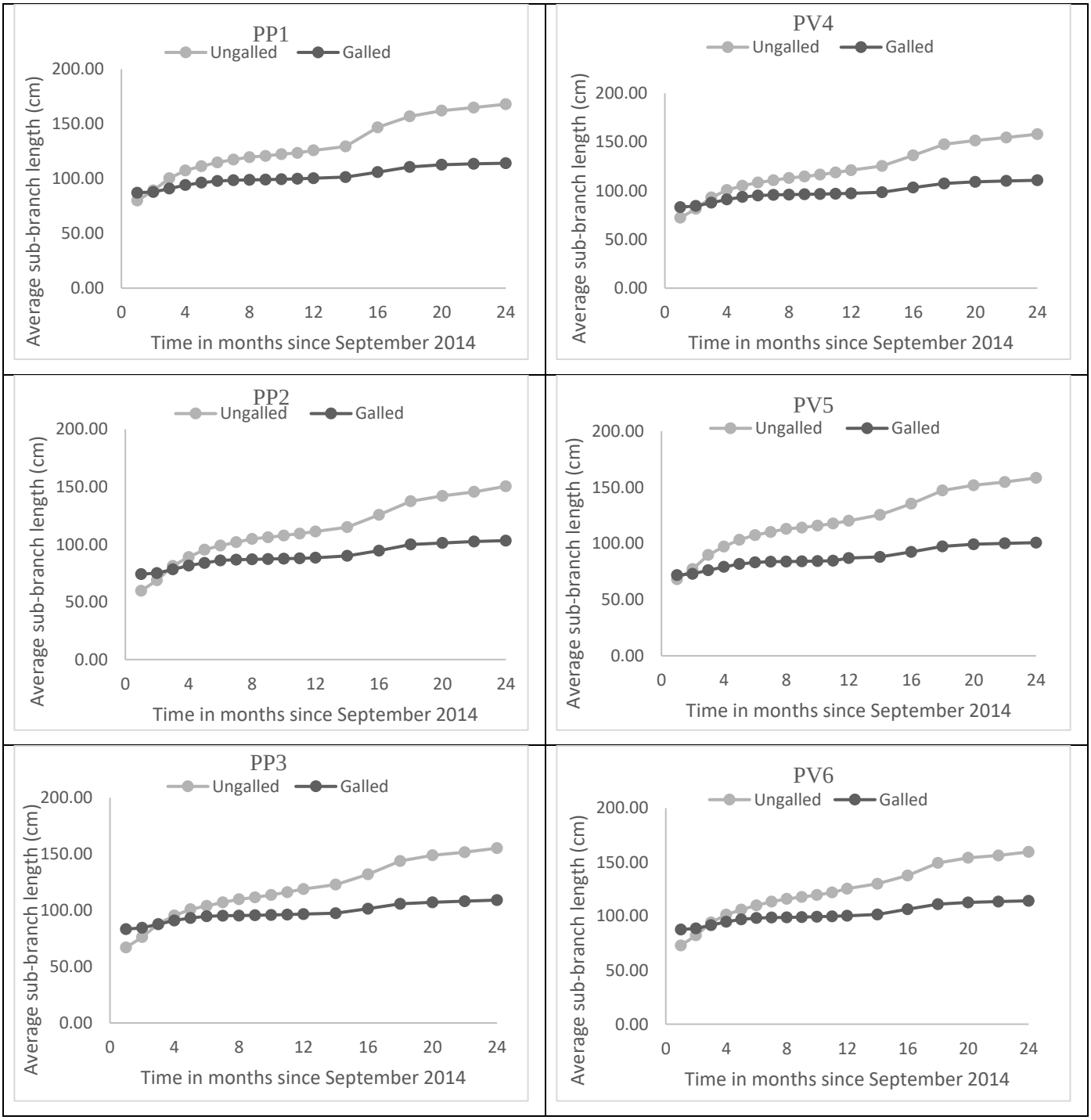

Figure 6. 1. Monthly variations in the average length of sub-branches of *A. l. longifolia* with galls formed by *T. acaciaelongifoliae* and of un-galled sub-branches during a two-year period from September 2014 to August 2016 at six different study locations (1-6, , PP= private property, PV= Parks Victoria and indicates the Grampians National Park) in the Greater Grampians Bioregion, Victoria, Australia.

The galls produced by *T. acaciaelongifoliae* reduced the vegetative growth of *A. l. longifolia.* Although galled sub-branches consistently showed lower growth rates than ungalled sub-branches, the extent of the difference in growth rate between galled and ungalled sub-branches varied across the six locations. Location PP1 supported older stands of *A. l. longifolia* compared to other locations (although there were some younger *A. l. longifolia* present). The initial (measured in September 2014) average length, of galled sub-branches at location PP1 was 87.59 cm (Figure 6.1). These galled sub-branches increased their average length by only 26.57 cm over the two year study period (Figure 6.1). On the other hand, the initial average length of un-galled sub-branches at location PP1 was 72.89 cm. These branches grew at a rate approximately three times higher than galled branches in the same location, reaching an average length of 159.40 cm by August 2016 (Figure 6.1).

The greatest differences in length between galled and ungalled sub-branches were found at locations PV5 and PV4, (57.6 cm and 53.90 cm) respectively, where younger *A. l. longifolia* were the most common. It is possible that the age of the plant may influence the rate of sub-branch growth; this was not tested here, and there was no bias in the age of plants selected for galled and ungalled groups in this study. Galled branches grow more slowly than those without galls and the reduced growth rate observed in galled branches is likely to be due to galls acting as nutrient sinks)Dennill, 1988(. Such effects have also been shown for other insect-plant relationships. For example, Shaw et al. (2009) showed that a psyllid, *Aphalara itadori* Shinji reduced vegetative growth of *Fallopia japonica* in the UK (Shaw, Bryner, & Tanner, 2009). *Fallopia japonica* is an invasive weed in the UK, North America and greater Europe.

The effect of galls on number of phyllodes

As in other members of the *Acacia* genus, the petioles of *A. l. longifolia*, are flattened and widened to form leaf-like structures known as phyllodes (Plate 6.3) (Boke, 1940).

Plate 6. 3. Phyllodes on the sub-branch of *A. l. longifolia* act as leaves in the photosynthetic process.

Phyllodes serve the same function as the leaves of other plants (i.e. photosynthesis). Anatomically, leaves comprise lamina, petiole and leaf base. In Acacias, the modified petiole performs as leaf. The number of phyllodes per sub-branch of *A. l. longifolia* did not differ significantly across the six study locations ($F(5, 24)=0.77$, $p=.58$, partial $\eta^2=0.14$) and there was no significant interaction between treatment (presence/absence of galls) and locations ($F(5, 24)=.79$, $p=.56$, partial $\eta^2=.14$); however the presence of galls had an effect on the number of phyllodes per sub-branch of *A. l. longifolia* $F(1, 24)=322.64$, $p<.001$, partial $\eta^2=0.93$. Considering all measurements made over a one year period, the average

number of phyllodes per sub-branch of *A. l. longifolia* was 1.7 times higher in ungalled sub-branches (M=26.44, SD=1.38) compared to galled sub-branches (M=16.00, SD=2.91). More phyllodes per ungalled sub-branch allowed more photosynthesis and the production of more enery resources for further growth of the plant.

Initially, the difference between the mean number of phyllodes per sub-branch for galled and ungalled sub-branches of *A. l. longifolia* was four, increasing to 28 after one full year of study (Figure 6.2). The number of phyllodes per sub-branch increased over time for both galled and ungalled plants. Post hoc comparisons using the LSD test revealed, for ungalled plants, differences between the initial number of phyllodes per sub-branch (measured in September 2014; M=26.44, SD=1.38) and the number of phyllodes per sub-branch measured in April 2015 (M=37.00, SD=2.72). During this period, *A. l. longifolia* started growing rapidly in response to weather factors. However, there was no significant difference between the initial number of phyllodes (M=19.00, SD=4.24) on sub-branches of galled *A. l. longifolia* compared to the number measured in April 2015 (M=16.00, SD=2.91) (Figure 6.2). The gall inducing wasps emerge from galls and galls dessicate during this period. After the month of April, the number of phyllodes increase in both cases due to weather factors, however it was comparatively low in number in galled branches than ungalled branches. Thus galled sub-branches did not increase their capacity for photosynthesis over time compared to ungalled sub-branches.

A combined effect of the presence of galls and time on the number of phyllodes per sub-branch of *A. l. longifolia* was found $F(11, 264)= .375$, $p<.001$, partial $\eta^2 =.85$. There is a difference in the average number of phyllodes over time. The highest number of phyllodes was found in the month of August 2015, which was followed by July and June 2015.

However, there was no significant interaction between time and locations $F(55, 264)= .38$, $p=1.00$, partial $\eta^2 =.07$; nor was there a significant interaction among time, treatments and locations $F(55, 264)=.64$, $p=.98$, partial $\eta^2 =0.12$ on the number of phyllodes of *A. l. longifolia*. The number of phyllodes per sub-branch of *A. l. longifolia* was affected by the presence of galls , as was the rate of increase in the number of phyllodes over time (Figure 5.2). It is likely that a reduced number of phyllodes in galled sub-branches has follow-on effects, possibly including reductions in other growth parameters, and in reproductive success.

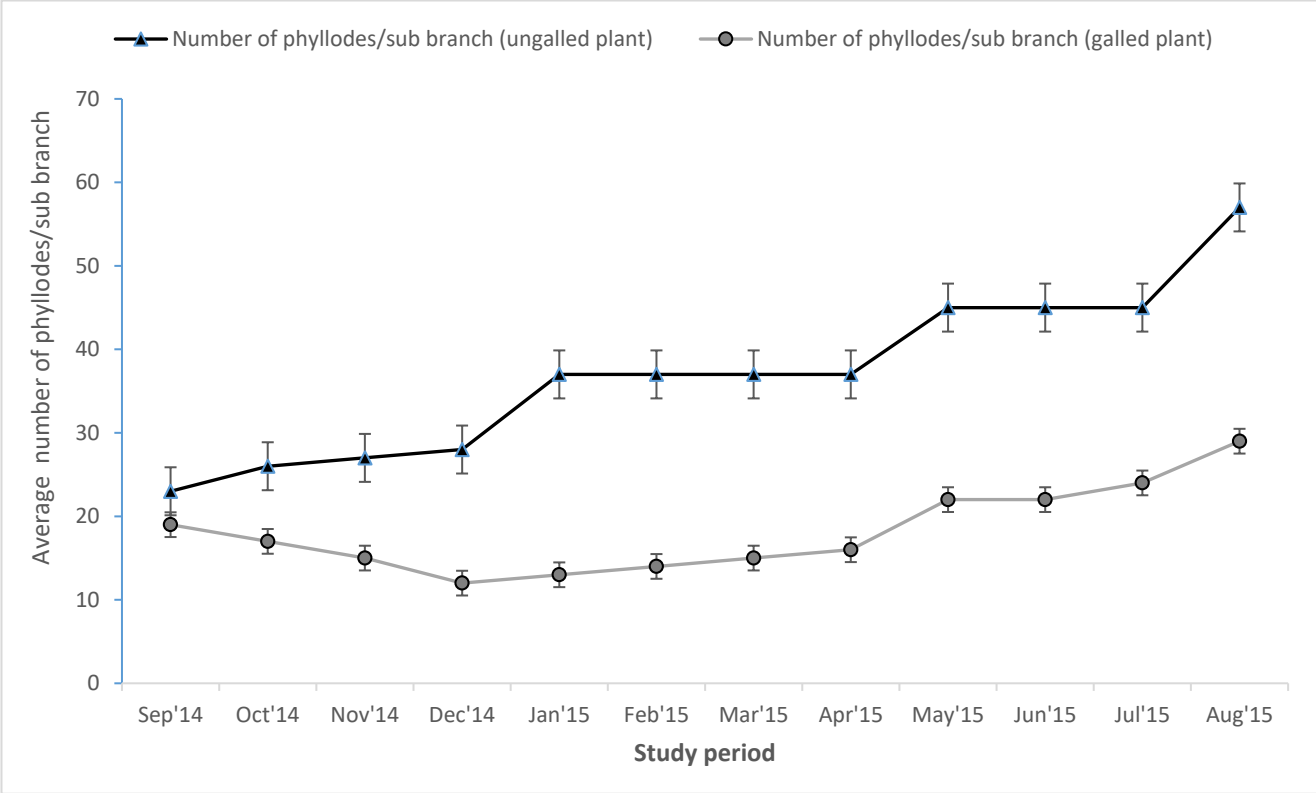

Figure 6. 2. Number of phyllodes on galled (by *T. acaciaeloguifoliae)* and ungalled sub-branches of *A. l. longifolia* from September 2014-August 2015.

Initially, the difference between the mean number of phyllodes per sub-branch for galled and ungalled sub-branches of *A. l. longifolia* was four, increasing to 28 after one full year

of study (Figure 6.2). The average number of phyllodes per sub-branch was significantly affected by the presence of galls over the time of study period. There was some indication of seasonal variability in the average number of phyllodes per sub-branch.

The average number of phyllodes per sub-branch on galled *A. l. longifolia* plants decreased gradually from September 2014–December 2014. The number of phyllodes per branch on galled branches then remained similar from January 2015 until April 2015 (Figure 6.2). At this time of year, the adult insects emerge from the galls; and the galls start to desiccate; and are no longer redirecting the plants' resources. The number of phyllodes per galled sub-branch increased in May 2015 but remained lower than in ungalled sub-branches. The number of phyllodes per sub-branch on ungalled sub-branches increased steadily over the period of the study (Figure 6.2).

The initial average number of phyllodes per sub-branch in September 2014 was 23 (ungalled) and 19 (galled). These numbers increased to 57 (ungalled) and 29 (galled) per sub-branch in August 2015 after one year (Figure 6.2). The galls have the potential to accumulate nutrients, which would otherwise be directed into the formation of phyllodes. G. Dennill (1985) observed similar results in South Africa, suggesting that galls have an adverse effect on the number of phyllodes. Galls formed by *Dryocosmus kuriphihs,* a cynipid wasp, are known to significantly reduce the vegetative growth of the chestnut tree in Japan (Kato & Hijii, 1997b).

Relationship between numbers of galls and twig mortality on branches of *A. l. longifolia*

There was a positive correlation (R2=.79, adjusted R2=.79; F(1,142)=526.49, r=.89, p<.001) between the number of galls present on the branch and twig mortality (the proportion of dead twigs on the branch) (Figure 6.3).

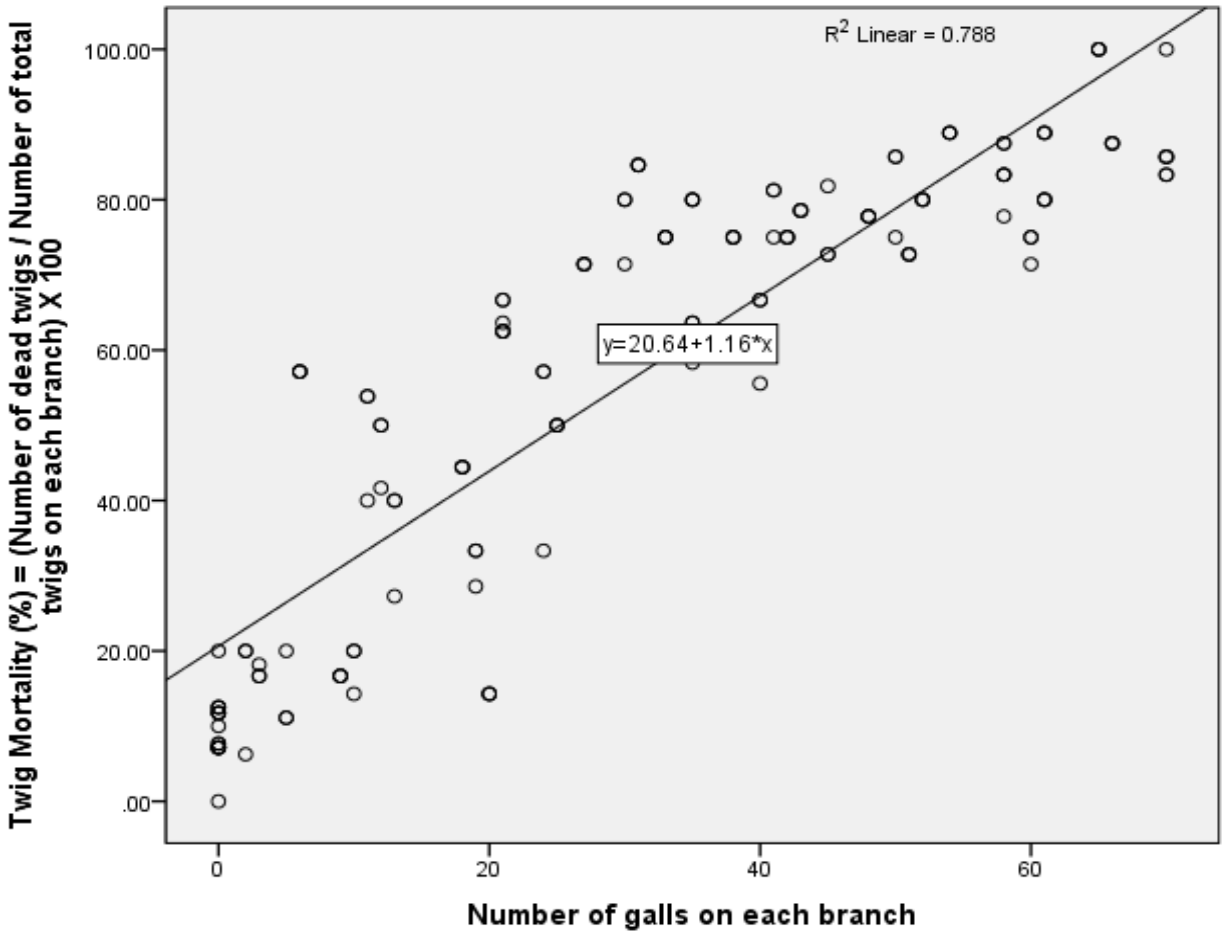

Figure 6. 3. The relationship between twig mortality and numbers of galls formed by *T. acaciaelogifoliae* on *A. l. longifolia*.

Branches with no galls showed little twig mortality. Twig mortality was around 80% for branches with galls at densities of around 60 per branch (Figure 6.3). These results support research by G. Dennill (1985), conducted in South Africa, which showed a positive linear

relationship between the proportion of dead growth tips and the number of galls. Dennill (1985) explained that the galls increased tip mortality and leaf abscission on infested branches, which obstructed the apical growth of the plants.

6.3.3 The effects of galls on reproduction

The presence and density of galls affects the reproductive capacity (as measured by the formation of seedpods) of *A. l. longifolia* (Figure 6.4).

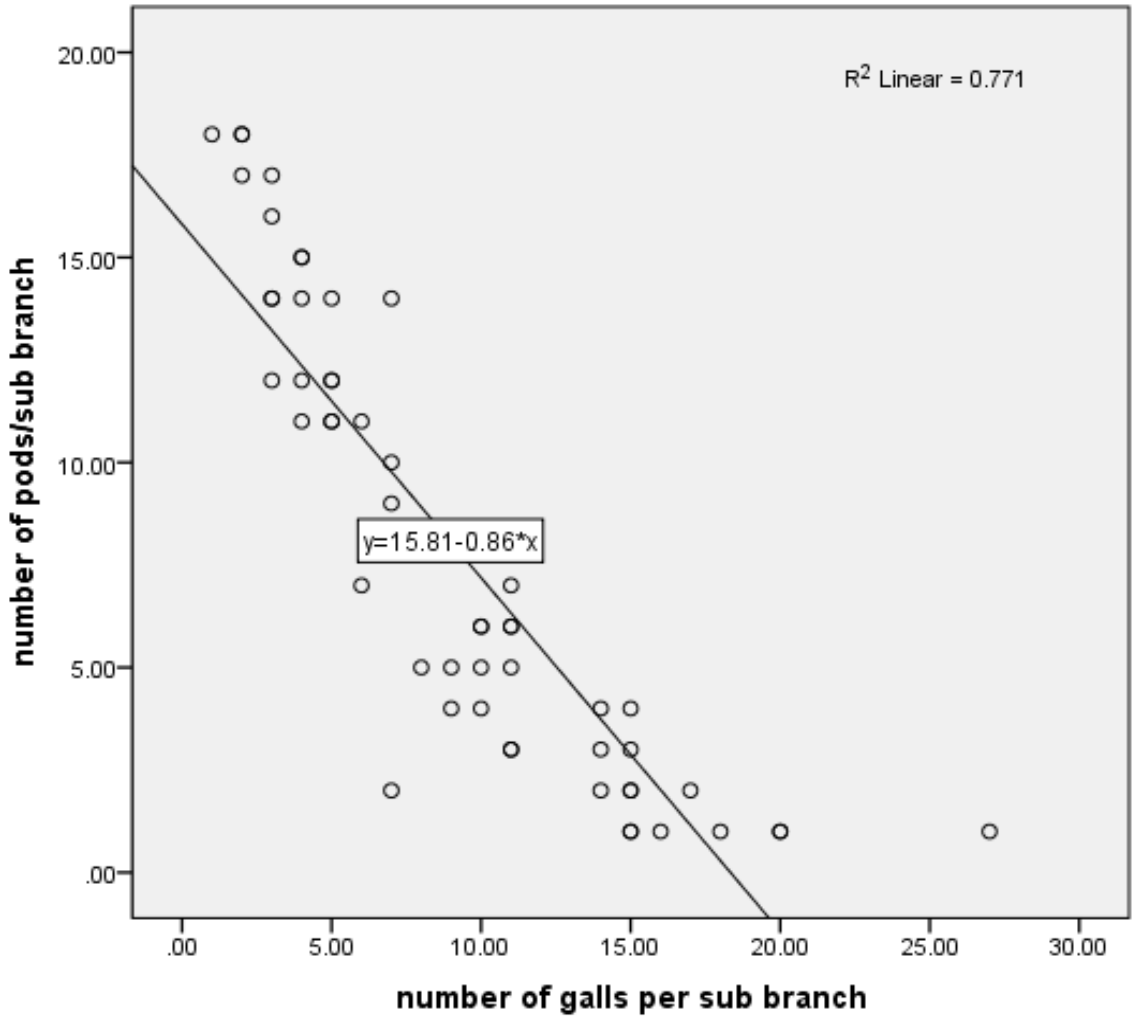

Figure 6. 4. The relationship between the average number of galls by *T. acaciaelongifoliae* and an average number of seed pods per sub-branch on *A. l. longifolia*.

The number of galls is negatively correlated with the number of seedpods, $F(1,52)=175.433$, $p<.001$). In fact, *Acacia l. longifolia* sub-branches with more than 20 galls per sub-branch produced no seedpods (Figure 6.4). The effect size between the two variables (the average number of galls per sub-branch and the number of seedpod per sub-branch) was large ($R^2=.77$).

T. acaciaelongifoliae has the potential to reduce seed production in *A. l. longifolia* (Plate 6.4). It is likely that the galls on gall-bearing sub-branches redirect resources that would otherwise be used for reproductive outputs, including the formation of seeds.

Plate 6. 4. Absence and presence of seed pods on a representative gall-bearing sub-branch (A) and a representative sub-branch with no galls (B).

G. Dennill (1985) also cited similar trends in his study of the same insect-plant system in South Africa, demonstrating that the number of galls adversely affected the seed production

of plants. The dipteran insect (*Dasineura dielsi*) is also effective in reducing number of seeds by developing galls on the ovary of *Acacia cyclops* (Post, Kleinjan, Hoffmann, & Impson, 2010).

Silky hakea (*Hakea sericea*) is another large shrub native to south-eastern Australia, which is considered invasive in some regions outside of its natural range. Its seed is released prolifically after fires and it is considered a problem weed in South Africa and New Zealand (F. Impson, Purcell, & Gordon, 2012). The hakea fruit moth (*Carposina autologa*) feeds on the developing seeds of this plant, reducing the number of viable seeds (Annecke & Neser, 1977; Stefanus Neser, 1968). R. L. Kluge (1983) reports that a weevil *(Erytenna consputa* Pascoe), is used as a biological control agent to reduce seed production of silky hakea. The weevil lays eggs in young developing fruits and the feeding activity of the larvae destroys the fruit (R. Kluge & Neser, 1991). Gall forming insects are also known to reduce viable seed numbers (Navie et al., 1998).

Acacia l. longifolia is considered an environmental weed nationally and internationally. In Australia, government and private land managers are concerned about its rapid invasion, particularly in its native range in south-eastern Australia (Adair, 2008; Thomson, 2016). This study indicates that galls formed by *T. acaciaelongifoliae* reduce vegetative growth (as measured by branch length, number of phyllodes and twig mortality) and reproductive capacity (as measured by the number of seedpods) of *A. l. longifolia*. However, since *Acacia l. longifolia* is invasive in parts of Victoria (Milkins, 2017), including in areas where *T. acaciaelongifoliae* is present, it is clear that these effects are insufficient to check the spread of *A. l. longifolia*.

Plate 6. 5. Branches of *A. l. longifolia* bearing galls and flowers (A), galls and seed pods (B). Photo is taken from study location PP1.

This chapter has also shown that with low numbers of galls the weed, *A. l. longifolia* is still able to produce a number of flowers and seeds on the same branch which allows the plant to continue to survive and to maintain the capacity to become invasive (Plate 6.5).

When discussing the challenges of controlling invasive plants, Harper (1977) cautioned that reliance on mechanisms which reduce seed production is problematic, since even a low percentage of viable seed can allow continued spread, especially of a species which produces seeds prolifically. This study shows that, even where *T. acaciaelongifoliae* can reduce seed production by 95-99%, a reduction in fecundity and therefore growth rates of current populations has not been sufficient to control *A. l. longifolia* invasions. Some gall-bearing branches on *A. l. longifolia* in the study location were still able to produce a number of flowers and seeds (shown in plate 6.5) which facilitated further invasion of areas in the following year. The wasp alone is effective in South Africa, however there are some other factors (abiotic, biotic factors) that might affect the performance of the wasp in different location. It has been said that the performance of any biological control agents can vary

with spatial and temporal changes and with abiotic and biotic conditions, plant resources, soil fertility, weather factors, increased resistance of the weed and, natural enemies (such as bird, predatory insects, parasitoids) of the biocontrol agent (Denno et al., 2002; Hovick & Carson, 2015; Stiling & Moon, 2005). It is clear that reliance only on *T. acaciaelongifoliae* as a biological control agent for *A. l. longifolia* is not effective in Australia.

The high abundance and persistence of *A. l. longifolia* seeds in the soil seed bank is likely to further reduce the effectiveness of *T. acaciaelongifoliae* as a control agent. F. A. Impson et al. (2013) also found similar results when considering the use of the gall-forming midge *Dasineura rubiformis* (Cecidomyiidae) to control *Acacia mearnsii* (black wattle) and recommended the use of the midge as part of an integrated approach to weed control. Therefore, an integrated management approach including *T. acaciaelongifoliae* might be effective to control *A. l. longifolia* in Australian ecosystems.

6.4. Conclusion

This chapter has demonstrated that galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* plants reduce vegetative growth and reproductive capacity of *A. l. longifolia* growing in the native home range of both species, although this does not appear to be sufficient to reduce the invasive spread of the plant. We conclude that effective control of the invasive weed *A. l. longifolia* will require an integrated weed management approach and recognise that while insufficient as a sole control agent, the role of *T. acaciaelongifoliae* in reducing vegetative and reproductive growth is likley to be an important component of an integrated approach.

CHAPTER SEVEN

Host preference of *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae), a gall inducer in Victoria, Australia

7.1. Introduction

T. acaciaelongifoliae is a hymenopteran wasp of the Pteromalidae family. *T. acaciaelongifoliae* induces galls on the flowers and vegetative buds of *A. l. longifolia*, redirecting nutrients used for growth and reproduction and affecting reproductive fitness of the plant (chapter 6). The wasp has been recognised as a useful biological control agent for *A. l. longifolia* growing outside of its native range in south eastern Australia (G. Dennill, 1987; Hélia Marchante, Freitas, & Hoffmann, 2011).

Acacia l. longifolia is considered invasive in many countries, for example: South Africa, Colombia, Portugal, Spain, Uruguay, Argentina and California, (Castro-Díez et al., 2011; Rascher et al., 2011). *Acacia l. longifolia* has also been recognised as a significant environmental weed in several Australian states, and is particularly problematic in the Greater Grampians Bioregion in Victoria (Milkins, 2017; Thomson, 2016), *A. l. longifolia* is threatening ecosystems within its native range (Milkins, 2017; Thomson, 2016). It out-competes other native flora and creates dense single- species stands, which change native plant and animal assemblages (Tunison, 1991).

Sustainable control of *A. l. longifolia* is difficult as the seeds of *A. l. longifolia* can remain dormant in the soil for decades (Milton & Hall, 1981; Pieterse & Cairns, 1988). Biological control of *A. l. longifolia* using *T. acaciaelongifoliae* has been shown to be effective in South Africa (Desneux et al., 2010; Richardson & Kluge, 2008; Shaw et al., 2009; Wilson

et al., 2011). In fact, control of *A. l. longifolia* plants using *T. acaciaelongifoliae* has been advocated as a method to reducing plant populations in South Africa with low cost active labour involvement from authorities or property owners (G. Dennill, 1985).

T. acaciaelongifoliae co-occurs with *A. l. longifolia* in its native range (south-east Australia) and significant impacts of gall formation by *T. acaciaelongifoliae* on the growth and reproduction of *A. l. longifolia* plants growing in their native range have been demonstrated (see details in chapter 6). However, the presence of the wasp is not sufficient to control *A. l. longifolia* populations in its native range, since *A. l. longifolia* continues to present a problem even in the presence of *T. acaciaelongifoliae*.

Biological control agents are generally relatively host specific, however, host specificity for gall forming wasps may vary in different ecological systems (Cullen, 1990; Dunn, 1976; Zwölfer & Harris, 1971). In South Africa *T. acaciaelongifoliae* is known to develop galls on *A. l. longifolia* and *A. l. floribunda* (McGeoch, 2000). A possible explanation for the ineffectiveness of *T. acaciaelongifoliae* in controlling populations of *A. l. longifolia* in Australia may be that there is a range of host plants for *T. acaciaelongifoliae* among taxonomically closely related plant species in Australian ecosystems. The purpose of this study was to determine host specificity of *T. acaciaelongifoliae* among ten plant species. These ten plant species were selected due to co-occurring with *A. l. longifolia* and their economic and biodiversity value in the study locations and other states of Australia (see table 7.1 about their distribution in Australia). We used evidence of oviposition behaviour and development of galls on host plants as indicators of use of the plant by the insect, following Marohasy (1998).

7.2. Materials and Methods

7.2.1. Study Locations

Adults of *T. acaciaelongifoliae* were collected from six study locations in the Greater Grampians Bioregion in Victoria, Australia. The study locations are described in detail in chapter 3.

7.2.1. Collection of adult of *T. acaciaelongifoliae* from the Greater Grampians Bioregion, Victoria

Collection of adult of *T. acaciaelongifoliae* from the six study locations followed the procedure described in chapter 3, section 3.3. For this experiment, a total of 30 galls were enclosed in this way in each year of the study, so that a total of 90 galls were enclosed over the duration of the study. A total 78 adults of *T. acaciaelongifoliae* were collected from those enclosed galls, among them 41 adults of *T. acaciaelongifoliae* were alive (on collection day) and 37 adults were dead (preserved in 70% ethanol) because they might emerged from enclosed galls few days before collection. Thirty-three adults out of the 41 live adults were introduced on the potted plants in the glasshouse at Federation University Australia and remainder were preserved in 70% ethanol for microscopy as they were less active in movement, assumed they emerged one or two days before collection.

7.2.2. Collection of adult of *T. acaciaelongifoliae* from galls kept in the laboratory

Sixty fresh, mature galls without exit holes (ten from randomly selected *A. l. longifolia* trees at each of the six locations in the Greater Grampians Bioregion), were collected during each of the twelve visits to the study area during the 'mature gall season' (September to February) in each year of the study. Of the 4,320 galls collected, 1,260 galls (randomly selected) were used in this experiment. From these galls, 257 adults of *T. acaciaelongifoliae*

emerged, of which 201 adult *T. acaciaelongifoliae* were introduced (known their emergence date) (by using insect aspirator) onto potted plants in the glasshouse at Federation University Australia. The collection procedure of adult *T. acaciaelongifoliae* from galls kept in the laboratory is described in chapter 3, section 3.2.

7.2.3. Origins of host plants and potential host plants

Ten different potential host plant species were tested (Table 7.1). The plant species were selected because of their economic value, seedling availability, distribution and they are co-occurring in the study area and other parts of Australia (ALA, 2017). These included *A. l. longifolia* and eight other species from the same genus (*Acacia*) and family (Fabaceae). The tenth species (*Eucalyptus obliqua*) was a common eucalypt found at the study location, co-occurring with *A. l. longifolia*.

Table 7.1. Ten different species used in host specificity tests for *T. acaciaelongifoliae*.

Species	Family	Distribution in Australia
Acacia longifolia	Fabaceae	WA, SA, VIC, NSW, QLD, TAS
Acacia mearnsii	Fabaceae	WA, SA, VIC, NSW, TAS
Acacia dealbata	Fabaceae	WA, SA, VIC, NSW, TAS
Acacia pycnantha	Fabaceae	WA, SA, VIC, NSW, TAS
Acacia paradoxa	Fabaceae	WA, SA, VIC, NSW, QLD, TAS
Acacia verticillata	Fabaceae	SA, VIC, NSW, TAS
Acacia genistifolia	Fabaceae	SA, VIC, NSW, TAS
Acacia melanoxylon	Fabaceae	WA, SA, VIC, NSW, QLD, TAS
Acacia provincialis	Fabaceae	SA, VIC, NSW, TAS
Eucalyptus obliqua	Myrtaceae	SA, VIC, NSW, QLD, TAS

(Note: WA=Western Australia, SA=South Australia, VIC=Victoria, NSW=New South Wales, QLD=Queensland, TAS=Tasmania) Source: ALA (2017)

Each species was represented by three young plants, approximately 30-60cm in height and with at least three branches. Seedlings of *A. l. longifolia* were not available in nurseries and

were collected from the study location in September 2014. The remaining specimens were purchased from local nurseries in the same month. All plants were kept in natural light conditions without artificial heating or cooling in the glasshouse at Federation University's Mount Helen campus for one month (September 2014) before being transferred to larger pots (25 cm diameter x 20 cm depth) in October 2014. Commercially available potting mix for native Australian plant species was used in the pots and no additional fertiliser was added. Plants were watered regularly and maintained throughout the whole study period.

7.2.4 Host specificity experiments

There were two types of host specificity experiments carried out.

In the 'Free choice' test experiments, one individual of each of the ten candidate host plant species was present within each of three large (120 cm x 80 cm x 90 cm) insect-proof net cages. Each cage was made of an aluminium frame and muslin netting, with a mesh size of 0.5mm (Plate 7.1). Six *T. acaciaelongifoliae* adults were introduced to each cage (thus a total of 18 adult wasps). These wasps were selected from a pool of field-collected and laboratory-reared adults. Of each group of six *T. acaciaelongifoliae* adults introduced to each cage, five were reared in the laboratory (sourced from galls collected in the field) and one was collected from the field post-emergence (the adult is selected from field, those one was active). The age of the adult were known, those emerged from laboratory (5 of 6 adults/cage). This experiment was initially carried out between November-December 2014 and was then repeated in November - December 2015 and November - December 2016.

In the 'No choice' test, individual plants of each of the ten candidate host species were placed inside individual insect-proof cages (made of same materials and measurement as described in the 'free choice test'). Each potential host plant species was represented by

three individually caged plants (Plate 7.2), so there was a total of thirty individually caged plants. Six *T. acaciaelongifoliae* adults were introduced into each cage. Five adult individuals were reared in the laboratory (sourced from galls collected in the field) and one active adult was collected from the field post-emergence. Use of both laboratory-reared and field collected adult insects enabled us to increase the number of adult insects used in this part of the study. The exact age of the laboratory-reared adults were known, whereas that of the field collected adults was not known. This experiment was also replicated across three consecutive years during the study period since a shortage of insect-proof cages and restricted availability of glasshouse space prevented concurrent replication. 'No choice' tests took place in November-December in each year of the study period.

7.2.5 Introduction of adult wasps to plants in the glasshouse and observations made

Adult of *T. acaciaelongifoliae* (obtained via the procedure detailed in chapter three, sections 3.2 and 3.3) were introduced to the insect-proof cages containing plants in the glasshouse at Federation University Australia immediately after emergence. Gall materials were collected every 15 days during the 'mature gall season' (September to February) in each year (12 visits) of the study to get adults in the laboratory. Individual wasp emergence from galls in the laboratory at different times on different days and wasp movement were observed using a magnifying glass and camera for each cage.

Immediately after introducing adult *T. acaciaelongifoliae* to plants, intensive observations were made to detect adult movements and gall formation on leaves, petioles, tendrils or stems, as described in chapter 3 section 3.5. For each of the two experiments (free choice and no choice), the following parameters were measured during observations:

i) Time spent by each adult insect on each plant species (hours),

ii) Number of times oviposition behaviour was observed on each plant species (frequency) per adult,

iii) Number of buds damaged per plant species (frequency) per adult,

iv) Number of galls developed per plant species (frequency)

Later, these parameters were converted to means per plant species.

7.3. Results and discussion

7.3.1. Time spent on individual plant species

The average time spent by each *T. acaciaelongifoliae* wasp on each plant species was recorded. Adult of *T. acaciaelongifoliae* spent most time on *A. l. longifolia* compared to any other candidate host plant species in both 'no choice' and 'free choice' tests. The mean number of hours spent by adult *T. acaciaelongifoliae* on *A. l. longifolia* plant species was 36.83(±3.44) hrs and 40.00(±3.14) hrs in 'free choice' and 'no choice' tests respectively (Table 2). In the 'no choice' tests where the preferred host plant was not available, the wasps remained for short periods (<1 hr) on all plant species other than *A. l. longifolia*. In such cases, the wasps rested more frequently and for longer on the walls or floor of the cage rather than on the plant.

7.3.2. Oviposition behaviour

Oviposition behaviour (described in chapter 3) and gall development were observed to occur only on *A. l. longifolia* plants and not on any other candidate host plant. In the free choice test experiment, the wasp stayed active (moving around the buds and stem) on *A. l. longifolia* and *A. melanoxylon*, though there was no oviposition behaviour observed on *A. melanoxylon* and staying time was only 1.41±0.37 hr (Mean±SE) (Table 7.2).

7.3.3. Bud damage

Although bud damage was observed on *A. melanoxylon* in the 'no choice' experiment, no gall developed on this species later on. The average number of damaged buds (flowers buds and leaf buds) was recorded as 0.67±0.21 (Mean±SE) and 0.83±0.30 (Mean±SE) on *A. melanoxylon* in 'free choice' and 'no choice' tests respectively (Table 2). In the case of *A. l. longifolia,* it was 4.66±0.56 and 5.00±0.58 in 'free choice' and 'no choice' tests, respectively (Table 2). No bud damage was observed in any other tested species except *A. l. longifolia* and *A. melanoxylon*.

7.3.4. Gall development

The results of the free choice tests showed that, of the ten potential host species tested, only *A. l. longifolia* was susceptible to gall induction. Individual adult wasps sometimes moved onto plants of other species for very short periods of time, but quickly moved on. No galls were formed on species other than *A. l. longifolia*. Oviposition behaviour and gall development were observed only on flower and leaf buds of *A. l. longifolia*.

Table 7. 2. Indications of host preference of *T. acaciaelongifoliae* on different plant species under 'free choice' and 'no choice' tests.

Species	Time spent on the plant per female wasp (hrs) (Mean±SE)		Oviposition behaviour Observed (frequency) (Mean±SE)		Number of flower/leaf buds damaged per plant (Mean±SE)		Number of galls developed per plant (Mean±SE)	
	Free choice (*N*=54)	**No choice (*N*=180)**	**Free choice (*N*=54)**	**No choice (*N*=180)**	**Free choice (*N*=54)**	**No choice (*N*=180)**	**Free choice (*N*=54)**	**No choice (*N*=180)**
Acacia longifolia	36.83±3.44	40.00±3.14	6.83±0.54	7.33±0.76	4.66±0.56	5.00±0.58	1.17±0.40	1.33±0.42
Acacia mearnsii	—	0.92±0.14	—	—	—	—	—	—
Acacia dealbata	—	0.96±0.18	—	—	—	—	—	—
Acacia pycnantha	—	0.95±0.18	—	—	—	—	—	—
Acacia paradoxa	—	0.13±0.08	—	—	—	—	—	—
Acacia verticillata	—	0.15±0.08	—	—	—	—	—	—
Acacia genistifolia	—	0.18±0.08	—	—	—	—	—	—
Acacia melanoxylon	1.41±0.37	1.75±0.44	—	—	0.67±0.21	0.83±0.30	—	—
Acacia provincialis	—	0.87±0.16	—	—	—	—	—	—
Eucalyptus obliqua	—	0.88±0.15	—	—	—	—	—	—

7.3.4. Summary

Where wasps had access to *A. l. longifolia*, they were consistently observed to spend more time on this plant. Furthermore, there was no oviposition behaviour, significant bud damage or gall development observed on any plants other than *A. l. longifolia* (Table 7.2). A previous study by McGeoch (2000) found that *T. acaciaelongifoliae* develops galls on *Acacia l. longifolia* and *Acacia l. floribunda* at rates of 10.2 and 4.39 galls per branch respectively. *A. l. floribunda* was not tested as a host in this present study, because, this present study primarily focused on potential host plant species co-occurring with *A. l. longifolia* in the study areas.

Plate 7. 1. 'Free choice' test experiment showing ten different potential host plant species within an insect proof net cage. Plants include *A. l. longifolia*. (*Acacia longifolia, Acacia dealbata, Acacia melanoxylon, Acacia verticillata, Acacia genistifolia, Acacia mearnsii, Acacia pycnantha, Eucalyptus obliqua, Acacia provincialis, Acacia paradoxa*).

Although McGeoch (2000)'s study showed some gall formation on an alternative host species by *T. acaciaelongifoliae*, it is clear that the wasp prefers *A. l. longifolia*. Generally, host specificity for gall forming wasps may vary in different ecological systems (Cullen, 1988; Dunn, 1976; Yukawa & Rohfritsch, 2005; Zwölfer & Harris, 1971). Gagné and Jaschhof (2004) found the majority species of the Cecidomyiidae family induce galls on a specific host plant species, however Yukawa and Rohfritsch (2005) noted some species of the Cecidomyiidae develop galls on a range of different plant species.

This study has shown that *T. acaciaelongifoliae* is highly host-specific; a feature common to biological control agents (Peter Harris and Shorthouse (1996). The presence of alternative host species for *T. acaciaelongifoliae* is therefore not the cause of the ineffectiveness of *T. acaciaelongifoliae* in controlling the weed *A. l. longifolia* in the study areas.

Plate 7. 2. 'No choice' test experiment ten different plant species in the separate cages. From top left: *Acacia longifolia, Acacia dealbata, Acacia melanoxylon, Acacia verticillata, Acacia genistifolia, Acacia mearnsii, Acacia pycnantha, Eucalyptus obliqua, Acacia provincialis, Acacia paradoxa*

7.4. Conclusion

T. acaciaelongifoliae is highly specific to a single host species, *A. l. longifolia*, and does not infest other native species available in the study areas. Thus the presence of other host species is not a sutiable explanation for the inability of the wasp to control the spread of *A. l. longifolia* in Australian ecosystems. *Acacia l. longifolia* is still expanding in the study area despite the presence of the wasp *T. acaciaelongifoliae*. Further investigation is recommended to investigate the ecology including predators, parasitoids of *T. acaciaelongifoliae* and *A. l. longifolia* in southeastern Australia performance of the wasp.

CHAPTER EIGHT

A new record of *Megastigmus* sp. (Torymidae: Megastigminae) associated with *Trichilogaster acaciaelongifoliae* from galls on *Acacia longifolia* subsp. *longifolia* Victoria, Australia

8.1. Introduction

Galls and gall inducing insects can be considered as part of a gall community. Galls can act as hosts for inquilines (insects that use a gall developed by another insect) and gall inducing insects can host parasitoids (insects that feed on, or in, the body of a host and ultimately kills the host) (Hayward & Stone, 2005; Sanver & Hawkins, 2000; J. D. Shorthouse, 1998; Stone et al., 2002). Information about ecological communities within plant galls adds to our understanding of the relationship between plants and insects in the plant–gall-former system (László & Tóthmérész, 2006; Price et al., 1980; Weis & Abrahamson, 1985).

Inquilines and parasitoids may influence the interactions between the host plant and gall-forming insects (László & Tóthmérész, 2006). Studies conducted on gall communities include work on galls formed on roses. Such galls are formed by the cynipid wasp, *Diplolepis rosae* (Hymenoptera: Cynipidae) and are used by hymenopteran inquilines and parasitoids (Nordlander, 1973; Stille, 1984). László and Tóthmérész (2006) also reported that parasitoids can kill the larvae of the gall inducing insect. Inquilines or parasitoids may affect the number of gall inducers which survive to adulthood and emerge. They can also influence the species richness and diversity of the gall-inhabiting community.

Moriya, Shiga, and Adachi (2003) showed that the parasitioids *Torymus sinensis* and *Torymus beneficus* (Hymenoptera: Torymidae) significantly reduced the number of chestnut gall wasps, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) in Japan. *T. sinensis* was introduced to Japan from China (Murakami, Ao, & CHANG, 1980) and *T. beneficus* is a native parasitoid of *Dryocosmus kuriphilus* in Japan (Moriya et al., 2003).

Communities of insects within galls can be quite diverse. Various species may be found within a single gall, representing gall inducers, parasitoids of gall inducers and inquilines (La Salle, 2005). Noyes (2003) reported that there are about 100 species of Chalcidoidea (Hymenoptera) known to be associated with eucalypt galls. Several species of *Quadrastichus* (Hymenoptera: Eulophidae) are found in the galls induced by dipteran (Cecidomyiidae), hymenopteran (Cynipidae) and coleopteran (Curculionidae and Buprestidae) insects (M. d. V. Graham, 1991; LaSalle, 1994; Noyes, 2003).

However, very little is known about the community ecology of galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* in Australia. While *T. acaciaelongifoliae* is effective in managing the *A. l. longifolia* in South Africa and Portugal (F. Impson et al., 2011; H Marchante et al., 2017), the plant is still invasive in Australia. Chapter 6 of this thesis has demonstrated the capacity of the wasp to affect the growth and reproduction of *A. l. longifolia*, but clearly this is not sufficient to check the invasive spreading of the plant in its native range. This chapter investigates whether parasitoids or inquilines within galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* in Australia are likely to affect the relationship between the gall forming insect

and the host plant in such a way that *T. acaciaelongifoliae* is not effective in controlling *A. l. longifolia* in Australia.

The chapter identifies and describes a second species of insect found inside several galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* and examines whether the second species may affect the gall-former/host plant relationship.

8.2. Materials and Methods

8.2.1. Collecting and processing specimens

Specimen collection from field

Four specimens of two unidentified insect species (temporarily named species A and B) were collected from *A. l. longifolia* plants, at study sites in the Greater Grampians bioregion, Victoria, Australia (see chapter 3 for locations of study sites and methods of collection of insects emerging from galls). The unidentified insects were collected by attaching muslin bags to branches during the early stages of gall formation on six randomly selected plants (with newly forming galls) from six different locations in the Greater Grampians bioregion (see detail in chapter 3). The muslin bags (N=90) were originally attached to capture adult *T. acaciaelongifoliae* in the field throughout the study period from September to February of each year (when galls were green and growing). They were replaced when damaged or lost (see detail in chapter 3). The frequency of observations of the galls within the bags was reduced to once a month from March–August of each year of 2014, 2015 and 2016 when galls were drying out or empty.

Specimen collection from the laboratory

Thirteen individual insects were also collected from mature galls of *T. acaciaelongifoliae* which had formed on *A. l. longifolia* in the field, where they were collected, and which had been brought to the laboratory. Mature galls (*N*=180) on twigs of *A. l. longifolia* were collected from the study locations and twigs were placed into glass jars containing Knop's solution and kept in the laboratory for close observation at Federation University Australia. Any insects/inhabitants observed emerging from the galls, were immediately collected using an aspirator and placed into vials containing 70% ethyl alcohol. The 13 individuals collected in this way were the same as one of the unidentified species (Species A) collected from the field.

A total of 19 unidentified insects representing two different species were collected. Of these, 17 individuals (four collected in the field and 13 collected while emerging from galls in the laboratory) were temporarily named species A and two individuals collected from the field were temporarily named labelled species B.

8.2.2. Scanning electron microscopy:

Ten of the 17 individuals of species A were processed for scanning electron microscopy. The procedure for scanning electron microscopy was described in chapter 3 section 3.7.2.

8.2.3. Identification of species A and B

The 17 individuals categorised as species A were carefully observed to confirm that they represented a single (as yet) unidentified species. Micrographs of diagnostic characters of different parts of the unidentified species were taken using a high-resolution 16MP USB3.0

digital camera attached to a compact stereo-microscope and to a scanning electron microscope. These micrographs were compared to descriptions of key characteristics (Gauld & Bolton, 1988) to identify species A to genus level. The identification was confirmed by Dr John LaSalle, former Director of the Australian National Insect Collection (ANIC), (Commonwealth Scientific and Industrial Research Organisation, Canberra).

The two individuals categorised as species B were examined and found to be of a single unidentified species, different from species A. Species B was tentatively identified to genus level with the assistance of Dr John LaSalle. As it was not certain that the individuals categorised as species B had emerged from galls, further investigation of this species did not occur. The method of collection (bagged branches of *A. l. longifolia* in the field) meant that these insects may have been present on the branches rather than associated with galls.

8.3. Results and Discussion

Species A was identified as a member of genus *Megastigmus*, in the subfamily Megastigminae. The species within this genus are poorly described (La Salle; pers. comm. 18 November, 2014). It was not possible to identify the specimens collected to species level.

Species B was considered likely to belong to the genus C*oelocyba* (Pteromalidae: Coelocybinae), of which there are nine Australian species (Bouček, 1988). One of these species, *C. nigrocincta,* is known to have been reared from several types of galls formed on *Eucalyptus* and *Acacia*–including galls formed by *Trichilogaster acaciaelongifoliae* (La Salle, pers. comm. 11 November, 2014). Species within the genus *Coelocyba* are poorly described

and it was not possible to identify the specimens collected to species level (La Salle, pers. comm. 11 November, 2014).

All of the 17 individuals identified as *Megastigmus* sp. emerged from galls between the months of October and January during the study period. One insect emerged from each of 17 galls; and no *T. acaciaelongifoliae* individuals emerged from any of the 17 galls which had hosted individuals of *Megastigmus* sp. Sixteen of the *Megastigmus* sp. specimens were female, identified by the presence of their long ovipositor (Figure 8.2). A single male emerged from a gall kept in the laboratory.

The genus *Megastigmus* belongs to the Torymidae (subfamily: Megastigminae) within the order Hymenoptera and was first described by Dalman (1820) as the subgenus *Torymus* with its type species being *Pteromalus bipunctatus*. The work of several entomologists resulted in a reclassification to the genus *Megastigmus* (Ashmead, 1904; Curtis, 1829). Gauld and Bolton (1988) described details of *Megastigmus* sp, which are consistent with features of the specimens from the galls of *T. acaciaelongifoliae* on *A. l. longifolia* as below.

The presence of a major identifying characteristic of the genus *Megastigmus*, the prominent circular black stigma vein in the forewing (Gauld & Bolton, 1988), in unidentified species A was key to its designation as a member of the genus *Megastigmus*. The following characteristics were also important in identifying unknown species A as belonging to *Megastigmus*. The descriptions below show how features present in the micrographs compare with the descriptions of *Megastigmus* by Gauld and Bolton (1988).

The fore-wings are membranous and fully developed with a short stigmal vein and uncus hardly separated from the postmarginal vein. The postmarginal vein is prominent and well-developed (Figure 8.1). The gaster has a one-segmented petiole (Figure 8.1). The average length of the insect is 3.7 mm without ovipositor and the tarsi are five-segmented. The female has a long well developed ovipositor projecting far beyond the apex of the gaster (Figure 8.2). The antennal toruli positioned are closer to each other than to the orbits and there is no vertical suture running adjacent to the inner orbits (Figure 8. 3). The hind femur is not swollen and has no ventral seta. The hind tibia are straight and the hind coxa is almost double the length of the fore coxa (Figure 8.2). The gaster has no rough sculpture but some smooth elongated bristles and the notauli is deeply impressed (Figure 8.1).

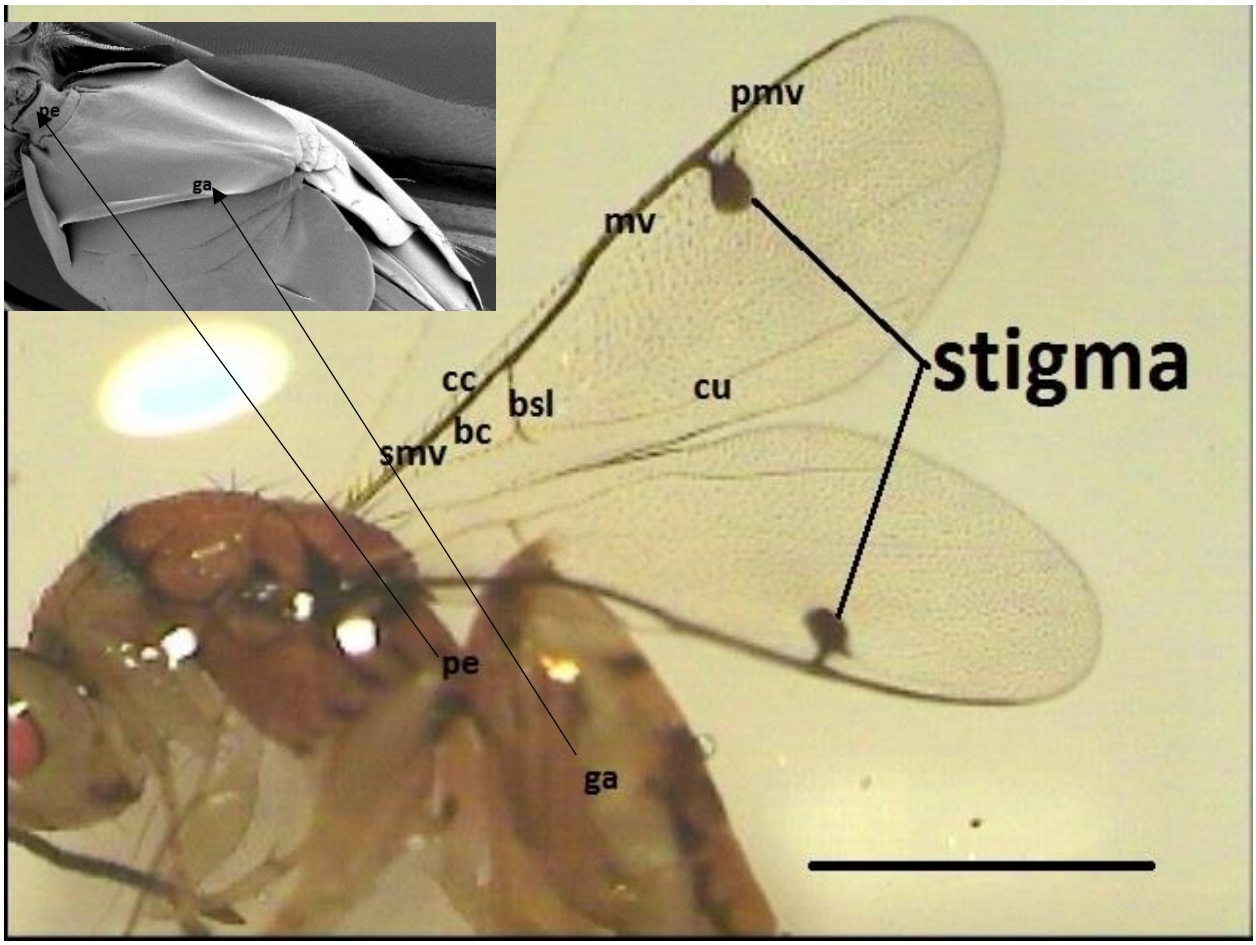

Figure 8. 1. Forewings of an adult of the *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia*, smv, submarginal vein; mv, marginal vein; pmv, postmarginal vein; bc, basal cell; cc, costal cell; bsl, basal setal line; cu, cubital vein; pe, petiole; ga, gaster; (Scale bar=1mm); ventral side of gaster with 1-segmented petiole (inset).

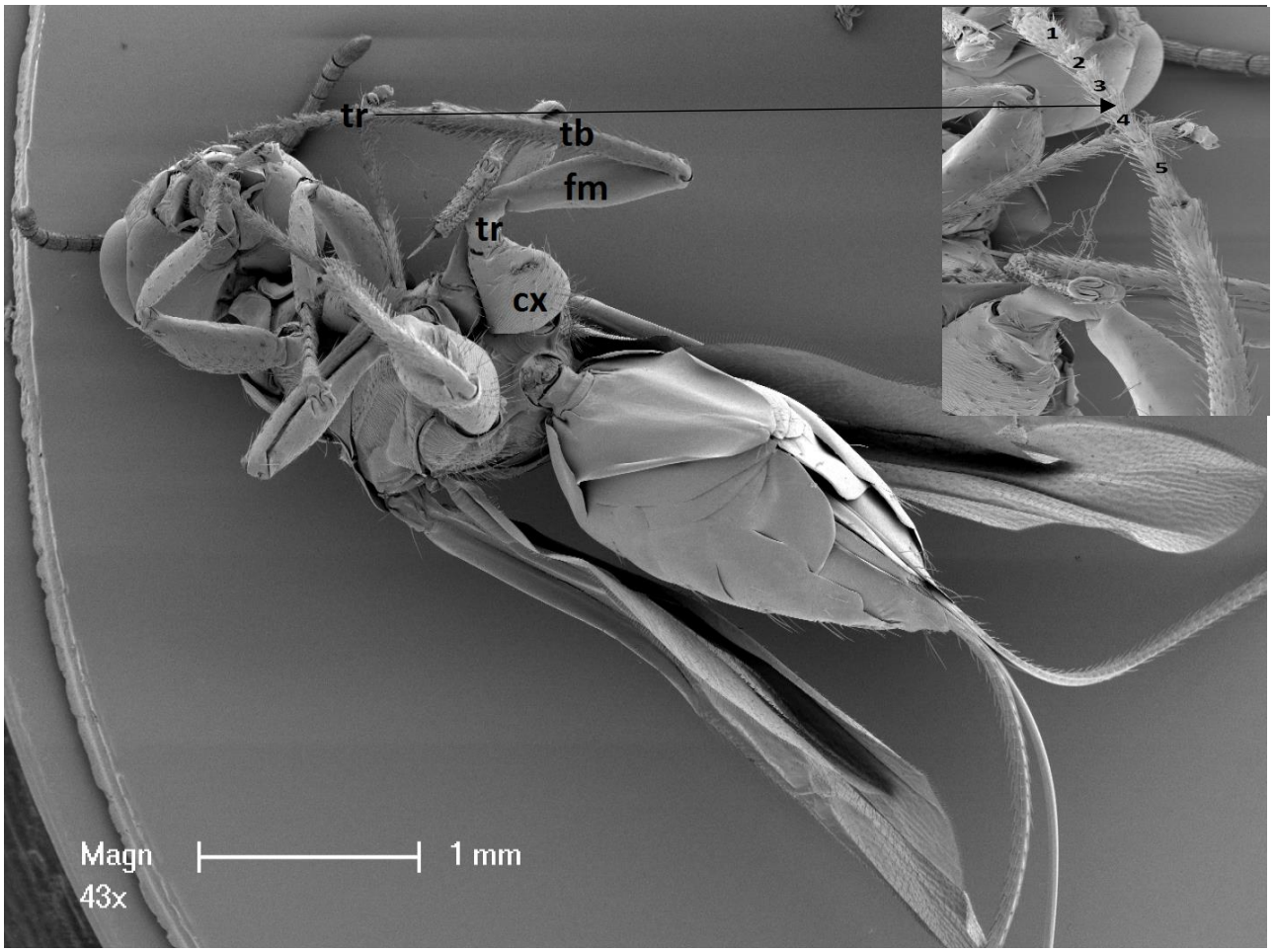

Figure 8. 2. Ventral side of a female adult of *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia* showing different parts of hind leg, cx, coxa; tr, trochanter; fm, femur; tb, tibia; tr, tarsus; 5 segmented tarsus (inset).

The antenna is clavate in shape, with a long flagellum. The antennal clava is long and unsegmented and the funicle is composed of two strongly transverse segments (Figure 8.4).

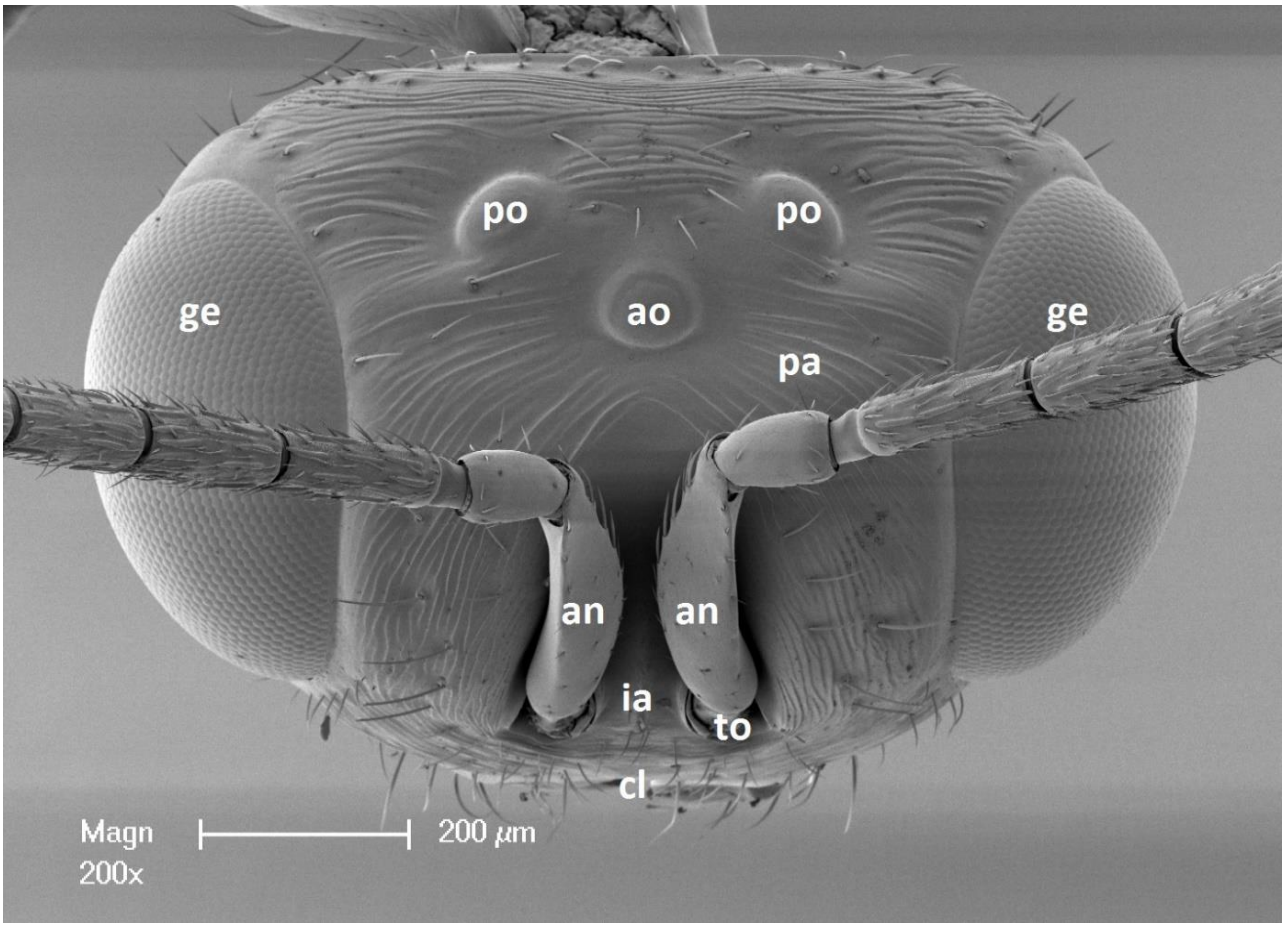

Figure 8. 3. Dorsal view of the head with antenna of *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia*. po, posterior ocellus; ao, anterior ocellus; gen, gena; psa, parascrobal area; an, antenna; ia, interantennal area; tor, torulus; cly, clypeus.

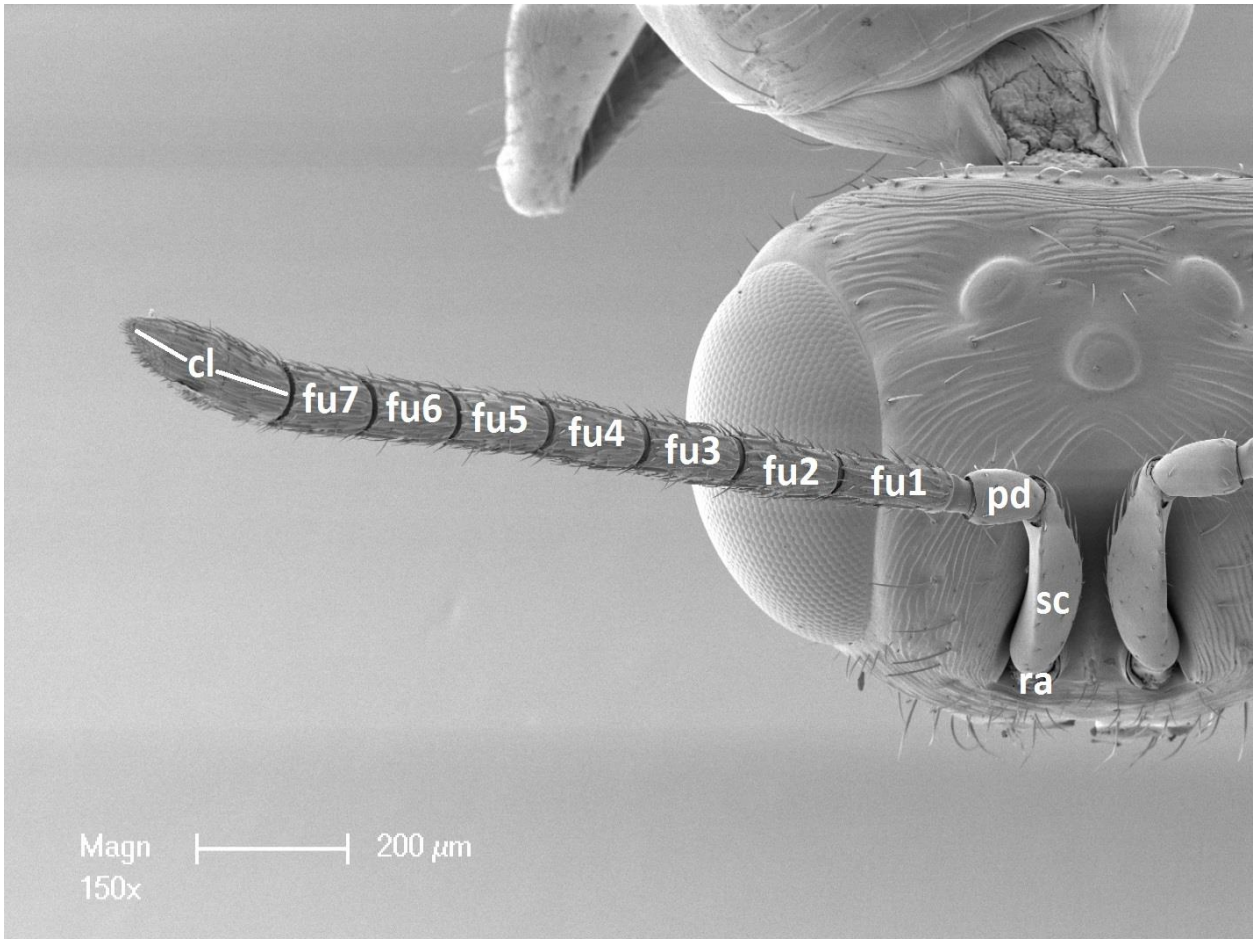

Figure 8. 4. An entire antenna showing segments attached to the head of *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia*. ra, radicle; sc, scape; pd, pedicel; fu, funicular segment; cl, clava.

The scutellum is shield-shaped and broad (Figure 8.5). The beginning of the gaster is conspicuously petiolate and visibly constricted at the junction with the propodeum (Figure 8.2 and 8.5). The pronotal collar at the begging of the thorax is not large and it is subrectangular in shape (Figure 8.5). The gena does not have a sharp edge (Figure 8.3).

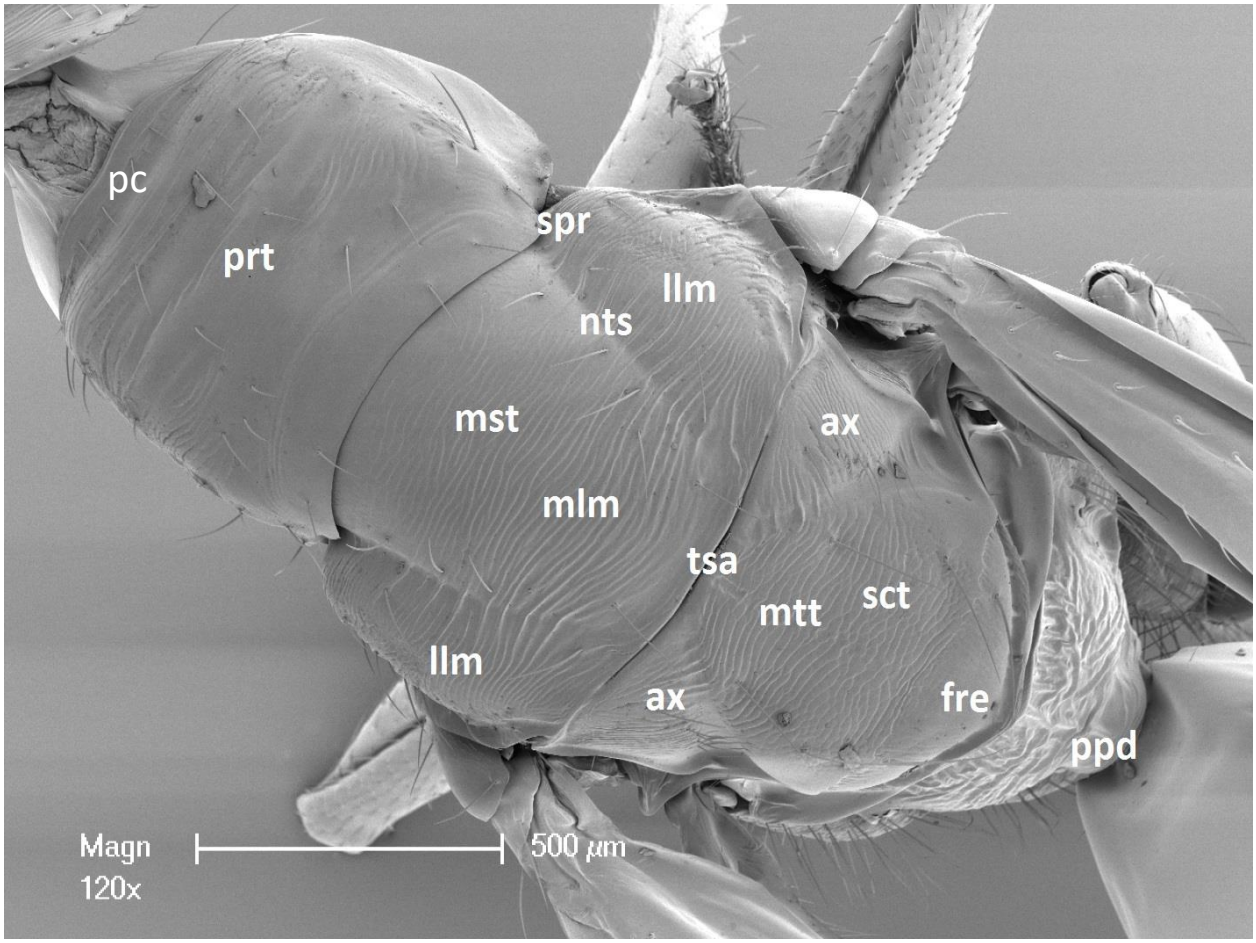

Figure 8. 5. Dorsal view of the thoracic region of *Megastigmus* sp. (Pronotum, mesonotum and metanotum) *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia*. pc, pronotal collar; prt, prothorax; mst, mesothorax; mtt, metathorax; spr, spiracle; nts, notaulus; llm, lateral lobe of mesoscutum; mlm, mid-lobe of mesoscutum; ax, axilla; tsa, transscutal articulation; sct, scutellum; ppd, propodeum; fre, frenum.

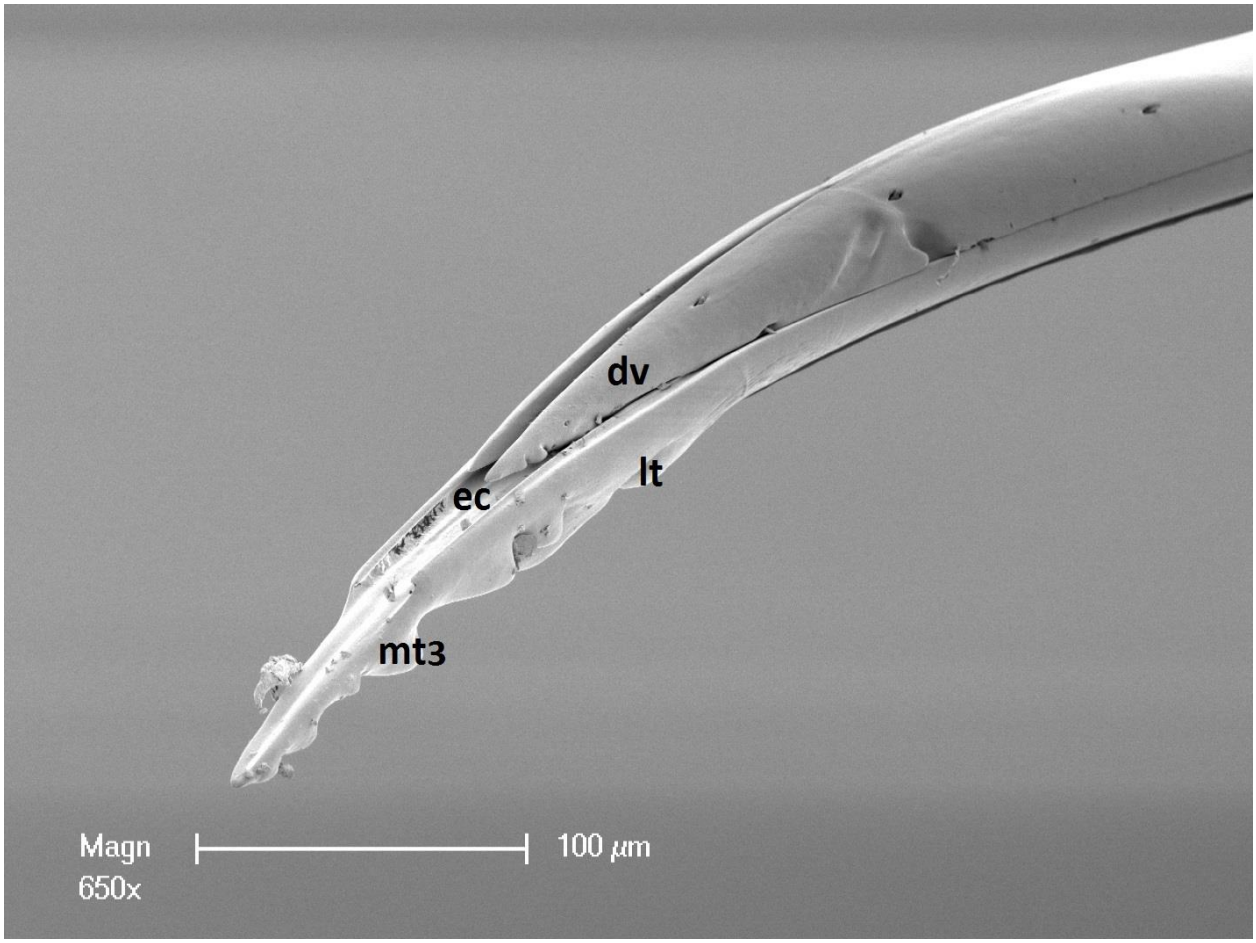

Figure 8. 6. Electron micrograph of ovipositor of *Megastigmus* sp. from the gall of *T. acaciaelongifoliae* on *A. l. longifolia*. mt3, third median tooth; ec, egg canal; lt, lateral teeth; dv, dorsal valve.

Female individuals bear the following characteristics:

The entire body is yellowish brown in colour with scattered black spots (Figure 8.1). There are some seta present on the black spots. The space between pronotum and mesonotum is black in colour. The average body length is 3.7 mm and the length of ovipositor is 3.9 mm. The body is yellowish brown in colour. The areas surrounding the ocellus are blackish in colour. The eyes are brownish red in colour with some short

hairs are present behind the eyes and on face. The antennal toruli, flagellum and inner margin of scape are black in colour. The antennal sockets are smooth and deep. The first segment of the flagellum is slightly longer than other segments, however, segments 2, 3 and 4 are subequal in length. Segments 5, 6 and 7 are roughly equal in length and slightly shorter than 4. The antennal scape is three times longer than the scape and the other segments (1-7) of the flagellum are elongated (Figure 8.4). The mandibles are yellowish-brown in colour and strong, broad and dentate with stiff hairs. A vertical black band is present on the scutellum. Sutures are present on mesosoma and black. The propodeum (basal part of the abdominal segment) is black, however the gaster is yellowish brown. The ovipositor is long and black and approximately 1.2 times as long as the body. The terminal part of the ovipositor bears small cutting teeth with the third median tooth relatively enlarged (Figure 8.6). The ovipositor sheath is black. The leg is yellowish. The margins of the wings wings are black in colour with blackish brown stigma and hind wings hyaline. The stigma are round in shape (Figure 8. 1). The apex of the hind wing is rounded.

Male individuals bear the following characteristics:

The body is similar to that of the female, with some exceptions: The average length of the body is 3.2 mm and colour of the body is light brown, with some black markings. Head is yellowish brown, however ocelli is surrounded by black bands. Scape and pedicel of the antenna are light brown. The flagellum are more elongated than female. A black marking found at the margin of the pronotum. The colour stigma on forewing is light brown. Aedeagus short and conical with teeth.

It was not possible to determine whether the members of the genus *Megastigmus* found in this study represented a new, or a previously described species. The key characteristics of the genus were also described by Bouček (1988) who noted that 44 species of *Megastigmus* are present in Australia, most of which are undescribed. Later, E. E. Grissell (1999) documented 133 species and 5 subspecies of *Megastigmus* globally, with many species still unidentified. A scarcity of information available at the species level meant that it was not possible to determine whether these insects, present in the galls of *T. acaciaelongifoliae* formed on *A. l. longifolia,* are a novel or a known species of the genus.

Roques and Skrzypczyńska (2003) investigated the European species of *Megastigmus* (native and alien species to the West Palearctic region) and provided identification keys to several species of the genus. A new species, *Megastigmus zebrinus* Gissell, associated with seed capsules of *Eucalyptus camaldulensis* Dehnhardt (Myrtales: Myrtaceae) in South Africa and Australia, was described by (E. Grissell, 2006). Doganlar and Hassan (2010) also found several new species of *Megastigmus* in the Palearctic region and in Australia which are also associated with *Eucalyptus*.

Since no adult or larval of *T. acaciaelongifoliae* emerged from the galls occupied by *Megastigmus* sp. in this study, it is hypothesised that this species is likely to be a parasitoid of *T. acaciaelongifoliae*. *Megastigmus* sp. might feed upon *T. acaciaelongifoliae* larvae and kill them inside the galls. Some species of the genus *Megastigmus* have displayed parasitoid characteristics in gall systems (La Salle; pers. comm. 18 November, 2014). Protasov,

Doğanlar, La Salle, and Mendel (2008), found that two species of *Megastigmus* act as parasitoids against the eucalyptus gall wasp, *Leptocybe invasa* (Hymenoptera: Eulophidae) in Turkey and Israel. Doğanlar, Zaché, and Wilcken (2013) also found another species of *Megastigmus*, which showed parasitoid characteristics against a gall former, *Leptocybe invasa* (Hymenoptera: Eulophidae), on *Eucalyptus camaldulensis* in Brazil. However, the species of *Megastigmus* responsible for killing the the gall inducer, *T. acaciaelongifoliae* in the galls on *A. l. longifolia* in Victoria, Australia remains unknown. The presence of *Megastigmus* sp. in galls formed by *T. acaciaelongifoliae* might be one reason that *T. acaciaelongifoliae* does not effectively control populations of *A. l. longifolia* in its native home range.

Since spatio-temporal variation in levels and rate of parasitism has been observed in several species of this group (Moriya et al., 2003), it is possible that investigation of the effect of *T. acaciaelongifoliae* on invasive populations of *A. l. longifolia* might be different in other locations in Australia. Moriya et al. (2003) found a native parasitoid, *Torymus beneficus* (Hymenoptera: Torymidae) effectively suppressed the population of the chestnut gall wasp (Hymenoptera: Cynipidae) in the southern part of Ibaraki Prefecture, in Japan, whereas *Torymus beneficus* was not effective in managing the chestnut gall wasp in other Prefectures in Japan.

8.4. Conclusion

A second species of wasp obtained from galls formed by *T. acaciaelongifoliae* on *A. l. longifolia* in Australia was identified and described as *Megastigmus* sp, and hypothesised to be a parasitoid of *T. acaciaelongifoliae*. The presence of this parasitoid might explain why *T.*

acaciaelongifoliae does not effectively control *A. l. longifolia* in its native range. It is likely that there are some other factors acting in the native range which affects the performance of *T. acaciaelongifoliae* in Australia. For example: other natural enemies of *T. acaciaelongifoliae*, such as bird, predatory insects, other parasitoids, weather factors, and nutrient elements in the ground for *A. l. longifolia* to recover from stresses. All possible factors should be investigated in future experiments to understand the unlike performance of *T. acaciaelongifoliae* in controlling *A. l. longifolia* in Australia. This study certainly contribute about natural enemies of *T. acaciaelongifoliae*, however, further studies are needed to study their life cycle and parasitism rates of *Megastigmus* sp. under laboratory and field condition.

CHAPTER NINE

CONCLUSIONS AND RECOMMENDATIONS

This chapter summarises the key findings embedded in the study. This study has addressed the overarching research goal to contribute knowledge about the relationship between the gall inducing insect, *Trichilogaster acaciaelongifoliae* and its host plant, *Acacia longifolia* (Andrews) Willd. subsp. *longifolia* in Australia.

In order to achieve the research goal, there were five experiments conducted in the study. These are presented in chapters four to eight of this thesis as follows: Mechanism of gall formation by *Trichilogaster acaciaelongifoliae* on *Acacia longifolia* subsp. *longifolia* (chapter 4), Presence of secondary metabolites in galled and ungalled tissue in *Acacia longifolia* spp. *longifolia* (chapter 5), The effect of galls formed by *Trichilogaster acaciaelongifoliae* on the vegetative growth and reproduction of invasive *Acacia longifolia* subsp. *longifolia* in Australia (chapter 6), Host preference of *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae), a gall inducer on the invasive species *Acacia longifolia* subsp. *longifolia* (Fabales: Fabaceae), in Victoria, Australia (chapter 7), and A new record of *Megastigmus* sp. (Torymidae: Megastigminae) associated with *Trichilogaster acaciaelongifoliae* from galls on *Acacia longifolia* subsp. *longifolia* (chapter 8).

In chapter four, the primary events of the gall development were investigated on galls of *A. l. longifolia* caused by the wasp *T. acaciaelongifoliae.* The findings of the study in chapter four have revealed that there are three major stages of the gall development: induction of gall, growth and maturation of gall, shrinking and desiccation of gall. Another key finding

revealed the feeding activity of the larvae of the wasp, *T. acaciaelongifoliae* is responsible for gall induction and development which is uncommon in hymenopteran group. However, the findings of this study are supported by the nutrition hypothesis suggested by Takei et al. (2015). This result is a novel research contribution since other hymenopteran gall-inducing insects typically induce galls through their ovipositional process. Therefore, the findings of the chapter four contribute to an understanding of the relationship between the wasp *T. acaciaelongifoliae* and their host, *A. l. longifolia* in terms of how the galls are developed by *T. acaciaelongifoliae* on *A. l. longifolia.*

In chapter five, the chemistry of galls was examined to explore the quantity of chemicals of gall tisssue and other plant tissue of *A. l. longifolia* induced by *T. acaciaelongifoliae* in understanding of the relationship between gall inducing agent and their host plant. This study showed higher amounts of antioxidants, phenolic compound and anthocyanin in the early stage gall development, which might contribute to the defence of the host plant from further infestation by microorganisms and arthropods. However, the highest amount of total antioxidant capacity (823mg/g), total phenols (12.13mg/g) and total anthocyanin (3.508mg/L) were discovered in the tissue of early stage of galls (6-15mm) of *A. l. longifolia* by *T. acaciaelongifoliae*, when the larvae of the wasp were more active in feeding behaviour, which indicates the feeding action by the larvae of the wasp *T. acaciaelongifoliae* triggers the amount of antioxidants, phenolic compound and anthocyanin in the gall tissue of *A. l. longifolia*. The study also demonstrates a positive correlation among the antioxidants, phenolic compound and anthocyanin in the gall tissue and plant samples of *A. l. longifolia*. The phenolic compounds in the galls by *T. acaciaelongifoliae* might be useful as a natural substitutes for artificial phenolics in food

processing and pharmaceuticals and further research to investigate the type and nature of these chemicals is suggested.

In chapter six, a study was conducted to understand the effect of galls formed by *T. acaciaelongifoliae* on the growth and reproduction of *A. l. longifolia*. This study demonstrated that the galls reduce vegetative growth and reproductive capacity of *A. l. longifolia*, however the wasp alone is unable to control invasive populations of the weed in the study area. An integrated weed management strategy is likley to be more appropriate to effectively control the plant *A. l. longifolia* in Australia, and *T. acaciaelongifoliae* is likley to be an important component of the integrated approach.

In chapter seven, an investigation of the host preference of *T. acaciaelongifoliae* considered ten plant species co-existing with *A. l. longifolia* in the study locations. The results from this study indicated that the wasp develops galls only on *A. l. longifolia* and does not induce galls on any other native species available in the study areas. The presence of other host plant species was therefore not affecting the performance of the wasp in suppressing the weed *A. l. longifolia* in the study areas.

Chapter eight presents a key finding: another insect species associated with galls formed by the wasp *T. acaciaelongifoliae* on *A. l. longifolia*. The insect species was indentified as a species of the genus *Megastigmus*, which is from a parasitoid group, Chalcidioidea. No adult or larval *T. acaciaelongifoliae* emerged from the galls occupied by *Megastigmus* sp. Thus, the findings of the study suggest that *Megastigmus* sp. is likely to be a natural enemy of *T. acaciaelongifoliae* in Australia, and may affect the performance of the wasp in controlling *A. l. longifolia* in Australian ecosystems. Further investigation about the life

cycle and parasitism rates of *Megastigmus* sp. is recommended, under laboratory and field conditions.

To conclude, this PhD research has contributed to a deeper understanding of the relationship between the wasp, *T. acaciaelongifoliae* and the invasive weed, *A. l. longifolia*. Several aspects of the study may help to explain why the wasp does not control the growth of the plant in Australian ecosystems, even though this has occurred in other places (such as South Africa).

REFERENCES

Abdel-lateif, K. S., Eldeab, H., & Maghrabi, I. (2016). The plant natural products: their antioxidants, free radical scavengers, DNA protection and antimicrobial activities. *Journal of Bioprocessing & Biotechniques, **6***(293), 10.4172.

Abrahamson, W., & Weis, A. (1987). Nutritional ecology of arthropod gall makers. In "Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates"(F. Slansky and JG Rodriguez, Eds.). Wiley, New York. United States of America.

Abrahamson, W. G., & Weis, A. E. (1997). Evolutionary ecology across three trophic levels: goldenrods, gallmakers, and natural enemies (Vol. **29**): Princeton University Press. Princeton, New Jersey, United States of America.

Adair, R. J. (2008). Biological control of Australian native plants, in Australia, with an emphasis on *Acacia* 's. *Muelleria, **26***(1), 67-78.

Adair, R. J., & Groves, R. H. (1997). Impact of environmental weeds on biodiversity: a review and development of a methodology. doi:53ff7ff4-3a61-417d-b55c-b0470558e46d. Available at: http://hdl.handle.net/102.100.100/222788?index=1.

Agourram, A., Ghirardello, D., Rantsiou, K., Zeppa, G., Belviso, S., Romane, A., Oufdou, K., & Giordano, M. (2013). Phenolic content, antioxidant potential, and antimicrobial activities of fruit and vegetable by-product extracts. *International Journal of Food Properties, **16***(5), 1092-1104.

Ahmed, Z. S., & Abozed, S. S. (2015). Functional and antioxidant properties of novel snack crackers incorporated with *Hibiscus sabdariffa* by-product. *Journal of Advanced Research, **6***(1), 79-87. doi:10.1016/j.jare.2014.07.002

Ak, T., & Gulcin, I. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions, 174*(1), 27-37. doi:10.1016/j.cbi.2008.05.003

Akhtar, M., & Malik, A. (2000). Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: a review. *Bioresource Technology*, ***74***(1), 35-47.

ALA. (2017). Atlas of Living Australia. Sharing biodiversity knowledge to shape our future. GPO Box 1700, CSIRO, Black Mountain, Canberra, ACT, 2601, Australia.

Alberio, C., & Comparatore, V. (2014). Patterns of woody plant invasion in an Argentinean coastal grassland. *Acta Oecologica*, ***54***, 65-71.

Allen, P., Bennett, K., & Heritage, B. (2014). SPSS statistics version 22: a practical guide: Cengage Learning Australia Pty Limited. South Melbourne, Australia.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.

Ananthakrishnan, T. N. (1984). *Biology of gall insects*. Edward Arnold. London. United Kingdom.

Annecke, D., & Neser, S. (1977). On the biological control of some Cape pest plants. Paper presented at the Proceedings of the National Weeds Conference of South Africa. Stellenbosch, South Africa. 303-319.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of agricultural and food chemistry*, *52*(26), 7970-7981.

Arrowsmith, C., & Inbakaran, R. (2002). Estimating environmental resiliency for the Grampians National Park, Victoria, Australia: a quantitative approach. *Tourism Management*, *23*(3), 295-309.

Ashmead, W. H. (1904). Descriptions of new Hymenoptera from Japan. II. Journal of the New York Entomological Society, *12*(3), 146-165.

Askew, R. (1984). Biology of gall wasps. Biology of gall insects/editor TN Ananthakrishnan. Oxford and IBH Publishing Company, New Delhi, Bombay, Calcutta, 223–271.

AVH. (2014). Australia's Virtual Herbarium, Council of Heads of Australasian Herbaria. Department of Environment and Primary Industries, The State of Victoria. Find *Acacia longifolia* subsp. *longifolia* in Victorian Biodiversity Atlas (published Dec. 2014). available at: http://avh.chah.org.au

Bagatto, G., Paquette, L. C., & Shorthouse, J. D. (1996). Influence of galls of *Phanacis taraxaci* on carbon partitioning within common dandelion, *Taraxacum officinale*. *Entomologia Experimentalis et Applicata*, *79*(1), 111-117.

Bailey, R., Schonrogge, K., Cook, J. M., Melika, G., Csoka, G., Thuroczy, C., & Stone, G. N. (2009). Host niches and defensive extended phenotypes structure parasitoid wasp communities. *PLoS Biology*, *7*(8), e1000179. doi:10.1371/journal.pbio.1000179

Bascompte, J., & Jordano, P. (2007). Plant-animal mutualistic networks: the architecture of biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, 567-593.

Bebawi, F., Vitelli, J., Campbell, S., Vogler, W., Lockett, C., Grace, B., Lukitsch, B., & Heard, T. (2007). The biology of Australian weeds 47. *Jatropha gossypiifolia* L. *Plant Protection Quarterly*, *22*(2), 42-58.

Bell, E. (1981). The physiological role (s) of secondary (natural) products. *The Biochemistry of Plants*, *7*, 1-19.

Benvenuti, S. (2007). Weed seed movement and dispersal strategies in the agricultural environment. *Weed Biology and Management*, *7*(3), 141-157.

Bhattacharya, A., Sood, P., & Citovsky, V. (2010). The roles of plant phenolics in defence and communication during *Agrobacterium* and *Rhizobium* infection. *Molecular Plant Pathology, 11*(5), 705-719. doi:10.1111/j.1364-3703.2010.00625.x

Blanche, K. (2000). Diversity of insect-induced galls along a temperature–rainfall gradient in the tropical savannah region of the Northern Territory, Australia. *Austral Ecology, 25*(4), 311-318.

Blanche, R. (2012). Life in a gall: The biology and ecology of insects that live in plant galls: CSIRO publishing. Collingwood, Clayton South VIC 3169, Australia.

Boke, N. H. (1940). Histogenesis and morphology of the phyllode in certain species of *Acacia*. *American Journal of Botany*, 73-90.

BOM. (2020). Bureau of Meteorology. Commonwealth of Australia 2020. GPO Box 1289, Melbourne VIC 3001, Australia.

Bouček, Z. (1988). Australasian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of fourteen families, with a reclassification of species: Cab International. Wallingford, Oxfordshire, United Kingdom.

Brito, L. M., Reis, M., Mourão, I., & Coutinho, J. (2015). Use of acacia waste compost as an alternative component for horticultural substrates. *Communications in Soil Science and Plant Analysis, 46*(14), 1814-1826.

Bronner, R. (1973). Proprietes lytiques des aeufs de Biorchiza pallida Ol. *Acad Sci Paris CR Ser D*. 276, 189-192.

Brooks, S. E., & Shorthouse, J. D. (1998). Developmental morphology of stem galls of *Diplolepis nodulosa* (Hymenoptera: Cynipidae) and those modified by the inquiline *Periclistus pirata* (Hymenoptera: Cynipidae) on *Rosa blanda* (Rosaceae). *Canadian Journal of Botany, 76*(3), 365-381.

Cai, Y., Luo, Q., Sun, M., & Corke, H. (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sciences, 74*(17), 2157-2184. doi:10.1016/j.lfs.2003.09.047

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*(11), 3426-3431.

Caresche, L., & Wapshere, A. (1974). Biology and host specificity of the Chondrilla gall mite *Aceria chondrillae* (G. Can.) (Acarina, Eriophyidae). *Bulletin of Entomological Research, 64*(2), 183-192.

Carr, G., Yugovic, J., & Robinson, K. E. (1992). Environmental weed invasions in Victoria: conservation and management implications. Department of Conservation and Environment and Ecological Horticulture Pty Ltd. Melbourne, Australia.

Castro-Díez, P., Godoy, O., Saldaña, A., & Richardson, D. M. (2011). Predicting invasiveness of Australian acacias on the basis of their native climatic affinities, life history traits and human use. *Diversity and Distributions, 17*(5), 934-945.

Caujape-Castells, J., Tye, A., Crawford, D. J., Santos-Guerra, A., Sakai, A., Beaver, K., Lobin, W., Florens, F. V., Moura, Mo., & Jardim, R. (2010). Conservation of oceanic island floras: present and future global challenges. *Perspectives in Plant Ecology, Evolution and Systematics, 12*(2), 107-129.

Chapin, F. S. (2003). Effects of plant traits on ecosystem and regional processes: a conceptual framework for predicting the consequences of global change. *Annals of Botany, 91*(4), 455-463. doi:10.1093/aob/mcg041

Cheeke, P. R. (1989). *Toxicants of plant origin: alkaloids* (Vol. 1): CRC Press. Boca Raton, Florida, United States of America.

Cock, M., Bennett, F. D., Hughes, I., Simmonds, F., & Yaseen, M. (1985). A review of biological control of pests in the Commonwealth Caribbean and Bermuda up to

1982 (No. 632.96 R4). Commonwealth Institute of Biological Control. London, United Kingdom.

Colvin, J., Omongo, C., Govindappa, M., Stevenson, P. C., Maruthi, M., Gibson, G., Seal, S., & Muniyappa, V. (2006). Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. *Advances in Virus Research, 67*, 419-452.

Cook, D. C., Sheppard, A., Liu, S., & Lonsdale, W. M. (2015). Predicting the economic impacts of invasive species: the eradication of the giant sensitive plant from Western Australia. *Pest Risk Modelling and Mapping for Invasive Alien Species, 7*, 145.

Cornell, H. V. (1983). The secondary chemistry and complex morphology of galls formed by the Cynipinae (Hymenoptera): why and how? *American Midland Naturalist*, 225-234.

Correia, M., Montesinos, D., French, K., & Rodríguez-Echeverría, S. (2016). Evidence for enemy release and increased seed production and size for two invasive Australian acacias. *Journal of Ecology, 104*(5), 1391-1399.

Costermans, L. F. (1981). Native trees and shrubs of south-eastern Australia. Native trees and shrubs of south-eastern Australia. Rigby Publishers Ltd. London, United Kingdom.

Crespi, B., & Abbot, P. (1999). The behavioral ecology and evolution of kleptoparasitism in Australian gall thrips. *Florida Entomologist*, 147-164.

Cronk, Q., & Fuller, J. (1995). Plant Invaders. The Threat to Natural Ecosystems. WWF International. In: UNESCO & Kew Gardens. Citado por: Andrade, G. I,. Lagos y humedales del Altiplano de Cundinamarca y Boyacá. Chapman & Hall. London, United Kingdom.

Csoka, G. (1998). *The Biology of Gall-inducing Arthropods* (Vol. 199): United States. Department of Agriculture, Forest Service, North Central Research Station. United States of America.

Csurhes, S. (1995). List of declared and non-declared plant species considered to pose a serious threat to native bushland and/or wetlands in Queensland. Internal report, Land Protection Branch, Queensland Department of Natural Resources, Brisbane, Australia.

Csurhes, S., & Edwards, R. (1998). National weeds program, potential environmental weeds in Australia, Candidate species for preventative control. *National Parks and Wildlife Biodiversity Group, Environment Australia, Canberra*, 208.

Cullen, J. (1988). *Current problems in host-specificity screening.* Paper presented at the Proceedings of the VII International Symposium on Biological Control of Weeds. Istituto Sperimentale per la Patologia Vegetale, Ministero dell'Agricoltura e delle Foreste. Rome, Italy. 27-36.

Curtis, J. (1829). Insects. Descriptions of the insects brought home by Commander James Clark Ross, RN, FRS, & C. Appendix to the narrative of a second voyage in search of a north-west passage and of a residence in arctic regions during the years, *1830*(1831), 1832.

D'Antonio, C. M. (2000). Fire, plant invasions, and global changes. In 'Invasive species in a changing world', (Eds H Mooney, RJ Hobbs), Island Press, Washington, DC, United States of America, 65-93.

Dakora, F., & Phillips, D. (1996). Diverse functions of isoflavonoids in legumes transcend anti-microbial definitions of phytoalexins. *Physiological and Molecular Plant Pathology*, *49*(1), 1-20.

Dalman, J. W. (1820). Forsok till Uppstallning af Insekfamilien Pteromalini, I synnerhet medafeende pa di I sverige funne arter. *Kongliga Vetenskaps-Akademiens Handlingar, 41*, 123–182.

Datta, S., Sinha, B., Bhattacharjee, S., & Seal, T. (2019). Nutritional composition, mineral content, antioxidant activity and quantitative estimation of water soluble vitamins and phenolics by RP-HPLC in some lesser used wild edible plants. *Heliyon, 5*(3), e01431.

Davies, P. J. (2010). The plant hormones: their nature, occurrence, and functions. In *Plant Hormones* (pp. 1-15): Springer, Dordrecht, The Netherlands.

Day, J., McGregor, G., & Johnstone, P. (1984). Grampians National Park: inventory of resources and uses: National Parks Service, Victoria.

De Wit, M., Crookes, D., & Van Wilgen, B. (2001). Conflicts of interest in environmental management: estimating the costs and benefits of a tree invasion. *Biological Invasions, 3*(2), 167-178.

DELWP (Department of Environment, L., Water and Planning). (2004). Bioregions and EVC benchmarks. State Government of Victoria. Available at: https://www.environment.vic.gov.au/biodiversity/bioregions-and-evc-benchmarks

Dennill, G. (1985). The effect of the gall wasp *Trichilogaster acaciaelongifoliae* (Hymenoptera: Pteromalidae) on reproductive potential and vegetative growth of the weed *Acacia longifolia*. *Agriculture, Ecosystems & Environment, **14***(1-2), 53-61.

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

Dennill, G., & Donnelly, D. (1991). Biological control of *Acacia longifolia* and related weed species (Fabaceae) in South Africa. *Agriculture, Ecosystems & Environment, **37***(1-3), 115-135.

Dennill, G. B., Donnelly, D., Stewart, K., & Impson, F. A. C. (1999). Insect agents used for the biological control of Australian *Acacia* species and *Paraserianthes lophantha* (Willd.) Nielsen (Fabaceae) in South Africa. *African Entomology, **1***, 45-54.

Denno, R. F., Gratton, C., Peterson, M. A., Langellotto, G. A., Finke, D. L., & Huberty, A. F. (2002). Bottom-up forces mediate natural-enemy impact in a phytophagous insect community. *Ecology, 83*(5), 1443-1458.

Desneux, N., Wajnberg, E., Wyckhuys, K. A., Burgio, G., Arpaia, S., Narváez-Vasquez, C. A., González-Cabrera, J., Ruescas, D. C., Tabone, E., & Frandon, J. (2010). Biological invasion of European tomato crops by Tuta absoluta: ecology, geographic expansion and prospects for biological control. *Journal of Pest Science, **83***(3), 197-215.

Dhileepan, K., Neser, S., & De Prins, J. (2014). Biological control of bellyache bush *(Jatropha gossypiifolia)* in Australia: South America as a possible source of natural enemies. Paper presented at the Proceedings of the XIV International Symposium on Biological Control of Weed. Kruger National Park, South Africa, March 2014.

Didham, R. K., Tylianakis, J. M., Gemmell, N. J., Rand, T. A., & Ewers, R. M. (2007). Interactive effects of habitat modification and species invasion on native species

decline. *Trends in Ecology & Evolution, 22*(9), 489-496. doi:10.1016/j.tree.2007.07.001

Dietz, H., & Steinlein, T. (2004). Recent advances in understanding plant invasions. *Progress in Botany, 65*, 539–573.

Doganlar, M., & Hassan, E. (2010). Review of Australian species of *Megastigmus* (Hymenoptera: Torymidae) associated with *Eucalyptus*, with descriptions of new species. *Australian Journal of Basic and Applied Sciences, 4*(10), 5059-5120.

Doğanlar, M., Zaché, B., & Wilcken, C. F. (2013). A new species of *Megastigmus* (Hymenoptera: Torymidae: Megastigminae) from Brazil. *Florida Entomologist, 96*(1), 196-199.

Dorchin, N., Cramer, M. D., & Hoffmann, J. H. (2006). Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. *Ecology, 87*(7), 1781-1791.

Dorchin, N., Cramer, M. D., & Hoffmann, J. H. (2006). Photosynthesis and sink activity of wasp-induced galls in *Acacia pycnantha*. *Ecology, 87*(7), 1781-1791. doi:10.1890/0012-9658(2006)87[1781:pasaow]2.0.co;2

Dorchin, N., Scott, E., Clarkin, C., Luongo, M., Jordan, S., & Abrahamson, W. (2009). Behavioural, ecological and genetic evidence confirm the occurrence of host-associated differentiation in goldenrod gall-midges. *Journal of Evolutionary Biology, 22*(4), 729-739.

DPI. (2008). Victorian Resources Online. Ecological Vegetation Class. Department of Primary Industries. Available at: http://www.land.vic.gov.au/DPI/Vro/vrosite.nsf/pages/veg_evc

Dreger-Jauffret, F. (1992). Diversity of gall-inducing insects and their galls. In 'Biology of insect-induced galls'.(Eds JD Shorthouse, O Rohfritsch) pp. 8–33. In: Oxford University Press: Oxford, United Kingdom.

Dreistadt, S. H., & Hagen, K. S. (1994). Classical biological control of the acacia psyllid, *Acizzia uncatoides* (Homoptera: Psyllidae), and predator-prey-plant interactions in the San Francisco Bay area. *Biological Control, **4***(4), 319-327.

DSE. (2003a). Flora Information System (database). Department of Sustainability and Environment, East Melbourne 3002, Victoria, Australia.

Dube, N., Zachariades, C., Uyi, O., & Munyai, T. C. (2019). Life history traits and host suitability of a gall-forming fly, *Polymorphomyia basilica* (Diptera: Tephritidae), for the biological control of *Chromolaena odorata* (Asteraceae) in South Africa. *Arthropod-Plant Interactions*, 1-14.

Dukes, J. S., & Mooney, H. A. (1999). Does global change increase the success of biological invaders? *Trends in Ecology & Evolution, 14*(4), 135-139.

Dunn, P. (1976). Shortcomings in the Classic Tests of Candidate Insects. Paper presented at the Proceedings of the IV International Symposium on Biological Control of Weeds. 30 August - 2 September 1976, Gainesville, Florida. Vol. 30, 51-56.

Egan, S. P., Hood, G. R., Martinson, E. O., & Ott, J. R. (2018). Cynipid gall wasps. *Current Biology, 28*(24), R1370-R1374.

Epstein, F. H., Diaz, M. N., Frei, B., Vita, J. A., & Keaney Jr, J. F. (1997). Antioxidants and atherosclerotic heart disease. *New England Journal of Medicine, 337*(6), 408-416.

Espírito-Santo, M. M., & Fernandes, G. W. (2007). How many species of gall-inducing insects are there on earth, and where are they? *Annals of the Entomological Society of America, 100*(2), 95-99.

Fay, P. A., Hartnett, D. C., & Knapp, A. K. (1996). Plant tolerance of gall-insect attack and gall-insect performance. *Ecology, 77*(2), 521-534.

Fernandes, G. W., Carneiro, M. A., & Isaias, R. M. (2012). Gall-inducing insects: from anatomy to biodiversity. Insect bioecology and nutrition for integrated pest management. Edited by AR Panizzi and JRP Parra. CRC Press, Boca Raton, Florida, United States of America. 369-395.

Ferreira, R. O., de Carvalho Junior, A. R., da Silva, T. M., Castro, R. N., da Silva, T. M., & de Carvalho, M. G. (2014). Distribution of metabolites in galled and non-galled leaves of Clusia lanceolata and its antioxidant activity. *Revista Brasileira de Farmacognosia, 24*(6), 617-625.

Fu, L., Xu, B.-T., Xu, X.-R., Gan, R.-Y., Zhang, Y., Xia, E.-Q., & Li, H.-B. (2011). Antioxidant capacities and total phenolic contents of 62 fruits. *Food Chemistry, 129*(2), 345-350. doi:10.1016/j.foodchem.2011.04.079

Gaertner, M., Den Breeyen, A., Hui, C., & Richardson, D. M. (2009). Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. *Progress in Physical Geography, 33*(3), 319-338.

Gagné, R. J., & Jaschhof, M. (2004). A catalog of the Cecidomyiidae (Diptera) of the world: Entomological Society of Washington Washington, DC, United States of America.

Gassmann, A., & Shorthouse, J. D. (1990). Structural damage and gall induction by *Pegomya curticornis* and *Pegomya euphorbiae* (Diptera: Anthomyiidae) within the stems of leafy spurge (*Euphorbia pseudovirgata*) (Euphorbiaceae). *The Canadian Entomologist, 122*(3), 429-439.

Gatjens-Boniche, O. (2019). The mechanism of plant gall induction by insects: revealing clues, facts, and consequences in a cross-kingdom complex interaction. *Revista de Biología Tropical, 67*(6), 1359-1382.

Gauld, I., & Bolton, B. (1988). *The Hymenoptera*: Oxford University Press in association with British Museum (Natural History). Oxford University Press. Oxford, United Kingdom.

Giron, D., Huguet, E., Stone, G. N., & Body, M. (2016). Insect-induced effects on plants and possible effectors used by galling and leaf-mining insects to manipulate their host-plant. *Journal of Insect Physiology, 84*, 70-89.

Gomez, K. A., Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research: John Wiley & Sons. 155 Cremorne St, Richmond VIC 3121, Australia.

Graham, M. d. V. (1991). A reclassification of the European Tetrastichinae (Hymenoptera: Eulophidae): revision of the remaiming genera. *Memoirs of the American Entomological Institute, 49*, 1-322.

Graham, S. A. (1995). Gall makers on flowers of *Cuphea* (Lythraceae). *Biotropica, 27*(4), 461-467.

Grissell, E. (2006). A new species of Megastigmus Dalman (Hymenoptera: Torymidae), galling seed capsules of Eucalyptus camaldulensis Dehnhardt (Myrtaceae) in South Africa and Australia. *African Entomology, 14*(1), 87.

Grissell, E. E. (1999). Hymenopteran biodiversity: some alien notions. *American Entomologist, 45*(4), 235-244.

Groves, R., & Willis, A. (1999). Environmental weeds and loss of native plant biodiversity: some Australian examples. *Australian Journal of Environmental Management, 6*(3), 164-171.

Guerrero, J., Ciampi, L., Castilla, A., Medel, F., Schalchli, H., Hormazabal, Bensch, E., & Alberdi, M. (2010). Antioxidant capacity, anthocyanins, and total phenols of wild

and cultivated berries in Chile. *Chilean Journal of Agricultural Research, 70*(4), 537-544.

Guerrieri, E., & Digilio, M. C. (2008). Aphid-plant interactions: a review. *Journal of Plant Interactions, 3*(4), 223-232.

Gülçin, İ. (2010). Antioxidant properties of resveratrol: a structure–activity insight. *Innovative Food Science & Emerging Technologies, 11*(1), 210-218.

Gullan, P. J. (1984). A revision of the gall-forming coccoid genus Apiomorpha Rübsaamen (Homoptera: Eriococcidae: Apiomorphinae). *Australian Journal of Zoology Supplementary Series, 32*(97), 1-203.

Gurevitch, J., & Padilla, D. K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution, 19*(9), 470-474. doi:10.1016/j.tree.2004.07.005

Gutterman, Y., & Chauser-Volfson, E. (2000). The distribution of the phenolic metabolites barbaloin, aloeresin and aloenin as a peripheral defense strategy in the succulent leaf parts of Aloe arborescens. *Biochemical Systematics and Ecology, 28*(9), 825-838.

Hagemann, G., & Rose, P. (1988). Leaf spot and blight on Acacia longifolia caused by *Cylindrocladium scoparium*: a new host record. *Phytophylactica, 20*(3), 311-316.

Haiden, S., Hoffmann, J., & Cramer, M. (2012). Benefits of photosynthesis for insects in galls. *Oecologia, 170*(4), 987-997. doi:10.1007/s00442-012-2365-1

Harper, J. L. (1977). Population biology of plants. Academic Press, London, United Kingdom.

Harris, M., Stuart, J., Mohan, M., Nair, S., Lamb, R., & Rohfritsch, O. (2003). Grasses and gall midges: plant defense and insect adaptation. *Annual Review of Entomology, 48*(1), 549-577. doi:10.1146/annurev.ento.48.091801.112559

Harris, P. (1980). Establishment of *Urophora affinis* Frfld. and *U. quadrifasciata* (Meig.)(Diptera: Tephritidae) in Canada for the biological control of diffuse and spotted knapweed. *Zeitschrift für Angewandte Entomologie, 89*(1-5), 504-514.

Harris, P., & Shorthouse, J. D. (1996). Effectiveness of gall inducers in weed biological control. *The Canadian Entomologist, 128*(6), 1021-1055.

Hartley, S. E. (1998). The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall-former? *Oecologia, 113*(4), 492-501.

Hartley, S. E. (1999). Are gall insects large rhizobia? *Oikos*, 84(2), 333-342.

Hartnett, D. C., & Abrahamson, W. G. (1979). The effects of stem gall insects on life history patterns in *Solidago canadensis*. *Ecology, 60*(5), 910-917.

Hausenblas, H. A., Schoulda, J. A., & Smoliga, J. M. (2015). Resveratrol treatment as an adjunct to pharmacological management in type 2 diabetes mellitus—systematic review and meta-analysis. *Molecular nutrition & food research, 59*(1), 147-159.

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

Hejda, M., Pyšek, P., & Jarošík, V. (2009). Impact of invasive plants on the species richness, diversity and composition of invaded communities. *Journal of Ecology, 97*(3), 393-403.

Hellmann, C., Sutter, R., Rascher, K. G., Máguas, C., Correia, O., & Werner, C. (2011). Impact of an exotic N 2-fixing *Acacia* on composition and N status of a native Mediterranean community. *Acta Oecologica, 37*(1), 43-50.

Henrik, H., & Biedermann, P. H. (2012). Patterns of functional enzyme activity show that larvae are the key to successful fungus farming by ambrosia beetles. *Frontiers in Zoology, 9(1),* 1-11.

Hertog, M. G., Hollman, P. C., & Van de Putte, B. (1993). Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *Journal of Agricultural and Food Chemistry, 41*(8), 1242-1246.

Hoffmann, J., Impson, F., Moran, V., & Donnelly, D. (2002a). Biological control of invasive golden wattle trees (*Acacia pycnantha*) by a gall wasp, *Trichilogaster* sp. (Hymenoptera: Pteromalidae), in South Africa. *Biological Control, 25*(1), 64-73.

Hoffmann, J., Impson, F., Moran, V., & Donnelly, D. (2002b). Biological control of invasive golden wattle trees (*Acacia pycnantha*) by a gall wasp, *Trichilogaster* sp.(Hymenoptera: Pteromalidae), in South Africa. *Biological Control, 25*(1), 64-73.

Hori, K. (1974). Plant growth-promoting factor in the salivary gland of the bug, Lygus disponsi. *Journal of Insect Physiology, 20*(8), 1623-1627.

Horner, T. A. (2002). Field release of *Cecidochares* (*Procecidochares*) *connexa* Macquart (Diptera: Tephritidae), a non-indigenous, gall-making fly for control of Siam weed, *Chromolaena odorata* (L.) King and Robinson (Asteraceae) in Guam and the Northern Mariana Islands. Environmental Assessment, United State Department of Agriculture, DAPHIS, Riverdale, United State of America.

Hovick, S. M., & Carson, W. P. (2015). Tailoring biocontrol to maximize top-down effects: on the importance of underlying site fertility. *Ecological Applications, 25*(1), 125-139.

Howe, G. A., & Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review Plant Biology, 59*, 41-66. doi:10.1146/annurev.arplant.59.032607.092825

Huang, W.-y., Zhang, H.-c., Liu, W.-x., & Li, C.-y. (2012). Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *Journal of Zhejiang University Science B, 13*(2), 94-102.

Huang, W. Y., Zhang, H. C., Liu, W. X., & Li, C. Y. (2012). Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *Journal of Zhejiang University Science B, 13*(2), 94-102. doi:10.1631/jzus.B1100137

Humphries, S. E., Groves, R. H., & Mitchell, D. S. (1991). *Plant invasions of Australian ecosystems: a status review and management directions*: Australian National Parks and Wildlife Service. Australia.

Hutangura, P., Mathesius, U., Jones, M. G., & Rolfe, B. G. (1999). Auxin induction is a trigger for root gall formation caused by root-knot nematodes in white clover and is associated with the activation of the flavonoid pathway. *Functional Plant Biology, 26*(3), 221-231.

Impson, F., Kleinjan, C., Hoffmann, J., Post, J., & Wood, A. (2011). Biological control of Australian *Acacia* species and *Paraserianthes lophantha* (Willd.) Nielsen (Mimosaceae) in South Africa. *African Entomology, 19*(2), 186-208.

Impson, F., Purcell, M., & Gordon, A. (2012). Biological control of Australian plants: a South African and US perspective. *Biological Control of Weeds in Australia*, CSIRO Publishing, Melbourne, 1-17.

Impson, F. A., Post, J. A., & Hoffmann, J. H. (2013). Impact of the flower-galling midge, *Dasineura rubiformis* Kolesik, on the growth of its host plant, *Acacia mearnsii* De Wild, in South Africa. *South African journal of botany, 87*, 118-121.

Inbar, M., Wink, M., & Wool, D. (2004). The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on *Pistacia Molecular Phylogenetics and Evolution, 32*(2), 504-511.doi:10.1016/j.ympev.2004.01.006

Jankiewicz, L., Plich, H., & Antoszewski, R. (1970). Preliminary studies on the translocation of 14C--labelled assimilates and 32P03-towards the gall evoked by *Cynips* (*Diplolepis*) *quercusfolii* L. on oak leaves. *Marcellia*. *36*, 163-174.

Johansen, D. A. (1940). *Plant microtechnique*. McGraw-Hill Book Company, Inc: London. United Kingdom, 530.

Johnson, E. T., & Dowd, P. F. (2004). Differentially enhanced insect resistance, at a cost, in Arabidopsis thaliana constitutively expressing a transcription factor of defensive metabolites. *Journal of Agricultural and Food Chemistry, 52*(16), 5135-5138.

Karageorgou, P., & Manetas, Y. (2006). The importance of being red when young: anthocyanins and the protection of young leaves of Quercus coccifera from insect herbivory and excess light. *Tree Physiology, 26*(5), 613-621.

Kato, K., & Hijii, N. (1997a). Effects of gall formation by Dryocosmus kuriphilus Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. *Journal of Applied Entomology, 121*(1-5), 9-15.

Kato, K., & Hijii, N. (1997b). Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera, Cynipidae) on the growth of chestnut trees. *Journal of Applied Entomology, 121*(1-5), 9-15.

Kirst, G. O., & Rapp, H. (1974). Zur Physiologie der Galle von Mikiola fagi Htg. auf Blaettern von Fagus silvatica L.: 2. Transport 14C-markierter Assimilate aus dem befallenen Blatt und aus Nachbarblaettern in die galle. *Biochemie und Physiologie der Pflanzen, 165*(5), 445-455.

Kloft, W. (1951). Insekten steuern das Wachsen von Pflanzen. *Umschau, 51*, 40-42.

Klöppel, M., Smith, L., & Syrett, P. (2003). Predicting the impact of the biocontrol agent *Aulacidea subterminalis* (Cynipidae) on growth of *Hieracium pilosella*

(Asteraceae) under differing environmental conditions in New Zealand. *Biocontrol Science and Technology, 13*(2), 207-218.

Kluge, R., & Neser, S. (1991). Biological control of *Hakea sericea* (Proteaceae) in South Africa. *Agriculture, Ecosystems & Environment, 37*(1-3), 91-113.

Kluge, R. L. (1983). The *Hakea* fruit weevil, *Erytenna consputa Pascoe* (Coleoptera: Curculionidae), and the biological control of *Hakea sericea* Schrader in South Africa. Doctoral dissertation, RL Kluge). Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa.

La Salle, J. (2005). Biology of gall inducers and evolution of gall induction in Chalcidoidea (Hymenoptera: Eulophidae, Eurytomidae, Pteromalidae, Tanaostigmatidae, Torymidae). *Biology, Ecology and Evolution of Gall-inducing Arthropods.* Science Publishers, Inc., Enfield, United States of America. *Volume 1 and 2*, 507-537.

Lalonde, R., & Shorthouse, J. (1984). Developmental morphology of the gall of *Urophora cardui* (Diptera, Tephritidae) in the stems of *Canada thistle* (Cirsium arvense). *Canadian Journal of Botany, 62*(7), 1372-1384.

Larson, K. C., & Whitham, T. G. (1991). Manipulation of food resources by a gall-forming aphid: the physiology of sink-source interactions. *Oecologia, 88*(1), 15-21.

LaSalle, J. (1994). North American genera of Tetrastichinae (Hymenoptera: Eulophidae). *Journal of Natural History, 28*(1), 109-236.

László, Z., & Tóthmérész, B. (2006). Inquiline effects on a multilocular gall community. *Acta Zoologica Academiae Scientiarum Hungaricae, 52*(4), 373-383.

Lattanzio, V., Lattanzio, V. M., & Cardinali, A. (2006). Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. *Phytochemistry: Advances in Research, 661*(2), 23-67.

Le Maitre, D., Versfeld, D., & Chapman, R. (2000). Impact of invading alien plants on surface water resources in South Africa: A preliminary assessment. *Water Research Commission*. **26**(3), 397-408.

Leigh, J., & Briggs, J. D. (1992). Australia's Nationally Rare or Threatened Flora. Appendix 4.1 and 4.4. Threatened Australian plants: overview and case studies (ed. by J.H. Leigh and J.D. Briggs). Australian National Parks and Wildlife Service, Canberra, Australia.

Leopold, A. C. (1964). *Plant growth and development*: Mcgraw-Hill Book Company, New York, United States of America.

Levine, J. M., Vilà, M., Antonio, C. M. D., Dukes, J. S., Grigulis, K., & Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 270*(1517), 775-781. doi:10.1098/rspb.2003.2327

Li, H.-B., Wong, C.-C., Cheng, K.-W., & Chen, F. (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT-Food Science and Technology, 41*(3), 385-390.

Li, J., Li, C., & Smith, S. M. (2017). *Hormone Metabolism and Signaling in Plants*: Academic Press. Cambridge, Massachusetts, United States of America.

Liu, S., Norris, D. M., Hartwig, E. E., & Xu, M. (1992). Inducible phytoalexins in juvenile soybean genotypes predict soybean looper resistance in the fully developed plants. *Plant Physiology, 100*(3), 1479-1485.

Luke, H., Zed, S., & Craig, B. (2008). 'Integrated Vegetation Management Plan for the Bells Beach Recreation Reserve, Torquay, Victoria'. Ecology Partners report prepared for Surfcoast Shire. Ecology Partners Pty Ltd, Australia.

Mack, R. N. (1981). Invasion of Bromus tectorum L. into western North America: an ecological chronicle. *Agro-ecosystems, 7*(2), 145-165.

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

Maffei, M. E., Mithöfer, A., & Boland, W. (2007). Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry, 68*(22-24), 2946-2959. doi:10.1016/j.phytochem.2007.07.016

Mani, M. (1964). Ecology of plant galls. *The Hague, W. Junk, 640*.

Marchante, E., Kjøller, A., Struwe, S., & Freitas, H. (2008a). Invasive *Acacia longifolia* induce changes in the microbial catabolic diversity of sand dunes. *Soil Biology and Biochemistry, 40*(10), 2563-2568.

Marchante, E., Kjøller, A., Struwe, S., & Freitas, H. (2008b). Short-and long-term impacts of *Acacia longifolia* invasion on the belowground processes of a Mediterranean coastal dune ecosystem. *Applied Soil Ecology, 40*(2), 210-217.

Marchante, E., Kjøller, A., Struwe, S., & Freitas, H. (2009). Soil recovery after removal of the N 2-fixing invasive *Acacia longifolia*: consequences for ecosystem restoration. *Biological Invasions, 11*(4), 813-823.

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

Marchante, H., Freitas, H., & 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. doi:10.3732/ajb.1000091

Marchante, H., López-Núñez, F., Freitas, H., Hoffmann, J., Impson, F., & Marchante, E. (2017). First report of the establishment of the biocontrol agent *Trichilogaster acaciaelongifoliae* for control of invasive *Acacia longifolia* in Portugal. *EPPO Bulletin, 47*(2), 274-278.

Marchante, H., Marchante, E., & Freitas, H. (2003). Invasion of the Portuguese dune ecosystems by the exotic species *Acacia longifolia* (Andrews) Willd.: effects at the community level. *Plant Invasions: Ecological Threats and Management Solutions*, Backhuys Publishers, Leiden, The Netherlands, 75-85.

Marchante, H. S. D. C. (2011). Invasion of portuguese dunes by *Acacia longifolia*: present status and perspective for the future. University of Coimbra, Coimbra, Portugal. doi:http://hdl.handle.net/10316/18181

Marohasy, J. (1998). The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. *Biocontrol News and Information, 19*, 13-20.

Martel, J. (1995). Performance of *Eurosta solidaginis* (Diptera: Tephritidae) and *Epiblema scudderiana* (Lepidoptera: Tortricidae), two gall-formers of goldenrod, in roadside environments. *Environmental Entomology, 24*(3), 697-706.

Mason, T., & French, K. (2008). Impacts of a woody invader vary in different vegetation communities. *Diversity and Distributions, 14*(5), 829-838.

McCrea, K. D., Abrahamson, W. G., & Weis, A. E. (1985). Goldenrod ball gall effects on *Solidago altissima*: 14C translocation and growth. *Ecology, 66*(6), 1902-1907.

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

Mendel, Z., Protasov, A., Fisher, N., & La Salle, J. (2004). Taxonomy and biology of *Leptocybe invasa* gen. & sp. n.(Hymenoptera: Eulophidae), an invasive gall inducer on Eucalyptus. *Australian Journal of Entomology, 43*(2), 101-113.

Metcalf, R. L., & Kogan, M. (1987). Plant volatiles as insect attractants. *Critical Reviews in Plant Sciences, 5*(3), 251-301.

Meyer, A. (1987). Phenotypic plasticity and heterochrony in Cichlasoma managuense (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution, 41*(6), 1357-1369.

Meyer, J. (1987). *Plant galls and gall inducers*. Stuttgart, German Federal Republic: Gebrüder Borntraeger, Germany.

Meyer, J., & Maresquelle, H. J. (1983a). *Anatomie des galles*. Schweizerbart Science Publishers. Johannesstraße 3A, D-70176 Stuttgart, Germany.

Meyer, J., & Maresquelle, H. J. (1983b). Anatomie des galles. Schweizerbart science publishers. Gebrüder Borntraeger, Berlin, Germany.

Miguel, M. G. (2011). Anthocyanins: antioxidant and/or anti-inflammatory activities. *Journal of Applied Pharmaceutical Science, 1*(6), 7-15.

Miles, P. W. (1999). Aphid saliva. *Biological Reviews, 74*(1), 41-85.

Milkins, K. (2017). New 3D vision technique to revolutionise conservation efforts. Parks Victoria media centre. News and media releases. Parks Victoria, Australia. 21 March, 2017. https://www.parks.vic.gov.au/news/2019/11/13/02/47/new-3d-vision-technique-to-revolutionise-conservation-efforts.

Milton, S., & Hall, A. (1981). Reproductive biology of Australian acacias in the south-western Cape Province, South Africa. *Transactions of the Royal Society of South Africa, 44*(3), 465-487.

Moriya, S., Shiga, M., & Adachi, I. (2003). Classical biological control of the chestnut gall wasp in Japan. Paper presented at the Proceedings of the 1st international symposium on biological control of arthropods. January 14-18, 2002, Honolulu, Hawaii. USDA Forest Service, Washington, United States of America.

Morris, T. L., Esler, K. J., Barger, N. N., Jacobs, S. M., & Cramer, M. D. (2011). Ecophysiological traits associated with the competitive ability of invasive Australian acacias. *Diversity and Distributions, 17*(5), 898-910.

Murakami, Y., Ao, H.-B., & CHANG, C.-H. (1980). Natural Enemies of the Chestnut Gall Wasp in Hopei Province, China: Hymenoptera: Chalcidoidea. *Applied Entomology and Zoology, 15*(2), 184-186.

Muyt, A. (2001). Bush invaders of South-East Australia: a guide to the identification and control of environmental weeds found in South-East Australia: RG and FJ Richardson. Meredith, Victoria, Australia.

Myer, V. G. (1987). Charlotte Brontë: truculent spirit: Barnes & Noble Imports, United States of America.

Myers, J. H., Risley, C., & Eng, R. (1988). The ability of plants to compensate for insect attack: why biological control of weeds with insects is so difficult. Paper presented at the Proceedings of VII International Symposium on the Biological Control of Weed. 16-11 March 1988. Rome, Italy.

Navie, S., Priest, T., McFadyen, R., & Adkins, S. (1998). Efficacy of the Stem-Galling Moth *Epiblema strenuana* Walk.(Lepidoptera: Tortricidae) as a Biological Control Agent for Ragweed *Parthenium* (*Parthenium hysterophorus* L.). *Biological Control, 13*(1), 1-8.

Neser, S. (1968). Studies on Some Potentially Useful Insect Enemies of Needle-bushes (*Hakea* Spp.-Proteacae): PhD Thesis. Australian National University. Canberra, Australia, 194.

Neser, S. (1984). A most promising bud-galling wasp, *Trichilogaster acaciaelongifoliae* (Pteromalidae), established against *Acacia longifolia* in South Africa. Paper presented at the Proceedings. VI International Symposium on the Biological Control of Weed. 19-25 August 1984, University of British Columbia, Vancouver, B.C., Canada.

Noble, N. (1940). *Trichilogaster acaciaelongifoliae* (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,* 13-38.

Nordlander, G. (1973). Parasitsteklar i galler av *Diplolepis rosae* (L.) och *D. mayri* Schlec htd. (Hymenptera, Cynipidae)(Hymenoptera, Ichneumonoidea, Chalcidoidea, Cynipoidea). *Entomologisk Tidskrift, 94*(3), 148-176.

Noyes, J. S. (2003). Universal Chalcidoidea Database. World Wide Web electronic publication. Food and Agriculture Organization of the United Nations, Rome, Italy. available at: *http://www. nhm. ac. uk/research-curation/projects/chalcidoids/*.

Old, K. M. (2002). *Acacia* spp. *Food and Agriculture Organization of the United Nations/ International Plant Genetic Resources Institute.* Technical Guidelines for the Safe Movement of Germplasm: Bioversity International. No. 20. Rome, Italy.

Onagbola, E. O., & Fadamiro, H. Y. (2008). Scanning electron microscopy studies of antennal sensilla of *Pteromalus cerealellae* (Hymenoptera: Pteromalidae). *Micron, 39*(5), 526-535. doi:http://dx.doi.org/10.1016/j.micron.2007.08.001

Orchard, A. E. (2001). Flora of Australia (Vol. 11): CSIRO Publishing. Melbourne, Australia.

Parks Victoria (1998). Grampians National Park: Parks Victoria. PO Box 18 HALLS GAP, Victoria, Australia.

Parks Victoria. (2003). Management plan, Grampians national park. Parks Victoria Information Centre, Level 10, 535 Bourke Street. Melbourne-3000, Victoria, Australia.

Parks Victoria. (2006). Australia's national heritage, Grampians National Park (Gariwerd). Parks Victoria Information Centre, Level 10, 535 Bourke Street. Melbourne-3000, Victoria, Australia.

Pejchar, L., & Mooney, H. A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution, 24*(9), 497-504. doi:10.1016/j.tree.2009.03.016

PennState, E. (2017). Introduction to Weeds: What are Weeds and Why do we Care? The Pennsylvania State University, 323 Agricultural Administration Building University Park, PA 16802, United States of America.

Penzes, Z., Tang, C.-T., Stone, G. N., Nicholls, J. A., Schweger, S., Bozso, M., & Melika, G. (2018). Current status of the oak gallwasp (Hymenoptera: Cynipidae: Cynipini) fauna of the Eastern Palaearctic and Oriental Regions. *Zootaxa, 4433*(2), 245-289.

Pickering, C., & Mount, A. (2010). Do tourists disperse weed seed? A global review of unintentional human-mediated terrestrial seed dispersal on clothing, vehicles and horses. *Journal of Sustainable Tourism, 18*(2), 239-256.

Pieterse, P., & Cairns, A. (1988). The population Dynamics of the Weed *Acacia longifolia* (Fabacae) in the Absence and Presence of Fire. *South African Forestry Journal, 145*(1), 25-27.

Pimentel, D. (2001). Agricultural invasions. In Levin, S.A., editor, Encyclopaedia of biodiversity, San Diego: Academic Press. Cambridge, Massachusetts, United States of America. *1*, 71-85.

Pimentel, D., Lach, L., Zuniga, R., & Morrison, D. (2000). Environmental and economic costs of nonindigenous species in the United States. *BioScience, 50*(1), 53-65.

Post, J., Kleinjan, C., Hoffmann, J., & Impson, F. (2010). Biological control of *Acacia cyclops* in South Africa: the fundamental and realized host range of *Dasineura dielsi* (Diptera: Cecidomyiidae). *Biological Control, 53*(1), 68-75.

Price, P. W., Bouton, C. E., Gross, P., McPheron, B. A., Thompson, J. N., & Weis, A. E. (1980). Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual review of Ecology and Systematics, 11*(1), 41-65.

Price, P. W., Fernandes, G. W., & Waring, G. L. (1987). Adaptive nature of insect galls. *Environmental Entomology, 16*(1), 15-24.

Protasov, A., Doĝanlar, M., La Salle, J., & Mendel, Z. (2008). Occurrence of two localMegastigmus species parasitic on the eucalyptus gall waspLeptocybe invasa in Israel and Turkey. *Phytoparasitica, 36*(5), 449-459.

Raman, A. (2003). Cecidogenetic behavior of some gall-inducing thrips, psyllids, coccids, and gall midges, and morphogenesis of their galls. *Oriental Insects, 37*(1), 359-413.

Raman, A., & Abrahamson, W. G. (1995). Morphometric relationships and energy allocation in the apical rosette galls of *Solidago altissima* (Asteraceae) induced by *Rhopalomyia solidaginis* (Diptera: Cecidomyiidae). *Environmental Entomology, 24*(3), 635-639.

Raman, A., & Dhileepan, K. (1999). Qualitative evaluation of damage by *Epiblema strenuana* (Lepidoptera: Tortricidae) to the weed *Parthenium hysterophorus* (Asteraceae). *Annals of the Entomological Society of America, 92*(5), 717-723.

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

Ravn, H., Andary, C., Kovács, G., & Mølgaard, P. (1989). Caffeic acid esters as in vitro inhibitors of plant pathogenic bacteria and fungi. *Biochemical systematics and Ecology, 17*(3), 175-184.

Redfern, M., & Shirley, P. (2011). *British Plant Galls*. Field Studies Council Publications. Preston Montford, Shrewsbury, Shropshire, SY4 1HW. United Kingdom.

Rey, L. (1992). Developmental morphology of two types of hymenopterous galls. *Biology of insect-induced galls* (eds J.D. Shorthouse, & O. Rohfritsch). Oxford University Press, Oxford, New York, United States of America, 87-101.

Richardson, D. M., Bond, W. J., Dean, W. R. J., Higgins, S. I., Midgley, G., Milton, S. J., Powrie, L. W., Rutherford, M. C., Samways, M. & Schulze, R. (2000). Invasive alien species and global change: a South African perspective. Invasive species in a changing world. Island Press, Washington, DC, United States of America, 303-349.

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

Richardson, D. M., & Kluge, R. L. (2008). Seed banks of invasive Australian *Acacia* species in South Africa: role in invasiveness and options for management. *Perspectives in Plant Ecology, Evolution and Systematics, 10*(3), 161-177.

Richardson, D. M., & Rejmánek, M. (2011). Trees and shrubs as invasive alien species–a global review. *Diversity and Distributions, 17*(5), 788-809.

Roberts, W. G., & Gordon, M. H. (2003). Determination of the total antioxidant activity of fruits and vegetables by a liposome assay. *Journal of Agricultural and Food Chemistry, 51*(5), 1486-1493. doi:10.1021/jf025983t

Rohfritsch, O. (1981). A "defense" mechanism of *Picea excelsa* L. against the gall former *Chermes abietis* L. (Homoptera, Adelgidae). *Zeitschrift für Angewandte Entomologie, 92*(1-5), 18-26.

Rohfritsch, O. (1992). Patterns in gall development. *Biology of insect-induced galls*, Oxford University Press, Oxford, United Kingdom, 60-86.

Ronquist, F., & Liljeblad, J. (2001). Evolution of the gall wasp-host plant association. *Evolution, 55*(12), 2503-2522.

Roques, A., & Skrzypczyńska, M. (2003). Seed-infesting chalcids of the genus *Megastigmus* Dalman, 1820 (Hymenoptera: Torymidae) native and introduced to the West Palearctic region: taxonomy, host specificity and distribution. *Journal of Natural History, 37*(2), 127. doi:10.1111/j.1440-1827.1997.tb04530.x

Saeung, A., Hempolchom, C., Yasanga, T., Otsuka, Y., Thongsahuan, S., Srisuka, W., Chaithong, U., Taai, K., Somboon, P., & Choochote, W. (2014). Scanning electron microscopy of *Anopheles hyrcanus* group (Diptera: Culicidae) eggs in Thailand and an ultrastructural key for species identification. *Parasitology Research, 113*(3), 973-981. doi:10.1007/s00436-013-3729-0

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

Seeram, N. P. (2008). Berry fruits: compositional elements, biochemical activities, and the impact of their intake on human health, performance, and disease. *Journal of Agricultural and Food Chemistry, 56*(3), 627-629.

Sellappan, S., & Akoh, C. C. (2002). Flavonoids and antioxidant capacity of Georgia-grown *Vidalia* onions. *Journal of Agricultural and Food Chemistry, 50*(19), 5338-5342.

Sellappan, S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry, 50*(8), 2432-2438. doi:10.1021/jf011097r

SGA (Sustainable Gardening Australia). (2014). Environmental Weeds: Native Invaders and Eager Exotics. 6 Manningham Road West, Bulleen, Victoria, 3105 available at: http://www.sgaonline.org.au/environmental-weeds-native-invaders-and-eager-exotics/

Shaw, R. H., Bryner, S., & Tanner, R. (2009). The life history and host range of the Japanese knotweed psyllid, *Aphalara itadori* Shinji: potentially the first classical biological weed control agent for the European Union. *Biological Control, 49*(2), 105-113.

Shorthouse, J. (1982). Resource exploitation by gall wasps of the genus *Diplolepis*. Paper presented at the Proceedings of the 5th International Symposium of Insect-Plant Relationships, Wageningen. Pudoc, Wageningen, the Netherlands. 1-4 March 1982.

Shorthouse, J. (1993). Adaptations of gall wasps of the genus *Diplolepis* (Hymenoptera: Cynipidae) and the role of gall anatomy in cynipid systematics. *The Memoirs of the Entomological Society of Canada, 125*(S165), 139-163.

Shorthouse, J., West, A., Landry, R., & Thibodeau, P. (1986). Structural damage by female *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) as a factor in gall induction on lowbush blueberry. *The Canadian Entomologist, 118*(3), 249-254.

Shorthouse, J. D. (1998). Role of *Periclistus* (Hymenoptera: Cynipidae) inquilines in in leaf galls of *Diplolepis* (Hymenoptera: Cynipidae) on wild roses in Canada. United States Department of Agriculture Forest Service General Technical Report Nc, United States of America. 61-81.

Shorthouse, J. D., & Gassmann, A. (1994). Gall induction by *Pegomya curticornis* (Stein) (Diptera: Anthomyiidae) within the roots of spurges *Euphorbia virgata* Waldst. and Kit. and *E. esula* L.(Euphorbiaceae). *The Canadian Entomologist, 126*(2), 193-197.

Shorthouse, J. D., & Rohfritsch, O. (1992). *Biology of insect-induced galls*: Oxford University Press. Oxford, United Kingdom.

Shorthouse, J. D., Wool, D., & Raman, A. (2005). Gall-inducing insects–Nature's most sophisticated herbivores. *Basic and Applied Ecology, 6*(5), 407-411.

Sinden, J., Jones, R., Hester, S., Odom, D., Kalisch, C., James, R., Cacho, O., & Griffith, G. (2005). The economic impact of weeds in Australia. *Plant Protection Quarterly, 20*(1), 25.

Sliva, M. D., & Shorthouse, J. D. (2006). Comparison of the development of stem galls induced by *Aulacidea hieracii* (Hymenoptera: Cynipidae) on hawkweed and by *Diplolepis spinosa* (Hymenoptera: Cynipidae) on rose. *Botany, 84*(7), 1052-1074.

Soulé, M. E. (1991). Conservation: tactics for a constant crisis. *Science (Washington), 253*(5021), 744-750.

Speisky, H., Peña, A., Gómez, M., Fredes, C., Hurtado, M., Gotteland, M., & Brunser, O. (2005). Antioxidants in Chilean berries. Paper presented at the IX International Rubus and Ribes Symposium 777. 1 December 2005. 485-492.

Stellatelli, O. A., Block, C., Vega, L. E., & Cruz, F. B. (2015). Responses of two sympatric sand lizards to exotic forestations in the coastal dunes of Argentina: some implications for conservation. *Wildlife Research, 41*(6), 480-489.

Stiling, P., & Moon, D. C. (2005). Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. *Oecologia, 142*(3), 413-420.

Stille, B. (1984). The effect of hosptlant and parasitoids on the reproductive success of the parthenogenetic gall wasp *Diplolepis rosae* (Hymenoptera, Cynipidae). *Oecologia, 63*(3), 364-369.

Stone, G. N., & Schönrogge, K. (2003). The adaptive significance of insect gall morphology. *Trends in Ecology & Evolution, 18*(10), 512-522.

Stone, G. N., Schönrogge, K., Atkinson, R. J., Bellido, D., & Pujade-Villar, J. (2002). The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology, 47*(1), 633-668.

Su, M. S., & Chien, P. J. (2007). Antioxidant activity, anthocyanins, and phenolics of rabbiteye blueberry (*Vaccinium ashei*) fluid products as affected by fermentation. *Food Chemistry, 104*(1), 182-187.

Swarbrick, J. T., & Skarratt, D. B. (1994). 'Bushland 2 Database of environmental weeds in Australia'. University of Queensland, Gatton College, Gatton, Queensland, Australia.

Takei, M., Yoshida, S., Kawai, T., Hasegawa, M., & Suzuki, Y. (2015). Adaptive significance of gall formation for a gall-inducing aphids on Japanese elm trees. *Journal of Insect Physiology, 72*, 43-51.

Thomson, L. (2016). Loss of biodiversity as a result of the spread of Coast Wattle (*Acacia longifolia* subsp. *sophorae*) and Sallow Wattle (*Acacia longifolia* subsp. *longifolia*) into areas outside its natural range. Potentially Threatening Process. Flora and

Fauna Guarantee Act 1988, Scientific Advisory Committee. Nomination no. 877. 2/8 Nicholson St. (PO Box 500), East Melbourne 3002, Australia.

Thuiller, W., Richardson, D. M., & Midgley, G. F. (2008). Will climate change promote alien plant invasions? In *Biological Invasions* (pp. 197-211): Springer, Berlin, Heidelberg, Germany.

Tipping, P. W., Martin, M. R., & Gettys, L. A. (2016). A gall-forming biological control agent suppresses vegetative growth of an invasive tree. *Biocontrol Science and Technology, 26*(11), 1586-1589.

Tjia, B., & Houston, D. (1975). Phenolic constituents of Norway spruce resistant or susceptible to the eastern spruce gall aphid. *Forest Science, 21*(2), 180-184.

Tooker, J. F., & Helms, A. M. (2014). Phytohormone dynamics associated with gall insects, and their potential role in the evolution of the gall-inducing habit. *Journal of Chemical Ecology, 40*(7), 742-753.

Tunison, T. (1991). Element stewardship abstract for *Psidium cattleianum*. The Nature Conservancy. Arlington, Virginia, United States of America.

Turner, P. J., & Downey, P. O. (2010). Ensuring Invasive Alien Plant Management Delivers Biodiversity Conservation: Insights from an Assessment of*'Lantana Camara*'in Australia. *Plant Protection Quarterly, 25*(3), 102.

Vencill, W. K. (2002). *Herbicide Handbook*. Weed Science Society of America. Lawrence, United States of America.

Vilà, M., Basnou, C., Pyšek, P., Josefsson, M., Genovesi, P., Gollasch, S., Nentwig, W., Olenin, S., Roques, A., & Roy, D. (2010). How well do we understand the impacts of alien species on ecosystem services? A pan-European, cross-taxa assessment. *Frontiers in Ecology and the Environment, 8*(3), 135-144.

Vilà, M., Corbin, J. D., Dukes, J. S., Pino, J., & Smith, S. D. (2007). Linking plant invasions to global environmental change. In *Terrestrial Ecosystems in a Changing World* (pp. 93-102): Springer, Berlin, Heidelberg, Germany.

Vinson, J. A., Su, X., Zubik, L., & Bose, P. (2001). Phenol antioxidant quantity and quality in foods: fruits. *Journal of Agricultural and Food Chemistry, 49*(11), 5315-5321. doi:10.1021/jf0009293

Vitousek, P. M. (1994). Beyond global warming: ecology and global change. *Ecology, 75*(7), 1861-1876.

Vitousek, P. M., Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist, 84*(5), 468.

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

Waring, G. L., & Price, P. W. (1989). Parasitoid pressure and the radiation of a gallforming group (Cecidomyiidae: *Asphondylia* spp.) on creosote bush (*Larrea tridentata*). *Oecologia, 79*(3), 293-299.

Weber, E. (2017). Invasive plant species of the world: a reference guide to environmental weeds: Cabi. Wallingford, Oxfordshire, United Kingdom.

Weis, A. E., & Abrahamson, W. G. (1985). Potential selective pressures by parasitoids on a plant-herbivore interaction. *Ecology, 66*(4), 1261-1269.

Weis, A. E., Walton, R., & Crego, C. L. (1988). Reactive plant tissue sites and the population biology of gall makers. *Annual Review of Entomology, 33*(1), 467-486.

Werner, C., Zumkier, U., Beyschlag, W., & Máguas, C. (2010). High competitiveness of a resource demanding invasive *acacia* under low resource supply. *Plant Ecology, 206*(1), 83-96.

West, A., & Shorthouse, J. (1989). Initiation and development of the stem gall induced by *Hemadas nubilipennis* (Hymenoptera: Pteromalidae) on lowbush blueberry, *Vaccinium angustifolium* (Ericaceae). *Canadian Journal of Botany, 67*(7), 2187-2198.

West, R., & Shorthouse, J. (1982). Morphology of the balsam fir needle gall induced by the midge *Paradiplosis tumifex* (Diptera: Cecidomyiidae). *Canadian Journal of Botany, 60*(2), 131-140.

Whitham, T. (1992). Ecology of Pemphigus gall aphids. *Biology of lnsect-Induced Galls*, (J. D. Shorthouse & O. Rohfritsch, eds.), pp. 225-237. Oxford Univ. Press, New York. United States of America.

Williams, J. A., & West, C. J. (2000). Environmental weeds in Australia and New Zealand: issues and approaches to management. *Austral Ecology, 25*(5), 425-444.

Williams, M. (1994). Plant Galls: Organisms. *Interactions, Populations*. Oxford University Press, Oxford, United Kingdom.

Wilson, J. R., Gairifo, C., Gibson, M. R., Arianoutsou, M., Bakar, B. B., Baret, S., Celesti-Grapow, L., DiTomaso, J. M., Dufour-Dror, J. M., & Kueffer, C. (2011). Risk assessment, eradication, and biological control: global efforts to limit Australian *acacia* invasions. *Diversity and Distributions, 17*(5), 1030-1046.

Wink, M. (2017). The role of quinolizidine alkaloids in plant-insect interactions. In *Insect-Plant Interactions (1992)* (pp. 139-174). CRC Press, Boca Raton, Florida, United States of America.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of Agricultural and Food Chemistry, 51*(3), 609-614. doi:10.1021/jf020782a

Wool, D., Aloni, R., Ben-Zvi, O., & Wollberg, M. (1999). A galling aphid furnishes its home with a built-in pipeline to the host food supply. *Entomologia Experimentalis et Applicata, 91*(1), 183-186.

Yelenik, S., Stock, W., & Richardson, D. (2004). Ecosystem level impacts of invasive *Acacia saligna* in the South African fynbos. *Restoration Ecology, 12*(1), 44-51.

Yukawa, J., & Rohfritsch, O. (2005). Biology and ecology of gall-inducing Cecidomyiidae (Diptera), Biology, ecology, and evolution of gall-inducing arthropods. Science Publishing, New York, United States of America. 273-304.

Zhang, Q., Zhai, J., Shao, L., Lin, W., & Peng, C. (2019). Accumulation of Anthocyanins: An Adaptation Strategy of *Mikania micrantha* to Low Temperature in Winter. *Frontiers in Plant Science, 10*(1049). doi:10.3389/fpls.2019.01049

Zheng, L. X., Wu, W. J., Liang, G. W., & Fu, Y. G. (2014). Nymphal antennae and antennal sensilla in *Aleurodicus dispersus* (Hemiptera: Aleyrodidae). *Bulletin of Entomological Research, 104*(5), 622-630. doi:10.1017/S000748531400039X

Zheng, Y., Wang, C. Y., Wang, S. Y., & Zheng, W. (2003). Effect of high-oxygen atmospheres on blueberry phenolics, anthocyanins, and antioxidant capacity. *Journal of Agricultural and Food Chemistry, 51*(24), 7162-7169. doi:10.1021/jf030440k

Zimdahl, R. L. (2013). *Fundamentals of weed science*: Academic press, Cambridge, Massachusetts, United States of America.

Zwölfer, H., & Harris, P. (1971). Host specificity determination of insects for biological control of weeds. *Annual Review of Entomology, 16*(1), 159-178.