Seed-reducing Cecidomyiidae as potential biological control agents for invasive Australian wattles in South Africa, particularly *Acacia mearnsii* and *A. cyclops*

by

Robin John Adair

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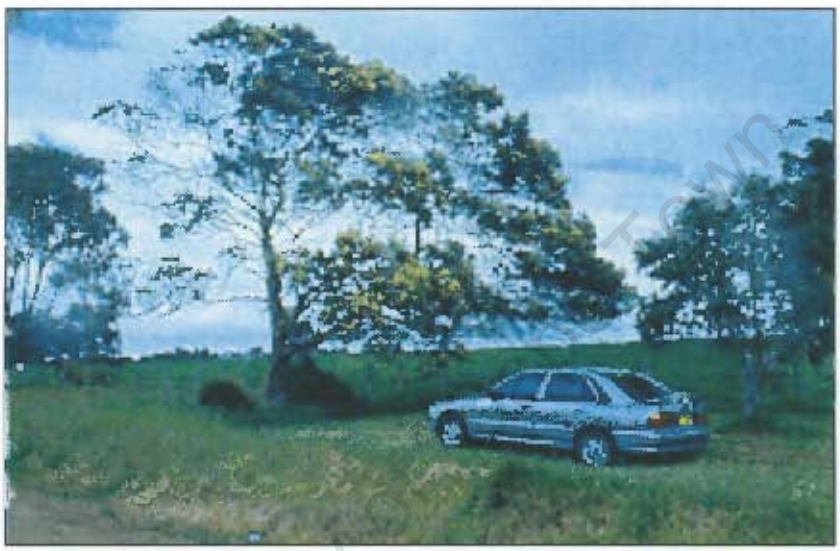


Frontispiece – 'Tree of Revelation'. One of a trio of *A. mearnsii* on a roadside at Albany, Western Australia. On the 3 November 1998 at 17:32 masses of *Dasineura* sp. (Tiny Floret Galler) were seen resting on flowers amongst branches with huge numbers of galls from the previous season. It was then that the taxonomic status of this midge was recognized, its dispersal from eastern Australia discovered and the potential for biological control of *A. mearnsii* in South Africa foreseen.

PREFACE

The collection of gall-forming Cecidomyiidae specimens and their parasitoids from Australia and South Africa during this project produced a large and valuable assemblage of material, most of which will be or has been lodged with the National Insect Collection (Pretoria) (parasitoids) or the South Australian Museum Adelaide (cecidomyiids).

Records of all insect and host plant accessions are recorded in a Microsoft Access database to facilitate data recovery and report development. Each accession is provided with a four-digit collection number between 2570 and 3305. These collection numbers are often used throughout this thesis and provide a reference point for access to additional collection information such as site locality, date of collection, host plant records, host phenology, type of specimens, and field notes. This information may be important to future investigations by entomologists interested in understanding the biology or ecology of cecidomyiids in Australia or South Africa. The provision of collection numbers has allowed brevity in the provision of descriptive information with some collections referred to in this thesis. A copy of the database 'Seed-reducing Cecidomyiidae (Diptera) of Australian and African *Acacia*' is included with this thesis. While standard botanical and zoological terminology has been adhered to, several terms require clarification due the specialized nature of this research.

The flowers of acacias consist of small florets clustered on a common receptacle and this unit is referred to as a flower-head. Florets are mostly bisexual, but a small proportion of unisexual florets may also occur. In many species, pedunculate flower-heads are borne on a common rachis (stalk), which may be unbranched, forming a racemose inflorescence or a branched rachis, forming a paniculate inflorescence. In others, including all African *Acacia* species, pedunculate flower-heads may occur singly or in clusters arising from a node. When an inflorescence completes flowering and becomes a fruit or gall bearing structure it is referred to as an infructescence. Where *Acacia* florets are utilized by cecidomyiids and produce abnormal, enlarged ovaries that are the developmental equivalent of fruit, these structures are called 'floret galls'. All Australian *Dasineura* seen in this study induce floret galls on host *Acacia*. Where one or more floret galls occur on a common receptacle this is referred to as a galled flower-head or abbreviated to a 'gall'. Other cecidomyiid genera can also produce floret galls, but this terminology is reserved strictly for those species that form galls on ovaries of the host plant. Galls, however, are used as a general term for any abnormal host organ that serves for the development of immature Cecidomyiidae.

In this study, the taxonomy of Australian acacias follows Orchard & Wilson (2001) and African acacias follows Smit (1999).

CHAPTER 1

An introduction to gall-forming Cecidomyiidae as seed-reducing biological control agents: issues associated with control of *Acacia mearnsii* and *A. cyclops* in South Africa

Australian acacias in South Africa

Thirteen Australian *Acacia* species (*A. baileyana, A. cyclops, A. dealbata, A. decurrens, A. elata, A. implexa, A. longifolia, A. mearnsii, A. melanoxylon, A. paradoxa, A. podalyriifolia, A. pycnantha, A. saligna*) are invasive in South Africa (Henderson 2001) and cause harm to natural and agricultural ecosystems. Most have been utilized in South Africa since the 1820s for stabilization of drift sands, garden ornamentals, timber and pulp production, and tannin extraction (Shaughnessy 1980). Naturalization has been widespread and most now form a conspicuous part of the South African landscape. *Acacia mearnsii* (black wattle), *A. melanoxylon* (black wood) and to a lesser extent *A. decurrens* (green wattle) form the basis of the present-day wattle industry in South Africa. However, all three species are invasive weeds in non-plantation areas and are responsible for reduced water production, loss of biodiversity, altered ecosystem functions and reduced agricultural productivity (Versfeld & van Wilgen 1986, Versfeld *et al.* 1998). *Acacia mearnsii* contributes most to the economy of South Africa as a commercial forest crop, but also ranks among the most problematic weeds of national significance. Similarly, in coastal to near-coastal habitats of southern South Africa, *A. cyclops* is an aggressive invader and displaces indigenous vegetation causing loss of biodiversity, increased fire intensity and frequency, and increased risk of dune erosion.

Acacia mearnsii is indigenous to southeastern Australia where it is a common component of *Eucalyptus* forests and woodlands (Costermans 1981) and a pioneer of disturbed ground. In South Africa, *A. mearnsii* was utilized initially as an ornamental in the Cape (Shaughnessy 1980), but in 1864 was first exploited for tannin production in the summer-rainfall areas of KwaZulu-Natal (Sherry 1971). *Acacia mearnsii* bark is a rich source of water-soluble tannins (31-51% dry weight of bark), primarily 3,7,3',4,'5'-pentahydroxy-2-phenyl chrom, which is used for tanning leather and the manufacture of water resistant resins and adhesives for reconstituted wood products (New 1984, Searle 2000). Black-wattle wood is also an important export product of South Africa with the majority being processed as pulpwood for the production of paper and paperboard products. In 2001, South Africa exported 1.2 million tonnes of black-wattle wood product worth around R360 million (US$31.5 million) (SAWGU pers. comm. 2002) from 130,000 ha of managed plantations centred in the provinces of Mpumalanga and KwaZulu-Natal in northeast South Africa, and

as by-product from infestations removed during control programmes. In 1998, the industry directly employed between 10,800 and 13,000 people, mostly unskilled labourers (de Wit *et al.* 2000). In addition to this, black wattle is a source of firewood utilized for cooking and heating in lower-income rural communities, where it is also used for informal housing and building construction.

Ecology and impact of A. mearnsii *and* A. cyclops

Acacia mearnsii is naturalized over 2.5 million hectares in South Africa (Versfeld *et al.* 1998), mostly in the southern and eastern sectors of the country where mean annual rainfall exceeds 500 mm, and is rated as an acute environmental threat (Macdonald and Jarman 1984, Versfeld *et al.* 1998), particularly in riparian habitats where infestations of the *A. mearnsii* reduced surface stream flow worth about R16,285 million (US$1425 million) (Versfeld *et al.* 1998, de Wit *et al.* 2001). Loss of biodiversity, increased soil erosion, increased fire damage and reduced grazing potential are additional negative environmental impacts, but are difficult to quantify economically (de Wit *et al.* 2001).

Acacia mearnsii invades grassland, fynbos, savanna and forest biomes in South Africa (Versfeld *et al.* 1998, Macdonald 1991) and is a threat to many of the biodiversity 'hot spots' of South Africa (Cowling & Hilton-Taylor 1994, de Wit *et al.* 2000). The potential for species reduction and loss is therefore substantial. Key ecological traits that contribute to the success of *A. mearnsii* in South Africa are high seed production, formation of large soil-stored banks of long-lived seeds that are triggered to germinate *en masse* by fire (Hendry & van Staden 1982, Pieterse & Boucher 1997b), strong competitive ability (Milton 1980, Rutherford *et al.* 1986) and the development of a large, structurally-dominating crown.

Biological control of A. mearnsii *and* A. cyclops

Biological control of *A. mearnsii* in South Africa commenced in 1973 with explorations in Australia that concentrated on finding host-specific seed-reducing agents (Neser & Annecke 1973). Organisms that are capable of causing damage to vegetative parts of *A. mearnsii* cannot be considered as biological control agents because they would reduce yields in wattle plantations and thus cause a conflict of interest with the wattle industry of southern Africa. Concerns within the wattle industry on the impact of seed-feeding insects on seed orchard production impeded the biological control programme on *A. mearnsii* for more than a decade, until it was demonstrated that insecticides could be used to protect seed orchards if the need arose (Donnelly *et al.* 1992). This cleared the way for the release of a seed-feeding curculionid *Melanterius maculatus* Lea in 1993 as a biological control agent for *A. mearnsii* (Dennill *et al.* 1999). *Melanterius maculatus* has become abundant in the vicinity of the original release sites in the Western Cape, and although seed damage levels

can be high, natural dispersal has been slow (Impson & Moran 2003). Similarly, the related beetle, *M. servulus* Pascoe, released in 1991 as a seed-feeding biological control agent for *A. cyclops* has achieved high levels of seed-destruction on this host in some areas of the southwestern Cape (Dennill *et al.* 1999, Impson *et al.* 2000).

However, *A. mearnsii* and *A. cyclops* are difficult targets for biological control. The trees inhabit a broad eco-climatic range in South Africa, have a high fecundity, sometimes aseasonal flowering and fruiting with high intra- and inter-population variation, and an early sexual maturation period. These attributes make it unlikely that any single seed-reducing biological control agent will achieve satisfactory control. Although *Melanterius* is causing increasing levels of seed damage, sufficient seed reduction across the full range of *A. mearnsii* and *A. cyclops* in South Africa will probably only be achieved by the additive effect of compatible seed-reducing insects or pathogens. The invasive South American tree, *Sesbania punicea* (Cav.) Benth. has been successfully suppressed in South Africa by the combined effects of three classical biological control agents, but not by any of these agents alone (Hoffmann & Moran 1998, 1999).

Near-complete seed destruction may be required for the suppression of an invasive African *Acacia* in Australia (Kriticos *et al.* 1999), highlighting difficulties in using seed-feeding agents alone as biological control agents for perennial plants. Seed-reducing insects have been regarded with scepticism as effective biological control agents (Huffaker 1964, Harris 1973, Goeden 1983), but increased predispersal seed destruction may reduce rate of spread thereby contributing to weed control efforts (Janzen 1970, Harper 1977, Macdonald & Jarman 1984, Harley 1985, Neser & Kluge 1986, Moody & Mack 1988). In the case of *A. mearnsii* in South Africa, biological control of seeds integrated with manual control methods that are already implemented on a broad-scale are anticipated to reduce the impact of infestations. Options involving biological damage to vegetative tissue similar to that achieved for the non-commercial species *A. longifolia* and *A. saligna* (Dennill 1988, Morris 1999) are not acceptable while a viable forestry industry based on *A. mearnsii* remains in South Africa.

Gall-forming organisms from acacias

Australia has a rich Cecidomyiidae fauna (Froggatt 1907, Gagné 1986), but only two species are described from *Acacia*, *Dasineura dielsi* (Rübsaamen 1916) and *D. acaciaelongifoliae* (Gagné & Marohasy 1993) from *A. cyclops* and *A. longifolia*, respectively. These and other Cecidomyiidae present on Australian *Acacia* were recognized as potential natural enemies of invasive Australian *Acacia* in South Africa (Neser 1984), but difficulties with identification and doubts regarding their potential impact delayed further evaluation. A preliminary census, based on gall morphology and biology, revealed at least 17 cecidomyiid

species from the reproductive organs of *Acacia* from southern Australia, including four from *A. mearnsii* and two from *A. cyclops*, hosts that are now subject to active biological control efforts utilising gall-forming Cecidomyiidae (Adair *et al.* 2000, Adair 2001, Adair 2002a).

Gall-forming biological control agents have had considerable success in the suppression of invasive weeds. In South Africa, the pteromalid wasps, *Trichilogaster acaciaelongifoliae* Froggatt and *Trichilogaster* spp., have had spectacular effects on *A. longifolia* (Dennill 1988) and *A. pycnantha* (Hoffmann *et al.* 2002) respectively, both highly invasive trees. Gall-formation reduces both vegetative growth and reproductive capacity of the host plants when resources committed to gall production are far in excess to those normally allocated to the production of buds, flowers and fruits, a process termed 'forced commitment' (Dennill 1988). A similar process may occur with the gall-forming rust fungus *Uromycladium tepperianum* (Sacc.) McAlp. that significantly reduces the vigour of *A. saligna* in South Africa.

Cecidomyiidae gall-formers tend to be susceptible to attack by parasitoids with parasitoid-inquiline induced mortality levels of 50% or more (Hawkins 1988), which limits the abundance of the host flies (Briggs & Latto 1996). Attack by parasitoids has compromised the effectiveness of cecidomyiids released for the biological of weeds in several countries (Goeden & Louda 1976, McFadyen 1985, Wehling & Piper 1988, Carlson & Mundal 1990, Harris & Shorthouse 1996). Despite their apparent vulnerability to parasitoids, the Cecidomyiidae continue to be used for biological control and are recent candidates for use against the herbaceous composite *Tripleurospermum perforatum* (Mérat) Laínz in Canada (Shuhravá & Hinz 2000) and *Euphorbia esula* L. in North America (Sobhian *et al.* 2000).

Significantly, *Dasineura* sp. (Tiny Floret Galler), an ovary-deforming gall midge of *A. mearnsii*, occurs in outbreak populations that have been sustained over many seasons and reduce fruit formation of *A. mearnsii* in Western Australia, where the midge has extended its range along with its host plant into an apparently low-parasitoid environment (Adair *et al.* 2000). The ability of the Tiny Floret Galler to avoid debilitating attack by indigenous Western Australian parasitoids and reach high population densities is a major impetus for evaluation of this insect as a biological control agent for *A. mearnsii* in South Africa. Conversely, in eastern Australia, the Tiny Floret Galler can be abundant, but undergoes dramatic population density fluctuations, probably mediated by changes in parasitoid activity. Environmental and biotic factors, including susceptibility to attack by South African pathogens and parasitoids, may govern the success of Tiny Floret Galler, if released in South Africa as a biological control agent for *A. mearnsii*.

Impact of parasitism

Predicting the outcome of new host-parasitoid associations and their impact on biological control programs is a difficult (Shaw & Askew 1986) and undeveloped science, but could help prioritize potential agents for evaluation, or gauge the likely success of agents before release. Hill & Hulley (1995) suggest that no biological control agent should be excluded based on expectations of susceptibility to parasitoid attack, as native parasitoids have not strongly influenced populations of weed biological control agents in South Africa. Gall-forming Cecidomyiidae are generally subject to high levels of parasitism in their native and introduced ranges. Comparative study of cecidomyiid parasitoids between continents may help to identify the reasons for the susceptibility of gall-forming Cecidomyiidae to parasitism and perhaps clarify their potential as biological control agents for weeds. As many cecidomyiids are important pests of commercial crops e.g. *Mayetiola destructor* (Say), *Contarinia sorghicola* (Coquillett), despite the presence of parasitoids and control attempts using insecticides, weed biological control science needs to be capable of identifying those species with the potential to have a high impact on their host plant.

Efficacy of potential biological control agents

Most *Acacia* species produce large numbers of flower-heads, each made up of few to many florets, on simple, racemose or sometimes paniculate inflorescences. *Acacia mearnsii* is an out-crossing species with partial self-compatibility (Raymond 1997). Despite high levels of flower production in Australia, only a small proportion of florets (0.6-2%) produce fruit (Moncur *et al.* 1991). Excessive flower production may be an adaptation to attract insects for pollination (New 1984). Biological control agents aimed at effectively reducing seed production by feeding on pre-fruiting stages of *Acacia* inflorescences must attain very high numbers each flowering season and be well synchronized with their host's phenology to be effective, as most flower-heads need to be utilised to achieve a high probability that those destined to produce fruit will be galled instead. This is a 'shot-gun' approach to seed-reduction compared to the target specific feeding behaviour of insects that attack developing fruits. Nearly all cecidomyiids on southern Australian *Acacia* inflorescences feed on floret buds (bud gallers) or floret ovaries (floret gallers, groove gallers, pouch gallers, fluted gallers, hollow gallers, inflated gallers), and a few attack developing seeds (seed gallers). Galling insects that sequester resources to primordial reproductive tissues and invade organs that are mass-produced, but most of which would normally abscise, are strong biological control candidates when high impact damage to reproductive and vegetative host tissues is desirable. This is mainly due to the insects' potential to 'force' the host plant to 'commit' resources to gall formation at levels that are much greater than normally required for development of

ungalled tissues (Dennill 1988) and is an approach suitable for weeds with no commercial importance.

However, to avoid a major conflict of interest with the wattle industry in South Africa, gall-forming biological control agents selected for *A. mearnsii* and other commercially important *Acacia* species must not induce resource allocation above that normally allocated to fruit production. Therefore, comparative assessments of biomass and nutrient status of galls induced by Australian cecidomyiids with fruits of *A. mearnsii* is a critical component of the evaluation process for agents being considered for *A. mearnsii*. Ideal gall-forming biological control candidates for *A. mearnsii* would induce galls with: a calorific value the same or lower than fruits, occur over a shorter period than normal fruit development, induce galls with the same as or lower masses than fruits, and cause negative growth impacts that are confined to inflorescences. Additionally, the insect should be synchronized with its host's phenology over a range of climate types and have acute host finding abilities.

Biological control and commercial wattle industries in South Africa

Commercial growers of *A. mearnsii* in South Africa re-establish plantations in cleared areas either by stimulating seed that has accumulated in the soil to germinate (25% of growers), or by line-sowing hot-water treated seeds collected from plantations (35% of growers), or by planting seedlings grown from seed produced in seed orchards (40% of growers) (R. Dunlop pers. comm. 2002). Seeds are therefore essential to the *A. mearnsii* industry and although seed destroying agents have been accepted for biological control nationally, their introduction is a source of contention between industry and weed control efforts in South Africa. Seed-reducing biological control agents for *A. mearnsii* must therefore be capable of being suppressed using insecticides, preferably with those currently registered for pest control in wattle crops.

Synthetic pyrethroids are most widely used for control of foliage damaging pests, mostly Lepidoptera, in South African wattle crops (Nel *et al.* 1999). In addition, the systemic insecticide acephate is registered for control of mirids on wattle seedlings. Synthetic pyrethroids and systemic insecticides control cecidomyiid pests on a range of other trees and herbaceous crops with mixed success. Seed-reducing cecidomyiids of conifer seed-orchards in the Northern Hemisphere have been controlled since the 1980s with insecticides applied as foliar-applications, stem injections and implantation techniques (e.g. Reardon *et al.* 1985). Conventional control techniques, as well as internal insecticide application methods that reduce non-target damage and chemical usage, needed to be evaluated for their potential to control seed-reducing cecidomyiids of *A. mearnsii*.

Components of this thesis

In order to identify which of the cecidomyiid species associated with *A. mearnsii* and *A. cyclops* best fulfilled the criteria raised in the preceding text, this study aimed to:

1. Survey *Acacia* species in southern Australia and South Africa to obtain the entire suite of seed-reducing cecidomyiids associated with *A. mearnsii* and *A. cyclops*;
2. locate and identified the cecidomyiid fauna on indigenous African *Acacia* species;
3. test the host specificity of Australian cecidomyiid species under consideration as biological control agents for *A. mearnsii* and *A. cyclops*;
4. determine the biology, parasitoid assemblages and impact of the candidate insects on their host plant to indicate which species were most likely to be successful as biological control agents in South Africa;
5. measure the resource-loading impact of gall-forming cecidomyiids from *A. mearnsii* to affirm that the insects would not affect vegetative growth of the host plants and thereby reduce their commercial value or productivity;
6. investigate whether insecticides could be used to exclude the Tiny Floret Galler from seed orchards and thus ensure a supply of seeds for commercial growers even if the insects became widespread and extremely damaging.
7. In 2001, the Small Fluted Galler (*D. dielsi*), an Australian gall-forming midge, was confirmed as naturalised on *A. cyclops*. In this study, the rate of spread of *D. dielsi* in South Africa and level of parasitism of *D. dielsi* in populations in the Western Cape was determined. Impacts on the biological control program for *A. cyclops* are predicted and implications for the biological control program for *A. mearnsii* are discussed.

CHAPTER 2

Gall-forming Cecidomyiidae on reproductive organs of *Acacia* from southern Australia and South Africa

Abstract

Five hundred sites in Australia, incorporating 146 species of *Acacia* were surveyed for gall-forming Cecidomyiidae. Thirty cecidomyiid species were identified and categorised into seven groups based on gall morphology. All were undescribed species except for *Dasineura dielsi* from *Acacia cyclops* and *D. acaciaelongifoliae* from the *A. longifolia–A. sophorae* complex. *Dasineura* and *Asphondylia* were the only cecidomyiid genera collected from the reproductive organs of *Acacia* in Australia. In South Africa, at 346 sites with 43 species of *Acacia*, 16 undescribed cecidomyiids were found galling inflorescences, nodes or pinnae. All were species of *Aposchizomyia*, *Acacidiplosis*, *Contarinia* or *Asphondylia* and these formed four groups based on gall morphology. The cytochrome *b* gene isolated from mitochondrial DNA provided a useful segregation of accessions of Australian *Asphondylia*. The same gene had limited variability in *Dasineura* and, although useful in separating major groups of *Dasineura*, it could not be used for reliable segregation of species. Two species, *Dasineura* sp. (Tiny Floret Galler) from *A. mearnsii* and *D. dielsi* from *A. cyclops*, were selected as the most promising candidates for detailed evaluation as potential biological control agents of their hosts in South Africa.

Introduction

Acacia is Australia's largest plant genus with around 960 described species (Maslin 2001) with representatives occurring in all major climate zones and in most vegetation associations. Extensive post-Miocene species radiation and diversification of the *Acacia* subgenus *Phyllodineae* (= *Heterophyllum*) in Australia (Wood 1959) has allowed the reciprocal development of a rich phytophagous fauna (New 1984). Gall-forming cecidomyiids are a common component of the phytophagous fauna of Australian *Acacia,* particularly on reproductive organs of their host plants. There is considerable variation in gall morphology and in the biology of the insects, suggesting a rich assemblage of allied taxa (Adair *et al.* 2000). The presence of damaging seed-reducing cecidomyiids on Australian acacias that are invasive in South Africa and elsewhere, suggests they have potential as classical biological control agents.

Determination of the natural host range of species under consideration as biological control agents is an important early step in the selection of organisms for more detailed evaluation. Monophagous organisms pose the lowest non-target risk, while polyphagous organisms pose the greatest risk. In this study, rapid survey techniques were developed to provide a cost-effective method of measuring the host ranges and distributions of gall-inducing Cecidomyiidae across the vast expanse of *Acacia*-rich southern Australia. The study was problematic because accurate natural host range determinations require the ability to recognize species in the field, but the Australian cecidomyiid fauna associated with *Acacia* is almost completely undescribed. Adult stages are difficult to collect so a system of species identification based on gall morphology and biological traits was developed. Gall structures are regarded as extensions of the cecidogenic agent rather than the host (Mani 1964, Stone & Cook 1998, Stone & Schönrogge 2003) and can been used for species recognition. However, in species with similar gall morphology, this approach is inadequate and additional diagnostic analyses are required (Barnes 1953).

DNA fingerprinting techniques have been applied to resolve species identification problems in insects (Finnerty & Collins 1988, Tang *et al.* 1998), including the Cecidomyiidae (Behura *et al.* 1999, Thongphak *et al.* 1999, Naber *et al.* 2000, Katiyar *et al.* 2000). However, DNA fingerprinting data does not allow for the robust reconstruction of the evolutionary relationships among species (Swofford *et al.* 1996). Therefore, in order to validate the classification of species based on gall morphology, and to examine the evolutionary relationships among representative species of Australian gall-forming cecidomyiids, molecular phylogenies were reconstructed using cytochrome *b* sequences from the mitochondrial genome. Phylogenetic reconstruction of species relationships using mitochondrial gene sequences is a robust technique, and has been widely used to resolve species relationships in a phylogenetically diverse array of insect genera (Thompson *et al.* 2000, Torres *et al.* 2001, Guryev *et al.* 2001). Molecular phylogenies may also reveal cryptic species that induce similar gall structures on different hosts, or indicate where a single cecidomyiid species utilizes different host organs and induces dissimilar gall characteristics. The cytochrome oxidase II and cytochrome *b* genes, both present on the rapidly evolving mitochondrial genome have proved useful for similar problems associated with species identification of insects (Beckenbach *et al.* 1993, Simon *et al.* 1994, Shirota *et al.* 1999, Simmons & Weller 2001) and have been applied to the Cecidomyiidae (Plantard *et al.* 1998, Widenfalk *et al.* 2002).

The southern African *Acacia* flora contains 52 described taxa (Arnold and De Wet 1993) and is species'-poor compared to the Australian flora. The cecidomyiid fauna of South

African *Acacia* is completely undescribed and few accessions from reared material occur within the South African National Insect Collection (M. Mansell pers. comm. 1999). However, gall-forming cecidomyiids occur on South African *Acacia* and several have been described from Kenyan *Acacia* (Gagné & Marohasy 1993). A comparison of the South African and Australian cecidomyiid fauna from *Acacia* was made to determine whether any of the Australian species have invaded or are co-present in South Africa, perhaps eliminating the need for their deliberate importation as biological control agents. Additionally, comparative studies of Australian and South African cecidomyiids from *Acacia* species may identify evolutionary trends between continents and reveal the effect of host plant radiation on speciation of dependant phytophages.

Materials and Methods

Survey technique

Surveys were made in Australia and South Africa for gall-forming cecidomyiid fauna associated with *Acacia* species in each region.

In Australia, study sites were restricted to latitudes south of 25^0S as this is approximately the northern limit of problematic Australian *Acacia* infestations in South Africa (Henderson 2001). Five hundred sites were sampled between 1998-2003 incorporating 141 Australian *Acacia* taxa from six sections, three African *Acacia* species and one Central American *Acacia* species.

In South Africa, 346 field sites and 47 Mimosaceae species were surveyed for gall-forming cecidomyiids between 1999-2002. Survey sites included African *Acacia* (24 species), Australian *Acacia* (19 species) and other African Mimosaceae (four species). Australian acacias surveyed were predominantly *A. mearnsii* and *A. cyclops* as these species are targets for biological control in South Africa. Sites were selected where at least three sexually mature *Acacia* trees of the sample species were present. A rapid survey technique was utilised to enable a large number of sites to be sampled. At each site, selected plants were visually searched for up to 10 minutes, depending on size, for the presence of cecidomyiid galls. Binoculars were used to search the upper canopy of large trees.

The searched plants were identified and their phenological stage was recorded. If galls were located, gall condition and stage of development was recorded, and voucher samples collected where the gall-forming agent could not be positively identified. Cecidomyiid genera were identified by Dr P. Kolesik (University of Adelaide) when adults were available. Site locations and altitudes were recorded using a Global Positioning System. The information was entered into a Microsoft Access database for storage and analysis.

Gall morphology

At selected sites, representative samples of cecidomyiid galls were collected from several trees of the same host species. Fresh, mature galls were used to annotate gall size, number of floret galls, shape, colour, indumentum (hair types) and external and internal architecture. The arrangement of immature cecidomyiid stages within galls was recorded and samples were either preserved in 70% ethanol or were reared to adults for later identification. The host organs affected were recorded. Gall size (length, width, lobe dimensions) was measured with digital callipers that were accurate to two decimal places of a millimeter. Standard botanical terminology was used to describe gall morphology.

Gall morphology was used to segregate accessions into groups with similar internal structure. In many cases, gall morphology could also be used to segregate candidate cecidomyiid species. In cases where similar gall types were found from a range of hosts, comparison of DNA from larvae was used to help segregate taxa.

Where possible, voucher specimens of cecidomyiids were preserved and mounted and specimens lodged with the South Australian Museum (SAMA), Adelaide. In nearly all cases, collections were of undescribed species and identification to species levels was not possible.

DNA sequencing

1. DNA extraction

Whole cecidomyiid larvae and pupae were dissected from galls on the reproductive organs of *Acacia*. In all accessions, gall morphology was described, the host identified and larval stages were preserved in 70% ethanol. Adults were reared from the same gall collection where possible. Total DNA was extracted from immature stages or adults using a standard phenol-chloroform extraction protocol (Maniatis *et al.* 1992). For very small specimens for which phenol-chloroform extraction was considered unfeasible, the entire pupa or larva was added to the PCR reaction tube. DNA pellets were re-suspended in 150 µl molecular grade water (Sigma).

2. Amplification conditions

Cytochrome *b* insect-specific primers CB1 and CB2 (Simon *et al.* 1994) were used to amplify a 450 base pair fragment of cytochrome *b* using the polymerase chain reaction (PCR). PCR reactions were performed in 0.5 ml thin-walled eppendorfs (Whitehead Scientific) using 25 µl reaction volumes overlaid with mineral oil. The reaction mixture consisted of each dNTP at 0.2mM, BIOTAQ DNA polymerase (Bioline, Whitehead Scientific) at 0.02U/µl, 0.2 µM of forward and reverse primer, 2.5mM $MgCl_2$, and

magnesium-free 10 X reaction buffer (Whitehead Scientific) was added to a 1 x final concentration. A PCR 'blank' tube where the template DNA was replaced by sterile distilled water was included in each experiment to control for possible PCR contamination of reagents.

PCR reactions were performed with the following cycling conditions: 1 cycle for 3 min at 94°C, followed by 35 cycles of 45 sec at 94°C, 45 sec at 40°C and 1 min at 72°C, and a final extension step at 72°C for 10 min. Five microlitres of the resulting PCR product was electrophoresed on a 1.5% agarose gel containing 0.5 μg/mL ethidium bromide and visualized under UV light. If amplification was successful, the remaining 20μl from each PCR reaction was purified using Qiagen QIAquick PCR purification kit columns (Southern Cross Biotechnology, Cape Town). Purified PCR product was eluted in 30 μl sterile distilled water pH 8.0. The concentration of eluted PCR product was estimated by running 3 μl of the Qiagen-purified product on a 1.5% agarose gel and comparing the intensity of the band to pGEM®f(+) double-stranded DNA Control Template (the ABI Prism® BigDye™ Terminator Cycle Sequencing Ready Reaction kit v1.0, Applied Biosystems) loaded on the gel at 20ng, 40ng, 60ng and 80ng.

Sequencing reactions were carried out directly on purified PCR products using BigDye terminator chemistry according to the recommendations of the manufacturer (Perkin-Elmer Applied Biosystems). The reactions were electrophoresed on an ABI 3100 capillary sequencer and manually checked and edited in Chromas 2.23 (Technelysium Pty Ltd). Sequences were aligned using Clustal X (Thompson *et al.* 1994). Preliminary phylogenetic analysis with *Asphondylia* and *Contarinia* as outgroups to *Dasineura* indicated that they were too distantly related to the in-group *Dasineura* specimens and might therefore distort in-group relationships (Hutcheon *et al.* 1998). Therefore, *Mayetiola destructor* (*Oligotrophini*) was chosen as an outgroup for the *Dasineura* data set. *Contarinia loti* (De Geer) (*Cecidomyiidi*) (Genbank accession number AY01735) and *Dasineura* sp. (RJA3304) (Genbank accession number AY278736) were designated as outgroups for *Asphondylia.* Separate sequence alignment data sets were constructed for *Asphondylia* and *Dasineura* as the primary purpose of this work was to examine intra- rather than inter-generic relationships. Sequences have been deposited in Genbank (accession numbers AY277743–AY277768 for *Asphondylia* and AY278680 – AY278738 for *Dasineura*).

Phylogenetic analysis

Phylogenetic analyses were executed in PAUP*4 version beta 10 (Swofford 2002) using maximum parsimony and maximum likelihood optimality criteria. Bayesian analyses of

the sequences were performed using MrBayes Version 3.0. As phylogenetic accuracy can be substantially improved by selection of appropriate models of evolutionary change (Swofford *et al.* 1996), several analyses were performed to define the characteristics of the cytochrome *b* sequence data. Saturation analysis of the cytochrome *b* sequences was performed by plotting the number of transitions (Ti) and transversions (Tv) against the uncorrected percentage sequence divergence. Base frequency stationarity was evaluated using a chi-squared test implemented in PAUP.

For MP analyses, all parsimony-uninformative characters were removed prior to the analyses. The remaining parsimony informative characters were given equal weights in the analysis. Trees were generated using the heuristic search option with TBR branch swapping and stepwise addition using 10 random sequence addition replicates. Due to the large size of the *Dasineura* data set and time constraints, the MaxTree limit was set to 1000 trees for the *Dasineura* data set. For the ML analysis, the appropriate nucleotide substitution model was estimated using MODELTEST Version 3.06 (Posada & Crandall 1998) using the Akaike Information Criterion following Buckley *et al.* (2001). The General Time Reversible model with variable sites assumed to follow a discrete gamma distribution (GTR+ Γ model, Yang *et al.* 1994) was selected as the best-fit model for the *Asphondylia* cytochrome *b* sequence data by MODELTEST. A TVM + I + Γ model was found to be optimal for the *Dasineura* sequence data. For both data sets, a heuristic ML analysis was implemented in PAUP with the starting tree obtained by stepwise addition of taxa and TBR branch swapping under the optimal model suggested by MODELTEST, using the default setting of four discrete rate categories for the gamma distribution. For both data sets, nodal support for the MP and ML analyses was assessed from 1000 and 100 non-parametric bootstrap replicates (full heuristic search; 'as is' stepwise addition of taxa). The MaxTree limits were set to 1000 for ML bootstrap analysis of the *Dasineura* data set due to time constraints. In this study, bootstrap values < 50% are considered as not supported, bootstrap values (BS) between 50% and 70% as weakly supported, and bootstrap values > 70% as strongly supported (Daniels *et al.* 2002). Bayesian phylogenetic analyses were conducted with MrBayes 3.0 (Huelsenbeck & Ronquist 2001) using a GTR + Γ model of nucleotide evolution for the *Asphondylia* data set, and a GTR + I + Γ model for the *Dasineura* data set as indicated by MODELTEST. Specific nucleotide substitution model parameter values were not defined *a priori*, but rather treated as unknown variables with uniform priors and were estimated during the analysis. A random tree generated by MrBayes was used as a starting tree for each Markov chain. For the analysis, four Markov chains were run for one million generations. Tree sampling was performed every 50 generations, generating 20,000 sample points. To ensure that the Markov

chain had reached stationarity, the fluctuating value of the likelihood was graphically monitored. Five thousand trees (corresponding to 250,000 generations) were discarded as 'burn-in'. This included sample points in the apparently stationary region of the chain where it is better to be cautious and discard useful samples, rather than inadvertently include burn-in samples when estimating Bayesian posterior probabilities (Leache & Reeder 2002). A 50% majority-rule consensus of the remaining 15,000 trees were used to generate posterior probability approximations for each clade. If >95% of sampled trees contained a given clade, this clade was considered to be significantly supported by the data. Clades contained in 90%-94% of all sampled trees were considered as strongly supported (Leache & Reeder 2002).

Bio-morphological gall characteristics were superimposed on the resulting phylogenetic trees to determine whether these were informative for recognition of cecidomyiid species.

Results

Surveys in Australia and South Africa

Australia

In southern Australia, 500 field sites (figure 2.1a) and 141 *Acacia* species (table 2.1) were surveyed for gall-forming cecidomyiids between 1998 and 2003. Surveyed *Acacia* were predominantly from the sections *Phyllodineae* (72 species), *Botrycephalae* (27 species) and the *Juliflorae* (19 species) with lesser numbers from the *Plurinerves* (11 species), *Pulchellae* (5 species) and the *Alatae* (1 species) (table 2.1). *Acacia* from the *Lycopodiifolia* were not seen in the field. Three African *Acacia* species (*A. karroo, A. xanthophloea, Acacia.* sp.) and one Central American *Acacia* (*A. caven* (Mol.) Mol.) were found in horticultural plantings at Melbourne, Canberra and Perth in the presence of Australian *Acacia* and were also surveyed for gall-forming cecidomyiids. No cecidomyiids were found on African *Acacia* in Australia.

Eight morphological gall-types were found on Australian *Acacia* induced by *Dasineura* galling ovaries in open florets, and *Asphondylia* galling floret buds and ovules in association with a fungal symbiont (table 2.2). A total of 22 *Dasineura* and eight *Asphondylia* species were recognized on southern Australian *Acacia* with fluted gallers (nine *Dasineura* species) and seed gallers (five *Asphondylia* species) being most species diverse (tables 2.4-2.10). Several are illustrated (figure 2.2).

The *Botrycephalae* was host to 14 species of gall-forming cecidomyiid with the 'Floret Gallers' and 'Hollow Galler' restricted to this section. *Acacia mearnsii* and *A. irrorata* supported more gall-forming cecidomyiids than any other Australian *Acacia* with eight cecidomyiid species each (table 2.11, figure 2.3). Ten gall-forming cecidomyiid species

were recorded from the *Phyllodineae*, eight from the *Plurinerves*, seven from the *Juliflorae*, three from the *Pulchellae* and two from the *Alatae*.

Twenty-four gall-forming cecidomyiids were recorded in eastern Australia and nine indigenous species in Western Australia with three species (*D. dielsi, Dasineura* sp. (Western Fluted Galler), *Dasineura* sp. (Groove Galler)) were common between both regions. Two eastern gall-forming cecidomyiids (*Dasineura* sp. (Tiny Floret Galler), *Dasineura* sp. (Hairy Inflated Galler)) are naturalized on eastern Australian *Acacia* species present in Western Australia.

In eastern Australia, six of the eight gall-forming cecidomyiids present on *A. mearnsii* were dispersed over the natural latitudinal range of *A. mearnsii* (figures 2.4, 2.5). The Groove Galler (*Dasineura* sp.) was rare and located at a single location in central Victoria (3220), while the Egg Galler (*Dasineura* sp.) was not recorded in Tasmania (figure 2.4). The altitudinal range of cecidomyiids from *A. mearnsii* was variable (table 2.12). The Egg Galler and Elongate Fluted Galler were present on *A. mearnsii* at its highest recorded altitude of 1069 m on the northern Tablelands at Armidale, New South Wales, while the Common Fluted Galler was restricted to altitudes below 365m.

On *A. cyclops*, the Small Fluted Galler, *Dasineura dielsi* (figures 2.2a, 2.3p) was present throughout the host's range and occurred in abundance in populations on the Yorke Peninsula, South Australia. The seed galler (*Asphondylia* sp.) and bud galler (*Asphondylia* sp.) from *A. cyclops* were rare and recorded only from the Perth region (figure 2.5).

Several host range patterns were present in gall-forming cecidomyiids from Australian *Acacia*. Monophagy (a single host) was uncommon, but with four clear examples, the Mossy Floret Galler and Tuberculate Floret Galler from *A. irrorata*, the Small Inflated Galler from *A. oshanesii*, and the Narrow Fluted Galler from *A. sclerophylla*. Most cecidomyiids were stenophagous (few hosts) within *Acacia* and several were clearly polyphagous (many hosts), but restricted to *Acacia*, e.g. *D. acaciaelongifoliae*, Common Fluted Galler, Hairy Inflated Galler, Hollow Galler, Pouch Galler, Pubescent Bud Galler, Bipinnate Seed Galler and the Eastern Bud/Seed Galler.

The majority of gall-forming cecidomyiids were univoltine, but several had undetermined voltinism. There were four confirmed cases of multivoltinism in *Dasineura* (Common Fluted Galler, *D. dielsi*, Grey Fluted Galler, Small Inflated Galler) all from hosts with either perpetual flowering or flowering extending across a large proportion of the year. Multivoltinism was present in two *Asphondylia* species where adults are either polyphagous and can switch between hosts (Pubescent Bud Galler) or can alternate between host organs (buds and ovules) of their hosts (Eastern Bud/Seed Galler).

South Africa

On the 43 *Acacia* species surveyed in South Africa (table 2.13, figure 2.1b), four gall-types were found on African *Acacia* (table 2.3): *Asphondylia* galling floral buds, *Contarinia* and *Aposchizomyia* galling ovaries, a *Contarinia* galling receptacles and *Acacidiplosis* galling node buds. Gall morphology and life cycle characteristics were used to segregate accessions into tentative species. Eleven gall-forming cecidomyiids were recorded from the inflorescences of African *Acacia* (table 2.14) and an additional five species forming galls on buds in stem nodes (tables 2.15). No Australian *Acacia* in South Africa were found galled by cecidomyiids. The African Mimosaceae shrub *Dichrostachys cinerea* (L.) Wright & Am. was the only non-*Acacia* Mimosaceae found with gall-forming Cecidomyiidae in South Africa, where two undetermined species were found galling florets in northern South Africa, and elsewhere in southern Africa. However, more extensive surveys of non-*Acacia* Mimosaceae in South Africa are required.

Twelve gall-forming cecidomyiids were recorded from African *Acacia* in the subgenus *Acacia* and four cecidomyiids from the subgenus *Aculeiferum*. There were no gall-forming cecidomyiid species common to both the subgenera *Acacia* and *Aculeiferum* in South Africa. All but two cecidomyiids (the bud galling *Asphondylia* sp. and node galler *Acacidiplosis* sp. from *A. karroo*) were restricted to northern South Africa, where the highest diversity of African acacias occurs. In this survey, *Acacia karroo* was host to more gall-forming cecidomyiids (five species) than other African *Acacia* species. Most gall-forming cecidomyiids from *A. karroo* were widespread except for the Node Galler (*Acacidiplosis* sp.), which was only found at two locations in the Western Cape, and the Receptacle Galler, which was only found in Gauteng, but may have been overlooked due to its cryptic gall symptoms. Several gall-forming cecidomyiids from African *Acacia* are illustrated (figure 2.6).

Unlike Australian cecidomyiids from *Acacia*, African cecidomyiids were mostly monophagous (68%) or stenophagous (32%) and all were apparently univoltine. None were clearly polyphagous (tables 2.14, 2.15).

DNA sequencing

Sequence characteristics

A total of 410 bp of cytochrome *b* was obtained for 59 specimens of *Dasineura* representing 23 putative species, and for 27 specimens of *Asphondylia* representing eight species. As no frame shift or nonsense codons were apparent, and because all amplifications

Table 2.1 *Acacia* surveyed for gall-forming Cecidomyiidae in southern Australia.

Host	Section	No. survey sites	Host	Section	No. survey sites	Host	Section	No. of survey sites
A. acinacea	Phyllodineae	7	*A. irrorata*	Botrycephalae	26	*A. idiomorpha*	Phyllodineae	1
A. acuminata	Juliflorae	4	*A. iteaphylla*	Phyllodineae	3	*A. implexa*	Plurinerves	13
A. alata	Alatae	3	*A. kettlewelliae*	Phyllodineae	1	*A. prominens*	Phyllodineae	3
A. aneura	Juliflorae	1	*A. kulnurensis*	Botrycephalae	2	*A. pulchella* var. *goadbyi*	Pulchellae	2
A. andrewsii	Phyllodineae	1	*A. kybeanensis*	Phyllodineae	1	*A. pulchella* var. *pulchella*	Pulchellae	7
A. ausfieldii	Phyllodineae	1	*A. lasiocarpa* var. *lasiocarpa*	Pulchellae	6	*A. pycnantha*	Phyllodineae	26
A. baileyana	Botrycephalae	32	*A. lasiocarpa* var. *sedifolia*	Pulchellae	2	*A. ramulosa*	Phyllodineae	1
A. baileyana hybrid	Botrycephalae	2	*A. leioderma*	Pulchellae	1	*A. restiacea*	Phyllodineae	1
A. barringtonensis	Phyllodineae	1	*A. leprosa*	Phyllodineae	3	*A. retinoides* var. *retinoides*	Phyllodineae	17
A. baxteri	Phyllodineae	1	*A. leptoclada*	Botrycephalae	1	*A. retinoides* var. *uncifolia*	Phyllodineae	3
A. bidentata	Phyllodineae	1	*A. leucoclada*	Botrycephalae	5	*A. rostellifera*	Phyllodineae	14
A. binervata	Phyllodineae	3	*A. ligulata*	Phyllodineae	3	*A. rubida*	Phyllodineae	3
A. binervia	Phyllodineae	3	*A. linearifolia*	Phyllodineae	2	*A. salicina*	Phyllodineae	5
A. blayana	Botrycephalae	1	*A. lineata*	Phyllodineae	2	*A. saligna*	Phyllodineae	26
A. boormannii	Phyllodineae	1	*A. linifolia*	Phyllodineae	4	*A. schinoides*	Botrycephalae	3
A. brachybotra	Phyllodineae	1	*A. littorea*	Phyllodineae	8	*A. scirpifolia*	Phyllodineae	3
A. buxifolia	Phyllodineae	5	*A. longifolia*	Juliflorae	12	*A. sclerophylla*	Plurinerves	5
A. cardiophylla	Botrycephalae	2	*A. longissimma*	Juliflorae	1	*A. sertiformis*	Phyllodineae	1
A. caroleae	Juliflorae	2	*A. lunata*	Phyllodineae	1	*A. signata*	Juliflorae	1
A. celastrifolia	Phyllodineae	2	*A. luteola*	Phyllodineae	1	*A. silvestris*	Botrycephalae	1
A. chinchillensis	Botrycephalae	1	*A. maidenii*	Juliflorae	5	*A. sophorae*	Juliflorae	30
A. browniana	Pulchellae	1	*A. mearnsii*	Botrycephalae	123	*A. spathulifolia*	Phyllodineae	2
A. chrysocephala	Phyllodineae	1	*A. meissneri*	Phyllodineae	1	*A. spectabilis*	Botrycephalae	6
A. chrysotricha	Botrycephalae	1	*A. melanoxylon*	Plurinerves	43	*A. sphenophylla*	Phyllodineae	1
A. cochlearis	Plurinerves	2	*A. mollifolia*	Botrycephalae	2	*A. stereophylla*	Juliflorae	1
A. concurrens	Juliflorae	3	*A. monatana*	Phyllodineae	1	*A. stricta*	Phyllodineae	7
A. constablei	Botrycephalae	2	*A. mucronata*	Juliflorae	4	*A. stricta x A. oxycedrus*	Phyllodineae	1
A. coolgardiensis	Juliflorae	1	*A. muellerana*	Botrycephalae	1	*A. suaveolens*	Phyllodineae	6
A. convenyi	Phyllodineae	1	*A. myrtifolia*	Phyllodineae	10	*A. subulata*	Phyllodineae	1
A. cremifolia	Phyllodineae	2	*A. neriifolia*	Phyllodineae	3	*A. terminalis*	Botrycephalae	13
A. cyclops	Plurinerves	33	*A. neurophylla*	Juliflorae	2	*A. tetragonophylla*	Phyllodineae	1
A. dealbata	Botrycephalae	74	*A. notabilis*	Phyllodineae	1	*A. trachyphloia*	Botrycephalae	4
A. deanei	Botrycephalae	18	*A. obtusifolia*	Juliflorae	2	*A. truncata*	Phyllodineae	5
A. decora	Phyllodineae	2	*A. oldfieldii*	Juliflorae	6	*A. ulicifolia*	Phyllodineae	3
A. decurrens	Botrycephalae	36	*A. omalophylla*	Juliflorae	1	*A. ulicina*	Phyllodineae	1
A. divergens	Phyllodineae	1	*A. oshanesii*	Botrycephalae	4	*A. urophylla*	Phyllodineae	4
A. dodinaerfolia	Phyllodineae	1	*A. oswaldii*	Plurinerves	2	*A. venulosa*	Plurinerves	2
A. drummondii	Pulchellae	5	*A. oxycedrus*	Juliflorae	2	*A. verniciflua*	Phyllodineae	2
A. elata	Botrycephalae	7	*A. papyrocarpa*	Plurinerves	2	*A. verticillata*	Phyllodineae	8
A. elongata	Plurinerves	1	*A. paradoxa*	Phyllodineae	13	*A. vestita*	Phyllodineae	1
A. ephredoides	Juliflorae	1	*A. parramattensis*	Botrycephalae	9	*A. viscidula*	Plurinerves	1
A. extensa	Phyllodineae	4	*A. parvipinnula*	Botrycephalae	6	*A. xanthina*	Phyllodineae	2
A. falciformis	Phyllodineae	1	*A. pendula*	Plurinerves	4			
A. filicifolia	Botrycephalae	13	*A. penninervis*	Phyllodineae	2	AFRICAN SPECIES	Subgenus	Sites
A. fimbriata	Phyllodineae	5	*A. pentadenia*	Pulchellae	2	*A. karroo*	Acacia	2
A. floribunda	Phyllodineae	9	*A. perangusta*	Phyllodineae	1	*A.* sp.	Acacia	1
A. falcata	Phyllodineae	4	*A. podalyriifolia*	Phyllodineae	8	*A. xanthophloia*	Acacia	1
A. genistifolia	Phyllodineae	8	*A. polybotrya*	Botrycephalae	2			
A. granitica	Juilflorae	1	*A. pravissima*	Phyllodineae	5	AMERICAN SPECIES		
A. hakeoides	Phyllodineae	3	*A. howittii*	Phyllodineae	1	*A. caven*	Acacia	1

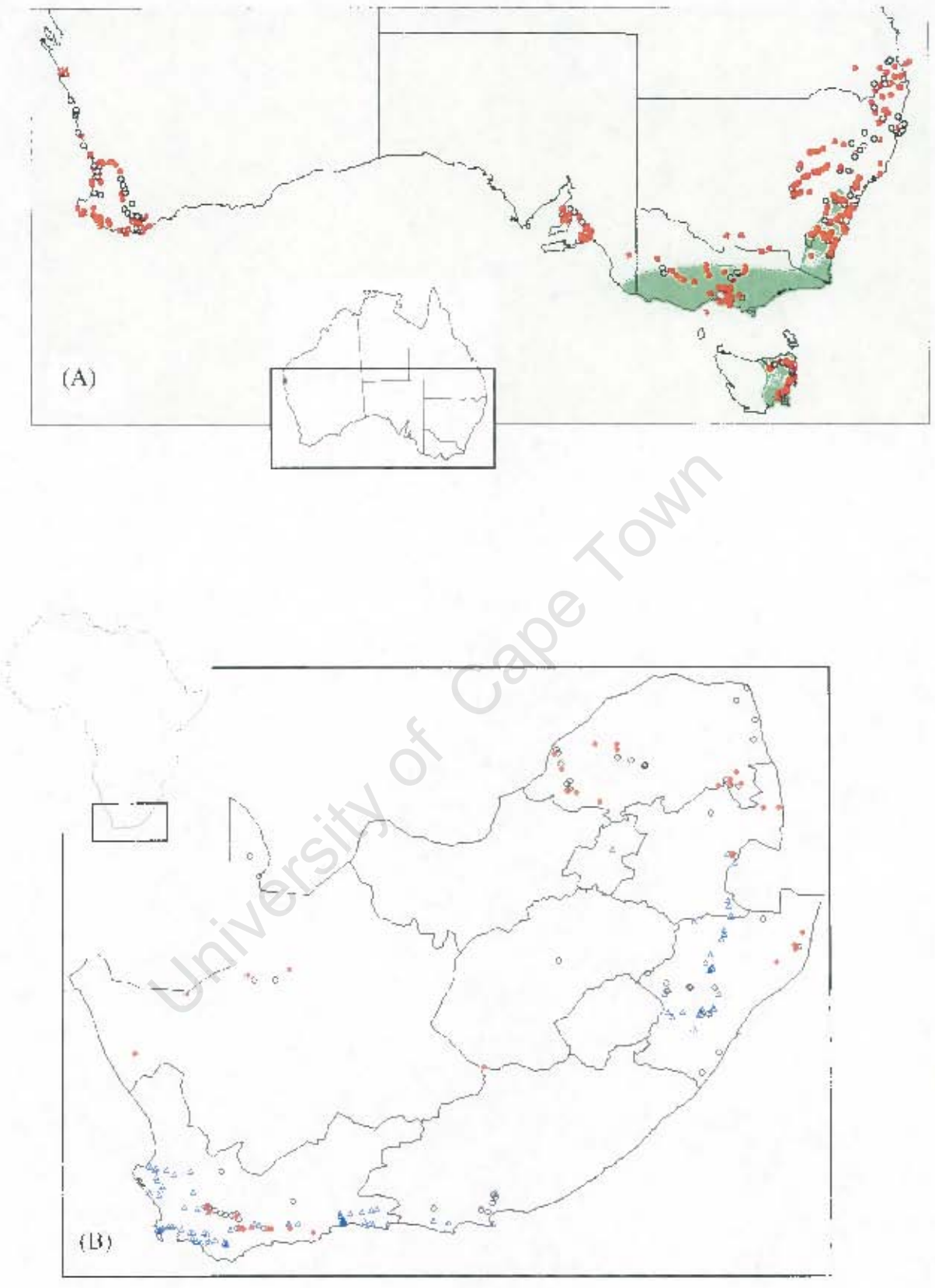


Figure 2.1. Survey site locations for gall-forming Cecidomyiidae from *Acacia* (a) southern Australia, ○ survey sites with no cecidomyiids, ● survey sites with cecidomyiids , the green shaded area is the natural distribution of *A. mearnsii*; (b) South Africa, ○ African *Acacia* with no cecidomyiids, ● African *Acacia* with cecidomyiids, Δ Australian *Acacia* with no cecidomyiids.

resulted in one appropriately sized PCR product, these sequences were assumed to be of mitochondrial origin rather than nuclear mitochondrial pseudogenes (Bensasson *et al.* 2000).

As expected for insect mitochondrial DNA (Thompson *et al.* 2000, Torres *et al.* 2001), sequences were A-T rich (*Asphondylia* A+T = 78.6%, *Dasineura* A+T = 78.6%) with a pronounced A-T bias at third codon positions (*Asphondylia* 94%, *Dasineura* 96%). One hundred and thirty-four sites were parsimony-informative at the nucleotide level for *Asphondylia* and 115 sites were parsimony informative for *Dasineura*. No significant differences in base composition across taxa in *Asphondylia* ($\chi^2 = 21.37$, df = 84, P = 1.00) or *Dasineura* ($\chi^2 = 17.04$, df = 177 P = 1.00) were found. Saturation plots for *Asphondylia* and *Dasineura* showed no transitional or transversional saturation for within-group comparisons when all codon positions were considered (data not shown), therefore no differential weighting of transitions relative to transversions was applied to the DNA sequence data prior to parsimony analysis.

Phylogenetic relationships

Asphondylia

Maximum parsimony analyses recovered 12 equally parsimonious trees (tree length = 272, CI = 0.59, RI = 0.79). Sixteen resolved nodes were common to all most parsimonious trees, with 14 having bootstrap support >50%. Maximum likelihood analysis recovered one tree (-lnL = 2126.63) with bootstrap support present for 16 nodes.

Although all methods of reconstruction (maximum parsimony (MP), maximum likelihood (ML) and Bayesian analysis (BA)) resulted in slightly different topologies significantly or strongly supported nodes in the Bayesian consensus topology were recovered by all methods (figure 2.7). These nodes correspond to the following six distinct clades: clade A (BA PP = 100%; ML BS = 66%; MP BS = 99%) containing bud and seed gallers from eastern Australia, predominantly from the *Phyllodineae*; clade B (BA PP = 99%, ML BS = 84%, MP BS = 95%) comprising bud gallers predominantly from Western Australian *Phyllodineae*; clade C (BA PP = 90%, ML BS = 67%, MP BS = 90%) with Pubescent Bud Gallers from eastern Australian *Botrycephalae*; clade D (BA PP = 100%, ML BS = 94%, MP BS = 100%) with Seed Gallers from eastern Australian *Botrycephalae*; clade E (BA PP= 98%, ML BS = 99%, MP BS = 100%) with the Glabrous Bud Gallers from eastern Australian *Botrycephalae*; and clade F (BA PP = 91%, ML BS = 76%, MP BS = 57%) with seed gallers from three sections of *Acacia* from south-west Western Australian.

In clade F, sequence divergence between the three accessions ranged from 6.6-10.3%, indicating three distinct taxa. Sequence divergence within all other clades ranged from 0.2-

Table 2.2. Morphological groups of gall-forming Cecidomyiidae from inflorescences of southern Australian *Acacia*

Group	Diagnostic characters	Host organ	Development biology	Illustration
Fluted Gall	Semi-woody to woody floret galls in irregular clusters. Floret galls usually distinctly lobed. Ostioles terminal on lobes and dorsal-lateral orientated. Chambers generally elongate and parallel. Chambers with solitary larvae. Floret galls often persistent after maturity.	ovary	Pupation occurs within chamber in white cocoon. Adults emerge through preformed ostiole.	Figures 2.2a,d,e Figures 2.3g,h,k,n,p
Floret Gall	Clusters of semi-woody floret galls. Floret galls simple and unlobed. Chambers basal or lateral with solitary larvae. Larvae emerge through preformed ostioles that are ventral-lateral orientated.	ovary	Larvae emerge from floret galls and pupate in soil within white cocoons.	Figures 2.2b-c Figures 2.3a-f
Groove Gall	Small soft-tissued floret galls. Larvae develop in a shallow groove or depression at base of ovary near pedicel. Larvae mostly solitary. Floret galls fall readily at maturity.	ovary	Larvae pupate external to floret gall either in perianth remains or in soil.	Figures 2.3i-j
Pouch Gall	Soft-fleshy floret galls. Chamber a single broad cavity occupying basal half of floret gall. Larvae usually gregarious. Ostiole is a circular rim surrounding pedicel and opening ventrally.	ovary	Larvae emerge from floret galls and pupate in soil.	
Inflated Gall	Compact clusters of appressed, soft-tissued floret galls. Floret galls often hollow and inflated. Larvae gregarious developing in shallow basal depression on exterior surface of floret galls.	ovary	Larvae pupate in soil or in cocoons around base of pedicel.	Figures 2.2g-i
Hollow Gall	Soft-tissued, near spherical floret galls. Hollow. Ostiole with a narrow closed lip near base of floret gall. Larvae solitary to gregarious developing within floret gall. Floret galls fall readily at maturity.	ovary	Pupation occurs externally often amongst dry stamens or in soil.	Figure 2.2f
Bud Gall	Expanded buds. Solitary larvae. Adults emerge through excised hole in wall of gall Associated with a fungal symbiont.	bud	Full development within galls.	Figures 2.2j-l Figures 2.3l,m,o
Seed Gall	Stunted or distorted fruits. Larvae develop on and around ovules within pods. Associated with fungal symbiont. Larvae solitary.	ovule	Full development within galls.	Figures 2.2m-n

Table 2.3. Morphological groups of gall-forming Cecidomyiidae from South African *Acacia*

Group	Diagnostic characters	Host organ	Development biology	Illustration
Bud Gall	Swollen buds. Soft to woody. Fungal symbiont present. Larvae solitary. Adults emerge through excised ostiole.	bud	Complete development occurs within gall.	Figures 2.6a,b
Floret Gall	Clusters of swollen, distorted ovaries. Ostioles rarely present. Larvae mostly emerge through excised holes or hair tufts. Larvae solitary.	ovary	Complete development within galls.	Figures 2.6d,g,h
Receptacle Gall	Slightly distorted buds or flowers. Single or united chambers with solitary larvae within receptacle.	receptacle	Larvae emerge from gall to pupate in some species.	Figure 2.6f
Node Gall	Swollen and grossly distorted node tissue. Single or united chambers. Larvae emerge through excised holes.	vegetative or floral buds	Complete development within galls.	Figures 2.6c,e

Table 2.4. Comparison of gall morphology and biology of tentative *Asphondylia* species associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No [1]	Common Name (code) [2]	Primary Hosts [3]	Gen.[4]	Mean gall cluster size (mm) length x width	Fresh gall colour	Gall shape [7]	External indumentum	Mean gall size (mm) length x width	Floret gall texture	Larvae per chamber	Host organ	Adult/larvae emergence Comments
Asphondylia sp. 3247, 3118	Glabrous Bud Galler (GBG)	*A. mearnsii* *A. irrorata* *A. parramattensis* *A. deanei*	U	6.3 x 7.6 n=23	green	Galls clusters spherical with tightly appressed swollen buds. Bud galls globose contracting abruptly into distinct "nipple-like" apex. Slight lobes due to several longitudinal furrows. Up to 28 buds/gall.	Glabrous to sparsely pubescent. Hairs appressed and white.	3.2 x 3.1 n=36	semi-soft	1	B	Adults emerge Sept.- October *A. schinoides* & *A. decurrens* are secondary hosts. Co-occurs with Pubescent Bud Galler Figure 2.3m
Asphondylia sp. 3151, 3146, 3141, 3135	Pubescent Bud Galler (PBG)	*A. mearnsii* *A. baileyana* *A. irrorata* *A. deanei* *A. dealbata* *A. decurrens*	M	4.1 x 7.0 n=9	green – frosty green	Bud galls oblong-elliptic, obovate, orbiculate. Apex broadly obtuse, minute "nipple-like" mucro present. Surface smooth to faintly ridged longitudinally.	Hoary-pubescent to densely pubescent. Hairs white and appressed.	4.6 x 2.5 n=36	soft	1	B	Adults emerge most months. Polyphagous with numerous alternative hosts: *A. cardiophylla, A. convenyei, A. filicifolia, A. leucoclada, A. mollifolia, A. oshanesii, A. parramattensis, A. parvipinnula, A. polybotrya, A. pubescens, A. spectabilis, A. trachyphloia* Figure 2.2k, figure 2.3l,o
Asphondylia sp. 2597, 2664, 2648, 2575	Bipinnate Seed Galler (BSG)	*A. mearnsii* *A. irrorata* *A. decurrens* *A. baileyana* *A. dealbata*	U	N/A	green	Galls forming in fruit. Pod wall surrounding ovule slightly swollen and thickened. Sometimes slight discolouration on surface above ovule.	Normal	Pods normal size apart from distortion of infected ovules.	semi-woody	1	F	Adults emerge Sept.-December. Secondary hosts: *A. parvipinnula, A. deanei, A. constablei, A. muellerana* Figure 2.2m
Asphondylia sp. 2829	Western Bud Galler	*A. littorea* *A. truncata* *A. xanthina*	?U	N/A	green	Bud galls elliptic –oblong, obovate. Apex acute to broadly obtuse with "nipple-like" mucro.	Variable. Glabrous to densely pubescent or hirsute.	3.7 x 2.8 n=5	soft-thickened	1	B	Polyphagous. Additional hosts: *A. alata, A. divergens, A. drummondii, A. cochlearis, A. pulchella, A. ramulosa.* Additional collections required to establish if separate taxa occur on *Pulchellae* and *Phyllodineae.* The bud galler on *A. urophylla* may be a distinct entity as galls are thick-walled with very pronounced and thickened mucros.
Asphondylia sp 3132.	Eastern Bud-Seed Galler	*A. sophorae* *A. melanoxylon* *A. verticillata* *A. floribunda* *A. buxifolia*	M	N/A	green	Oblong-elliptic, oblate, obovate. Apex with nipple-like mucro.	Generally glabrous, sparsely pubescent.	2.2 x 2.9 n=15	soft	1	B/S	Adults emerge Polyphagous. Numerous additional hosts including: *A. binervia, A. concurrens, A. cremiflora, A. decora, A. dodinaefolia, A. falcata, A. frimbriata, A. gladiiformis, A. implexa, A. leprosa, A. linearifolia, A. longisima, A. lunata, A. maidenii, A. mucronata, A. obtusifolia, A. oxycedrus, A. pravissima, A. pycnantha, A. retinoides, A. rubida, A. salicina, A. stricta, A. ulicifolia* Utilizes both buds and fruits. Figure 2.2l
Asphondylia sp. 3087	Seed Galler	*A. pentadenia*	U	N/A	green	Pod wall surrounding ovule slightly swollen and thickened. Fruit often stunted. Apex of fruit splitting shortly. Remaining green out-of –season.	Normal	Pods shorter than normal	semi-hard	1	F	Adults not reared. Restricted to Western Australia. DNA sequence data recognises this a distinct from 2 other Western Australian seed gallers (below)
Asphondylia sp. 3209	Seed Galler	*A. rostellifera*	U	N/A	green	Pods stunted, slightly thickened. Remaining green out-of-season.	Normal	Pods shorter than normal	semi-hard	1	F	Restricted to Western Australia. Possibly the same species on *A. cyclops, A. littorea, A. truncata, A. sphenophylla, A. meissneri.* DNA sequence data recognises this a distinct from 2 other Western Australian seed gallers. Figure 2.2n
Asphondylia sp. 3210	Seed Galler	*A. ramulosa*	U	13.8 x 23.3 n=12	grey	Pods stunted, elliptic, compressed. Remaining green out-of-season.	Normal	Pods stunted. 12.4 x 4.3 n=20	semi-hard	1	F	Restricted to Western Australia. DNA sequence data recognises this a distinct from 2 other Western Australian seed gallers (above).

Table 2.5. Comparison of gall morphology and biology of tentative 'fluted galler' species associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host Organ	Adult/larvae emergence Comments
Dasineura dielsi 3023 2598	Small Fluted Galler (SFG)	*A. cyclops*	M	21.5 x 24.6 n=45	mid green	Floret gall narrowly elliptic, irregularly lobed. Lobes 1-4, tubular, straight to gently recurved. Length variable, mostly diverging from posterior half of floret gall. Floret gall occasionally keeled with vestigial pod.	Body of floret gall glabrous. Ostiole entrance plugged with dense fringe of short white hairs, but not in throat of chamber.	9.6 x 6.2 n=126	At anterior end of lobes or body of floret gall.	woody	1	1-5 x = 2.5 ± 1.1 n=37	F	Adults emerge all months. Secondary host: *A. papyrocarpa, A. ligulata, A. sophorae, A. oswaldii* in the near vicinity of *A. cyclops* Figures 2.2a, 2.3p
Dasineura sp. 3022 3124 3021 3127	Elongate Fluted Galler (EFG)	*A. mearnsii* *A. irrorata*	U	21.0 x 26.8 n=80	dark green	Floret gall narrowly obovate-elliptic. Irregular short lobes or knobs in anterior half. Mostly irregularly keeled with vestigial pod. Slightly winged longitudinally.	Floret gall with sparse hoary indumentum of appressed whitish hairs. Ostiole with dense pubescent-cobwebby hairs at entrance. Inner surface of ostiole rim densely puberulous.	10.9 x 4.1 n=192	Usually inset within shallow rims or grooves in anterior section of floret gall or in terminal position on lobes or knobs.	woody	1	1-6 x = 2.2 ± 1.1 n=147	F	Adults emerge Sept.-Oct. Figures 2.3k,n
Dasineura sp. 2662	Fluted Galler (MFG)	*A. melanoxylon*	U	N/A	mid green	Floret gall oblong, elliptic-obovate. Irregularly lobed. Lobes straight and mostly forward to laterally projecting. Sometimes slightly recurved. Lobes often forked.	Glabrous. Ostiole with dense fringe of short white hairs plugging entrance.	11.0 x 8.7 n=14	Terminal on lobes	woody	1	2-11 x = 5 ± 3.1 n=14	F	Adults emerge Jun.-Aug. Small % of larvae diapause in chambers.
Dasineura sp.	Rams Horn Galler (RHG)	*A. neurophylla* *A. oldfieldii*	?U	N/A	green	Floret gall obovate. Irregularly lobed. Lobes strongly recurved to coiled.	N/A	N/A	Terminal on lobes	woody	1	N/A	F	Adults emerge August
Dasineura acaciaelongifoliae 3157 3134	Large Fluted Galler (LFG)	*A. longifolia* *A. sophorae* *A. implexa* *A. maidenii*	U-?B	*A. sophorae* 26.3 x 22.8 n=10 *A. implexa* 31.2 x 38.6 n=5	light-mid green	Floret galls large. Oblong to obovate, dorsally compressed. Irregularly, short, knobby, blunt lobes on anterior half of floret gall. Floret gall tapering to base. On *A. sophorae* and *A. longifolia* lobes tubular up to 10-12 mm, sometimes forked.	Sparsely puberulous. Densely puberulous at entrance to ostiole. Hairs plug ostiole entrance, sometimes present in throat.	Highly variable *A. sophorae* 13.9 x 15.7 n=10 *A. implexa* 17.3 x 15.3 n=10	Terminal on lobes or on anterior surface of floret gall.	woody	1	1-30 Highly variable	F	Reported as bivoltine Skuse (1891). Widespread in eastern Australia. Potential biocontrol agent of *A. longifolia* in Western Australia. Figure 2.2e
Dasineura sp. 3137	Common Fluted Galler (CFG)	*A. mearnsii,* *A. pycnantha* *A. melanoxylon* *A. retinoides* *A. deanei* *A. hakeoides*	B-M	12.3 x 14.5 n=107	pale green	Floret gall obovate, tapered towards base. Irregularly lobed. Lobes shallow and knobby, blunt. Sometimes with short keel of vestigial pod on anterior surface. Floret gall chamber always curved.	Pubescent to woolly-villose. Dense hairs in ostiole plugging entrance. Hairs present on lower surface of throat.	8.9 x 5.8 n=90	Terminal on knobby lobes or mostly anterior surface of floret gall	semi-woody	1	1-19 x = 5.8 ± 4.4 Highly variable	F	Adults emerge most months on *A. retinoides*, Aug.-Sept. on *A. mearnsii*. Polyphagous, but gall symptoms consistent across hosts. Figures 2.3f-g
Dasineura sp. 3147 2885 3211 2886	Western Grey Fluted Galler (WGFG)	*A. aneura* *A. coolgardiensis* *A. ramulosa*	?U	21.9 x 21.1 n=14	grey	Irregularly lobed. Lobes as tubular arms up to 8 mm distinct from body of floret gall or fused into main axis where forming longitudinal ridges. Lobes diverging laterally up to 90° from axis, straight. Floret galls often V or Y-shaped.	Densely-sparsely hoary. Hairs appressed. Dense white-ferruginous hairs at entrance to ostiole. Throat and upper section of chamber with pillose antrorsely projecting hairs.	8.6 x 7.7 n=56	Terminal on lobes	woody	1	1-10 x = 3.6 ± 1.9	F	Adults not reared. Figure 2.2d

Table 2.5 (continued). Comparison of gall morphology and biology of tentative 'fluted galler' species associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host Organ	Adult/larvae emergence Comments
Dasineura sp. 3144 2710	Grey Fluted Galler (GFG)	*A. pendula* *A. omalophylla*	M	15.3 x 22.1 n=10	grey-green	Highly irregularly lobed. Lobes as tubular arms to 8mm parallel or fused to main body or diverging up to 90°. Lobes often curved.	Sparsely pubescent. Densely pubescent around rim of ostiole where hairs are white-ferruginous.	12.9 x 10.3 n=10	Terminal on lobes	woody	1	1-12 x =3.2 ± 2.3	F	Adults emerge most months. Often abundant on *A. pendula*. Gall/floret gall size highly variable
Dasineura sp. 2913 3304	Narrow Fluted Galler (NFG)	*A. sclerophylla*	?U	N/A	green	Irregularly lobed. Lobes distinctly tubular, narrow, straight to slightly recurved. Lobes mostly free and widely divergent. Sometimes joined at base and appearing forked. Lobes terminating in a shallow slightly flanged cup around the ostiole.	Glabrous. Ostiole with dense plug of short white hairs.	8.7 x 1.4 (single chamber) Multi-chambered forked floret galls up to 13 mm wide. n=30	Terminal on lobes	woody	1	1- 4 x =1.4 ± 0.6	F	Adults not reared.

Table 2.6. Comparison of gall morphology and biology of tentative 'floret galler' species associated with southern Australian *Acacia*.

Cecidomyiid Species *Voucher No.* [1]	Common Name (code) [2]	Primary hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host organ	Larvae/adult emergence. Comments
Dasineura sp. 3195, 3185 3011, 3018, 3015	Tiny Floret Galler (TPG)	*A. mearnsii* *A. parramattensis* *A. deanei* *A. irrorata*	U	9.6 x 10.1 n=160	green	Galls a globose cluster of tightly packed floret galls. Floret galls obovate, pyriform to irregularly globose. Bulging asymmetrically at base. Apex broadly acute, often tapered with a short flattened keel.	Hoary, white, appressed hairs. Becoming pale yellow-orange at base of floret gall and perimeter of ostiole	4.2 x 3.1 n=55	Basal. Posterior surface around pedicel.	semi-hard	1	1-5 x =2.0 ± 1.0 n=55	F	Larvae emerge June/July Adults emerge Sept.-Nov. Naturalised in Western Australia. Floret galls up to 30/gall. Figures 2.2b, 2.3a-c
Dasineura sp. 3208/b 3245	Egg Galler (EG)	*A. mearnsii* *A. parramattensis*	U	4.3 x 6.6 n=25	green	Loosely arranged floret galls. Floret galls globose – elliptic. Obtuse apex and posterior. Smooth to slightly wrinkled surface. Simple hairs over chamber position.	White, sparsely pubescent.	2.6 x 2.5 n=24	Lateral. Floret galls erupting along T-Y shaped sutures running horizontally or diagonally across floret gall. No well defined ostiole.	semi-hard	1	1-3 x =1.5 ± 0.6 n=80	F	Larvae emerge April-June Adults emerge October. Co-occurs with TPG. No intermediates. Adults small. Wide latitudinal and altitudinal range. Floret galls up to 14 /gall. Figures 2.3d-f
Dasineura sp. 3195	Tuberculate Tiny Floret Galler (TTPG)	*A. irrorata*	U	5.9 x 6.5 n=17	green. Crimson -purple in basal region	Globose clusters of packed floret galls. Floret galls ovate, often laterally compressed. Tapering abruptly to an acute apex. Apex often curved and flattened forming part of a keel extending along the length of floret gall on one side. Conspicuously tuberculate.	Sparsely tomentose, becoming dense and silky at floret gall apex. Hairs white, sometimes ferruginous.	2.9 x 2.2 n=20	Basal. Posterior surface around pedicel.	semi-hard	1	1-2 x =1.1 ± 0.3 n=20	F	Larvae emerge June-July. Co-occurs with TPG on *A. irrorata*
Dasineura sp. 3196	Mossy Floret Galler (MPG)	*A. irrorata*	U	10.3 x 13.1 n=10	yellow-green	Floret galls loosely clustered. Globose-elliptic. Mostly with flattened keel and well developed in some. Dense, erect, thickened papillae over surface, longer around ostiole region. Papillae simple, irregularly lobed or branched.	Papillae with antrorse pubescent hairs but erect at apex, appearing frimbriate. Orange-yellow, particularly around ostiole region.	10.1 x 4.2 n=23	Lateral. Single central ostiole rupturing to reveal shallow chamber compartments.	semi-hard	1-several	N/A	F	Larvae emerge July-Sept. No adults reared. Highly distinctive. Floret galls up to 4 per gall cluster Figure 2.2c

Table 2.7. Comparison of gall morphology and biology of tentative 'inflated gallers' associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary Hosts[3]	Gen.[4]	Mean gall cluster size (mm) length x width	Fresh floret gall Colour	Floret gall[5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall[6]	Host organ	Adult/larvae emergence. Comments
Dasineura sp. 3138, 3149, 3160, 3148	Hairy Inflated Galler (HIG)	*A. baileyana* *A. decurrens* *A. dealbata* *A. filicifolia*	U	15.1 x 17.3 n=20	pale green	Galls globose cluster of tightly appressed floret galls. Floret galls irregularly convoluted, bullate, dorsally compressed. Hollow and inflated. Often with short flattened keel on apex. Tapered to pedicel. Up to 28 floret galls/gall cluster. Chamber a shallow depression on ventral surface of floret gall. Floret galls persistent at maturity.	Variable. Villose – woolly,sometimes sparsely pilose-pubescent. Indumentum most developed in basal half. Dorsal surface can be glabrous with slight glume.	6.4 x 7.8 n=45	absent	soft, spongy, inflated.	Gregarious on ventral surface of floret gall	None	F	Adults emerge May-August. Larvae pupate in gall or soil. Polyphagous. Naturalised in Western Australia. Numerous secondary hosts: *A. blayana, A. frimbriata, A. paramgusta, A. leucoclada, A. leptoclada, A. lineartifolia, A. prominens, A. polybotrya.* Figure 2.2g
Dasineura sp. 3155	Small Inflated Galler (SIG)	*A. oshanesii*	M	6.6 x 8.3 n=10	white-cream	Galls globose with tightly appressed floret galls. Obovate-globose. Sometimes with apical flattened keel. One chamber per floret gall. Shallow, sometimes a deep groove. Up to 20 floret galls/gall cluster.	Pillose-woolly. White when immature, becoming pale ferruginous.	4.5 x 4.2 n=10	basal.	soft. Slightly inflated	?single.	1	F	Adults emerge all months. Pupation occurs in indumentum around ostiole or amongst floret galls. Monophagous. Figure 2.2i
Dasineura sp. 3145	Paradox Inflated Galler (PIG)	*A. paradoxa*	?U	14.9 x 8.6 n=10	frosty green	Globose galls. Floret galls, appressed, ovate. Obtuse, centrally curved. Bluntly acute often with persistent style or short tapered keel. Up to 27 floret galls/gall cluster. Floret galls soft, with enlarged locus, but not inflated. Stamens persisting amongst floret galls. Chamber a horizontal groove or depression near pedicel. Floret gall readily deciduous at maturity.	Villose-woolly. White appearing frosty.	4.0 x 2.8 n=10	basal	soft	?gregarious	1-2	F	Adults not reared. Larvae migrate from gall and pupate in soil. Common in South Australia and occasional on western slopes, NSW. Monophagous. Figure 2.2h

Table 2.8. Description of gall morphology and biology of the 'hollow galler' associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary Hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host organ	Adult/larvae emergence Comments
Dasineura sp. 2776, 2787,2789, 2791, 3024, 3150, 2810	Hollow Galler (HG)	*A. baileyana* *A. spectabilis* *A. dealbata* *A. polybotrya* *A. leucoclada* *A. decurrens*	?U	4.7 x 5.2 n=4	green	Galls of loosely arranged floret galls Floret galls small, globose-spherical, hollow, thin-walled. Style often persistent or with rudimentary keel on apex. Stamens generally persistent amongst floret galls. Readily deciduous when mature. Up to 10 floret galls/gall cluster.	Glabrous to sparsely villous. Indumentum most developed around ostiole.	2.7 x 2.7 n=23	Lateral in basal 1/3 of floret gall. Narrow slit or lip-like projection, sometimes developing into short neck.	soft	1-4	1	F	Adults not reared. Larvae pupate in soil. Restricted to Botrycephalae in eastern Australia. Probably a single polyphagous species. Figure 2.2f

Table 2.9. Comparison of gall morphology and biology of tentative 'pouch galler' species associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary Hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host organ	Adult/larvae emergence Comments
Dasineura sp. 3131, 3140, 3152	Pouch Galler (PG)	*A. terminalis* *A. leucoclada* *A. rubida* *A. salicina* *A. buxifolia*	?U	A. terminalis 6.1 x 11.1 n=15 A. salicina/buxifolia 4.0 x 5.9 n=10	green-pale green. Can be red-tinged.	Galls with loose clusters of floret galls. Floret galls oblate, globose to broadly elliptic. Flattened keel along length of floret gall and extending onto apex. Style often persistent. Base cordate. Floret galls up to 8/gall cluster. Mature floret galls dehisce readily.	Usually glabrous. Smooth, shiny.	Highly variable, depending on host. *A. terminalis* 7.5 x 6.7 n=15 *A. salicina* 3.5 x 3.1 n=19	Ventral rim surrounding pedicel.	semi-soft to fleshy	1-4	1	F	No adults reared. Larvae emerge Sept.-Nov. Further collections may establish the presence of several taxa in this group.
Dasineura sp. 2570	Pouch Galler (PG)	*A. verticillata*	U	9.6 x 15.1 n=3	green	Floret galls irregularly globose-oblate. Often dorso-ventrally compressed. Irregularly bulging. Apex often with flattened keel.	Sparsely-densely pilose.	5.3 x 6.9 n=11	Ventral rim surrounding pedicel.	semi-soft	several	1	F	No adults reared. Larvae emerge Sept.-Oct. Further collections required to clarify status.
Dasineura sp. 2582	Western Pouch Galler (WPG)	*A. cochlearis* *A. littorea*	?U	N/A	green	Floret galls globose, irregularly bulged. Apex with flattened keel extending along lateral margin to form slight keel.	Sparsely pubescent. Hairs appressed.	7.5 x 6.2 n=4	Ventral rim surrounding pedicel	semi - soft	several	1	F	No adults reared. Larvae emerge Oct.-Dec.

Table 2.10. Comparison of gall morphology and biology of tentative 'groove gallers' associated with southern Australian *Acacia*.

Cecidomyiid Species Voucher No. [1]	Common Name (code) [2]	Primary Hosts [3]	Gen. [4]	Mean gall cluster size (mm) length x width	Fresh floret gall colour	Floret gall [5] shape	External indumentum	Mean floret gall size (mm) length x width	Ostiole position	Floret gall texture	Larvae per chamber	Number of chambers per floret gall [6]	Host organ	Adult/larvae emergence Comments
Dasineura sp. 3139, 2749, 2814	Groove Galler (GG)	polyphagous	?M	N/A. Gall is composed of florets with slightly swollen ovaries.	pale green	Ovary slightly swollen, thickened or deformed. Sometimes no deformation apart from basal groove or depression. Ovary persists amongst dry perianth. Readily deciduous at maturity.	Variable depending on ovary of host. No apparent modification to host indumentum.	2.1 x 1.7 n=23	Open groove or depression at base of ovary	-	1	1	F	Adults emerge during flowering of host. Polyphagous on non-*Botrycephalae*. Recorded hosts: *A. alata, A. drummondii, A. extensa, A. genistifolia, A. longifolia, A. linifolia , A. myrtifolia, A. saligna, A. sophorae, A. urophylla.*
Dasineura sp. 3200, 3220	Groove Galler (GG)	*A. mearnsii* *A. irrorata*	?U	As above	pale green	As above	As above	1.7 x 1.3 n=14	As above	-	1	1	F	Adults emerge ?April-June. DNA sequence data suggests this is a distinct taxon from above. Rare, but probably over looked. Figure 2.3I-j

Definitions and legend for tables 2.3 - 2.10

1 = Authors collection number, accessible in Access database where detailed collection information is provided,

2 = abbreviated common name used as a standard in text, figures and tables, 3 = Primary host=most commonly encountered host plant in field. Secondary host=hosts occasionally utilised,

4 = U=univoltine, M=multivoltine, B=bivoltine, 5 = Floret gall=modified ovary or receptacle,

6 = Range of chambers per floret gall. Mean (±SD)

7 = Gall=modified bud or pod (gall cluster=cluster of galled ovaries, buds, pods or growth nodes)

Table 2.11. Census of gall-forming Cecidomyiidae found on Australian *Acacia* that are invasive, or potentially so, in South Africa.

Acacia	Common name	*Dasineura*	*Asphondylia*
A. baileyana	Bailey's wattle	Hairy Inflated Galler, Hollow Galler	Pubescent Bud Galler, Bipinnate Seed Galler
A. cyclops	Rooikrans	*D. dielsi*	Seed Galler
A. dealbata	Silver wattle	Hairy Inflated Galler	Pubescent Bud Galler, Bipinnate Seed Galler
A. decurrens	Green wattle	Hairy Inflated Galler, Hollow Galler	Pubescent Bud Galler, Bipinnate Seed Galler, Glabrous Bud Galler
A. elata	Cedar wattle	Common Fluted Galler	-
A. implexa	Screw-pod wattle	*D. acaciaelongifoliae*	-
A. irrorata	Green wattle	Elongate Fluted Galler, Mossy Floret Galler, Tiny Floret Galler, Tuberculate Tiny Floret Galler, Groove Galler	Glabrous Bud Galler, Pubescent Bud Galler, Bipinnate Seed Galler
A. longifolia	Long-leaved wattle	*D. acaciaelongifoliae*, Groove Galler	Eastern Bud/Seed Galler
A. mearnsii	Black wattle	Elongate Fluted Galler, Tiny Floret Galler, Common Fluted Galler, Egg Galler, Groove Galler	Glabrous Bud Galler, Pubescent Bud Galler, Bipinnate Seed Galler
A. melanoxylon	Blackwood	Fluted Galler, Common Fluted Galler	Eastern Bud/Seed Galler
A. paradoxa	Kangaroo thorn	Paradoxa Inflated Galler	Eastern Bud/Seed Galler
A. pycnantha	Golden wattle	Common Fluted Galler	Eastern Bud/Seed Galler
A. saligna	Port Jackson willow	Groove Galler	-

Table 2.12. Altitudinal range of gall-forming Cecidomyiidae from *A. mearnsii*

Cecidomyiid	Common name	Altitudinal Range (m)	n
Dasineura sp.	Elongate Fluted Galler	24 - 1069	18
Dasineura sp.	Common Fluted Galler	48 - 364	14
Dasineura sp.	Tiny Floret Galler	17 - 970	52
Dasineura sp.	Egg Galler	80 - 1069	6
Dasineura sp.	Groove Galler	135	1
Asphondylia sp.	Glabrous Bud galler	54 - 637	30
Asphondylia sp.	Pubescent Bud Galler	55 - 637	19
Asphondylia sp.	Bipinnate Seed Galler	73 - 731	10

Figure 2.2 (A-G) Gall-forming cecidomyiids from southern Australian *Acacia*. (A) *D. dielsi* from *A. cyclops* at Moonta, SA; (B) Tiny Floret Galler on *A. mearnsii* at Jarrahdale, WA; (C) Mossy Floret Galler on *A. irrorata* at Boonoo Boonoo, NSW; (D) Western Grey Fluted Galler on *A. aneura* at Parkes, NSW; (E) *D. acaciaelongifoliae* on *A. longifolia* at Nowra, NSW; (F) Hollow Galler on *A. baileyana* at Canberra, ACT; (G) Hairy Inflated Galler on *A. baileyana* at Mt Barker, WA.

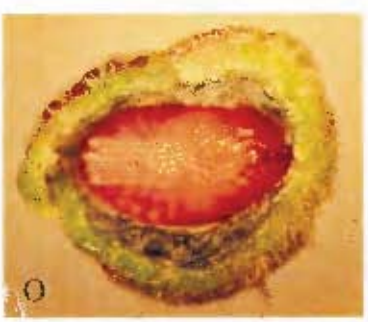


Figure 2.2 (H-O) Gall-forming cecidomyiids from southern Australian *Acacia*. (H) Paradoxa Inflated Galler on *A. paradoxa* at Goobang Creek, NSW; (I) Small Inflated Galler on *A. oshanesii* at Woolhi, NSW; (J) Eastern Bud-Seed Galler from *A. sophorae* at Bicheno, Tasmania; (K) Pubescent Bud Galler from *A. parvipinnula* in NSW; (L) Eastern Bud-Seed Galler on *A. melanoxylon* at Melbourne, VIC; (M) Seed Galler on *A. mearnsii* at Canberra, ACT; (N) Seed Galler on *A. rostellifera* at Moonta, SA; (O) dissected Glabrous Bud Gall from *A. irrorata*.

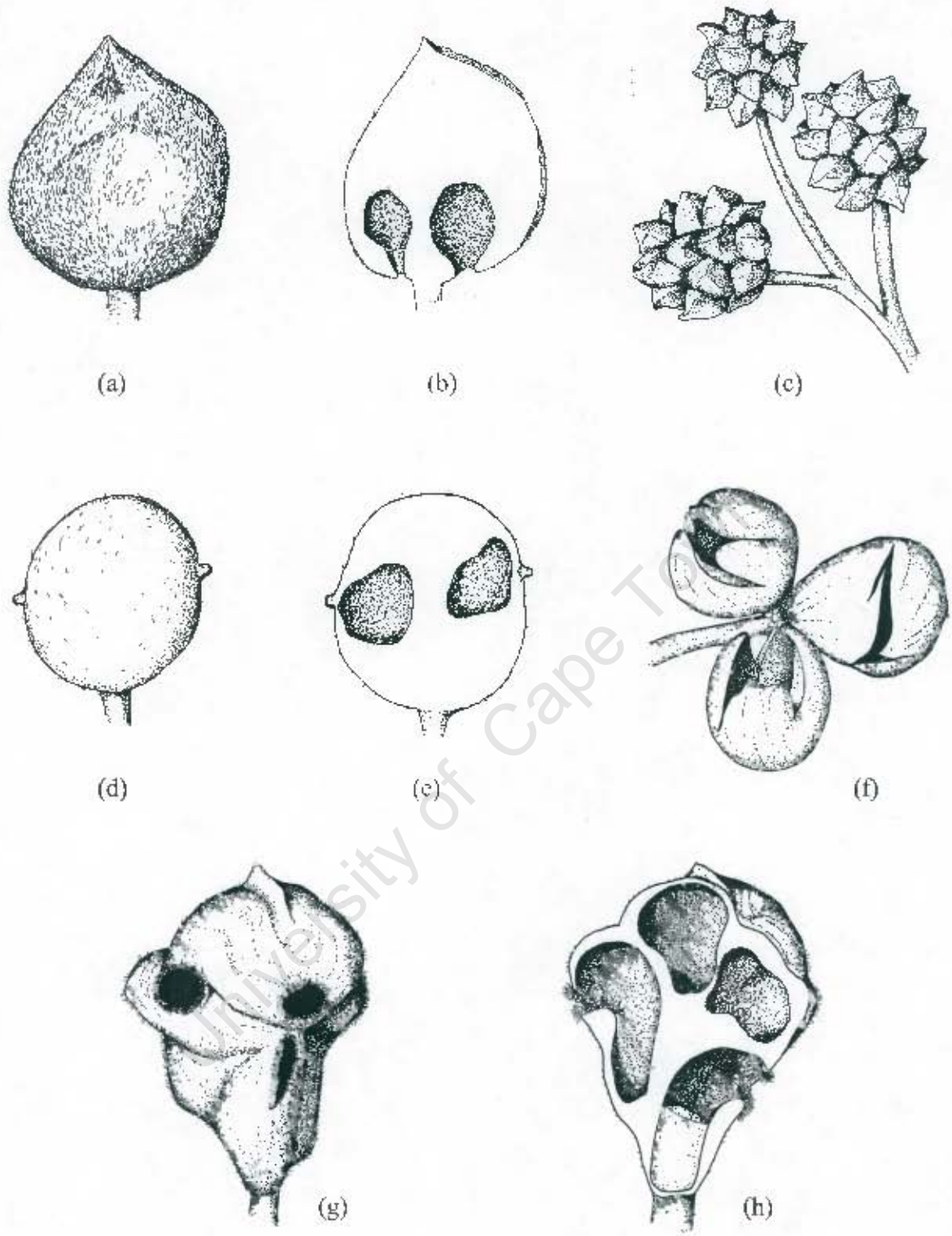


Figure 2.3. Cecidomyiid galls of *A. mearnsii* (a-o) and *A. cyclops* (p). Tiny Floret Galler (a-c): (a) floret gall (x9), (b) longitudinally dissected floret gall (x9), (c) gall cluster (x1.8); Egg Galler (d-f): (d) floret gall (x10), (e) longitudinally dissected floret gall (x10), (f) gall cluster (x8); Common Fluted Galler (g-h): (g) floret gall (x7), (h) longitudinally dissected floret gall (x7); Groove Galler (i-j): (i) floret gall (x24), (j) gall cluster; (k) Elongate Fluted Galler floret gall (x3.5); (l) Pubescent Bud Galler galls, LHS showing emergence hole (x12); (m) Glabrous Bud Galler galls (x8); (n) Elongate Fluted Galler, longitudinally dissected floret gall (x3.5); (o) Pubescent Bud Galler, longitudinally dissected galls (LHS an emerged gall, RHS showing enlarged stamens and larva) (x12); (p) *Dasineura dielsi* floret gall, longitudinally dissected showing cocoons in developmental chambers (x3).

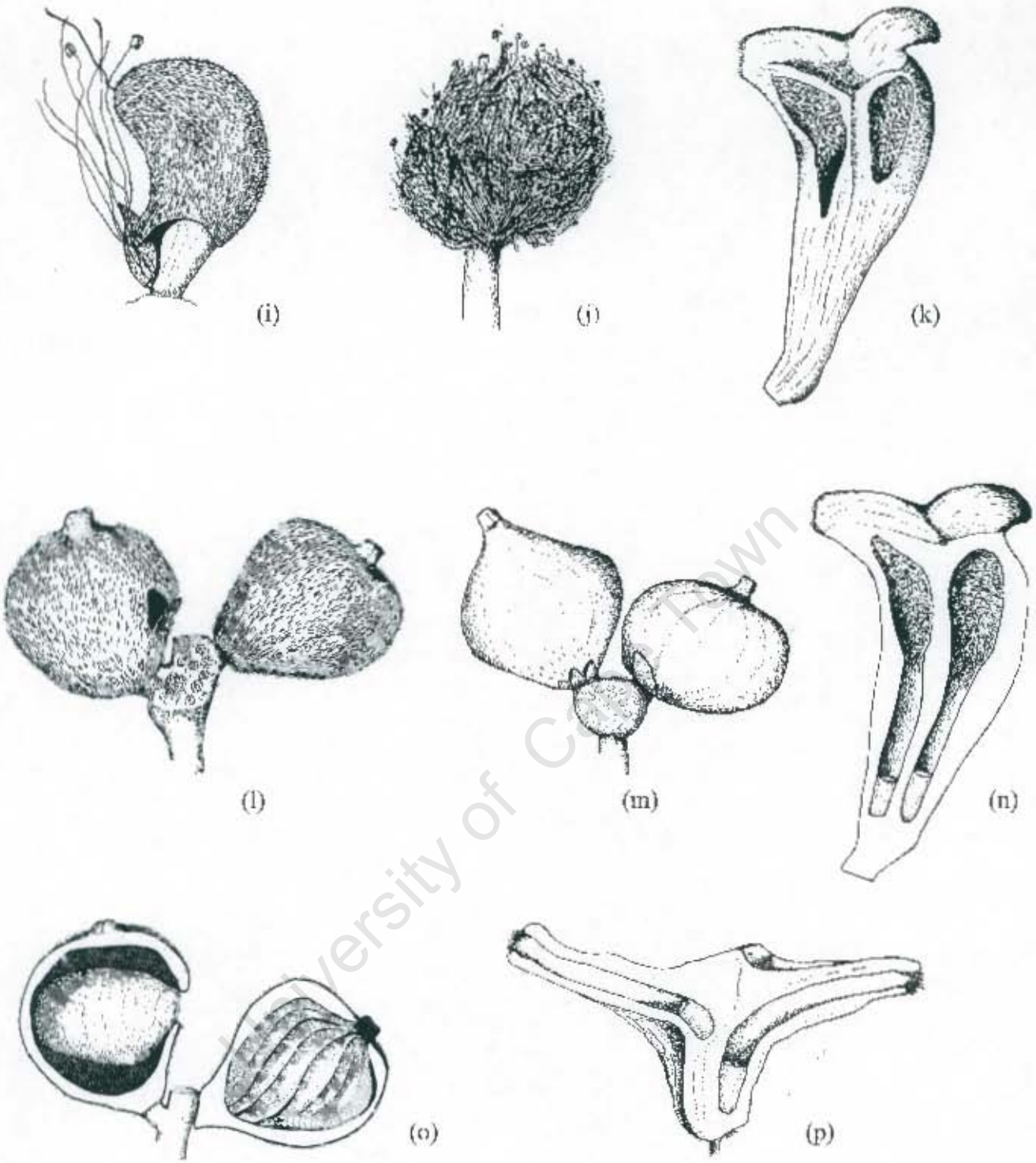


Figure 2.3. Cecidomyiid galls of *A. mearnsii* (a-o) and *A. cyclops* (p). Tiny Floret Galler (a-c): (a) floret gall (x9), (b) longitudinally dissected floret gall (x9), (c) gall cluster (x1.8); Egg Galler (d-f): (d) floret gall (x10), (e) longitudinally dissected floret gall (x10), (f) gall cluster (x8); Common Fluted Galler (g-h): (g) floret gall (x7), (h) longitudinally dissected floret gall (x7); Groove Galler (i-j): (i) floret gall (x24), (j) gall cluster; (k) Elongate Fluted Galler floret gall (x3.5); (l) Pubescent Bud Galler galls, LHS showing emergence hole (x12); (m) Glabrous Bud Galler galls (x8); (n) Elongate Fluted Galler, longitudinally dissected floret gall (x3.5); (o) Pubescent Bud Galler, longitudinally dissected galls (LHS an emerged gall, RHS showing enlarged stamens and larva) (x12); (p) *Dasineura dielsi* floret gall, longitudinally dissected showing cocoons in developmental chambers (x3).

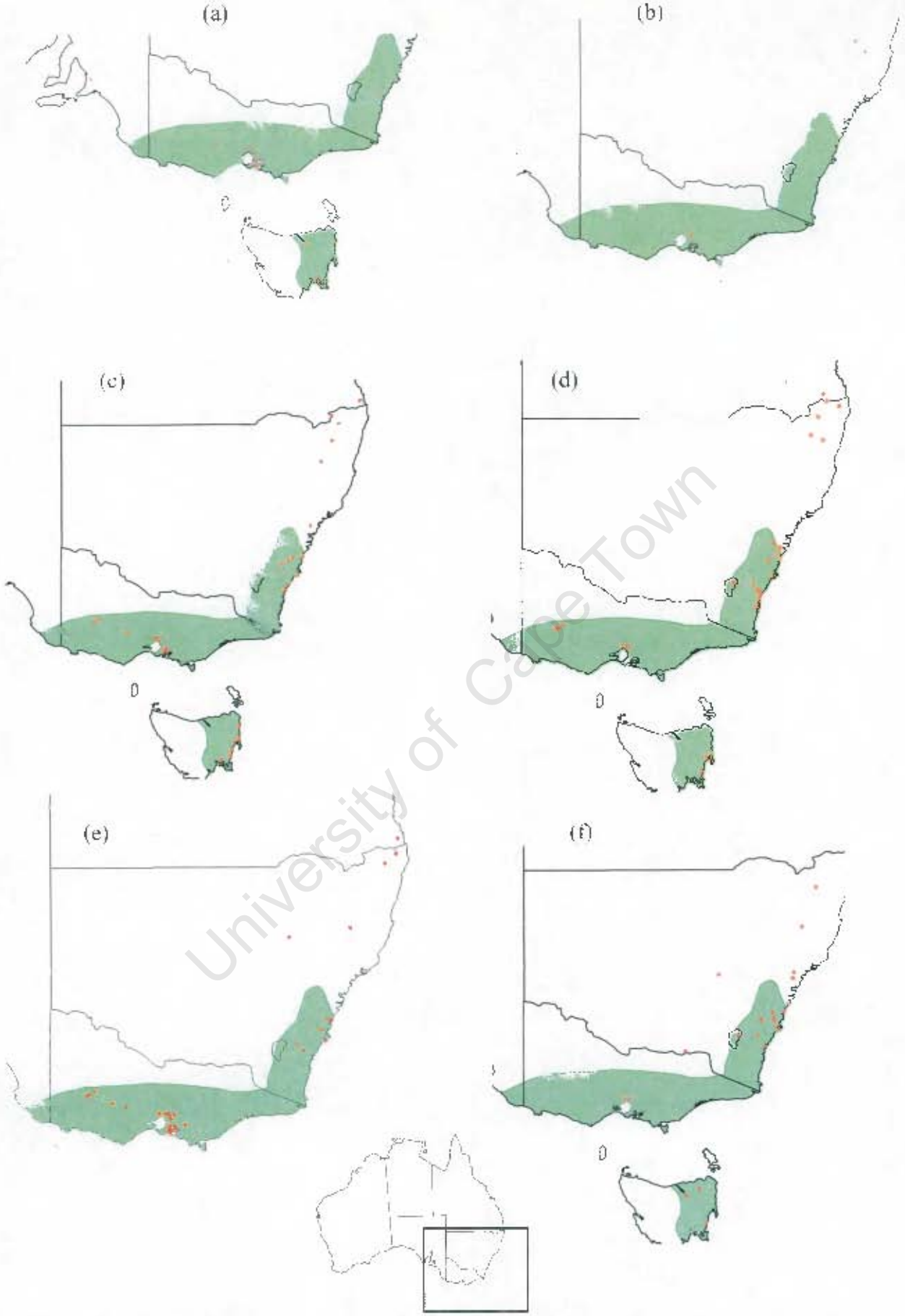


Figure 2.4 (a-f) Distribution of gall-forming cecidomyiids from *A. mearnsii* (a) *Dasineura* sp. (Common Fluted Galler), (b) *Dasineura* sp. (Egg Galler), (c) *Dasineura* sp. (Elongate Fluted Galler), (d) *Asphondylia* sp. (Glabrous Bud Galler), (e) *Asphondylia* sp. (Pubescent Bud Galler), (f) *Asphondylia* sp. (Bipinnate Seed Galler). Shaded area = natural distribution of *A. mearnsii*

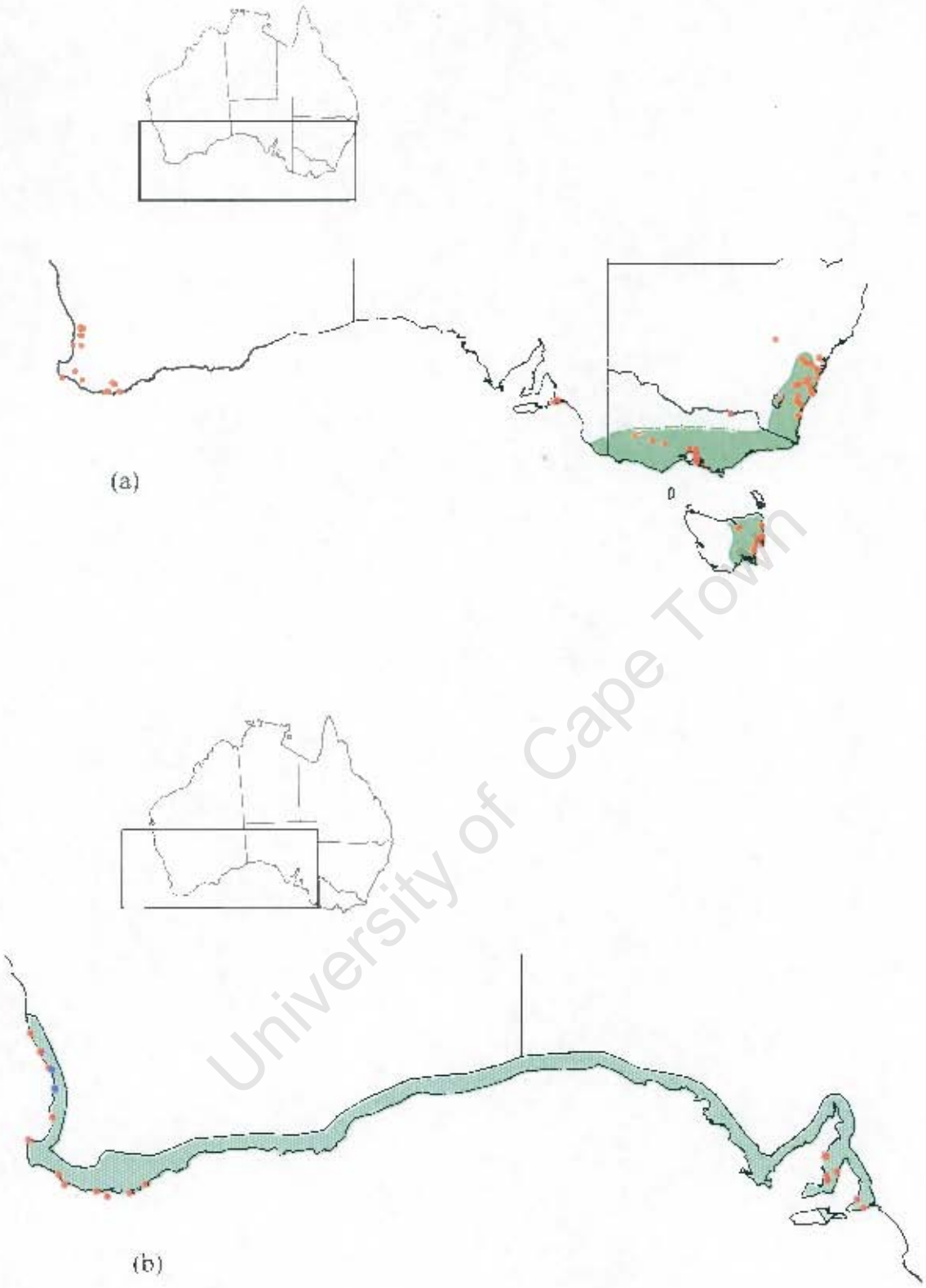


Figure 2.5 (a-b). Distribution of gall-forming cecidomyiids from *A. mearnsii and A. cyclops*. (a) *Dasineura* sp. (Tiny Floret Galler) and the distribution of *A. mearnsii*, (b) *Dasineura dielsi* (Small Fluted Galler) and the distribution of *A. cyclops*.

2.7%, except for clade C where the Pubescent Bud Galler from *A. baileyana* (3115, 2641) differed by 3.2-3.4% from other clade C accessions, including an additional collection of the Pubescent Bud Galler from *A. baileyana* (3116). The mean among-species sequence divergence value was 9.4%.

Dasineura

Maximum parsimony analyses recovered 962 equally parsimonious trees (tree length = 223; CI = 0.51, RI = 0.77). All methods of phylogenetic reconstruction produced similar topologies with most significantly or strongly supported nodes in the Bayesian majority rule consensus recovered by all methods (figure 2.8). Deeper nodes in the topology were unresolved polytomies, with the placement of many taxa unresolved (e.g. 3030, 2570).

The Bayesian and ML phylograms are characterized by short internal branches indicating limited sequence differences between most groups. However, several basic groupings are apparent. Clade A (BA PP = 100%, MP BS = 96%) contains Fluted Gallers and Groove Gallers (GG) clearly separated into several sub-clades grouped according to gall structure. Clade A1 (BA PP = 97%, MP BS = 85%) contains Fluted Gallers from *A. maidenii, A. omalophylla* and *A. pendula*. Clade A2 (BA PP = 100%, MP BS = 97%) contains the Common Fluted Galler (CFG) from several acacias. Clade A3 (BA PP = 70%, MP BS = 70%) groups the Elongate Fluted Galler (EFG) from *A. mearnsii* and *A. irrorata* whereas, Clade A4, (BA PP = 61%, ML = 52%, MP BS = 80%) which forms the sister group to all gallers in Clade A, contains all Groove Gallers (GG) from non-*Botrycephalae* acacias. The placement of several accessions, including *D. dielsi* from *A. cyclops* was unresolved. Accession 2574 had a 100% sequence match with galls with very similar morphological characters, including 2626 and 3157 from *A. sophorae,* which are recognized as *D. acaciaelongifoliae*. Clade B (BA PP = 89%) includes all accessions of the Tiny Floret Galler (TFG) and Egg Gallers (EG) grouped into subclade B1 (BA PP = 100%, ML = 71%, MP BS = 90%), and Mossy Floret Gallers (MFG) into subclade B2 (BA PP = 99%, ML = 97%, MP BS = 96%). Clade C (BA PP = 100%, ML BS = 84%,) contains most Hollow Gallers (HG) together with an Egg Galler from *A. parramattensis*, which is enigmatic given that all other Egg Gallers fall into clade B. Clade D (BA PP = 100%, ML BS = 100%, MP BS = 100%) is basal and contains a set of closely aligned Fluted Gallers that span southern Australia and are phylogenetically distant from fluted gallers with very similar morphology contained in clade A.

Inflated galls occur in three separate clades: accession 3155 in clade B which occurs as a distinct entity on *A. oshanesii*, accession 3145 on *A. paradoxa* in an unresolved position near clade C, and several accessions from *Botrycephalae Acacia* in eastern Australia that are

Table 2.13. *Acacia* and close relatives surveyed for gall-forming Cecidomyiidae in South Africa.

Host	Subgenus / Section	No. survey sites	Host	Section	No. survey sites	Host	Section	No. of survey sites
AFRICAN ACACIA			*A. nilotica*	Acacia / -	29	*A. dealbata*	Phyllodineae / Botrycephalae	1
A. ataxacantha	?	3	*A. permixta*	Acacia / -	2	*A. deanei*	Phyllodineae / Botrycephalae	1
A. brevipsica	Aculeiferum / Monacanthea	1	*A. robusta*	Acacia / -	6	*A. decurrens*	Phyllodineae / Botrycephalae	1
A. burkei	Aculeiferum / Aculeiferum	5	*A. schweinfurthii*	Aculeiferum / Monacanthea	1	*A. elata*	Phyllodineae / Botrycephalae	1
A. caffra	Aculeiferum / Aculeiferum	10	*A. sieberiana*	Acacia / -	9	*A. implexa*	Phyllodineae / Phyllodineae	1
A. erioloba	Acacia / -	5	*A. senegal*	Aculeiferum / Aculeiferum	1	*A. irrorata*	Phyllodineae / Botrycephalae	1
A. erubescens	Aculeiferum / Aculeiferum	8	*A. tortilis*	Acacia / -	17	*A. leucoclada*	Phyllodineae / Botrycephalae	1
A. exuvialis	Acacia /-	3	*A. xanthophloea*	Acacia / -	5	*A. mearnsii*	Phyllodineae / Botrycephalae	63
A. galpinii	Aculeiferum / Aculeiferum	2	AFRICAN MIMOSACEAE			*A. melanoxylon*	Phyllodineae / Plurinerves	5
A. gerrardii	Acacia / -	6	*Albizzia brevifolia*	-	1	*A. nanodealbata*	Phyllodineae / Botrycephalae	1
A. grandicornuta	Acacia / -	1	*A. harveyi*	-	2	*A. parramattensis*	Phyllodineae / Botrycephalae	1
A. hebeclada	Acacia / -	1	*Dichrostachys cinerea*	-	24	*A. parvipinnula*	Phyllodineae / Botrycephalae	1
A. hemitoxylon	Acacia / -	2	*Faidherbia albida*	-	3	*A. pycnantha*	Phyllodineae / Phyllodineae	1
A. karroo	Acacia/-	60	AUSTRALIAN ACACIA			*A. schinoides*	Phyllodineae / Botrycephalae	1
A. luederitzii	Aculeiferum / Aculeiferum	2	*A. binervata*	Phylloidineae / Phyllodineae	1	*A. sylvestris*	Phyllodineae / Botrycephalae	1
A. mellifera	Aculeiferum / Aculeiferum	7	*A. cyclops*	Phyllodineae / Plurinerves	36	*A. trachyphloia*	Phyllodineae / Botrycephalae	1
A. nigrescens	Aculeiferum / Aculeiferum	10	*A. dangarensis*	Phyllodineae / Botrycephalae	1			

Figure 2.6 (a-h) Gall-forming cecidomyiids from South African *Acacia*. (a) *Asphondylia* sp. (Bead Galler) on *A. caffra* near Potgieterus and (b) *A. luederitzii* near Thabazimbi; (c) Bracteose Node Galler on *A. tortilis* at Steenbok Pan; (d) *Contarinia* sp. (Lumpy Node Galler) (left) and ?*Contarinia* sp. (Barnacle Galler) (right) on *A. mellifera* near Warmbaths; (e) *Acacidiplosis* sp. (Durian Galler) on *A. tortilis* at Ben Alberts Nature Reserve; (f) *Contarinia* sp. (Receptacle Galler) on *A. erioloba* at Klein Pella; (g) *Aposchizomyia* sp. (Minute Floret Galler) on *A. nilotica* at Kruger National Park; (h) Knobby Hair Tuft Galler on *A. nigrescens* near Hoedspruit.

Table 2.14. Comparison of tentative gall-forming Cecidomyiidae species occurring on inflorescences of South African *Acacia*

Cecidomyiid Species *Voucher No.* [1]	Common Name	Primary Hosts [2] (section)	Gen.[3]	Fresh gall colour	Gall shape	External indumentum	Mean gall size (mm) length x width	Floret gall texture	Larvae per chamber	Host organ	Adult/larvae emergence Comments
Asphondylia sp. 3263, 3264	Bead Galler	*A. nigrescens* *A. caffra* *A. erubescens* *A. luederitzii* *(Aculeiferum)*	U	Grey-green	Pyriform. Globose base abruptly contracting to a short, stout apical mucro. Perianth remain often around base of gall.	Marginally flaky. Sparsely pubescent. Puberulous on *A. luederitzii*	6.3 x 3.9 n=14	woody	1	ovary	Adults emerge July-August. Restricted to lowveld. Galls closely resemble *A. napiformis* from *A. mellifera* (Gagne & Marohasy 1993). Figures 2.6a-b
Asphondylia sp. 3057, 3055, 3054	Bud Galler	*A. karroo* *(Acacia)*	U-?B	green	Swollen buds projecting conspicuously from uninfected buds. Broadly elliptic-orbiculate. Apex bluntly acute.	glabrous	2.5 x 0.9 n=5	soft	1	bud	Adults emerge December – January. Apparently restricted to Western Cape, but easily overlooked and could be more widely spread.
Asphondylia sp. 3305	Receptacle Galler	*A. karroo* *(Acacia)*	?U	-	Slightly swollen or disfigured flower-head buds	-	-	soft	1-3	buds	Adults emerge September. Galls symptoms cryptic and easily overlooked. May be widespread. Only found in Gauteng.
Aposchizomyia sp. 2853, 3229	Minute Floret Galler	*A. nilotica* *(Acacia)*	U	?	Swollen ovary. Broadly elliptic to orbiculate-globose. Slight lateral lobe dorsal half. Protruding slightly from perianth.	puberulous to woolly	1.8 x 1.7 n=10	soft-semi-soft	1	ovary	Adults emerge November. Secondary host: *A. exuvialis* Larvae pupate within chamber.
? 3094	Small Receptacle Galler	*A. grandicornuta* *(?)*	?U	?	Swollen, irregularly lobed receptacle. Floret scars present on exterior surface. One to 7 chambers united. Peduncle often bent or curved.	glabrous	3.7 x 4.0 n=10	woody	?1 per chamber.	receptacle	Adults not reared. Possible emerging Nov. –Dec. Larvae pupate within chamber.
Acacidiplosis sp. 3061, 2858, 2856, 3062, 3064	Durian Galler	*A. tortilis* *(Acacia)*	?U	green	Swollen spherical vegetative or floral buds. Conspicuously echinulate. Thickened bracts pubescent, slightly curved.	bracts pubescent	7.5 x 8.0 n=16	semi-soft	1	bud	Adults emerge November-February. Secondary hosts: *A. nilotica, A. gerradii* Occurs on vegetative and floral buds. Widespread. Larvae pupate within chamber. Possibly *A. verticillata* of Gagne & Marohasy (1993). Figure 2.6e
Contarinia sp.	Receptacle Galler	*A. erioloba* *(Acacia)*	?U	-	Slightly distorted buds or flowers.	normal	N/A	soft	?1	receptacle	Adults emerge October. White larvae pupate in soil. Figure 2.6f
Contarinia sp. 3216	Lumpy Floret Galler	*A. mellifera* *(Aculeiferum)*	U	grey-green, red tinged	Globose, irregularly lobed ovaries. Floret galls in irregular arranged clusters. One-18 united chambers per floret gall. Ostioles rimmed with recurved, thickened yellowish hairs and surrounding shorter erect hairs. Ostioles resemble sea anemones. Floret galls persistent.	glabrous	6.9 x 8.9 n=12	woody	1	ovary	Adults emerge August-September. Larvae pupate within chamber. Common on *A. mellifera* in Northern Cape. Figure 2.6d
?*Contarinia* sp. 3067	Barnacle Galler	*A. mellifera* *(Aculeiferum)*	U	reddish brown	Globose, irregularly lobed ovaries. Floret galls in irregularly arranged clusters. Floret galls with thickened, laterally compressed ± parallel aligned platelets, resembling barnacles. Floret gall chamber walls united.	glabrous	8.3 x 10.2 n=10	woody	1	ovary	No adults reared. Co-occurs with *Contarinia* sp. on *A. mellifera.* Figure 2.6d
? 3092, 3227	Knobby Hair Tuft Galler	*A. nigrescens* *(Aculeiferum)*	U	brown-green	Irregular clusters of swollen floret galls. Floret galls irregularly lobed. Lobes terminating in conspicuous tuft of dense, erect ferruginous hairs-bristles. Larvae develop in chamber beneath hairs. Chambers 1-several, walls united.	Densely villous around chamber. Flaky elsewhere.	Variable. 8.1 x 10.3 n=13	woody	1	ovary	Adults not reared but probably emerge August-September. Galls developing on ovaries, stem nodes and occasionally leaves. Figure 2.6h
? 3096. 3097	Pinebud Galler	*A. tortilis* *(Acacia)*	?U	grey, grey-brown	Globose clusters of distorted buds. Galls conical-oblong. Conspicuously bracteose; bracts imbricate, appressed, frimbriate. Resembling young pine buds.	Bracts fringed with white, shiny hairs. Sparsely hirsute-glabrous elsewhere.	3.8 x 2.0 n=8	semi-soft	1	bud	Adults not reared. Adults emerge at apex. Galls resemble *Apaschizomyia acuta* from *A. nilotica* (Gagne & Marohasy 1993).

Table 2.15. Comparison of tentative gall-forming Cecidomyiidae species occurring on nodes of South African *Acacia*

Cecidomyiid Species Voucher No. [1]	Common Name	Primary Hosts[2] (Section)	Gen.[3]	Fresh gall colour	Gall shape	External indumentum	Mean gall size (mm) length x width	Floret gall texture	Larvae per chamber	Host organ	Adult/larvae emergence Comments
Acacidiplosis sp. 2764	Node Galler	*A. karroo* *(Acacia)*	U	Green. Dark brown when dry.	Irregular clusters of elliptic-oval cells loosely coalesced over node surface. Cells thin-walled with single chamber. Adults emerge through apical lid.	glabrous	2.8 x 1.5 n=6	brittle	1	node buds	Adults emerge September. Rare.
Acacidiplosis sp. 2851, 2899	Lumpy Node Galler	*A. karroo* *(Acacia)*	U	green	Loosely united clusters of swollen node buds. Galls with finger-like projections, united basally, giving a pronounced knobby appearance. Chambers large, united 1- several per gall.	glabrous	9.2 x 9.8 n=9	woody	1	node buds	Adults emerge November-December. Larvae pupate in chamber, adults emerge through apex. Restricted to northern South Africa.
? 2860, 2855	Artichoke Galler	*A. nilotica* *(Acacia)*	?U	green	Swollen node buds. Galls oval-broadly elliptic. Obtuse. Bracteose. Bracts imbricate, appressed, thickened base with acute hyaline apex; frimbriate. Tomentose elsewhere.	tomentose on fleshy bract base.	7.7 x 5.6 n=5	woody	1	node buds	Adults not reared.
? 3074	Hedgehog Galler	*A. karroo* *A. robusta* *(??Acacia)*	?U	green	Swollen node buds. Globose, echinate, bristles straight, slightly ridged. Gall prickly. Single large central chamber.	glabrous	N/A	semi-woody	1	node buds	No adults reared. Distinguished from *Acacidiplosis* sp. on *A. tortilis* by long, stout bristles.
Acacidiplosis sp. 3065, 2857	Bracteose Node Galler	*A. tortilis* *A. gerradii* *A. exuvialis* *(??Acacia)*	?U	green	Swollen node buds. Galls elliptic, conical when immature. Bracteose. Bracts imbricate, appressed, erect, subulate-long acuminate. Woolly between bracts. Single large central chamber. Adults emerge from apex.	woolly between bracts in *A. tortilis*	5.2 x 3.2 n=9	semi-soft	1	node buds	Adults emerge February. Larvae pupate in chamber. *Dichrostachys cinerea* a possible secondary host. Galls node buds and occasional pinnae. Co-occurs with Durian galler on *A. tortilis*. Has gall similarities to *A. ananas* of Gagne & Marohasy (1993). Figure 2.6c

Definitions and legend for tables 2.14 - 2.15

1 = Authors collection number, accessible in Access database where detailed collection information is provided.

2 = Primary host=most commonly encountered host plant in field. Secondary host=hosts occasionally utilised,

3 = U=univoltine, M=multivoltine,

B = bivoltine.

united with Pouch Gallers (PG) at clade E. Inflated Gall morphology has clearly developed from several independent evolutionary events. The two accessions of Inflated Gall from *A. baileyana* (3148, 2602) were placed in separate subclades in clade E and had a sequence divergence of 3.7%. Similarly, two accessions of the Hollow Galler (HG) from *A. polybotrya* (3150, 2810) from the same geographical region of New South Wales were segregated into different clades with a sequence divergence of 6.6%.

Discussion

The gall-forming Cecidomyiidae of *Acacia* from Australia and South Africa share no common species. In Australia, *Dasineura* and *Asphondylia* have radiated on southern *Acacia* hosts exclusively, while in South Africa although both genera are present, *Dasineura* is uncommon and absent on *Acacia*, and *Asphondylia* is species poor and restricted to few hosts. The South African gall-forming cecidomyiid fauna is dominated by *Acacidiplosis*, a genus endemic to southern Africa, and *Contarinia*, which is cosmopolitan and occurs on *Acacia* elsewhere in Africa (Gagné & Marohasy 1993). Further segregation occurs at the supertribe rank with all southern African non-*Asphondylini* from *Acacia* occurring in the *Cecidomyiidi*, while the Australian non-*Asphondylini* fauna is restricted to the *Oligiotrophini* (*Dasineura*). Although the phylogenetic relationship of Australian and South African cecidomyiid species was not examined using molecular techniques, the taxonomic relationships based on morphological characteristics and distributions suggest that independent evolutionary development has occurred within the two continents.

Although south-west Western Australia has the highest diversity of *Acacia* (Hnatiuk & Maslin 1988), the region is relatively poor in indigenous gall-forming cecidomyiids with nine tentative species. Temperate south-eastern Australia has the highest diversity of cecidomyiid from *Acacia*, with the *Botrycephalae* supporting more cecidomyiid taxa (14) than other sections, despite encompassing a relatively small number (42) of *Acacia* species (Miller *et al.* 2003). However, field survey sites were biased towards the Botrycephalae and this may have had an influence on species ratios. Seven of the twenty-four cecidomyiids from eastern Australia were restricted to the *Botrycephalae* with the closely allied *A. mearnsii* and *A. irrorata* supporting more gall-forming cecidomyiids (8 species each) than other *Acacia* from southern Australia. This, together with the basal positioning of most of the *Botrycephalae* cecidomyiids in the DNA-derived phylograms, suggests this section may be the ancestral group for Australian gall-forming cecidomyiids on *Acacia*. However, *A. elata*, probably a basal taxon in the *Botrycephalae* (Ariati 2000) was almost devoid of gall-forming cecidomyiids. The larger host number/cecidomyiid number ratio for species feeding on inflorescences in Australia (101/24) compared to South Africa (13/12) suggests that the

spectacular diversity of Australian *Acacia* either has not been a strong factor in the evolution of this group of insects, or the Australian cecidomyiids on *Acacia* are a relatively young group still undergoing speciation. Poor differentiation within the mitochondrial cytochrome *b* gene of Australian *Dasineura* suggests the latter explanation is more likely.

Gall-forming arthropods manipulate their host's physiology and cell-growth processes to produce a vast array of organized gall structures, each generally regarded as an expression of the cecidogenic agent's genotype (Dawkins 1983, Stern 1995, Crespi *et al.* 1997, Stone & Schönrogge 2003). Molecular recognition of cecidomyiid species within highly variable and sometimes cryptic gall characters provides a useful framework for further investigation into biological, ecological and taxonomic properties. In this case, molecular sequence data from the cytochrome *b* gene of Australian *Asphondylia* and *Dasineura* from *Acacia* validate the use of gall characters for species recognition, particularly in *Asphondylia*, and identifies taxa that cannot be readily recognized by other means. However, apparent anomalies in placement of some *Dasineura* accessions, particularly an inflated gallers from *A. baileyana* (2602,3148), with the same gall morphology and no recognizable differences in adult and larval characteristics (P. Kolesik, pers. comm. 2002), and similarly, accessions of the Hollow Galler from *A. polybotrya* (3150,2810), Egg Gallers from *A. parramattensis* (3190,3187) and Common Fluted Galler (CFG) from *A. deanei* (two accessions of 3142) are puzzling (figure 2.8).

In the case of Hairy Inflated Gallers (HIG) from *A. baileyana*, 100% sequence alignment was found between a collection from this host in New South Wales and from *A. filifolia*, also from the same region. High sequence divergence (3.7%) between New South Wales (3148) and a Victorian accession of Hairy Inflated Galler (HIG) (2602) may be the result of genetic isolation by distance and reflect population extremes rather than affirming any systematic status. It is clear however, that inflated gall morphology has arisen on three separate occasions, typified by one taxon each on *A. paradoxa*, *Botrycephalae* acacias indigenous to eastern Australia, including *A. dealbata, A. decurrens, A. baileyana, A. filifolia* and *A. leucoclada*; and *A. oshanesii.* The latter two taxa in the process of being described using morphological data (P. Kolesik pers. comm. 2003).

Discrepancies in placement of Hollow Gallers, Egg Gallers and Common Fluted Galler are unlikely to be the result of PCR artefact or cross-contamination of samples. A more likely explanation is the presence of cecidomyiid inquilines that have displaced the primary gall inducer e.g. Kolesik & Shuhravá (1997), but this can only be confirmed by detailed examination of insect characters. *Dasineura* is used as a catch-all category (Gagné 1989) and does not necessarily represent a natural grouping of phylogenetically related taxa. However, the short branch lengths of the *Dasineura* phylogram suggest rapid genetic radiation of *Dasineura* on *Acacia* hosts stemming

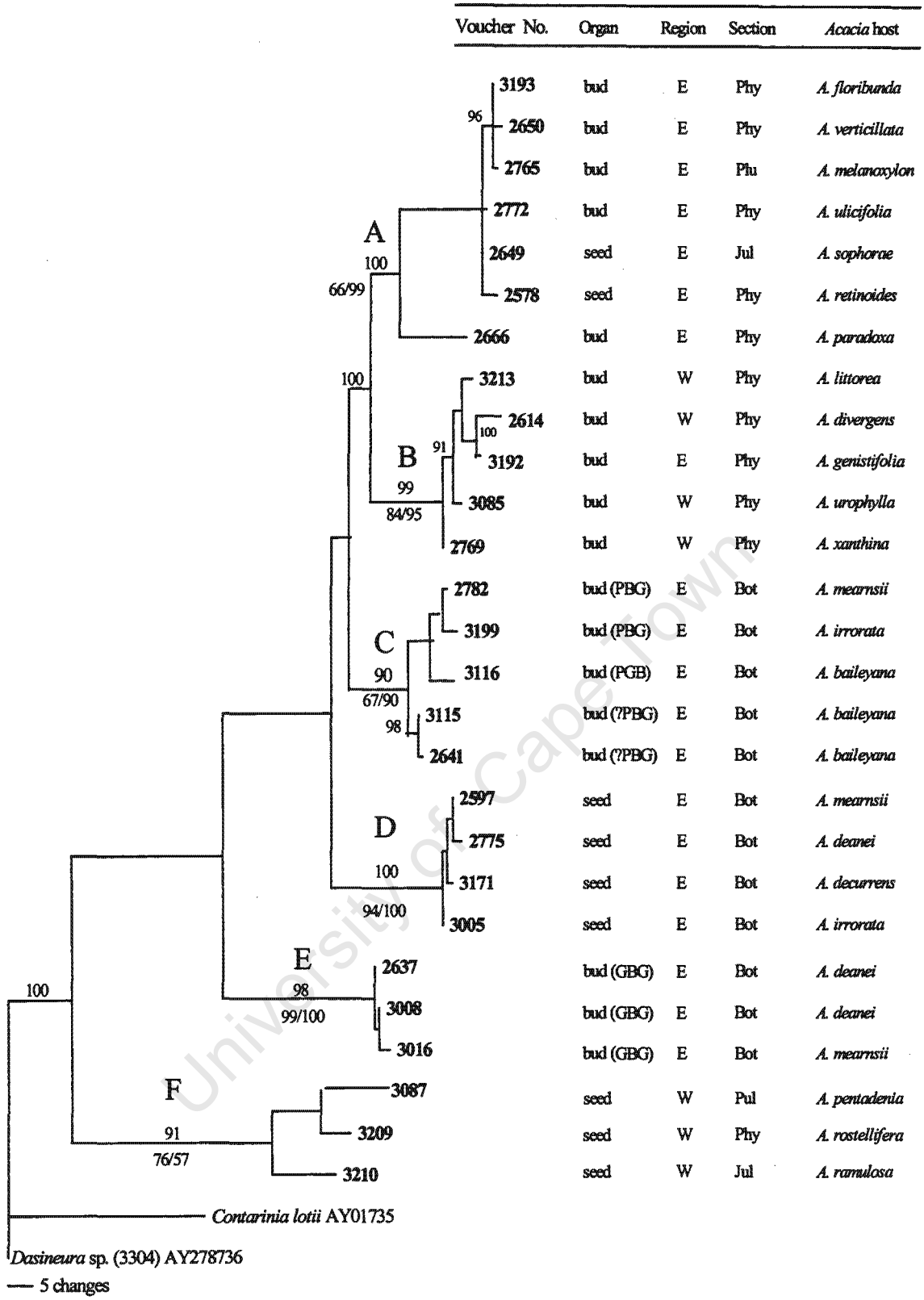


Figure 2.7. Bayesian phylogeny of relationships amongst *Asphondylia* based on cytochrome *b* sequences. Numbers above the nodes represent posterior probabilities from Bayesian analyses using a GTR + I + Γ model of nucleotide evolution. Values below each clade (A-F) are values ML/MP bootstrap values, respectively. Nodes with less than 50% support are not labelled. Branch lengths are drawn proportional to the number of changes as indicated by the scale bar. Voucher number=accession number for each cecidomyiid collection and is used to locate additional collection data in the Access database; Organ=host organ cecidomyiid was reared from, PBG=Pubescent Bud Galler, GBG=Glabrous Bud Galler; Region E=eastern Australia, W=Western Australia; Section=sections within the subgenus *Phyllodineae* that the host of the cecidomyiid collection is located following Pedley (1978) with Phy=*Phyllodineae*, Plu=*Plurinerves*, Jul=*Juliflorae*, Bot=*Botrycephalae*.

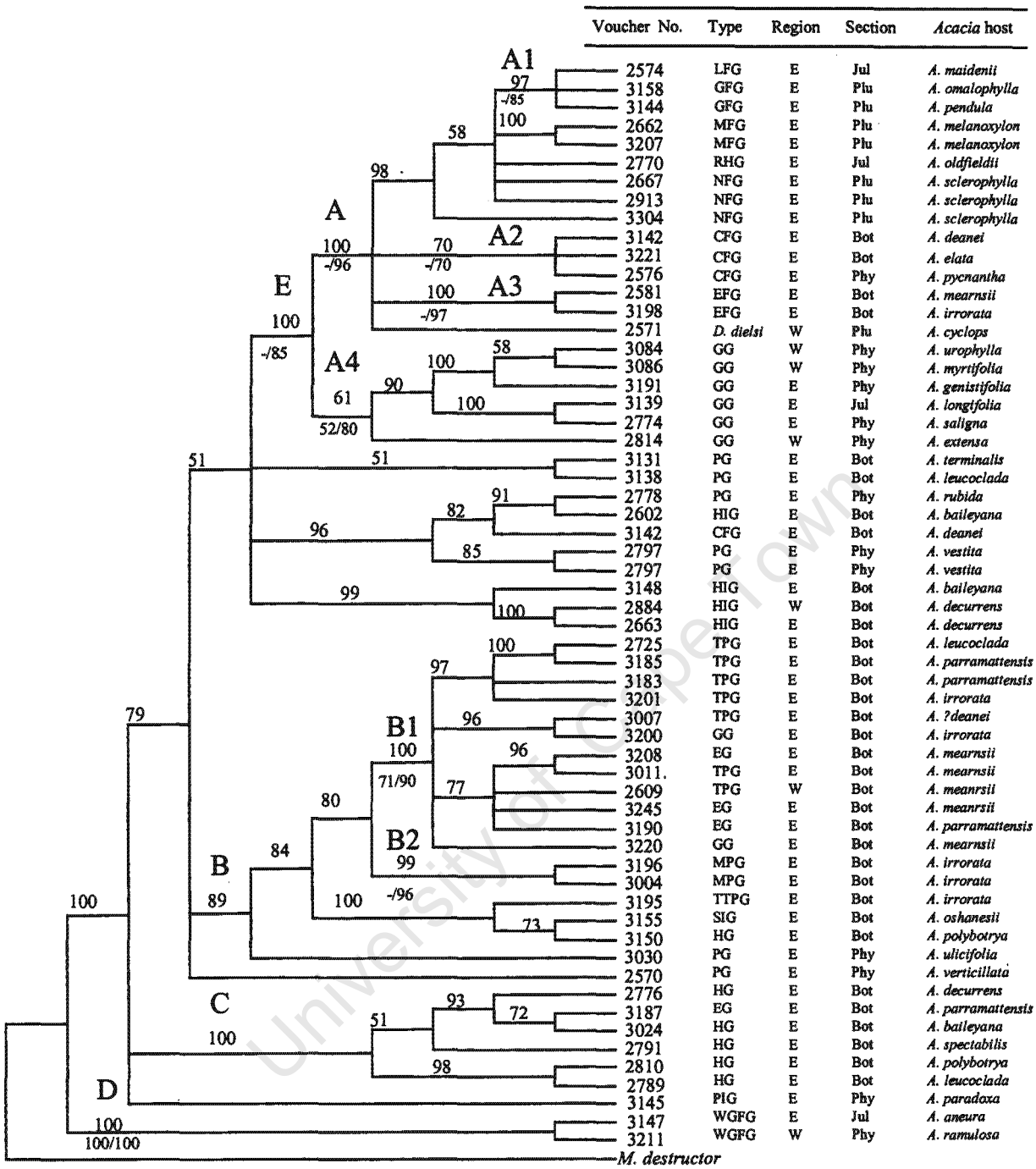


Figure 2.8. The 50% majority rule consensus tree from Bayesian analysis of *Dasineura* cytochrome *b* sequences. Numbers above the nodes represent posterior probabilities from Bayesian analyses using a GTR + I + Γ model of nucleotide evolution. Values below each clade (A-D) are values ML/MP bootstrap values, respectively. Nodes with less than 50% support are not labelled. Branch lengths are drawn proportional to the number of changes as indicated by the scale bar. Voucher number=accession number for each cecidomyiid collection and is used to locate additional collection data in the Access database; Type=common name code; Region E=eastern Australia, W=Western Australia; Section=sections within the subgenus *Phyllodineae* that the host of the cecidomyiid collection is located following Pedley (1978) with Phy=*Phyllodineae*, Plu=*Plurinerves*, Jul=*Juliflorae*, Bot=*Botrycephalae*.

from a recent common ancestor. A similar pattern was observed for *Diplolepis* (Cynipidae) using 12S and cytochrome *b* sequence data (Plantard *et al.* 1998). Although cytochrome *b* is a rapidly evolving gene, it appears that not enough mutations have accumulated in this gene to provide sufficient phylogenetic signal to resolve relationships amongst *Dasineura*. This is best seen in Egg Gallers (EG) (3245, 3208) and the Tiny Floret Gallers (TPG) (2609, 3011) accessions from *A. mearnsii* and *A. parramattensis*, which appear closely related in the phylogeny and are characterized by little sequence divergence despite life history, gall morphology and insect characters suggesting the occurrence of clearly distinct taxa.

Despite deficiencies in the cytochrome *b* sequence data to distinguish *Dasineura* taxa, several interesting patterns are apparent. The repeated independent development of fluted and inflated gall morphology suggests possible adaptive advantages. Several possible explanations exist, but the most plausible is increased protection against hymenopteran parasitoids, which can exert high mortality in gall forming Cecidomyiidae. Pronounced lignification and thickening of gall walls in fluted galls and the tightly appressed, expanded, hollow and often hairy galls of inflated gallers may restrict access to parasitoids, particularly in mature galls. If this hypothesis is correct, parasitism rates in these gall types will be lower than other gall forms that allow less restricted access to parasitoids. Alternatively, inflated and fluted gall types may ameliorate micro-climatic conditions within developmental chambers, but as all gall types come from temperate environments in southern Australia where climatic stresses are not extreme, this explanation is less acceptable. The separation of the Tiny Floret Galler (TPG) accessions into several subclades within clade B indicates genetic divergence between northern and southern populations of this cecidomyiid. The taxonomic status of this separation is unclear, but suggests that close host-ecoclimatic matching may be required if this insect is to be considered for release as a biological control agent for *A. mearnsii* in South Africa. The use of life history, morphological and DNA sequence data to clarify the status of sibling *Contarinia* on *Vincetoxicum* in Scandinavia has considerable merit (Widenfalk *et al.* 2002). Such an approach is strongly advocated to clarify species relationships in Australian *Dasineura* where ambiguity can occur in individual methodologies.

Wider sequence divergence and well-supported and resolved topologies in *Asphondylia* relative to *Dasineura* suggests the former is an older genus, at least within taxa utilising Australian *Acacia*. *Asphondylia* sequence data show close agreement with bio-morphological characters, supporting their use as indicators of taxa. *Asphondylia* exploit buds and seeds on Australian *Acacia*, while none has been found utilising flowers, which, in this study, are used exclusively as a food source by *Dasineura*. This is unlikely to be the result of competitive exclusion due the profuse production of flowers in most *Acacia*, and limited exploitation by gall-forming cecidomyiid

communities. *Asphondylia* show clear segregation into eastern and western Australian taxa, with Western Australian seed-feeding *Asphondylia* forming a basal group and by inference, more primitive group, to other assemblages. Present and historical geographical barriers between eastern and Western Australia dating from the Cretaceous (Heatwole 1987) have contributed to regionalized species radiation in many taxonomic groups, including Australian *Acacia* (Hnatiuk & Maslin 1988) and are likely to have contributed to diversification of *Asphondylia*.

Specialized feeding strategies occur in *Asphondylia* on *Acacia,* with seed-feeding developing independently on three occasions (figure 2.7), including three taxa on Western Australian hosts from different sections (clade G), an oligophagous taxon on *Botrycephalae Acacia* (clade D), and a polyphagous taxon utilising both buds and seeds from eastern Australia (clade A). Bud feeders show similar host restriction within sections of *Acacia.* Although sub-generic reclassification of *Acacia* is under consideration (Miller *et al.* 2003), host restriction in gall-forming cecidomyiids offers some peripheral support for natural groupings under the current divisions proposed by Pedley (1978) and Maslin & Stirton (1997). The cytochrome *b* gene in *Asphondylia* contains sufficient phylogenetic signal to clarify the identity and phylogenetic position of component species. Broader application is therefore advocated and may help resolve taxonomic boundaries in other difficult groups such as the *A. gennadii* (Marchal) - *websteri* Felt complex (Orphanides & Harris 1975, Gagné & Orphanides 1992), Japanese *Asphondylia* (Yukawa 1982, Yukawa *et al.* 1983) and *Asphondylia* from American *Atriplex* (Hawkins *et al.* 1986). *Asphondylia* co-inhabit galls with mutualistic fungi that provide a food source for developing larvae. As *Asphondylia* speciation on Australian *Acacia* has occurred, similar trends may have occurred in cecidomyiid-fungi relationships within galls. These relationships are explored in more detail in Chapter 4 where fungi isolated from *Asphondylia* galls from South Africa and Australia from a broad range of hosts are subjected to phylogenetic analysis

Australian and African cecidomyiids from reproductive organs of *Acacia* are predominantly univoltine with life cycles well synchronized with the limited temporal availability of host organs. Multivoltinism has arisen in several taxa and invariably these species exploit *Acacia* hosts that flower over most months of the year, or have at least one host that does. In Australia, the monophagous Small Inflated Galler from *A. oshanesii*, *D. dielsi* from *A. cyclops* and the Grey Fluted Galler from *A. pendula* are examples. African *Acacia* occur predominantly in the summer-rainfall region of northern South Africa, with strongly seasonal growth and flowering rhythms. Most are fully or at least partially deciduous in winter, an adaptation to low rainfall (Smit 1999). Consequently, African gall-forming cecidomyiids appear to be entirely univoltine, but the mode of survival from one growth season to the next is unknown and requires investigation. In contrast,

southern Australian *Acacia* are evergreen and gall-forming cecidomyiids transgress the inter-flowering period mostly as developing larvae within galls. This strategy is not apparent in African cecidomyiids from *Acacia*.

Although oligophagy is a general pattern in herbivorous insects (Jermy 1976, 1984), insects that attack and live in the reproductive parts of their host plants generally display a high degree of host specificity (Mani 1964, Janzen 1980, Auld 1983, Ananthakrishnan 1986, Dreger-Jauffret & Shorthouse 1992) as they are sensitive to small differences in host plant chemistry, physiology and biology (Floate *et al.* 1996). Cecidomyiidae are mostly restricted to a single host species or at the most a group of close relatives (Gagné 1989). The cecidomyiids from South African and Australian *Acacia* appear to be no exception with all taxa apparently restricted to *Acacia*, a few with multiple *Acacia* hosts, but most with one or a few closely related host species.

Narrow feeding range is an important requirement for the planned use of novel organisms for classical biological control of alien pests. Organisms with a narrow host range reduce the risk of non-target damage and possible conflicts of interest in the country of introduction. All gall-forming cecidomyiids from Australian *Acacia* found in this study appear to be sufficiently host specific for consideration as biological control agents for invasive Australian *Acacia* in South Africa. Polyphagous Australian cecidomyiids are unlikely to develop on any African *Acacia* because of the wide segregation between the African and Australian *Acacia* flora and concomitant differences in biochemistry, biology and anatomy (New 1984). African *Acacia* growing in the presence of cecidomyiid-infected Australian *Acacia* in Australia were devoid of galls supporting the absence of susceptibility, but as these sites were rare, fully replicated laboratory-based host testing trials are warranted. In South Africa, Australian cecidomyiids are absent on invasive Australian *Acacia*, justifying their consideration as potential biological control agents. Similarly, African cecidomyiids are absent on Australian *Acacia* in South Africa (Hepburn 1966, 1967, 1979) further indicating the confined feeding range of this family of insects and the very low risk of subgeneric host crossing.

All invasive Australian *Acacia* in South Africa have gall-forming cecidomyiids developing on reproductive organs in their native range in Australia, but cecidomyiid diversity is variable, a pattern consistent with all Australian *Acacia* sampled in this study. Forty-four species of Australian *Acacia* (30%) had no recorded cecidomyiids, highlighting the fortunate diversity on *A. mearnsii,* a principal target for biological control in South Africa. The eight gall-forming cecidomyiids on reproductive organs of *A. mearnsii* provide some flexibility in candidate agent selection to meet host specificity as well as 'minimal impact on growth' criteria (see Chapter 1). Cecidomyiids from *A. mearnsii* that produce galls with low mass and calorific values compared to fruit may avoid causing 'resource sinks' (Dennill 1988) that could restrict the vegetative growth of the plants. Use

of cecidomyiids as biological control agents with low impact on growth, but effective in reducing seed loads of *A. mearnsii* would minimize the conflict of interest between weed control objectives and productivity targets of the commercial wattle industries in South Africa. Five cecidomyiids on *A. mearnsii* could meet these criteria, the Tiny Floret Galler, Egg Galler, Pubescent Bud Galler, Glabrous Bud Galler and Bipinnate Seed Galler, but all require detailed evaluation.

The wide latitudinal and altitudinal distribution of several cecidomyiids from *A. mearnsii* in Australia indicate broad climatic tolerance within these taxa. As *A. mearnsii* in South Africa is naturalized and problematic over 12 degrees of latitude and occurs at altitudes up to 1700m, a broader range than its natural Australian distribution, climatically tolerant cecidomyiids offer higher prospects for effective biological control than those with narrow climatic tolerance. The Tiny Floret Galler, Egg Galler and Glabrous Bud Galler from *A. mearnsii* and allied hosts show broad latitudinal and altitudinal tolerance, indicating that suitable climate-adapted subspecies or races (Mayr 1971) may exist for possible use as biological control agents in South Africa. Similarly, the wide Australian distribution of the *D. dielsi* on *A. cyclops*, also a prime target for biological control in South Africa, indicates similar potential for use as a seed-reducing agent against this target.

The establishment and spread of the Tiny Floret Galler on *A. mearnsii* in south-west Western Australia highlights even further the biological control prospects of this cecidomyiid. It has naturalized in a region well matched climatically with significant *A. mearnsii* infestations in South Africa and has dispersed widely, locating isolated *A. mearnsii* individuals separated by up to 80 km of host-free bushland. Galling density can reach saturation levels with virtually no seed forming on some populations of *A. mearnsii* in some years at least (Adair pers. obser. 1998-2003). In addition to the Tiny Floret Galler, the occurrence of the Hairy Inflated Galler (HIG), an eastern Australian endemic, on *A. baileyana, A. decurrens* and *A. dealbata* in Western Australia raises questions regarding the mode of dispersal of eastern Australian cecidomyiids into Western Australia. These insects either have migrated across the formidable Nullarbor desert by natural means or have been introduced by human intervention. Wind movement patterns across the Nullarbor are variable, with surface winds predominantly from the east, particularly in summer, while at higher altitudes winds are invariably from the NW and SW (Bureau of Meteorology, pers. comm. 2003). Long distances between eastern populations of the Tiny Floret Galler and *A. mearnsii* trees in Western Australia, together with high diurnal temperatures and strong day/night temperature fluctuations in the Nullarbor region make natural dispersal of this insect across Australia unlikely. Whether the eastern Australian cecidomyiids present in Western Australia have been deliberately or accidentally introduced cannot be ascertained, but their arrival probably

occurred after the late 1980s, as early entomological surveys on *Acacia* undertaken by South African entomologists did not record these conspicuous insects.

CHAPTER 3

Host specificity, biology and ecology of seed-reducing Cecidomyiidae with potential for biological control of *Acacia mearnsii* and *A. cyclops*.

Abstract

The host specificity of three gall-forming *Dasineura* species from *A. mearnsii* (Tiny Floret Galler, Common Fluted Galler, Elongate Fluted Galler), an *Asphondylia* sp. (Glabrous Bud Galler) and *Dasineura dielsi* from *A. cyclops* was determined using caged, no-choice oviposition tests. All Australian cecidomyiids were only able to complete development on Australian *Acacia* species, and mostly only on those hosts that are utilized within the natural distribution of each species. No African *Acacia* species were susceptible to gall-formation by Australian cecidomyiids. Three of the cecidomyiid species (Tiny Floret Galler, Elongate Floret Galler, Glabrous Bud Galler) were univoltine, while *Dasineura* sp. (Common Fluted Galler) from *A. mearnsii* was bivoltine and *D. dielsi* from *A. cyclops* was multivoltine. Adults of all species were short-lived and oviposited in either flowers (*Dasineura*) or buds and seeds (*Asphondylia*). Tiny Floret Galler larvae emerged from galls in June-July and pupated in the surface soil-litter layer beneath host trees. All other cecidomyiids completed development within galls.

Introduction

Most gall-forming Cecidomyiidae have highly restricted host ranges and are confined to a single host plant species or a group of closely allied species from the same genus or family (Gagné 1989). Restricted host specificity is in part attributed to the gall inducer's intimate relationship with its host plant (Shorthouse 1982, Raman 1993) where host plant physiology, chemistry and phenology influence host selection and cecidogenesis. As galls induced by cecidomyiid species are mainly prosoplasmic organs (galls with typical and constant external form, size and tissue differentiation), host-cecidomyiid compatibility at a physiological level is probably the major driving force in determining narrow host specificity. Although uncommon, there are several notable cases of broad-spectrum polyphagy in herbivorous Cecidomyiidae, such as *Contarinia nasturtii* Kieffer (Barnes 1946a), *Contarinia pisi* Winnertz (Harris 1966) and the *Asphondylia gennadii* (Marchal) - *A. websteri* Felt complex (Barnes 1946a,1946b, Orphanides & Harris 1975, Gagné & Woods 1988, Gagné & Orphanides 1992, Gagné pers. comm. 2002) where seven, four, and six plant genera recorded as hosts, respectively.

Narrow host specificity is a mandatory requirement for biological control agents used against weeds. Insects feeding on seeds or seed-producing organs are often host specific due partially to the specialisation required to overcome plant defence mechanisms (Janzen 1971, 1978; Smith 1975) and temporal restrictions on availability of host resources requiring close host-insect synchronization. Gall-inducing cecidomyiids feeding on the reproductive organs of their host are affected by these same circumstances and therefore are expected to fulfil the specificity requirements for consideration as potential biological control agents. None the less, formal assessments of the susceptibility of African plants, particularly African *Acacia*, to attack are required before Australian cecidomyiids are used against Australian acacias in South Africa.

African and Australian *Acacia* represent two phylogenetic lineages and can be distinguished by a broad range of phytochemical and morphological characteristics. These form the basis for separation of the genus into three subgenera, with the *Phyllodineae* encompassing 99% of the rich Australian *Acacia* flora (Maslin 2001), and the subgenera *Acacia* and the *Aculeiferum* dominating the relatively species poor African *Acacia* flora. Indigenous acacias are keystone species in the ecology of the savanna biome of southern Africa and make significant contributions to the economy and socio-economy of that region (Smit 1999). Consequently, African acacias were the primary focus of host specificity tests with Australian cecidomyiids that are potential biocontrol agents for Australian acacias in South Africa. Although host records of cecidomyiids in Australia provide data on specificity, African *Acacia* species are rare in Australia and therefore they have had inadequate exposure to the Australian cecidomyiid fauna to provide meaningful evidence from a risk assessment point of view.

Host specificity testing is a rigorous means of determining the susceptibility of the African acacias to exploitation by Australian cecidomyiids and may help the relative importance of physiological, morphological, phenological cues and other processes in host selection. Finally, as insects can be 'reliable taxonomists', as demonstrated by the cynipid-oak associations of eastern America (Abrahamson *et al.* 1997), the cecidomyiid-*Acacia* association, with apparently narrow specificity in the field (Chapter 2), may provide clues into the relatedness and phylogeny of *Acacia*, which is poorly understood (Maslin & Stirton 1997).

In this chapter, the host ranges and biology of cecidomyiids from *Acacia mearnsii* and *A. cyclops* are compared and the relevance of the findings to the risk assessment process for biological control agents is considered.

Materials and Methods

Natural host range in Australia

Quantitative surveys were used to measure the natural host range of gall-forming cecidomyiids on *Acacia mearnsii* and *A. cyclops* in southern Australia. Field sites were selected where (1) host plants were sexually mature, (2) more than one species of *Acacia* co-occurred within the immediate area, (3) more than three individuals of each *Acacia* species were present and (4) at least one gall-forming cecidomyiid species was present. Survey trees were haphazardly selected within the population and the canopy up to a height of 2.5m was searched for galls for up to five minutes. In the case of small plants, less time was required for a complete search. Galls were collected and subsequently sorted into species based on gall morphology. The numbers of galls on each inflorescence and the numbers of floret galls per flower-head were recorded along with the stage of gall development.

Physiological host range for oviposition and feeding

No-choice oviposition tests in cages were used to determine the physiological host range of five Australian cecidomyiids. Galls were collected from wild populations in Australia and transferred to a quarantine insectary in South Africa where they were stored while adults emerged. *Dasineura dielsi* was collected at Moonta, South Australia (34^0.03'S, 137^0.33'E) and Cheyene Beach, Western Australia (34^0.49'S, $118^0$19'E) from *A. cyclops*. The Tiny Floret Galler was collected at Jarrahdale (32^0 20'S, 116^0 03'E) and Albany (34^0.58'S, 117^0.53'E) in Western Australia from *A. meanrsii*. The Common Fluted Galler was collected from Lake Burrumbeet in Victoria (37^0 29'S, 143^0 40'E), while the Elongate Fluted Galler was collected at Cranbourne in Victoria (38^0.07'S, 145^0.16'E), all from *A. meanrsii*. Cecidomyiids pupating within floret galls were retained as untreated galls within emergence cages, while those pupating within the soil (Tiny Floret Galler) where first collected as fully developed galls and placed on a layer of garden peat held in plastic containers within emergence cages. The garden peat served as a pupation medium and was misted with tap water every 3-5 days to prevent desiccation in the insectary, where the temperature ranged from 15-22^0C. Adult emergence was manipulated to coincide with the flowering of *Acacia* test plants in South Africa by maintaining galls at approximately 5^0C. Galls were removed from cool storage and held under the insectary temperatures when adults were required. Galls collected with final instar larvae could be held in cool storage for three to four months without noticeable detrimental effects. Adult cecidomyiids emerged around 1-3 weeks after removal of galls from cool storage.

Adult cecidomyiids were collected every 1-2 days by aspirating into plastic containers that were furnished with a basal pad of moist moss to reduce impact damage in the collection process. Containers were capped with a ventilated lid and wrapped in a moist cloth during handling to reduce static electricity that caused flies to adhere to the sides of the containers. Within 2 hr of collection, adults were used in host specificity tests.

Physiological host ranges could not be determined in laboratories as *Acacia* trees, particularly African *Acacia*, could not be maintained and induced to flower reliably in pots under artificial conditions. Instead, the insects were confined on branches in cylindrical sleeves (80 cm x 30 cm, 417μ gauge gauze) that were retained for 8-10 days on test plants growing outdoors. Immediately prior to sleeving, thorns or spines were removed from test branches and neighbouring branches were pruned to prevent damage to the sleeves. The number of adults released in each sleeve varied from 9-126 as the availability of each species varied over the emergence periods. Branches were labelled and retained on the host plant for 1-4 months, after which branches were removed and the number of galled flower-heads and component floret galls were counted. There were 3-6 replicates for most test plant species, but occasionally more or less (range 1-28) tests were performed depending on the availability of insects and host plants. Control branches of the main Australian host *Acacia* of each of the four cecidomyiid species tested were also sleeved with adult midges throughout the testing period. Host tests were undertaken, under permit conditions (Department of Agriculture), on African *Acacia* within the National Botanical Gardens at Kirstenbosch, Worcester (Karroo) and Pretoria, and at the Plant Protection Research Institute's Vredenburg laboratory, Stellenbosch. Tests with the *Dasineura* sp. (Elongate Fluted Galler) were undertaken in the field at Frankston, Australia.

Physiological host range tests were attempted using the Glabrous Bud Galler collected at Campbelltown, NSW (34^0.26'S, 150^0.28'E) and the Grampians, Victoria (37^0.03'S, 142^0. 21'E) from *A. mearnsii*. Tests were undertaken on branches of *A. mearnsii* in bud using field trees at Stellenbosch and Villiersdorp, following the methods described above. As no galls developed after several months, no further host testing on was undertaken with this cecidomyiid and no further results are reported in this thesis.

Oviposition tests

No-choice oviposition tests were used to determine the reproductive organs of the host and stage of development utilized by female cecidomyiids of each species. Newly emerged adult cecidomyiids were collected from emergence cages by aspirating them into plastic cylinders (12 cm

x 8.5 cm). Each cylinder contained a stem with 1-7 *Acacia* buds, flowers or young fruits of the cecidomyiid's primary host held in a small vial of water. Stems were categorised into six phenological stages of development: bud initials, early bud, mid bud, late bud, open flowers, and early fruit. There were 3-11 replicates for each phenological stage of the host for each of the cecidomyiid species tested. The number of replicates varied according to availability of host material at the time of emergence of adult cecidomyiids. Between eight and forty adults of mixed sex were added to each cylinder. Adults were retained for three days in a quarantine laboratory at 20^0C before removing the stems and sexing the test flies. In each flower-head, florets samples were removed, (15 per flower-head for tests with the Tiny Floret Galler and the Common Fluted Galler, 20 per flower-head for tests with the Elongate Fluted Galler and *D. dielsi*) dissected and the number of cecidomyiid eggs counted. In each fruit, the fruit surface and ovules were searched for evidence of oviposition.

In a separate experiment, oviposition specificity was determined for the Tiny Floret Galler, *D. dielsi* and the Glabrous Bud Galler using methods similar to those described above. Insufficient adults were available for testing the Common Fluted galler and the Elongate Fluted Galler. In plastic cylinders, bouquets of one or two flower-heads of a single test species were provided to 15-31 newly emerged adults of the Tiny Floret Galler and *D. dielsi.* Flower-heads in late bud were used for the Glabrous Bud Galler where three or four pairs of newly emerged adults were added to each cylinder. After two days, 20 florets were haphazardly selected, dissected and the number of cecidomyiid eggs counted. Adults were sexed after all had died. Eight *Acacia* species were tested with the Tiny Floret Galler, nine species with *D. dielsi* and three species with the Glabrous Bud Galler. There were 2-5 replicates for each *Acacia* species tested with each cecidomyiid species. The number of replicates varied because of difficulties in synchronising adult emergence with host availability in some instances.

Adult life span, egg duration and wing length

Adult cecidomyiids between 3-7 hrs old were collected by aspirating directly into ventilated plastic cylinders (12 cm x 8.5 cm). Each cylinder contained a stem, held in a small vial of water, with several mature flower-heads of the cecidomyiid's normal host. One to seven adults were added to each cylinder and between 20-30 adults were tested for each cecidomyiid species. As the life span of insects are generally temperature dependent, cylinders were maintained in controlled environment cabinets at 20^0C with a 12 h photoperiod from fluorescent lamps. Cylinders were misted daily on the capping gauze to provide moisture for the insects. Cylinders were inspected daily and the numbers and sex of dead flies were recorded.

The time to egg hatch was determined by allowing females to oviposit in flower-heads held in containers as above. After 1 h, adults were removed and bouquets left at 20^{O}C for two days before removing florets and dissecting eggs. Eggs were placed on fresh floret buds on moist filter paper in Petri dishes and were observed regularly until eclosion.

Wing lengths were determined by removing a wing from dead flies and measuring the length from the wing tip to the tegula under a calibrated eye-piece micrometer in a stereomicroscope.

A one-way ANOVA using untransformed data was used to determine the significance of differences in the life span of adult cecidomyiid species, and differences in the wing length of cecidomyiid species. A Tukey's HSD test was used for unplanned pair-wise comparison of means. A student's *t*-test was used to determine the significance of differences in wing length of male and female flies for each cecidomyiid species. Statistical analyses were undertaken using Systat® Version 10.

Fecundity

The number of eggs produced by female cecidomyiids was determined by counting either eggs from dissected ovaries (*D. dielsi*, Elongate Fluted Galler, Tiny Floret Galler, Egg Galler) or the number of eggs laid in florets (Common Fluted Galler). Egg counts from dissected ovaries were undertaken on freshly emerged females held in plastic cylinders for approximately 24 h without host bouquets, or from preserved specimens. Flies were cooled at 0^{0}C then killed by removing the head with a sharp scalpel. The abdomen was slit with a sharp blade and the ovaries were extruded in a drop of water on a glass microscope slide. Eggs were isolated by drawing the egg mass across the slide with a fine paintbrush. The slide was dried at room temperature and eggs were counted against a black background. Egg counts from natural oviposition were undertaken from bouquets with a single open flower-head held in plastic cylinders described above. A single female together with several males were added to each cylinder and maintained until the female died. All florets were then dissected and eggs counted. No egg counts were made for the Glabrous Bud Galler because females could not be induced to lay eggs in buds, and egg numbers within ovaries could not be determined accurately by dissection due to their small size and the immaturity of oocytes.

Adult emergence patterns

Mature galls collected in the field in Australia were transferred to a quarantine insectary in Stellenbosch, South Africa. Galls were considered mature when: the majority of larvae were in cocoons within floret galls of *D. dielsi*, Elongate Fluted Galler and Common Fluted Galler; third

instar larvae were about to emerge from floret galls of the Tiny Floret Galler; and late third instar larvae, prepupae or pupae were evident in the Glabrous Bud Galler and the Pubescent Bud Galler. All galls were held in emergence cages with a temperature range of 15-25^{0}C and natural lighting received through double glazed windows. Galls of *D. dielsi*, Elongate Fluted Galler, Common Fluted Galler and the Glabrous Bud Galler were kept in plastic trays within emergence cages. The galls of the Tiny Floret Galler were placed on a 2-4 cm layer of moist peat moss held in perforated plastic trays. Larvae emerged from floret galls and pupated in the peat moss or between floret galls of the flower-head. Emergence cages were inspected every 1-3 days and adult cecidomyiids and parasitoids collected. The cecidomyiids were sexed and parasitoids identified. The cages were maintained until emergence ceased. Galls were then dissected to check for diapausing stages that could result in emergence the following season.

Collections of *D. dielsi* were made at Moonta, South Australia (34^0.03'S, 137^0.33'E) from *A. cyclops*; Common Fluted Galler at Lake Burrumbeet, Victoria (37^0 29'S, 143^0 40'E) from *A. mearnsii*; Tiny Floret Galler at Jarrahdale, Western Australia (32^0 20'S, 116^0 03'E) from *A. mearnsii*; Elongate Fluted Galler at Cranbourne, Victoria (38^0.07'S, 145^0.16'E) from *A. mearnsii*; and *Asphondylia* sp. (Glabrous Bud Galler) at Wartook, Victoria (37^0 03'S, 142^0 21'E) from *A. mearnsii*. A chi-square goodness of fit calculation was used to test the null hypothesis that the proportion of female to male flies was equal for each species of cecidomyiid tested

The voltinism and duration of generations of *D. dielsi* was determined by confining newly emerged adults in gauze sleeves on flowering branches of *A. cyclops* at Stellenbosch, South Africa. Prior to the introduction of *D. dielsi*, buds and senesced flowers were removed from test branches. After 10 days, sleeves were removed and gall development was monitored on a regular basis. At pupation, galls were collected and held in cages in an insectary until adults emerged. Newly emerged adults were returned to flowering branches of *A. cyclops*. Cohorts of adults that emerged within 1-7 days of each other were used for sleeving on field plants. This process was continued for 12 months. In each sleeve, 7-50 *D. dielsi* adults (mean = 20.5, n = 33) were released. Mean weekly maximum and minimum temperature records were obtained from Fleurbaix weather station at Elsenberg, Stellenbosch (33^0.57'.31"S, 18^0.50'.03"E). A Pearson's correlation coefficient for mean weekly maximum and minimum temperatures and the number of days to first emergence of each generation was determined.

Depth of pupation of the Tiny Floret Galler in the soil profile

During September 2001 at Denmark, Western Australia (34^0.59'S, 117^0.13'E), cylindrical soil cores (7 cm x 7.5 cm) were collected with a soil corer beneath the canopy of *A. mearnsii* trees

with high densities of the Tiny Floret Galler. Prior to coring, the litter layer within the sampling area was removed and retained separately. The soil core was divided into 2 cm sections with a sharp knife and each section was bagged separately. Each soil sample was gently mixed and 50% by volume was removed and inspected under a stereomicroscope to count the number of Tiny Floret Galler cocoons. There were six replicates for each sample depth.

A one-way ANOVA (Systat® Version 10) was used to determine if the mean number of Tiny Floret Galler cocoons varied significantly between sample depths in the soil profile. Cocoon numbers were cube root transformed to improve normality before analysis.

Results

Natural host range in Australia

At four sites in Western and South Australia with sympatric populations of one or more *Acacia* species with *A. cyclops, D. dielsi* was most abundant on *A. cyclops*, but was also found on *A. sophorae* at low densities when these two *Acacia* occurred in close proximity. Four other cecidomyiids (two *Dasineura* spp. and two *Asphondylia* spp.) were each present on a single host at sites with more than one *Acacia* species (table 3.1).

At 10 sites in eastern Australia where *A. mearnsii* co-occurred with 2-6 other *Acacia* species (table 3.2), there were two host utilization patterns apparent for *Dasineura* and *Asphondylia*: utilisation of a single host or host species belonging to the same section, and utilization of multiple hosts across sections of *Acacia*. All *Dasineura* spp. were specific to sections of *Acacia*, and often to a single host species in a multiple-choice situation, except for the Common Fluted Galler, which was polyphagous (table 3.2a). Two *Asphondylia* spp. (Glabrous Bud Galler and Bipinnate Seed Galler) were also restricted to hosts in the same section of *Acacia*, although galling densities showed large within-site variation which may have been mediated by extraneous factors such as differential parasitism and predation on different hosts. *Asphondylia* spp. (Pubescent Bud Galler and Eastern Bud Seed Galler) utilized cross-sectional hosts and also had large within-site galling densities depending on the host utilized (table 3.2b).

Physiological host range for oviposition and feeding

In controlled oviposition tests, *Dasineura* species from *A. mearnsii* and *A. cyclops* displayed narrow host ranges and only induced gall formations on Australian *Acacia* species (tables 3.3-3.6). In laboratory tests, two *Dasineura* species, *D. dielsi* and the Tiny Floret Galler, oviposited in the flowers of African *A. robusta* (table 3.7), but the same *Acacia* showed no signs of gall development when exposed to these cecidomyiids in sleeve cages under field-test conditions (tables 3.3, 3.4).

The physiological host range of Australian *Dasineura* in sleeve tests was restricted to the sections, and in most cases the species, of *Acacia* utilized as hosts in their natural Australian range. However, *D. dielsi* induced one gall on *A. elata* (*Botrycephalae*), an eastern Australian wattle that is in a different section to the insect's normal host, *A. cyclops*. The Common Fluted Galler had the largest host range with gall formation occurring on four host plant species each from separate sections: *A. melanoxylon, A. baileyana, A. longifolia* and *A. saligna*. Only *A. saligna* is a novel host for this cecidomyiid with respect to field records. However, but the association is tenuous because *A. saligna* is an ungalled, common ornamental and naturalized species within the natural range of this insect in eastern Australia.

General Biology

Based on field observations in conjunction with laboratory studies the life cycles of cecidomyiids from *A. mearnsii* and *A. cyclops* can be summarised as follows.

Dasineura:

Eggs of the Tiny Floret Galler, Common Fluted Galler, Elongate Fluted Galler and *D. dielsi* are laid in the perianth tube of open *Acacia* florets (table 3.8), usually against the exterior surface of the ovary, but often amongst staminal filaments. Eggs are cigar-shaped, pinkish-brown and show significant interspecific variation in length ($F_{4,43}$ = 285.8, P <0.001) and width ($F_{4,43}$ = 59.09, P <0.001) (table 3.9). *Dasineura* eggs hatch within 1-2 days (table 3.9) and larvae lie appressed to the surface of the floret ovary.

Neonate larvae induce ovaries to evaginate rapidly and form developmental chambers thus creating floret galls. All larvae of *Dasineura* from *A. mearnsii* and *D. dielsi* from *A. cyclops* are solitary in developmental chambers, but chamber number per floret gall of each cecidomyiid varied from 1-28 (see table 2.5). The Tiny Floret Galler forms no more than five chambers per floret gall, while the Common Fluted Galler was found to have a maximum of 28 chambers. In fluted galls from *A. mearnsii* and *A. cyclops* (*D. dielsi*, Common Fluted Galler, Elongate Fluted Galler), larval development and pupation occurs within the chambers of the floret gall. In all other *Dasineura* (Tiny Floret Galler, Groove Galler, Egg Galler) pupation occurs either after larvae emerge from their chambers and fall to the ground (Tiny Floret Galler, Egg Galler) or amongst stamens and remnant perianth (Groove Galler). All *Dasineura* species, including those from other species of *Acacia*, pupate within white silky, elongate cocoons.

Table 3.1. Gall densities of five cecidomyiids on Australian *Acacia* at sites with *A. cyclops*

			Dasineura dielsi (Small Fluted Galler)				*Dasineura* sp. (Common Fluted Galler)				*Dasineura* sp. (Fluted Galler)				*Asphondylia* sp. (Bud Galler)				*Asphondylia* sp. (Seed Galler)			
SITE	ACACIA	N	MEAN[1] No. GALLS	MEAN[2] FLORET GALLS	MEAN[3] No. INFLOR.	RANGE[4]	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	RANGE	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	RANGE	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	RANGE	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	RANGE
Cervantes (WA)	*A. cyclops*	7	4.5 ± 3.0	9.08 ± 5.6	4.1 ± 2.9	2-26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. rostellifera	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. lasiocarpa	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Albany (WA)	*A. cyclops*	5	11 ± 5.4	9.9 ± 3.0	11 ± 5.4	1-37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. sophorae	5	3.6 ± 5.3	1	3.6 ± 5.3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. littorea	5	0	0	0	0	0	0	0	0	4.2 ± 6.5	1.1 ± 0.1	4.2 ± 6.5	1-2	0	0	0	0	0.4 ± 0.8	1.5 ± 0.7	0.4 ± 0.8	1-2
Moonta (SA)	*A. cyclops*	4	69.2 ±11.4	12.4 ±3.7	65 ± 8.8	1-43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. ligulata	6	0	0	0	0	0	0	0	0	0	0	0	0	0.2 ± 0.4	2	1	2	1.6 ± 4.0	0.3 ± 0.7	1.6 ± 4.0	1-4
Aldinga (SA)	*A. cyclops*	4	43.2 ± 41.3	9.1 ± 3.1	35.5 ± 29.3	1-35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. sophorae	4	1.0	NA	0.25 ± 0.5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. saligna	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	A. pycnantha	4	0	0	0	0	2	11.5 ± 4.9	0.5 ± 1	8 -15	0	0	0	0	0	0	0	0	0	0	0	0

1. Mean number of galls per tree ± standard deviation, 2. Mean number of floret galls per gall cluster ± standard deviation
3. Mean number of inflorescences with galls per tree ± standard deviation, 4. Range of floret gall number per gall cluster

Table 3.2a. Gall densities of seven *Dasineura* on Australian *Acacia* at sites with *A. mearnsii* (footnote captions as per table 3.1)

SITE	ACACIA	N	*Dasineura* sp. (Tiny Floret Galler)			*Dasineura* sp. (Common Fluted Galler)			*Dasineura* sp. (Elongate Fluted Galler)			*Dasineura* sp. (Hairy Inflated Galler)			*Dasineura acaciaelongifoliae*			*Dasineura* sp. (Groove Galler)			*Dasineura* sp. (Pouch Galler)		
			MEAN[1] No. GALLS	MEAN[2] FLORET GALLS	MEAN[3] No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN FLORET GALLS	MEAN No. INFLOR.
Mckenzie River	*mearnsii*		2 ± 1	3 ± 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	retinoides		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Braidwood East	*mearnsii*		18 ± 13	5 ± 1	11 ± 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	trachyphloia		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Budawangs	*mearnsii*		23	1.7	13	0	0	0	2	4	2	0	0	0	0	0	0	0	0	0	0	0	
	terminalis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5 ± 8	1 ± 0.02	4 ± 6
Batemans Bay	*mearnsii*		30 ± 5	5 ± 2	20 ± 2	0	0	0	2 ± 2	3 ± 3	2 ± 1	0	0	0	0	0	0	0	0	0	0	0	0
	implexa		0	0	0	0	0	0	0	0	0	0	0	0	18 ± 19	7 ± 5	12 ± 19	0	0	0	0	0	0
Batemansbay-Moruya	*mearnsii*		35 ± 12	2 ± 0.3	24 ± 5	0	0	0	12 ± 13	2 ± 1	6 ± 6	0	0	0	0	0	0	0	0	0	0	0	0
	implexa		0	0	0	0	0	0	0	0	0	0	0	0	8	16.5	5	0	0	0	0	0	0
	irrorata		11 ± 17	2 ± 0.3	10 ± 13	0	0	0	0.6 ± 1	1 ± 3	0.6 ± 1	0	0	0	0	0	0	0	0	0	0	0	0
Wollongong	*balleyana*		0	0	0	0	0	0	0	0	0	28 ± 26	3 ± 0.9	26 ± 30	0	0	0	0	0	0	0	0	0
	mearnsii		34 ± 19	6 ± 3	23 ± 12	0	0	0	1 ± 3	0.8 ± 1	1.4 ± 3.1	0	0	0	0	0	0	0	0	0	0	0	0
	fimbriata		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	sophorae		0	0	0	0	0	0	0	0	0	0	0	0	34 ± 27	2 ± 0.7	34 ± 27	0	0	0	0	0	0
	lineata		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	1	9	0	0	0
	saligna		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63 ± 40	2 ± 1	63 ± 40	0	0	0
Mittagong	*mearnsii*		2 ± 1	8 ± 9	2 ± 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	terminalis		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5 ± 2	2 ± 1	3 ± 1
	parramattensis		7± 6	8 ± 3	8 ± 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crossroads	*decurrens*		0	0	0	0	0	0	0	0	0	1 ± 1	2 ± 4	1 ± 1	0	0	0	0	0	0	0	0	0
	filicifolia		0	0	0	0	0	0	0	0	0	6 ± 6	4 ± 3	5 ± 6	0	0	0	0	0	0	0	0	0
	mearnsii		105 ± 94	8 ± 0.8	37 ± 18	0	0	0	38 ± 66	2 ± 3	8 ± 15	0	0	0	0	0	0	0	0	0	0	0	0
Burrumbeet	*mearnsii*		0	0	0	166 ± 147	9 ± 2	52 ± 29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	melanoxylon		0	0	0	38 ± 26	12 ± 10	33 ± 25	0	0	0	0	0	0	0.2 ± 0.5	1 ± 3	0.2 ± 0.5	0	0	0	0	0	0
	retinoides		0	0	0	13 ± 6	3 ± 0.6	8 ± 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glen Dhu	*retinoides*		0	0	0	166 ± 8	10 ± 0.2	97 ± 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	mearnsii		244 ± 187	8 ± 1	47 ± 25	9 ± 9	5 ± 4	3 ± 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	dealbata		0	0	0	0	0	0	0	0	0	1 ± 1	3 ± 5	1 ± 1	0	0	0	0	0	0	0	0	0
	balleyana		0	0	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	melanoxylon		0	0	0	30 ± 51	7 ± 9	19 ± 31	0	0	0	0	0	0	0.2 ± 0.4	0.8 ± 1.7	0.2 ± 0.4	0	0	0	0	0	0

Table 3.2b. Gall densities of four *Asphondylia* on Australian *Acacia* at sites with *A. mearnsii* (footnote caption as per table 3.1)

SITE	ACACIA	N	*Asphondylia* sp. (Glabrous Bud Galler)			*Asphondylia* sp. (Pubescent Bud Galler)			*Asphondylia* sp. (Eastern Bud-Seed Galler)			*Asphondylia* sp. (Bipinnate Seed Galler)		
			MEAN[1] No. GALLS	MEAN No. BUD GALLS	MEAN[2] No. INFLOR.	MEAN No. GALLS	MEAN No. BUD GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN No.BUD GALLS	MEAN No. INFLOR.	MEAN No. GALLS	MEAN No. BUD GALLS	MEAN No. NFLOR.
		5												
Mckenzie River	*mearnsii*		38.3 ± 32.0	3.6 ± 0.5	0	0	0		0	0	0	0	0	0
	retinoides		0	0	0	0	0	0	4 5 .3	2.2 1.9	?	0	0	0
Braidwood east	*mearnsii*		0	0	0	0.6 ± 1.1	0.6 ± 0.6	1.0 ± 1.0	0	0	0	0	0	0
	trachyphloia		0	0	0	174.6 ± 230.1	2.5 ± 1.1	29.6 ± 21.5	0	0	0	0	0	0
Budawangs	*mearnsii*		10	2	4	0	0	0	0	0	0	11	1	6
	botrycephala		0	0	0	0	0	0	0	0	0	0	0	0
Batemans Bay	*mearnsii*		21.6 ± 13.7	4.3 ± 0.3	7.0 ± 3.6	0	0	0	0	0	0	2.3 ± 2.5	0.7 ± 0.6	1.6 ± 1.5
	implexa		0	0	0	0	0	0	0	0	0	0	0	0
Bate/bay-Moruya	*mearnsii*		97.5 ± 74.3	5.9 ± 0.7	33.5 ± 16.3	0.5 ± 1.0	0.2 ± 0.5	0.5 ± 1.0	0	0	0	24.2 ± 24.6	1.2 ± 0.1	15.5 ± 12.4
	implexa		0	0	0	0	0	0	0	0	0	0	0	0
	irrorata		30.3 ± 23 0	5.3 ± 1.2	12.0 ± 8.6	0	0	0	0	0	0	1.3 ± 1.1	0.6 ± 1.6	1.3 ± 1.2
Wollongong	*baileyana*		0	0	0	226.5 ± 83.5	2.8 ± 0.5	49.2 ± 15.3	0	0	0	0	0	0
	mearnsii		4.6 ± 6.3	2.6 ± 2.4	3.2 ± 3.6	0	0	0	0	0	0	3.4 ± 3.7	1.2 ± 1.6	2.2 ± 2.7
	frimbriata		0	0	0	0	0	0	34.2 ± 38.6	1.3 ± 0.2	19.0 ± 14.3	0	0	0
	sophorae		0	0	0	0	0	0	21.4 ± 21.7	2.4 ± 1.4	21.0 ± 21.5	0	0	0
	lineata		0	0	0	0	0	0	0	0	0	0	0	0
	saligna		0	0	0	0	0	0	0	0	0	0	0	0
Mittagong	*mearnsii*		47.0 ± 74.1	3.6 ± 2.3	13.2 ± 19.6	0	0	0	0	0	0	0.6 ± 0.6	0.6 ± 0.5	0.6 ± 0.5
	botrycephala		0	0	0	0	0	0	0	0	0	0	0	0
	parramattensis		5.0 ± 6.2	3.5 ± 3.1	1.3 ± 1.1	0.6 ± 1.1	0.3 ± 0.5	0.6 ± 1.1	0	0	0	12.3 ± 9.6	1.0 ± 0.0	5.3 ± 4.0
Crossroads	*decurrens*		0.6 ± 1.1	1.3 ± 2.3	0.3 ± 0.5	26.3 ± 22.6	1.8 ± 1.5	11.0± 9.5	0	0	0	0	0	0
	filicifolia		0	0	0	1.3 2.3	1.4 2.4	0.8 1.1	0	0	0	0	0	0
	mearnsii		56.0 ± 6.2	5.3 ± 0.7	21.6 ±7.4	6.3 ±10.1	1.8 ± 2.1	3.0 ± 4.3	0	0	0	12.6 ± 6.4	1.1 ± 0.1	10.0 ± 4.0
Burrumbeet	*mearnsii*		0	0	0	5.0 ± 6.6	1.6 ± 1.1	5.5 ± 5.2	0	0	0	0	0	0
	melanoxylon		0	0	0	0	0	0	12.2 ± 11.2	1.1 ± 0.7	11.0 ± 10.0	0	0	0
	retinoides		0	0	0	0	0	0	0	0	0	0	0	0
Glen Dhu	*retinoides*		0	0	0	0	0	0	0	0	0	0	0	0
	mearnsii		0	0	0	0	0	0	0	0	0	0	0	0
	dealbata		0	0	0	2.5 ± 3.5	3.6 ± 5.1	0.5 ± 0.7	0	0	0	0	0	0
	baileyana		0	0	0	0	0	0	0	0	0	0	0	0
	melanoxylon		0	0	0	0	0	0	11.0 ±14.5	0.7 ± 0.7	10.4 ± 13.2	0	0	0

Table 3.3. No-choice host specificity test results for the Tiny Floret Galler (*Dasineura* sp.)

Test plant origin	*Acacia* subgenus	*Acacia* section	Test Species	*n*	Mean No. adults/test	Range of adults/test	Mean No. gall clusters/branch ± SE	Total No. gall clusters	Mean No. floret galls /gall cluster ± SE
Australia	Phyllodineae	Botrycephalae	*Acacia mearnsii*	28	48.0	18-100	20.0 ± 3.6	585	9.8 ± 0.36
			A. dealbata	4	100	100	0	0	0
			A. baileyana	4	37.5	35-45	0	0	0
			A. decurrens	4	36.2	30-45	0	0	0
		Phyllodineae	*A. cultriformis*	2	98	70-126	0	0	0
			A. podalyriifolia	3	35	35	0	0	0
			A. saligna	6	43.1	35-55	0	0	0
		Juliflorae	*A. longifolia*	6	40	30-55	0	0	0
		Plurinerves	*A. melanoxylon*	4	33.7	25-35	0	0	0
			A. cyclops	5	55	55	0	0	0
South Africa	Acacia	*Acacia*	*A. erioloba*	5	60.6	23-80	0	0	0
			A. karroo	3	58	29-85	0	0	0
			A. galpinii	4	50	50	0	0	0
			A. nilotica subsp. *kraussiana*	1	50	50	0	0	0
			A. robusta subsp. *robusta*	4	65	60-70	0	0	0
			A. robusta subsp. *clavigera*	8	39	22-50	0	0	0
			A. hebeclada subsp. *tristis*	4	60	60	0	0	0
			A. hebeclada subsp. *chombensis*	4	30	30	0	0	0
			A. xanthophloia	4	60	60	0	0	0
			A. sieberiana var. *woodii*	1	30	30	0	0	0
	Aculeiferum	*Aculeiferum*	*A. caffra*	4	60	60	0	0	0
			A. mellifera	4	72.5	70-80	0	0	0
			A. nigrescens	4	60	60	0	0	0
			A. senegal var. *leiorachis*	4	70	70	0	0	0
		Monacanthea	*A. schweinfurthii*	6	40.5	40-43	0	0	0
America	Acacia	"farnesiana group"	*A. farnesiana*	2	70	60-80	0	0	0
	Non-*Acacia*	Mimosaceae	*Paraserianthes lophantha*	4	35.5	35-37	0	0	0
		Rosaceae	*Cydonia oblonga*	4	50	50	0	0	0
			Prunus armeniaca	3	36.6	30-40	0	0	0
		Vitaceae	*Vitis vinifera*	3	33.3	30-40	0	0	0

Table 3.4. No-choice host specificity test results for *Dasineura dielsi*

Test plant origin	*Acacia* subgenus	*Acacia* section	Test Species	*n*	Mean No. adults/test	Range adults/test	Mean No. gall clusters/branch ± SE	Total No. gall clusters	Mean No. floret galls /gall cluster ± SE
Australia	Phyllodineae	*Plurinerves*	*Acacia cyclops*	8	23.7	9-40	5.5 ± 2.29	44	18.9 ± 2.5
			A. implexa	3	35	35	0.6 ± 1.1	2	6.5 ± 6.3
		Botrycephalae	*A. elata*	4	35	30-40	0.25 ± 0.5	1	1
			A. decurrens	4	51.5	40-62	0	0	0
			A. mearnsii	3	45	37-58	0	0	0
		Phyllodineae	*A. podalyriifolia*	4	34	17-45	0	0	0
			A. cultriformis	4	50	50	0	0	0
South Africa	Acacia	*Acacia*	*A. karroo*	7	25.1	17-40	0	0	0
			A. gerradii	4	45	40-50	0	0	0
			A. fleckii	3	50	40-60	0	0	0
			A. robusta subsp. *clavigera*	1	30	30	0	0	0
			A. sieberiana var *woodii*	4	25.7	10-40	0	0	0
	Aculeiferum	*Aculeiferum*	*A. ataxacantha*	7	22.5	20-25	0	0	0
			A. schweinfurthii	4	27.5	19-40	0	0	0

Table 3.5 No-choice host specificity test results for the Common Fluted Galler (*Dasineura* sp.)

Test plant Origin	*Acacia* subgenus	*Acacia* section	Test Species	*n*	Mean No. adults/test	Range of adults/test	Mean No. gall clusters/branch ± SE	Total No. gall clusters	Mean No. floret galls /gall cluster ± SE
Australia	Phyllodineae	*Plurinerves*	*Acacia melanoxylon*	6	58.3	50-100	14.5 ± 4.1	87	20.3 ± 0.9
			A. cyclops	4	50	50	0	0	0
		Botrycephalae	*A. mearnsii*	5	90	50-100	0	0	0
			A. baileyana	4	40	30-50	13.2 ± 9.6	53	2.8 ± 0.3
			A. decurrens	6	36.6	30-50	0	0	0
		Phyllodineae	*A. saligna*	4	50	50	34.5 ± 3.0	139	10.1± 0.7
			A. podalyriifolia	4	40	30-50	0	0	0
		Juliflorae	*A. longifolia*	6	50	50	41 ± 5.4	141	3.4 ± 0.23
South Africa	Acacia	*Acacia*	*A. robusta* subsp. *Clavigera*	4	40	35-50	0	0	0
			A. robusta subsp. *Robusta*	4	55.2	50-71	0	0	0
			A. galpinii	4	50	50	0	0	0
			A. nilotica	4	50	50	0	0	0
			A. erioloba	4	60	60	0	0	0
			A. hebeclada subsp. *Chombensis*	4	60	60	0	0	0
			A. xanthophloia	4	60	60	0	0	0
	Aculeiferum	*Aculeiferum*	*A. schweinfurthii*	2	45	40-50	0	0	0
			A. nigrescens	4	60	60	0	0	0
	Non-*Acacia*		*Paraserianthes lophantha*	4	40	30-50	0	0	0

Table 3.6. No-choice host specificity test results for the Elongate Fluted Galler (*Dasineura* sp.)

Test plant Origin	*Acacia* subgenus	*Acacia* section	Test species	*n*	Mean No. adults/test	Range of adults/test	Mean No. gall clusters /branch ± SE	Total No. gall clusters	Mean No. floret galls /gall cluster ± SE
Australia	Phyllodineae	*Plurinerves*	*Acacia melanoxylon*	11	50	50	0	0	0
		Botrycephalae	*A. mearnsii*	3	50	50	65 ± 53.8	195	5.12 ± 3.5
		Phyllodineae	*A. verticillata*	3	36.6	30-50	0	0	0
			A. calamifolia	3	50	50	0	0	0
		Juliflorae	*A. longifolia*	2	40	30-50	0	0	0

Table 3.7. Number of eggs laid by Australian cecidomyiids in flowers of Australian and a South African *Acacia* (*A. robusta*) in no-choice oviposition tests. Data are mean (± se) number of eggs/female/20 florets. *n* = number of replicates.

Cecidomyiid	*A. mearnsii*	*A. cyclops*	*A. melanoxylon*	*A. saligna*	*A. longifolia*	*A. decurrens*	*A. pycnantha*	*A. robusta*	*A. dealbata*	*Paraserianthes lophantha*
Tiny Floret Galler *Dasineura* sp.	3.9 ± 1.0 *n*=4	-	0 *n*=4	0 *n*=4	0 *n*=4	2.5 ± 2.4 *n*=4	0.09 ± 0.09 *n*=4	0 *n*=4	-	0 *n*=4
D. dielsi.	0.70 ± 0.70 *n*=4	28.6 ± 2.3 *n*=3	8.1 ± 3.4 *n*=3	6.9 ± 4.3 *n*=4	15.9 ± 1.2 *n*=2	0.31 ± 0.20 *n*=4	1.2 *n*=1	5.6 ± 2.4 *n*=4	0 *n*=3	-
Glabrous Bud Galler *Asphondylia* sp.	0 *n*=5	-	-	0 *n*=5	-	-	-	0.04 ± 0.04 *n*=5	-	-

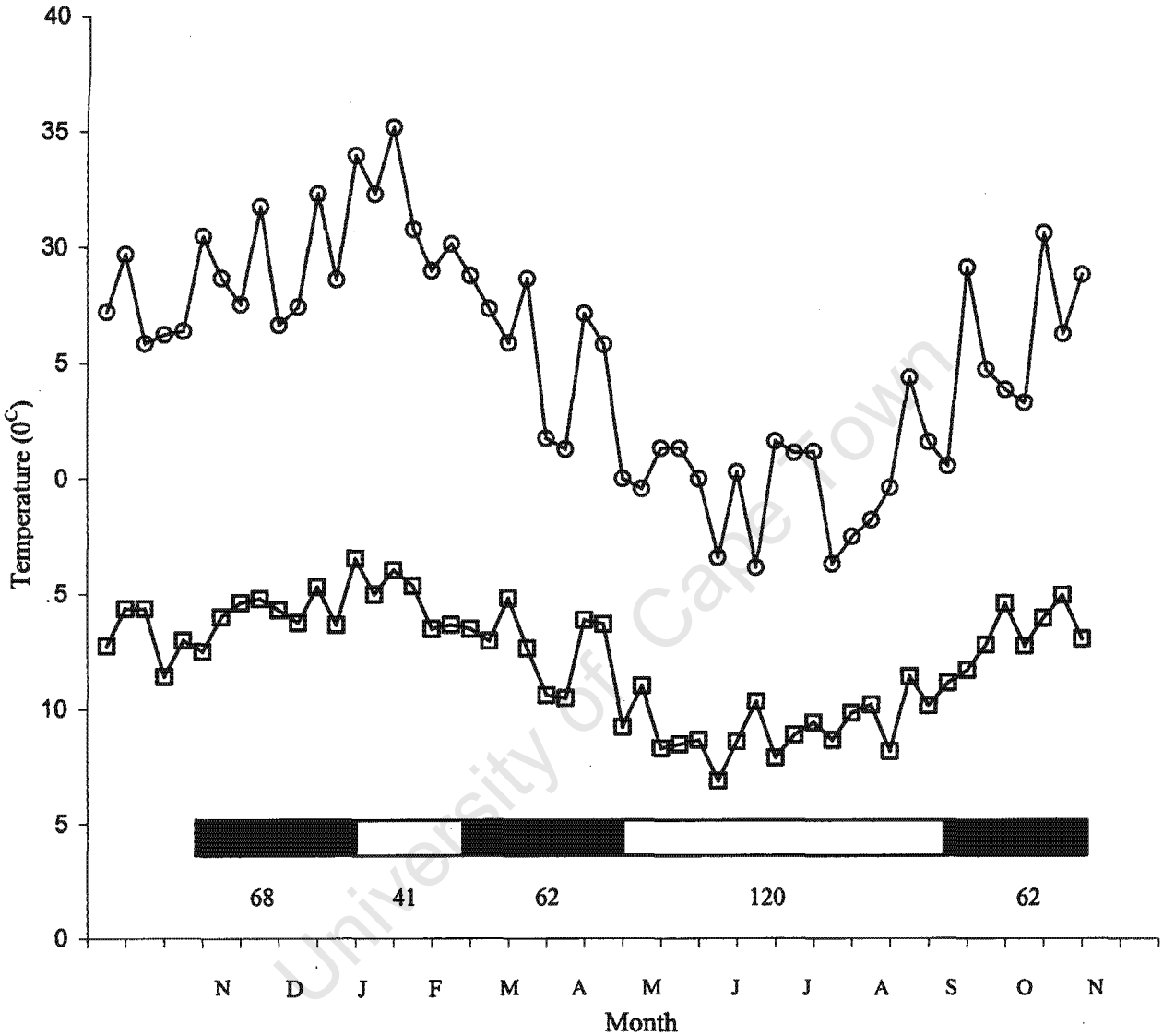


Figure 3.1 Mean maximum (○) and minimum (□) weekly temperatures at Stellenbosch between 2000 and 2001 and duration of generations of *D. dielsi* on *A. cyclops*. The horizontal bars show the duration of consecutive generations and the number of days of each generation.

Dasineura species from *A. mearnsii* are univoltine, except for the Common Fluted Galler, which is mostly bivoltine. The Common Fluted Galler shows an unusual pattern of voltinism and polyphagy within *Acacia* (see table 2.5) by predominantly utilising *A. mearnsii* from adults emerged in July, followed by oviposition on *A. melanoxylon* among adults that emerge in September and return to flowering *A. mearnsii* or *A. pycnantha* in October (see Chapter 5, figure 5.1g-h). The Common Fluted Galler may also develop without host switching *on A. retinoides*, a widespread species that produces flowers most of the year and may allow multivoltine development of the Common Fluted Galler (Adair pers. obser. 1999). *Dasineura dielsi* from *A. cyclops* is multivoltine with five generations per annum. The duration of development of *D. dielsi* generations was correlated with mean weekly maximum temperature ($r = -0.938$, $P<0.001$, $n=5$) and minimum temperature ($n=5$, $r = -0.886$, $P<0.01$, $n=5$).

Adult *Dasineura* are short-lived with a mean life span of 1.7-2.6 days (table 3.9). Wing length varied significantly between *Dasineura* species for both male ($F_{6,188} = 381.8$, $P<0.001$) and female flies ($F_{6,252} = 539.9$, $P<0.001$). Female *Dasineura* had longer wing lengths than males in each of the five species measured (Tiny Fluted Galler $P<0.001$, Common Fluted Galler $P<0.001$, Elongate Fluted Galler $P<0.001$, Egg Galler $P = 0.03$, *D. dielsi* $P = 0.03$ (table 3.9). Female *Dasineura* oviposit within a few hours of emergence and produce a mean of 85-233 eggs female^{-1} (table 3.9). After emergence, galls desiccate rapidly, but woody galls may persist for up to two years. Soft- to semi-soft tissued galls (Common Fluted Galler, Groove Galler, Egg Galler) are generally shed from the trees within several months.

Asphondylia:

Bud-galling *Asphondylia* from *A. mearnsii* (Glabrous Bud Galler and Pubescent Bud Galler) oviposit in immature buds, mostly in the late afternoon, and deposit eggs singly amongst stamens, usually in the distal region between the top of the anthers and the inner perianth wall. In the field, females were observed to oviposit on buds at a range of developmental stages, but mostly at the mid bud stage where the flower-head was 1/2-2/3 full size and floret buds were discernable, but tightly packed together. Under caged conditions, *Asphondylia* readily performed oviposition behaviour on the buds of African and Australian *Acacia*, but on dissection of seemingly-utilized buds only one egg was found (table 3.7), indicating very low levels of egg deposition, and most egg-laying behaviour occurred as 'pseudo-oviposition' events.

Table 3.8. Number of eggs laid by four *Dasineura* spp. on different phenological stages of their host *Acacia*. TFG= Tiny Floret Galler, CFG=Common Fluted Galler, EFG=Elongate Fruit Galler, *n*=number of replicates. Number proceeding sex symbol is mean number of insects used in test.

Cecidomyiid	Code	Host	bud initials	early bud	mid bud	late bud	flower	early fruit
Dasineura sp.	TFG	*A. mearnsii*	0 0 0	0 0 0	0 0 0	0 0 0	0.49 ± 0.5* 0.10 ± 0.1** 77***	0 0 0
			n=4 ♂ 14.2 ♀ 8.2	*n*=3 ♂ 11.3 ♀ 7.6	*n*=3 ♂ 11.6 ♀ 10.3	*n*=3 ♂ 12.3 ♀ 9.3	*n*=6 ♂ 14.6 ♀ 8	*n*=5 ♂ 5.75 ♀ 12.2
Dasineura sp.	CFG	*A. mearnsii* *A. melanoxylon*	0 0 0	0 0 0	0 0 0	0 0 0	5.28 ± 4.3 1.19 ± 0.9 1376	0 0 0
			n=5 ♂ 19.6 ♀ 5	*n*=3 ♂ 16.6 ♀ 13.6	*n*=5 ♂14.4 ♀ 13.8	*n*=3 ♂ 17 ♀ 5.6	*n*=11 ♂ 13.9 ♀ 3.5	*n*=6 ♂ 22.5 ♀ 3.3
Dasineura sp	EFG	*Acacia mearnsii*	0 0 0	0 0 0	0 0 0	0 0 0	8.94 ± 6.2* 1.73 ± 1.4** 895***	0 0 0
			n=3 ♂ 9.3 ♀ 10	*n*=1 ♂ 5 ♀ 14	*n*=3 ♂ 6.5 ♀ 29.5	*n*=4 ♂ 14.3 ♀ 7.6	*n*=5 ♂ 15.4 ♀ 9.2	*n*=3 ♂ 11.6 ♀ 8
Dasineura dielsi	SFG	*A. cyclops*	0 0 0	0 0 0	NT	0 0 0	12.92 ± 6.3* 1.03 ± 0.45** 1875***	0 0 0
			n=3 ♂ 5.3 ♀ 16	*n*=4 ♂ 7.7 ♀ 12.5		*n*=4 ♂ 6.2 ♀ 9.75	*n*=7 ♂ 6.7 ♀ 4.99	*n*=3 ♂ 6.6 ♀ 14

*mean number of eggs/floret ± standard deviation, **mean number of eggs/floret/female ± standard deviation, *** total number of eggs.
NT=not tested.

Table 3.9. Life span, fecundity and size of *Dasineura* and *Asphondylia* from *A. mearnsii and A. cyclops*. Data are means ± SD. NA=not available. *n* = number of replicates, TFG = Tiny Floret Galler, CFG = Common Fluted Galler, EFG = Elongate Fluted Galler, Eg = Egg Galler, GBG = Glabrous Bud Galler, PBG = Pubescent Bud Galler. Column values followed by the same letter are not significantly different (P>0.05).

Cecidomyiid	Common name Accession No.	Host	Adult life span (days)	Egg hatch (days)	Eggs/♀	Wing length (mm) ♂	Wing length (mm) ♀	Egg size (mm) Length	Egg size (mm) Width
Dasineura sp.	TFG 3018	*A. mearnsii*	1.82 ± 0.71	na	85.8 ± 43.6	2.19 ± 0.11a	2.36 ± 0.18a	0.12 ? ± 0.008a	0.03 ± 0.003a
			n=29		*n*=15	*n*=22	*n*=37	*n*=10	*n*=10
Dasineura sp.	CFG 2660, 3019	*A. mearnsii* *A. melanoxylon*	1.73 ± 0.87	2.7 ± 0.1	210.2 ± 136.7	3.22 ± 0.19b	3.46 ± 0.17b	0.29 ± 0.01b	0.07 ± 0.008b
			n=19	*n*=17	*n*=10	*n*=103	*n*=148	*n*=15	*n*=15
Dasineura dielsi	SFG 3023	*A. cyclops*	1.83 ± 0.94		233.5 ± 52.0	2.32 ± 0.19a	2.43 ± 0.15a	0.14 ± 0.008c	0.03 ± 0.003a
			n=30		*n*=16	*n*=21	*n*=25	*n*=10	*n*=10
Dasineura sp.	EFG 3127	*A. mearnsii*	2.1 ± 0.78	na	204 ± 78.5	2.15 ± 0.06a	2.37 ± 0.07a	0.22 ± 0.07d	0.07 ± 0.01a
			n=18		*n*=5	*n*=15	*n*=15	*n*=10	*n*=10
Dasineura sp.	EG 3208	*A. mearnsii*	na	na	102. 7 ± 18.0	1.31± 0.13c	1.47± 0.10c	0.11 ± 0.02c	0.04 ± 0.001a
					n=4	*n*=9	*n*=5	*n*=3	*n*=3
Asphondylia sp.	GBG 3118, 3120, 3252	*A. mearnsii*	2.3 ± 0.80	na	na	3.50 ± 0.11d	4.04 ± 0.11d	0.12 ± 0.01-	0.08 ± 0.012-
			n=44			*n*=15	*n*=15	*n*=9	*n*=9
Asphondylia sp.	PBG 3121, 3119	*A. baileyana*	2.6 ± 0.83	na	na	3.68 ± 0.20d	3.96 ± 0.10d	na	na
			n=70			*n*=5	*n*=15		

The eggs of *Asphondylia* (Glabrous Bud Galler) are translucent and globose. The eggs of other *Asphondylia* species were never observed. The seed-galling *Asphondylia* of *A. mearnsii* (Bipinnate Seed Galler) and *A. cyclops* (Seed Galler) oviposit in developing fruits of their hosts. All *Asphondylia* larvae on Australian *Acacia* are bright yellow and occur with a fungal symbiont, which forms a white mycelial mantle over infected tissue (see Chapter 4). In *Asphondylia* developing in buds, the mycelial mantle develops on stamens, while in *Asphondylia* developing in fruits, the fungal mantle develops over ovules. In both bud- and seed-feeding *Asphondylia*, a single larva occurs in each gall and pupation is completed within the developmental chamber. *Asphondylia* pupae use their antennal horns to cutemergence holes in the wall of the gall and after emergence usually leave their exuvia in the emergence hole.

Asphondylia from *A. mearnsii* are either univoltine (Glabrous Bud Galler, Bipinnate Seed Galler) or multivoltine (Pubescent Bud Galler). The Seed Galler from *A. cyclops* appears to be univoltine, but further observations are required for confirmation. Adult *Asphondylia* from *A. mearnsii* are short-lived with a mean life span of 1–5 days (Glabrous Bud Galler: mean ± SD=2.3 ± 0.80, Pubescent Bud Galler: mean ± SD=2.6 0.03). Female *Asphondylia* have a longer wing span than males (Glabrous Bud Galler, $P<0.001$; Pubescent Bud Galler, $P = 0.03$) (table 3.9).

Asphondylia bud galls are soft-tissued and desiccate several weeks after emergence of adults when they fall readily from the host tree.

Adult emergence patterns

The duration of emergence of adult *Dasineura* species from galls (Common Fluted Galler, Elongate Fluted Galler, *D. dielsi*) or subterranean cocoons (Tiny Floret Galler) ranged from 25 days for the Elongate Fluted Galler to 166 days for field collected galls of *D. dielsi* (figure 3.2, 3.3. 3.4). Male and female *Dasineura* from *A. mearnsii* and *D. dielsi* commenced emergence simultaneously, but with higher female to male ratios for all taxa: *D. dielsi* (1.5:1, $\chi^2 = 19.7$, $P<0.001$, $n = 497$), Tiny Floret Galler (1.8:1, $\chi^2 = 89.1$, $P<0.001$ $n = 1113$), Common Fluted Galler (1.4:1, $\chi^2 = 78.2$, $P<0.001$, $n = 2656$), Elongate Fluted Galler (1.3:1, $\chi^2 = 5$, $0.025 <P<0.05$, $n = 256$) (figures 3.2, 3.3, 3.4).

Hymenopteran parasitoids mainly emerged shortly after that of their cecidomyiid host (figures 3.3, 3.4), except for the Tiny Floret Galler, where parasitoid emergence seemed to coincide with emergence of the cecidomyiid (figure 3.3a). In field-collected galls of the Elongate Fluted Galler, delayed emergence of the parasitoid *Aprostocetus* sp.2 (3127/e) (Eulophidae) occurred three months after peak emergence of this cecidomyiid (figure 3.4a).

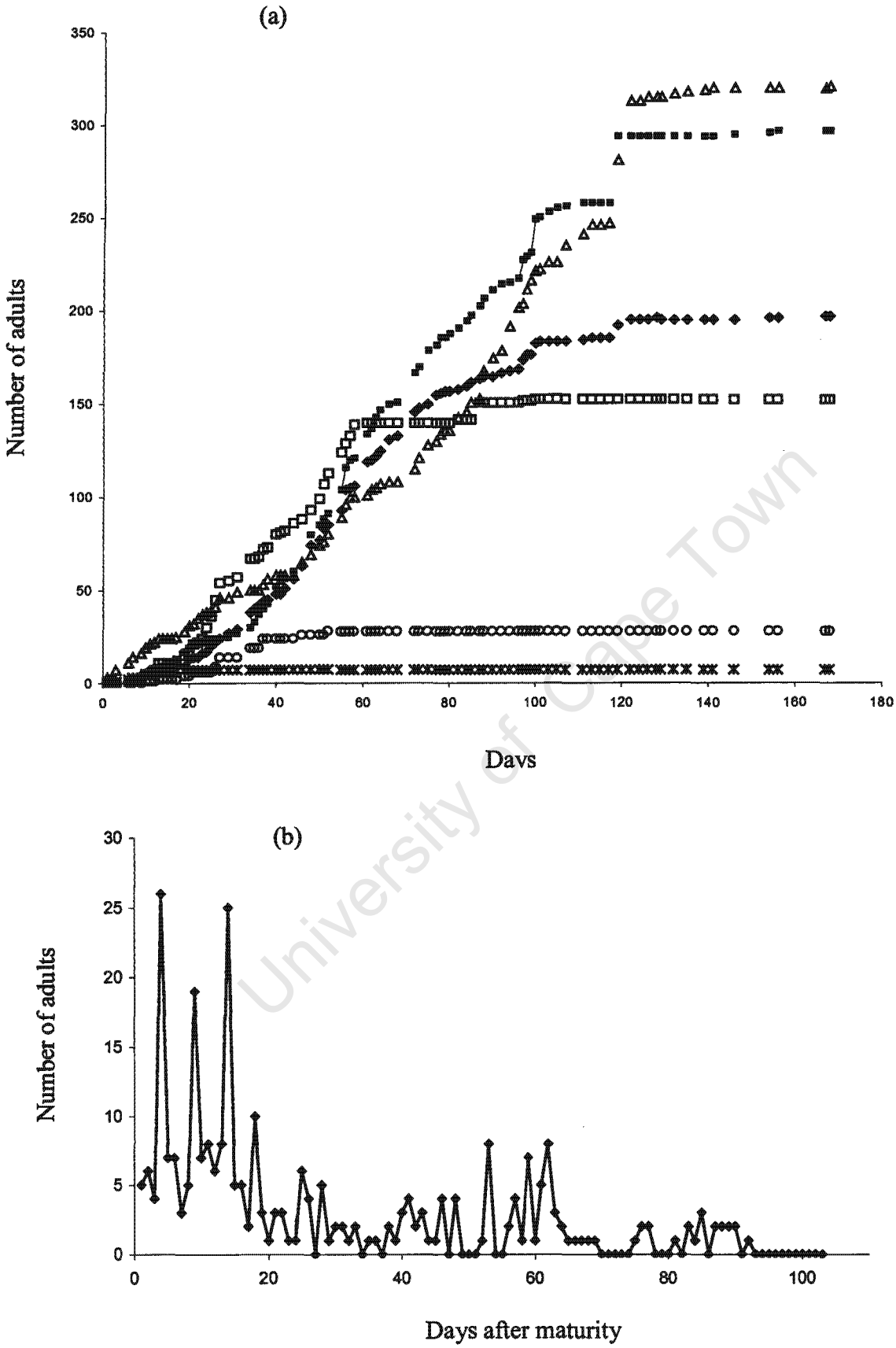


Figure 3.2. (a) Accumulative emergence of *D. dielsi* and parasitoids from galls collected at Moonta, South Australia. ♦= male *D. dielsi*,■ = female *D. dielsi*, △= *Systasis* sp., □= ?*Synopeas* sp. , * = *Megastigmus* sp., ○=?*Inostemma* sp. (b) emergence of *D. dielsi* from galls reared on *A. cyclops* in South Africa.

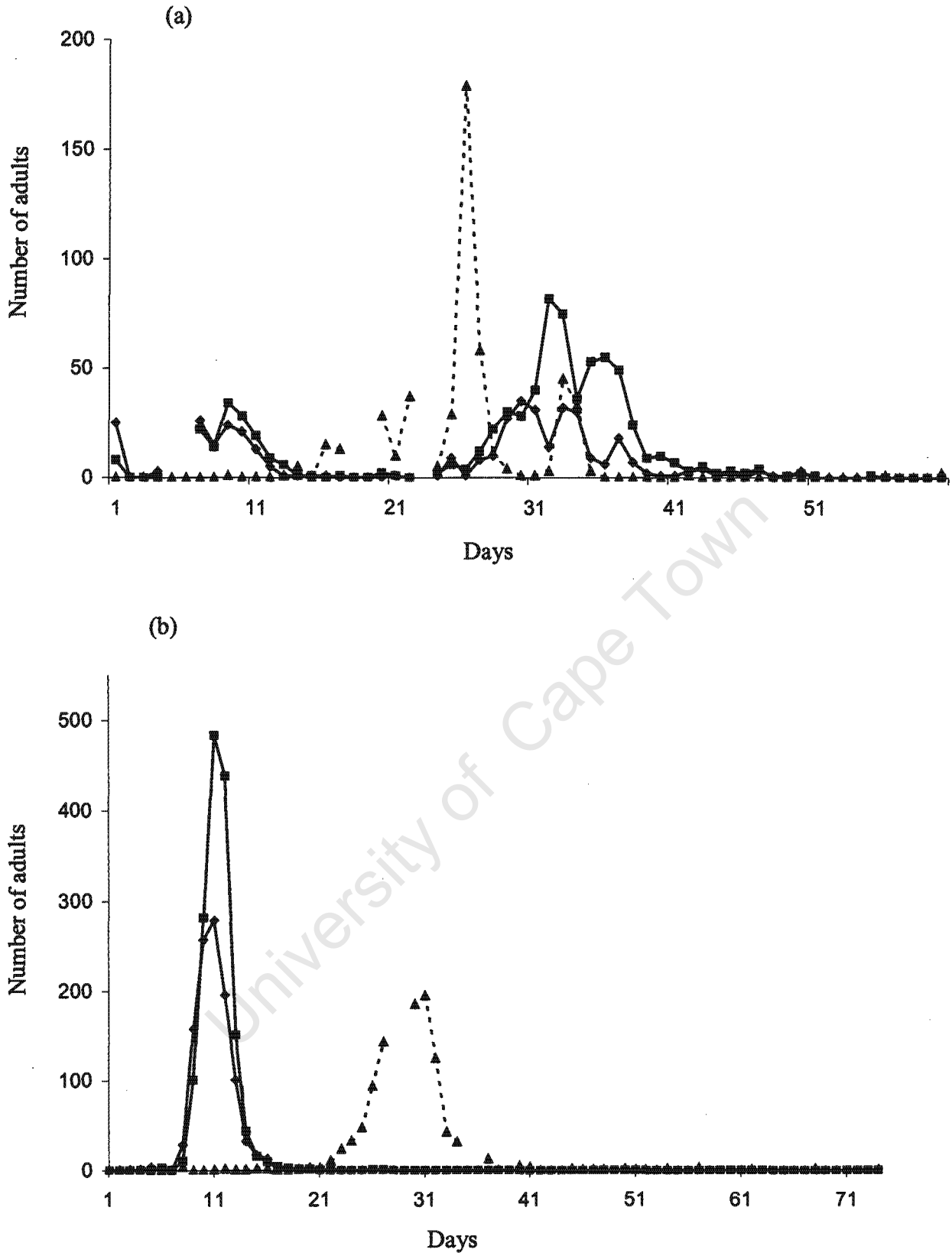


Figure 3.3 (a) Emergence of *Dasineura* sp. (Tiny Floret Galler) and parasitoids collected at Jarrahdale, Western Australia, and (b) *Dasineura* sp. (Common Fluted Galler) and parasitoids collected at Lake Burrumneet, Victoria from *Acacia mearnsii*. ■= female *Dasineura*, ♦=male *Dasineura*, ▲= hymenopteran parasitoids

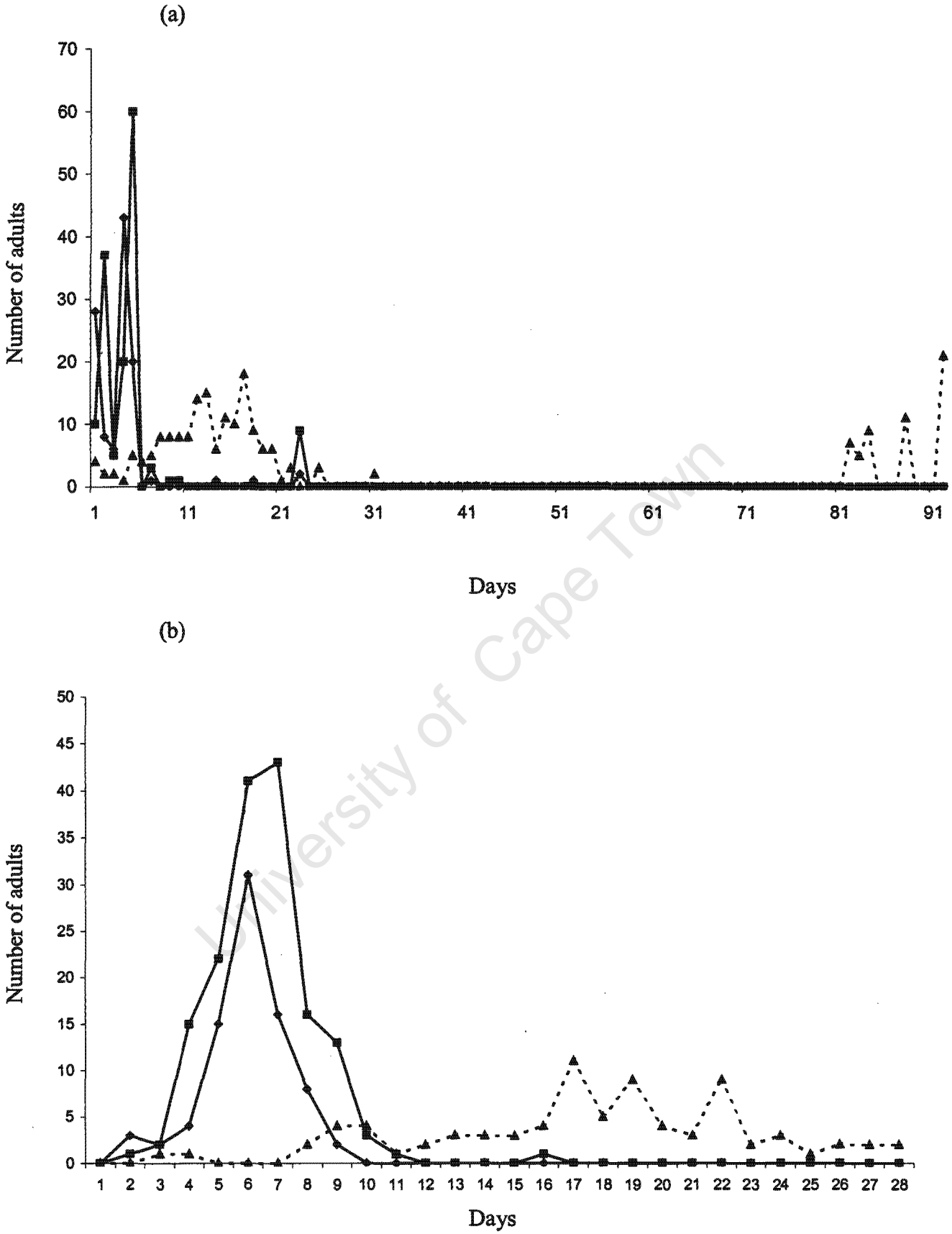


Figure 3.4. (a) Emergernce of *Dasineura* sp. (Elongate Fluted Galler) and parasitoids from galls collected at Cranbourne, Victoria; (b) emergence of *Asphondylia* sp. (Glabrous Bud Galler) and parasitoids collected at Wartook, Victoria from *A. mearnsii* ■ = female *Dasineura,* ♦ =male *Dasineura,* ▲= hymenopteran parasitoids

Similarly, emergence of small numbers of the endoparasitoid ?*Synopeas* sp. (Platygastridae) from *D. dielsi* galls held in a quarantine laboratory for 21 months after collection from an Australian field site, together with *D. dielsi* adults, indicated developmental diapause occurred in both the parasitoid and the host cecidomyiid. The composition of the parasitoid fauna of Tiny Floret Galler, Common Fluted galler, Elongate Fluted Galler, Egg Galler, Glabrous Bud Galler, Bipinnate Seed Galler and *D. dielsi* is discussed in detail in Chapter 5.

Emergence of *Asphondylia* sp. (Glabrous Bud Galler) occurred over 15 days with a higher female to male ratio (1.9:1, $\chi^2 = 24.8$, $P<0.001$, $n = 239$). Male and female Glabrous Bud Galler emerged simultaneously. The main parasitoids of Glabrous Bud Galler were *Ormyromorpha* sp.2 (3118/c) (Pteromalidae) and *Torymoides* sp.5 (3118/d) (Torymidae) and these emerged over three weeks and after the main emergence period of this cecidomyiid (figure 3.4b).

Depth of pupation of the Tiny Floret Galler in the soil profile

At Denmark, Western Australia, Tiny Floret Galler cocoons occurred up to 6 cm in the soil profile. There were significantly ($P<0.05$) more cocoons in the litter layer and 0-2 cm than at deeper depths in the soil profile, but no difference in cocoon number was detectable between the litter layer and 0-2 cm ($P = 0.98$) (figure 3.5).

Discussion

Indigenous South African and southern Australian *Acacia* belong to separate non-monophyletic subgenera (Miller *et al.* 2003, Miller & Bayer 2003, Murphy *et al.* 2003) with well-differentiated morphological, phytochemical and physiognomic characteristics (New 1984). Subgeneric segregation of *Acacia* based on molecular sequences is supported by host utilization patterns of gall-forming Cecidomyiidae, and support the Floate *et al.* (1996) concept that insects can provide independent tests for plant taxonomic traits.

Australian gall-forming cecidomyiids lack the capacity to utilize African *Acacia* as hosts, despite the acceptance of African acacias by some Australian midges for oviposition under caged conditions. This, together with oviposition in non-compatible Australian host *Acacia* species in cages, suggests that adult choice in Australian cecidomyiids from *Acacia* is either secondary to host-insect physiological compatibility as a determinant in gall induction or was an artefact induced by confined laboratory no-choice conditions. Examination of oviposition patterns of Australian cecidomyiids under field conditions could resolve this seeming anomaly.

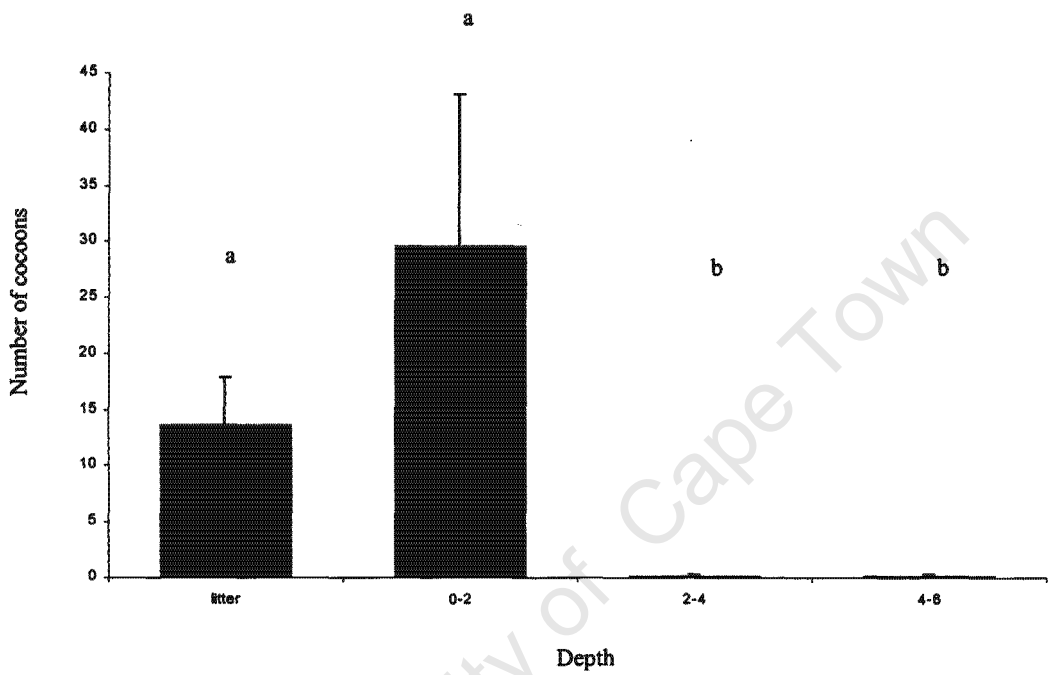


Figure 3.5. Number of Tiny Floret Galler (*Dasineura* sp.) cocoons at four soil depths (cm) at Jarrahdale, Western Australia. Data are mean ± standard deviation. Number of cocoons were cube root transformed to improve normality before analysis by ANOVA. Columns with the same letter are not significantly different (P>0.05).

In Australia, most gall-forming cecidomyiids from *Acacia* have host ranges restricted to one or a few closely related host species, with polyphagy within *Acacia* occurring rarely (Chapter 2). *Acacia* is widespread in natural Australian ecosystems with most plant communities containing a number of sympatric *Acacia* species, which often have similar reproductive phenologies. Although phenological asynchrony may explain why some Australian *Acacia* are not utilized by cecidomyiids that gall reproductive organs, host range in Australian cecidomyiids mostly regulated by physiological constraints between the host and the insect (Floate *et al.* 1996).

The *Dasineura* species, Egg Galler, Tiny Floret Galler and the Elongate Fluted Galler, together with the *Asphondylia* species, Glabrous Bud Galler and Bipinnate Seed Galler, are confined to the *Botrycephalae*, indicating a close pheno-physiological relationship with hosts in this section. Host testing, at least on Tiny Floret Galler and Elongate Fluted Galler, confirmed the stenophagous host range of these insects. In contrast, the bivoltine Common Fluted Galler occurs on at least nine *Acacia* species from four sections in eastern Australia. In host tests, the Common Fluted Galler induced large numbers of well-formed galls on *A. saligna*, a Western Australian *Acacia* that is widespread in eastern Australia, but has not been found to be susceptible to the Common Fluted Galler in field surveys in eastern Australia. In this case, asynchrony between flowering of *A. saligna* and adult emergence of the Common Fluted Galler seems to be a mechanism inhibiting galling on this host in Australia.

In Western Australia, *D. dielsi* is restricted to *A. cyclops* where galling occurs sporadically mostly at low densities, but sometimes in abundance. At several sites in South Australia where other Australian *Acacia* species were planted in shelterbelts in close proximity with *A. cyclops*, *D. dielsi* was found on four *Acacia* species (*A. sophorae, A. papyrocarpa, A. oswaldii, A. ligulata*) not previously recorded as hosts. Host testing showed that *D. dielsi* has the capacity to form galls on hosts other than those normally utilized in its natural range, suggesting that *D. dielsi* shows a strong preference for *A. cyclops*, but that other susceptible species are utilized opportunistically on occasion. All of the alternate hosts of *D. dielsi* are unlikely to support this insect in isolation as *D. dielsi* is multivoltine and requires the perpetual presence of flowers for its persistence. *A. cyclops* produces flowers throughout the year and is synchronised with the life history of *D. dielsi*. All known alternate hosts of *D. dielsi* and those found to be susceptible under laboratory conditions have a single, short, flowering pulse, usually in spring or after sufficient rain, and may support *D. dielsi* at low densities in areas where they occur in close proximity to *A. cyclops*.

Since the release of *D. dielsi* in South Africa as a biological control agent for *A. cyclops*, superficial galling on *A. melanoxylon*, closely allied to *A. cyclops*, has been recorded

at several localities. Minor galling is also expected to occur on *A. longifolia*, but has not yet been recorded.

In addition to *D. dielsi*, multivoltinism occurs in several other cecidomyiids that utilise Australian *Acacia* species with continuous flowering periods, (e.g. Small Inflated Galler on *A. oshanesii*) (figure 2.2), or those that utilize host-switching, such as the Common Fluted Galler. The Pubescent Bud Galler, a common cecidomyiid on *A. mearnsii*, is multivoltine and switches from one *Botrycephalae* host to another depending on availability of buds. *Asphondylia* sp. (Eastern Bud-Seed Galler) appears to alternate between buds and fruits of its host as well as switching between host species, thus providing this cecidomyiid with considerable choice in host selection. Consequently, it is a widespread cecidomyiid on Australian *Acacia*.

Univoltine host specialists such as cecidomyiids from inflorescences of *Acacia* risk missing reproduction if development of host organs is skipped or prevented over a season. This is likely to occur in the advent of fire, where host populations are killed; widespread masting; or during unfavourable climatic conditions, such as drought, which may lead to reduced or aborted flowering. Developmental diapause is a common risk-reducing life strategy in insects, and known from other cecidomyiids (Barnes 1956, Takasu & Yukawa 1984, Koziol 1998, Redfern & Cameron 1998). It is surprising that diapause was only recorded for *D. dielsi* from *A. cyclops*, a cecidomyiid with a very low risk from host-insect asynchrony. More intensive observations, particularly in species pupating in the soil, may reveal a more common occurrence of diapause in cecidomyiids from Australian acacias. Developmental diapause would favour the establishment success of cecidomyiids considered for biological control of Australian acacias because infestations in South Africa are subject to unpredictable and frequent fires and widespread cutting for domestic fuel consumption. In addition, Australian cecidomyiids are likely to be subject to attack from indigenous parasitoids (Chapter 5), therefore diapause that delays emergence of adults over several seasons or years is likely to enhance the survival prospects and impact of these insects.

The adaptive significance of variation in pupation sites of Australian cecidomyiids from *Acacia* is unclear. Three general patterns occur: complete larval development within chambers of the gall; subterranean development of non-feeding third instar larvae; and pupation within galls, but external to larval chambers or partial subterranean pupation. Larvae that emerge from developmental chambers to pupate within other parts of the galls or within in the soil may do so to avoid the risk of attack by predators or parasitoids or to locate favourable micro-climatic conditions for the insect's final stages of development. Dispersal from the galls may reduce the rate of parasitism by making larvae harder to locate by

potential predators or parasitoids. However, during migration larvae may face increased susceptibility to foraging predators. All lignified, thick-walled, woody galls (Fluted Gallers) utilize the 'within-chamber' pupation strategy suggesting a correlation between gall wall thickness and this form of larval development. Gall wall thickening together with dense antrorse hairs that plug the ostiole of woody galls may deter predators from entering galls, but as the most common cecidomyiid parasitoids appear to attack the very early stages of development before gall formation has advanced, only limited protection from this guild of parasitoids seems to have occurred.

On *A. mearnsii*, Tiny Floret Galler larvae emerge from larval chambers in June-July and pupate in the soil surface before ecdysis in September-November when flowering of *A. mearnsii* commences. The Tiny Floret Galler is a potential biological control agent of *A. mearnsii* in South Africa, but this insect may need to be suppressed in commercial plantations where yields of seed are important. The migration of larvae from galls in the host's canopy to subterranean positions beneath the tree may render them susceptible to insecticides applied to the soil surface during the transition period. Insecticides with sufficient residual activity in the field to endure the entire period of larval emergence are more likely to be effective than those that degrade rapidly, e.g. some synthetic pyrethroids. This possible control technique, together with canopy-applied insecticides that target adults or immature larvae, a pest control method currently used by wattle growers for other foliage-feeding insects, may increase the range of control options for the Tiny Floret Galler, if it is utilized as a classical biological control agent. The efficacy of several canopy and soil-applied insecticides for suppression of the Tiny Floret Galler are investigated in Chapter 7.

CHAPTER 4

Fungal associations in *Asphondylia* galls from Australia and South Africa: implications for biological control of invasive *Acacia* in South Africa

Abstract

Asphondylia in Australia and South Africa are associated with communities of fungi in their galls. In Australia, *Camarosporium* sp. is the most abundant and sometimes the only fungus present in *Asphondylia* galls from a broad range of host plant species. Using DNA sequences from the ITS region of the 5.8S gene extracted from rRNA cistron, the same genotype of *Camarosporium* was found in *Asphondylia* galls on the seeds of *Rhoicissus digitata* (L.f.) Gilg *et* Brandt. in South Africa, but other South African *Asphondylia* species were associated with more distantly related isolates of *Camarosporium*, and other genera of fungi. In the southern hemisphere, an *Asphondylia-Camarosporium* association is proposed as the dominant association and may have a Gondwanan ancestry. Female *Asphondylia* carry *Camarosporium* spores in mycangia located on abdominal sternites, but spores are only present on field-collected specimens. The mechanism of spore collection remains unresolved, but needs to be understood if *Asphondylia* species are to be utilized for biological control of invasive Australian acacias in South Africa. Artificial inoculation of *Acacia mearnsii* buds, a primary host of the Glabrous Bud Galler (*Asphondylia* sp.), with concentrated spore suspensions of *Camarosporium* failed to induce gall formation suggesting either an insect-fungus interaction, or that the presence of *Asphondylia* eggs or larvae serves as the stimulus for gall development.

Introduction

Asphondylia galls are invariably associated with fungi, usually *Macrophoma, Macrophomopsis* and *Botryosphaeria* or closely related anamorphs (Bissett & Borkent 1988), where mutualistic symbiosis may occur (Bissett & Borkent 1988, Gagné 1989), although some authors have argued to the contrary (Ross 1932, Batra & Lichtwardt 1963). The fungi serve as a food source for developing cecidomyiid larvae (Haridass 1987). Female Asphondyliini possess membranous cavities or pockets (mycangia) on the posterior sternites which are reputedly adapted for spore collection and transmission, suggesting that the female cecidomyiid is a vector of the fungal associates (Borkent & Bissett 1985, Bissett & Borkent 1988, Rohfritsch 1992, 1997). However, fungi within insect galls can be endophytes of the host that exploit gall formation as pathogens, saprophytes or fungal inquilines (Wilson 1995).

They may also be implicated with disease symptoms of their host, for example, *Botryosphaeria,* which are often isolated from cecidomyiid galls from the Northern Hemisphere and are saprophytes or weak parasites. In cecidomyiid galls, *Botryosphaeria* mycelium is intracellular and nutrition appears to be biotrophic (Bissett & Borkent 1988).

Asphondylia is a large cosmopolitan genus with six species described from Australia (Kolesik *et al.* 1997) and 16 species from the Afrotropics (Harris 1980, Gagné & Marohasy 1993), but many more undescribed species occur in both regions. *Asphondylia* species occur on the buds or fruits of Australian and African *Acacia* species (Gagné & Marohasy 1993, Adair *et al.* 2000). Eight undescribed *Asphondylia* occur on Australian acacias (see table 2.4) with three of these (Glabrous Bud Galler, Pubescent Bud Galler, Bipinnate Seed Galler) recorded from *A. mearnsii,* and one from *A. cyclops* (Seed Galler). The Glabrous Bud Galler is a univoltine, stenophagus cecidomyiid capable of causing high mortality of *A. mearnsii* buds. Larvae disrupt development of floret buds and populations can reach 'outbreak' proportions within their natural distribution of eastern Australia, suggesting this insect has potential as a biological control agent for *A. mearnsii* in South Africa.

The cecid-fungus relationship in Australian *Acacia* and the taxonomic status of fungal associates in both Australia and South Africa requires clarification before Australian *Asphondylia* can be utilized as biological control agents. In particular, the process of gall initiation, whether by the fungus, insect, or both needs to be determined to develop reliable host-specificity testing techniques. A mutualistic fungus from Australian *Asphondylia* species will require an independent risk assessment, if found to be non-indigenous in South Africa. However, mutualistic gall fungi may be less diverse than their insect co-inhabitants (Bissett & Borkent 1988) and South African and Australian fungi from *Asphondylia* galls may be synonymous. If *Asphondylia* symbionts are endophytic in *A. mearnsii*, their pathogenic status and distribution in South Africa needs to be determined. Although the questions raised here are essentially pragmatic from a biological control point of view, the answers could provide insights into evolutionary processes at a tri-trophic level involving a tree, a gall-forming insect and a fungal associate.

In this chapter, the relationship between *Asphondylia* from *A. mearnsii* and their fungal associates is determined and the implications for biological control of this weed in South Africa are discussed. The findings have broader utility because other *Asphondylia* species are known from *Chromolaena odorata* (L.) King & Robinson, *Ligustrum* spp., *Prosopis* spp., *Lantana camara sensu lato, Ulex europaeus* L., *Sida acuta* N.L. Burm., *S. rhombifolia* L. and *Cytisus scoparius* (L.) Link (Barnes 1946b Gagné 1977, 1989, 1994,

Ohsako *et al.* 1981, Yukawa 1982, Coetzer & Hoffmann 1997) and could be considered as seed-reducing biological control agents of these weeds in South Africa and elsewhere.

Materials and Methods

Gall development and anatomy

Tissue arrangement and structure of *Asphondylia* sp. (Glabrous Bud Galler) galls from *A. mearnsii* and *A. irrorata* were prepared by sectioning of wax-embedded galls, following a slightly modified procedure of Bancroft & Stevens (1990). Fresh galls from a range of developmental stages were preserved in glutaldehyde/paraformaldehyde fixative prior to sectioning. Galls were soaked in 70%, 90% and 100% ethanol consecutively for 1 hr to dehydrate tissue before clearing in xylol for 1 hr. Galls were then infiltrated with liquid paraffin wax at 60^{0}C for 2 hrs then embedded in wax before cutting 5 μ sections with a rotary microtome. Sections were then reheated to 60^{0}C for 1 hr before dewaxing in xylol for 10 min and hydrated (100%, 90%, 70% alcohol) to water before staining with Mayer's hematoxylin and eosin. Sections were then dehydrated (70%, 90%, 100% alcohol) and cleared in xylol before mounting with Entellen medium and covering with a glass coverslip. Tissue structure was examined using an optical microscope.

Fungi from Asphondylia *galls*

Fungi associated with the galls of *Asphondylia* were isolated by culturing hyphae on Petri dishes containing potato dextrose agar (PDA) (20g potato, 20g Biolab agar, 1000ml H_2O) or water agar (WA) (20g Biolab agar, 1000ml H_2O) or WA at 27^{0}C for 2-4 weeks. Galls were double surface sterilized by rinsing in 70% ethanol for 30 sec, followed by 1% NaOCL for 2 min, then again in 70% ethanol for 15 sec before fungal material was dissected from the inner lining of the gall chamber under a stereomicroscope. After 1-5 days mycelial growth was hyphal tipped and transferred to fresh PDA plates for sporulation and identification.

Fungi in buds of A. mearnsii

Ungalled buds of *A. mearnsii* were sampled in Australia and South Africa to determine if mutualistic fungi in *Asphondylia* galls were present as endophytes in the host tree. At nine sites in Australia, five inflorescences with ungalled buds were haphazardly selected from each of five *A. mearnsii* trees. Five buds from each inflorescence were removed and double surface sterilized using the methods described above. Buds were cut in half with a sterile scalpel and plated onto PDA or WA and cultured at 25-27^{0}C. Mycelial growth was

hyphal-tipped onto PDA and cultured until sporulation. Cultures were identified to genera using fruiting body and mycelial characteristics. Fungal identifications were made by M. Serdani, Weeds Division (pathology) and Isabel Rong, Biosystematics Division ARC-Plant Protection Research Institute. Five sites of *A. mearnsii* in the Western Cape, South Africa were also sampled using exactly the same techniques.

Fungi from Asphondylia *adults*

To determine the risk of *Asphondylia* acting as vectors of plant pathogens, adults of the Glabrous Bud Galler (3118, 3119, 3120, 3256, 3259) and Pubescent Bud Galler (3121) were reared under quarantine conditions from fresh galls collected in Australia. Galls of the Glabrous Bud Galler were collected at the Grampians, Victoria (37^0.03'S, 142^0.21'E), Cranbourne, Victoria (38^0.07'S, 145^0.16'E), Campbelltown, New South Wales (NSW) (33^0.59'S, 150^0.50'E) and Mittagong, NSW (34^0.26'E, 150^0.28'E) from *A. mearnsii*, and Pubescent Bud Galler was collected at Stawell, Victoria (37^0. 21'S, 143^0. 10'E) from *A. baileyana*. Galls were held in emergence cages that had been sterilized by washing in 1% NaOCL for 5 min. Freshly emerged adults were collected in sterile plastic tubes and killed using ethyl acetate or freezing. Adults were plated onto WA within a laminar flow cabinet. Mycelium growth was hyphal-tipped, aseptically transferred to PDA and cultured for identification as above.

To determine if female *Asphondylia* carry spores of fungi in mycangia, wild adult *Asphondylia* were aspirated from *A. mearnsii* and *A. baileyana* in Australia and preserved in either 70% ethanol or glutaldehyde/paraformaldehyde fixative. Adults were cleared and mounted on glass slides in Canada balsam following Gagné (1989). Mycangia were examined using differential interference contrast microscopy. Adult *Asphondylia* reared from galls held in sterilized cages under quarantine conditions were also examined for the presence of mycangial spores. Mycangia were carefully dissected from 47 dried, wild caught Glabrous Bud Galler (3252) collected at Campbelltown, NSW (33^0.59'S, 150^0.50'E) on *A. mearnsii* and plated, within a laminar flow cabinet, onto PDA and maintained at 25^0C. Mycelial growth was cultured as above.

The external anatomy of *Asphondylia* sp. (3171) mycangia reared from *Acacia decurrens* fruits from Holbrook, NSW (35^0.56'S, 147^0.08'E) were studied using SEM. Ethanol preserved abdomens were critical point dried, mounted on aluminium stubs with carbon glue and sputter-coated with gold palladium alloy then examined with a Leo S440 scanning electron microscope.

Asphondylia *behaviour in the presence of fungal spores*

The behaviour of *Asphondylia* in the presence of gall fungi was studied by caging freshly emerged laboratory-reared adults of the Glabrous Bud Galler (3120, 3118, 3259) collected from *A. mearnsii* at the Campbelltown, NSW, the Grampians, Victoria, and Mittagong, NSW with sporulating cultures of *Phoma* spp. and *Camarosporium* sp. These fungal cultures were isolated from Pubescent Bud Galler and Glabrous Bud Galler galls from *A. mearnsii*. Adult cecidomyiids were reared from galls held in cages in a quarantine laboratory. Freshly cut blocks of PDA with sporulating fungi were placed in plastic caps (11 mm x 7 mm) in the base of clear plastic cylinders. Cylinders were 12 cm x 8.5 cm and capped with a ventilated lid. Two male and three or four female Glabrous Bud Galler were added to each cylinder and provided with a fresh twig of young *A. mearnsii* buds held in a vial of water. *Phoma* (5 isolates) and *Camarosporium* (1 isolate) were selected for testing as these genera were strong candidates as primary gall symbionts. There were 2-5 replicates for each isolate. Control tests contained caps of uninoculated agar. Tests were undertaken in a quarantine glasshouse at 22-23^{0}C with natural lighting. Observations on midge behaviour were made continuously over 5 hrs. After 24 hrs, females were collected and preserved in 70% ethanol for examination for the presence of spores in their mycangia following the techniques described above.

Inoculation of A. mearnsii *buds with conidia of* Camarosporium

An isolate of *Camarosporium* sp. (3001) from Glabrous Bud Galler galls of *A. mearnsii* was cultured on PDA with an infusion of *A. mearnsii* leaves at 20^{0}C in a controlled environment chamber. Pycnidia were collected, emasculated then added to 5 ml of autoclaved distilled water to produce a spore suspension of 1x10^5 spores per ml. In a quarantine glasshouse, 52 inflorescences with small to mature buds of *A. mearnsii* were wiped with spore suspension using a paintbrush. Treated inflorescences were immediately enclosed in clear plastic bags for 24 hrs and maintained under glasshouse conditions with natural lighting at 21-25^{0}C. Control and treated inflorescences were inspected for gall formation after six weeks.

In a second experiment, emasculated pycnidia of *Camarosporium* sp. were prepared into a wet paste with a drop of autoclaved distilled water. Entomological micro-pins (6 mm x 0.23 mm) were mounted on toothpicks and used as needles to pierce small to large buds of *A. mearnsii* on 13 inflorescences. The tapered ends of the micro-pins were dipped in spore paste immediately prior to piercing individual buds several times by inserting the sharp point just

below the outer layer of fringed bracteoles. Inflorescences were bagged and maintained as above.

DNA sequencing of Camarosporium *from Australian and South African galls*

Relationships among the *Camarosporium* isolates from Australian and South African *Asphondylia* galls were compared by sequencing DNA from the ITS region (Internal Transcribed spacer 1, 5.8S gene, Internal Transcribed Spacer 2) of the nuclear-encoded rRNA cistron. DNA was extracted using a modified CTAB method (Gawel & Jarret 1991). The ITS region was amplified using primers ITS5 (forward) and ITS4 (reverse) (White *et al.* 1990). Amplified products were cleaned using Qiagen clean-up columns, and cycle sequenced using the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit (P. E. Applied Biosystems). Amplification primers were also used as sequencing primers, and products were resolved on an ABI 3900 automated sequencing machine.

There were relatively low levels of sequence divergence, therefore a statistical parsimony approach (Templeton *et al.* 1992) was used to reconstruct relationships among isolates. Haplotype networks were determined using the TCS programme (Clement *et al.* 2000). Gaps were treated as a fifth character-state, but were all trimmed to have a length of one position.

Results

Gall development and anatomy

Bud galls of *Asphondylia* sp. (Glabrous Bud Galler) from *A. mearnsii* are globose contracting abruptly into a flattened apex with a distinct nipple-like mucro formed from remnant perianth (see figure 2.3m). The exterior is glabrous to sparsely pubescent. Gall formation occurs within five weeks of adult emergence where slightly swollen buds occur amongst withered perianth. Galls expand rapidly reaching full size within several months, although larval development is slow in the early stages of gall development (Adair pers. obser. 2001-2003). Larvae undergo accelerated development one to two months before emergence.

Mycelia of the fungal co-associate are evident early in gall development, although mostly localized in the distal region, probably around the point of inoculation or entry (figure 4.1a). In young galls, staminal tissue appears normal, but soon undergoes dramatic changes that are associated with fungal development and appear as gross thickening of the filaments through cell proliferation. The gall wall becomes lignified through growth of sclerenchyma cells and simultaneous compaction of inner parenchyma tissue. Fungal mycelia ramify in the apex of the gall and extend across the inner gall surface and amongst the stamen bundle,

where cellular disintegration of staminal tissue and the outer parenchyma of the gall wall are evident (figure 4.1b-c). A larval cavity forms with advanced disintegration of staminal tissue (figure 4.1d), which is associated with compression of remnant staminal tissue against the inner gall wall amongst thin layers of mycelium.

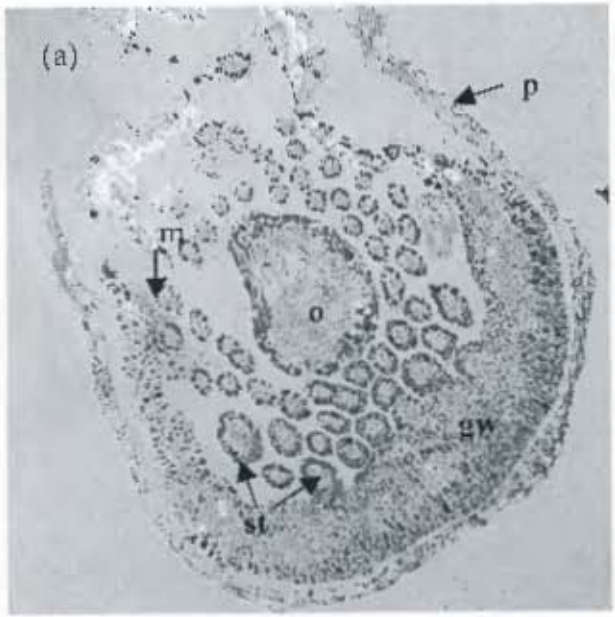
In a mature gall, the cecidomyiid larva occupies most of the gall cavity and is surrounded by a compacted layer of melanised fungal mycelium that forms a mantle over the entire inner gall wall (figure 4.1e). Partially disintegrated staminal remnants occur in the mantle, but intra-cellular penetration of intact host tissue by mycelium is absent in all stages of gall development. The pupa creates an emergence hole through the fungal mantle and gall wall by rotating and using its antennal horns as a cutting device. No sporulating structures of the fungal co-associate (*Camarosporium* sp.) were seen on or within galls at any stage of development, including fresh and dry galls after emergence of adults (figure 4.1e).

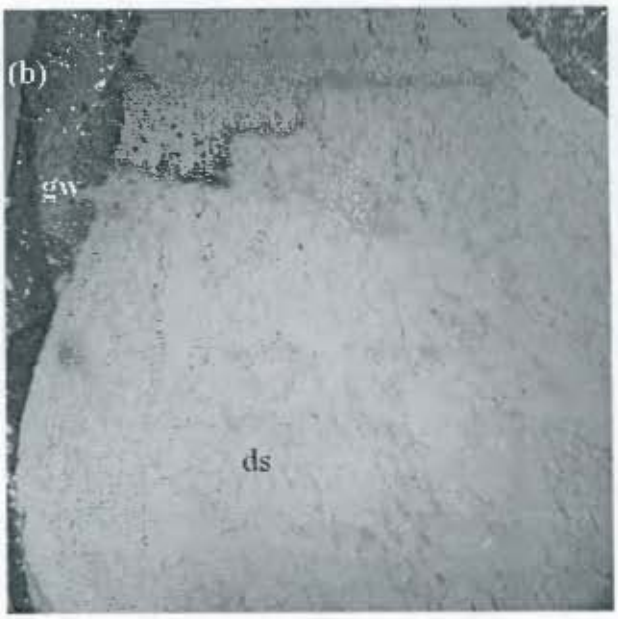
Fungi in buds of A. mearnsii

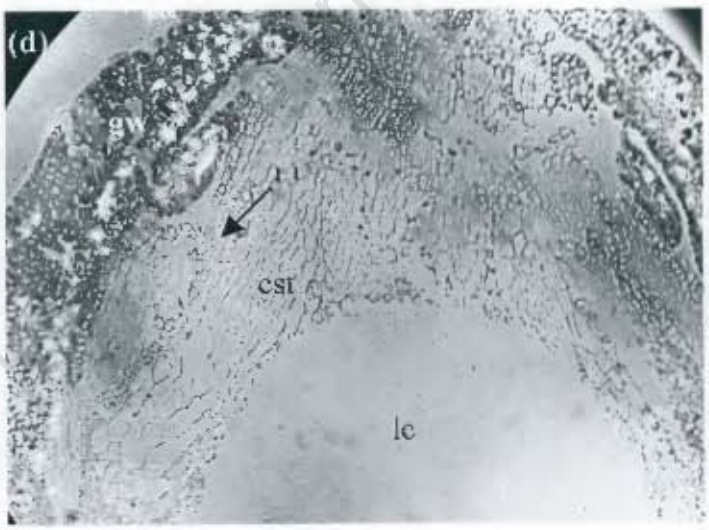
Twenty-seven fungal taxa were isolated from surface-sterilized buds of *A. mearnsii* collected in Australia with a mean (± SD) diversity of 7.6 ± 4.2 fungal taxa per site (table 4.1). Buds of *A. mearnsii* in South Africa had a less diverse fungal flora than buds from Australia, with nine fungal taxa recorded. There was a mean (± SD) of 2.2 ± 2.2 fungal taxa per site in South Africa (table 4.1). All but two South African fungal genera (*Pycnostysanus, Chaetomium*) were present in buds from Australian accessions of *A. mearnsii.* All fungi isolated from buds of *A. mearnsii* from Australia and South Africa occurred at low incidence levels. The predominantly pathogenic genera *Alternaria, Aureobasidium, Coniothyrium* and *Colletotrichum* were widespread in buds of *A. mearnsii* from Australia.

Fungi from Asphondylia *galls*

Sixteen accessions of *Asphondylia* galls were made in southern Australia between 1999-2003 from 11 host species and six plant families. Nine accessions were from *Acacia* and seven from non-Mimosaceae genera (table 4.2a). In South Africa, 10 *Asphondylia* accessions were made from 10 host species and nine families, with only one accession from *Acacia* (table 4.2b). Australian accessions of *Asphondylia* galls yielded 21 fungal taxa with 1-11 species occurring within galls of a single host. All inoculations yielded *Camarosporium* sp., most at high incidence levels and this was the only fungus isolated from *Dodonaea viscosa* Jacq. (Sapindaceae), *Acacia cyclops*, *A. irrorata* and fruit galls of *A. mearnsii.* Eighteen fungal taxa were reared from *Asphondylia* galls from Australian *Acacia*, nearly all from bud galls of *A. mearnsii* (table 4.2a).







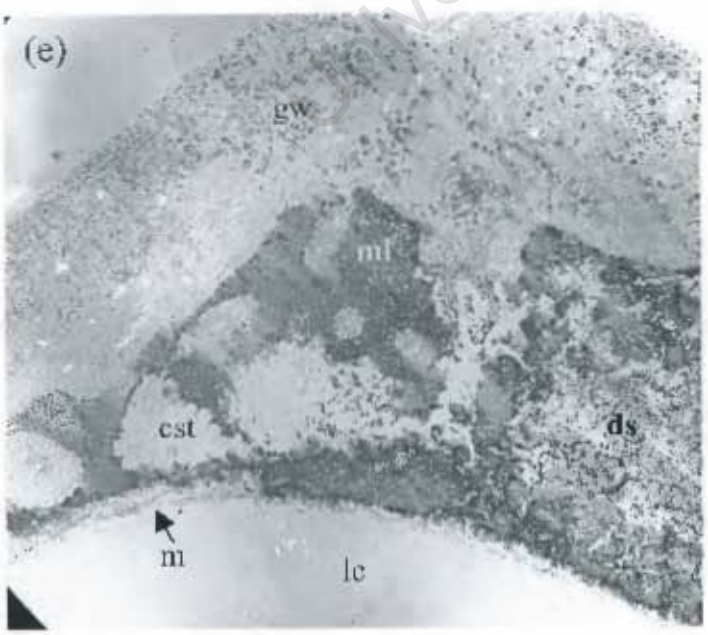


Figure 4.1 (a-e). Sections through *Asphondylia* sp. (GBG) galls from *A. mearnsii* and *A. irrorata*. (a) Five-week gall *from A. mearnsii* (3130) (transverse section (T.S.)), (b) 10-month gall from *A. mearnsii* (3001) (longitudinal section (L.S.)), (c-d) gall from *A. irrorata* (3006), (c) L.S., (d) T.S., (e) mature gall from *A. mearnsii* (3026) with pupa emerged (T.S.).
st= stamen, o=ovary, m= mycelium, p= perianth, gw= gall wall, lc= larval chamber, ml= melanized hyphal layer, ds= distintegrating enlarged stamens, cst= compacted stamen remnants.

South African accessions of *Asphondylia* galls yielded 11 fungal taxa with 1-5 species occurring within galls of a single host (table 4.2b). *Camarosporium* was a primary fungus reared from South African *Asphondylia* galls, although incidence levels were lower than that occurring in Australian galls. Five fungal genera were common to Australian and South African *Asphondylia* galls: *Camarosporium, Epicoccum, Alternaria, Phoma* and *Fusarium* (table 4.2).

Eleven of the 18 fungal genera isolated from *Asphondylia* galls from Australian *Acacia* were present in surface-sterilized buds, the remainder were only found in galls and were rare (*Bipolaris, Septoria, Botryocrea, Chaetomium, Drechslera, Heteropatella, Xylaria, Apiosordaria*).

Fungi isolated from Asphondylia *adults*

Seven genera of fungi were isolated from adult *Asphondylia* reared from bud galls of *A. mearnsii* under laboratory conditions (table 4.3) and all were deuteromycetes and predominantly cosmopolitan saprophytes. Only two fungal isolates from adult *Asphondylia* (*Camarosporium* and *Chaetomium*) were also present in *Asphondylia* galls on Australian *Acacia* and buds of *A. mearnsii* (tables 4.1, 4.2).

The mycangia of wild caught adults of *Asphondylia* from Australian *Acacia* contained fungal spores in conspicuous numbers (figure 4.2a, table 4.4), while nearly all laboratory-reared specimens were devoid of spores (figure 4.2b). Spores within the mycangia of wild-caught *Asphondylia* were elliptic-oval and were either undivided or uni- to multiseptate. Spores from mycangia were slightly smaller than those of *Camarosporium* conidia isolated from *Asphondylia* galls (tables 4.4, 4.5), but shared the characteristic irregularly partite, septal pattern characteristic of this fungal genus (figure 4.2c) (Barnett & Hunter 1998).

Seven fungi were isolated from excised mycangia from wild-caught *Asphondylia* from *Acacia,* but were dominated by *Camarosporium,* which occurred in 74% of individuals (table 4.6). Other fungi occurred at low levels and were either common endophytes (*Alternaria, Epicoccum*), potential pathogens (*Sphaeropsis, Botryodiplosis*) or common soil fungi (*Trichoderma*).

Mycangia of *Asphondylia* from Australian *Acacia* were similar to those described elsewhere by Borkent & Bissett (1985). The mycangium is a membranous sac located at the seventh abdominal sternite (Bissett & Borkent 1988). The sac extends laterally to the margin of the sternite and opens broadly along the posterior margin. The apex is widely obtuse and a hairless, longitudinally grooved pad subtends the entrance to the mycangium (figure 4.3).

Table 4.1. Fungi isolated from surface sterilized buds of *Acacia mearnsii* from Australia and South Africa. *n* = number of buds sampled per site. NSW =New South Wales, VIC=Victoria, WA=Western Australia, WC=Western Cape. Number in parenthesis is the number of isolations of each fungus. NI= no isolations

Country	Site	State or Province	Voucher. No.	*n*	Fungi
South Africa	Stellenbosch	WC	-	-	*Alternaria* sp. (1), *Colletotrichum gleosporioides* (1), *Nigrospora* sp. (1), *Phomopsis* sp. (1), *Pycnostysanus* sp. (1), sterile grey (1)
	Villiersdorp	WC	3272	25	sterile white (1)
	Grabouw	WC	3270	25	*Chaetomium* sp. (1), sterile white (1)
	Paarl	WC	3271	25	NI
	Kylemore	WC	3273	25	black yeast-like (1), sterile grey (1)
Australia	Campbelltown	NSW	3161	50	*Alternaria* sp. (2), *Aureobasidium* sp. (3), *Cladosporium* sp. (1), *Colletotrichum* sp. (1), *Nigrospora* sp. (1), *Truncatella* sp. (1), sterile grey (1)
	Armidale	NSW	3249	20	sterile grey (2)
	Marulan	NSW	3257	25	sterile white (1)
	Warrandyte	VIC	3123	25	*Camarosporium* sp. (3), *Cercospora* sp. (1), *Colletotrichum* sp. (2), *Coniothyrium* sp. (2), *Diplodia* sp. (2), *Epicoccum purpurascens* (1), *Graphium* sp. (2) , *Phomopsis* sp. (5) , *Torulla* sp. (1), *Ulocladium* sp. (2), sterile grey (1), sterile white (1), sterile brown (2)
	Bundoora	VIC	3124	25	*Alternaria* sp. (4), *Aureobasdium* sp. (2), *Camarosporium* sp. (3), *Colletotrichum* sp. (4), *Coniothyrium* sp. (2), *Fusarium* sp. (2), *Phoma* sp. (9), sterile grey (2), yeast-like (1)
	Brimbank	VIC	3125	25	*Alternaria* sp. (5), *Aureobasidium* sp. (4), *Phoma* sp. (3), *Steganosporium* sp. (1), *Stemphylium* sp. (1), sterile grey (1), sterile brown (1)
	Cranbourne	VIC	3127	25	*Alternaria* sp. (1), *Aureobasidium* sp. (2), *Curvularia* sp. (1), *Colletotrichum* sp. (2), *Coniothyrium sp.* (2), *Epicoccum purpurascens* (1), *Nigrospora* sp. (1), *Phoma sp.* (3), *Phomopsis* sp. (2), *Seimatosporium arbusti* (6), sterile brown (3)
	Lilydale	VIC	3128	25	*Alternaria* sp. (1), *Aureobasidium* sp. (1), *Coniothyrium* sp. (3), *Camarosporium* sp. (2), *Curvularia* sp. (1), *Diplodia* sp. (1), *Epicoccum purpurascens* (2), *Fusarium* sp. (2), *Phomopsis* sp. (3), sterile grey (6)
	Denmark	WA	3244	25	*Alternaria* sp. (1), *Colletotrichum* sp. (1), *Diplodia* sp. (1), *Phoma* sp. (5), *Seimatosporium* sp. (1), sterile white (2), sterile grey (4), black yeast (2), unidentified hyphomycete (8)

Table 4.2. Fungi reared from *Asphondylia* galls in (a) Australia and (b) South Africa. Organ is the host parts infected by the cecidiomyiid: L=leaves, F= fruits, B=buds, S=stems, ST=shoot tips. Numbers in parenthesis are the number of isolates obtained for each fungal species. NI=no fungi isolated from galls. GBG=Glabrous Bud Galler, PBG=Pubescent Bud Galler, SG=Seed Galler. *n* = number of galls sampled.

(a)

Cecidomyiid	Voucher No.	Host family	Host species	Organ	*n*	Fungi
Asphondylia dodonaeae	3253	Sapindaceae	*Dodonaea viscosa*	L, S	10	*Camarosporium* sp. (6)
Asphondylia anthocercidis	3294	Solanaceae	*Anthocersis littorea*	F	14	*Camarosporium* sp. (2), *Alternaria* sp. (1), sterile white (1), sterile grey (5)
Asphondylia inflata	3254	Chenopodiaceae	*Halosarcia pergranulata*	S	7	NI
Asphondylia sp.	3255	Chenopodiaceae	*Sarcocornia quinqueflora*	S	-	NI
Asphondylia sp.	3261	Pittosporaceae	*Billardiera variifolia*	ST	10	*Camarosporium* sp. (17), *Phragmotrichum* sp. (1)
Asphondylia sp.	2891	Pittosporaceae	*Sollya heterophylla*	B, F, ST	10	*Camarosporium* sp. (15), *Phoma.* sp. (1)
Asphondylia sp.	3253A	Violaceae	*Hybanthus floribundus*	B	14	*Camarosporium* sp. (9), *Cladosporium* sp. (1), *Phoma* sp. (1), sterile white (1)
Asphondylia sp.	3213	Mimosaceae	*Acacia littorea*	B	11	*Camarosporium* sp. (3), *Bipolaris* sp. (1), *Colletotrichum* sp. (1), *Phoma* sp. (1), *Septoria* sp. (2)
Asphondylia sp. (GBG)	3247	Mimosaceae	*Acacia irrorata*	B	11	*Camarosporium* sp. (9)
Asphondylia sp. (SG)	3248	Mimosaceae	*Acacia irrorata*	F	10	*Camarosporium* sp. (3)
Asphondylia sp. (SG)	3275	Mimosaceae	*Acacia cyclops*	F	10	*Camarosporium* sp. (3)
Asphondylia sp. (SG)	3126, 3129*	Mimosaceae	*Acacia mearnsii*	F	20	*Camarosporium* sp. (20)
Asphondylia sp. (GBG)	3161	Mimosaceae	*Acacia mearnsii*	B	20	*Camarosporium* sp. (2)
Asphondylia sp. (PBG)	3161	Mimosaceae	*Acacia mearnsii*	B	2	*Camarosporium* sp. (1)
Asphondylia sp. (GBG)	3001	Mimosaceae	*Acacia mearnsii*	B	40	*Camarosporium* sp. (2), *Alternaria* sp. (6), *Botryocrea* sp. (1), *Chaetomium globosum* (1), *Diplodia* sp. (6), *Drechslera biseptata* (1), *Fusarium lateritium* (6), *Heteropatella* sp. (1), *Phoma* sp. (2), *Xylaria* sp. (1), sterile grey (1)
Asphondylia sp. (PBG)	3002	Mimosaceae	*Acacia mearnsii*	B	40	*Camarosporium* sp. (2), *Alternaria* sp. (1), *Apiosordaria verruculosa* (1), *Coniothyrium* sp. (1), *Diplodia* sp. (2), *Epicoccum purpureum* (1), *Phoma* sp. (2), sterile grey (2), yeast (3)

* developing pycnidia isolated from loci of pods with advanced stages of *Asphondylia* sp.

(b)

Cecidomyiid	Coll. No.	Host family	Host species	Organ	*n*	Fungi
Asphondylia sp.	3242	Vitaceae	*Rhoicissus digitata*	F	5	*Camarosporium* sp. (5), *Alternaria* sp. (1)
Asphondylia sp.	3301	Fabaceae	*Psoralea oligophylla*	F	12	*Camarosporium* sp. (9)
Asphondylia sp.	3179	Fabaceae	*Virgillia oroboides*	B	19	*Camarosporium* sp. (8), *Fusarium* sp. (4), *Nigrospora* sp. (5), *Alternaria.* sp. (1), black yeast-like (1)
Asphondylia sp.	3038, 3278	Mimosaceae	*Acacia karroo*	B	13	*Alternaria* sp. (1), *Epicoccum pupurascens* (1), *Phoma* sp. (1), sterile grey (4)
Asphondylia sp.	3243/3274	Ebenaceae	*Diospyros glabra*	S	15	*Fusicoccum* sp. (?)
Asphondylia sp.	3302	Mesembryanthaceae	*Psilocaulon articulatum*	F	1	*Camarosporium* sp. (1)
Asphondylia ricini	3236	Euphorbiaceae	*Ricinus communis*	B	13	*Macrophoma* sp. (6)
Asphondylia sp.	3241	Tiliaceae	*Grewia occidentalis*	B	7	*Camarosporium* sp. (3), *Alternaria* sp. (1), *Phomopsis* sp. (1)
Asphondylia sp.	3302	Chenopodiaceae	*Salsola* sp.	ST	4	*Epicoccum pururascens* (3)
?Asphondylia sp.	3279	Santalaceae	*Osyris compressa*	B	38	*Camarosporium* sp. (1), *Epicoccum purpurascens* (1), sterile grey (7), sterile white (1), black yeast-like (3)

Asphondylia *behaviour in the presence of fungal spores*

Asphondylia bud gallers oviposited in small to medium buds of their host *Acacia* after performing a series of sequential acts commencing with short episodes of antennal probing at the potential egg-laying site. This was followed by foreleg tapping in a tight circle over the bud surface. Oviposition then commenced with the female acutely bending her abdomen beneath the thorax before probing and then inserting her ovipositor through bud tissue. In caged tests with sporulating cultures of *Phoma* and *Camarosporium*, female *Asphondylia* demonstrated full oviposition behaviour on buds of *A. mearnsii*, but did not make deliberate attempts to collect spores from the fungal source. Mycangia of laboratory-reared females contained no *Phoma* or *Camarosporium* conidia after 24-36 h exposure to the fungal source (table 4.7).

Inoculation of *A. mearnsii* buds with conidia of *Camarosporium*

Acacia mearnsii buds inoculated with concentrated *Camarosporium* spores in suspension or as a paste flowered normally within several weeks of treatment. After six weeks, no bud galls had been induced. Galls normally become apparent at field sites within five weeks of adults being present.

DNA sequencing of Camarosporium *from Australian and South African galls*

Maximum parsimony analysis established *Camarosporium* from Australian and South African *Asphondylia* galls and *Asphondylia* adults form a robust monophyletic group. As divergences among isolates were generally low, an unrooted statistical parsimony network was calculated for all isolates. Two haplotype groups with identical DNA sequences from the ITS region are apparent, Type 1 comprised of *Camarosporium* isolated from *Asphondylia* galls from four Australian host plants: *Acacia, Dodonaea* (Sapindaceae), *Billardiera* (Pittosporaceae), and *Sollya* (Pittosporaceae), and the South African liana *Rhoicissus digitata* (Vitaceae). Most isolates from *Asphondylia* from *A. mearnsii* were included in the type1 group. A second group, Type 2, included two accessions with identical ITS sequences, one from the mycangia of Glabrous Bud Galler from *A. mearnsii* and the other from the seed galls of *Asphondylia* sp. (Bipinnate Seed Galler). *Camarosporium* from *Asphondylia* galls in Western Australia from *A. littorea* and *A. cyclops* were closely related to Type 1 and Type 2 haplotypes. Most South African *Camarosporium* isolates varied from the Australian haplotypes by 2-4 indels and had greater variation between accessions than those collections from Australia (figure 4.4).

Table 4.3. Fungi reared from adult *Asphondylia* sp. (GBG) reared under caged conditions from buds of *Acacia mearnsii*. Numbers in parenthesis are the number of isolates from each fungal species. *n* = number of adult flies sampled.

Cecidomyiid	Voucher No.	Host	*n*	Fungi
Asphondylia sp.	3256	*A. mearnsii*	18	*Cladosporium* sp. (2), *Chaetomium* sp. (2), *Penicillium sp.* (1), sterile white (3)
Asphondylia sp.	3259	*A. parramattensis* *A. mearnsii*	24	*Camarosporium* sp. (8), *Penicillium* sp. (8), *Cladosporium* sp. (3)

Table 4.4. Incidence of spores present in mycangia *of Asphondylia* spp. from Australian *Acacia*. PBG = Pubescent Bud Galler, GBG = Glabrous Bud Galler, SG = Seed Galler. Source is the method of rearing:W = wild caught adults, R = adults reared from galls in emergence cages in quarantine. Mean conidia size (±SD): L = spore length, W = spore width. *n* = number of mycangia sampled.

Cecidomyiid	Voucher No.	Host	Source	*n*	% ♀'s with spores	Mean spore size (µm) L x W	*n*
Asphondylia sp. (PBG)	3119	*A. baileyana*	R	20	0	-	
Asphondylia sp. (GBG)	3120	*A. mearnsii*	R	20	0	-	
Asphondylia sp. (SG)	3171	*A. decurrens*	R	13	0	-	
Asphondylia sp. (GBG)	3113	*A. mearnsii*	W	21	90.4	10.8 x 7.7	34
Asphondylia sp. (PBG)	2670	*A. baileyana*	W	1	0	-	
Asphondylia sp. (GBG)	3252	*A. mearnsii*	W	19	94.7	12.7 x 8.1	17

Table 4.5. Dimensions of *Camarosporium* conidia isolated from *Asphondylia* galls from Australian *Acacia*. GBG = Glabrous Bud Galler, SG = Seed Galler, PBG = Pubescent Bud Galler. *n* = number of spores sampled.

Cecidomyiid	Voucher No.	Host	Mean conidia size (µm) L x W	*n*
Asphondylia sp. (GBG)	3259	*A. mearnsii*	19.5 x 6.6	10
Asphondylia sp. (GBG)	3001	*A. mearnsii*	16.8 x 8.1	10
Asphondylia sp. (PBG)	3002	*A. mearnsii*	16.0 x 7.6	10
Asphondylia sp. (SG)	3129	*A. mearnsii*	12.7 x 7.5	10

Table 4.6. Fungi isolated from mycangia of wild caught *Asphondylia* sp. (Glabrous Bud Galler) from *Acacia mearnsii*. Numbers in parenthesis are the number of isolates of each fungal species. *n* = number of mycangia sampled.

Cecidomyiid	Voucher No.	*n*	Fungi
Asphondylia sp.	3252	47	*Camarosporium* sp. (34), *Alternaria* sp. (3), *Botryodiplodia* sp. (1), *Epicoccum* sp. (2), *Sphaeropsis* sp. (2), *Trichoderma* sp. (2), unidentified hyphomycete (2)

Table 4.7 Results of oviposition behaviour experiments with *Asphondylia* sp. (Glabrous Bud Galler) (3120, 3118, 3259) from *Acacia mearnsii* in the presence of sporulating cultures of *Phoma* and *Camarosporium* isolated from cecidomyiid galls. *n* = number of female's in each experiment

Observation	Control $n = 6$	*Phoma* spp. $n = 28$	*Camarosporium* sp. $n = 35$
No. of copulations	0	0	0
No. of oviposition events	1	7	12
No. casual contacts with fungal culture	1	7	3
No. spore collection events	0	0	0
No. females with spores in mycangium	0	1*	2*

* elongate, multi-septate conidia of undetermined fungus. Not *Phoma* spp. or *Camarosporium* sp.

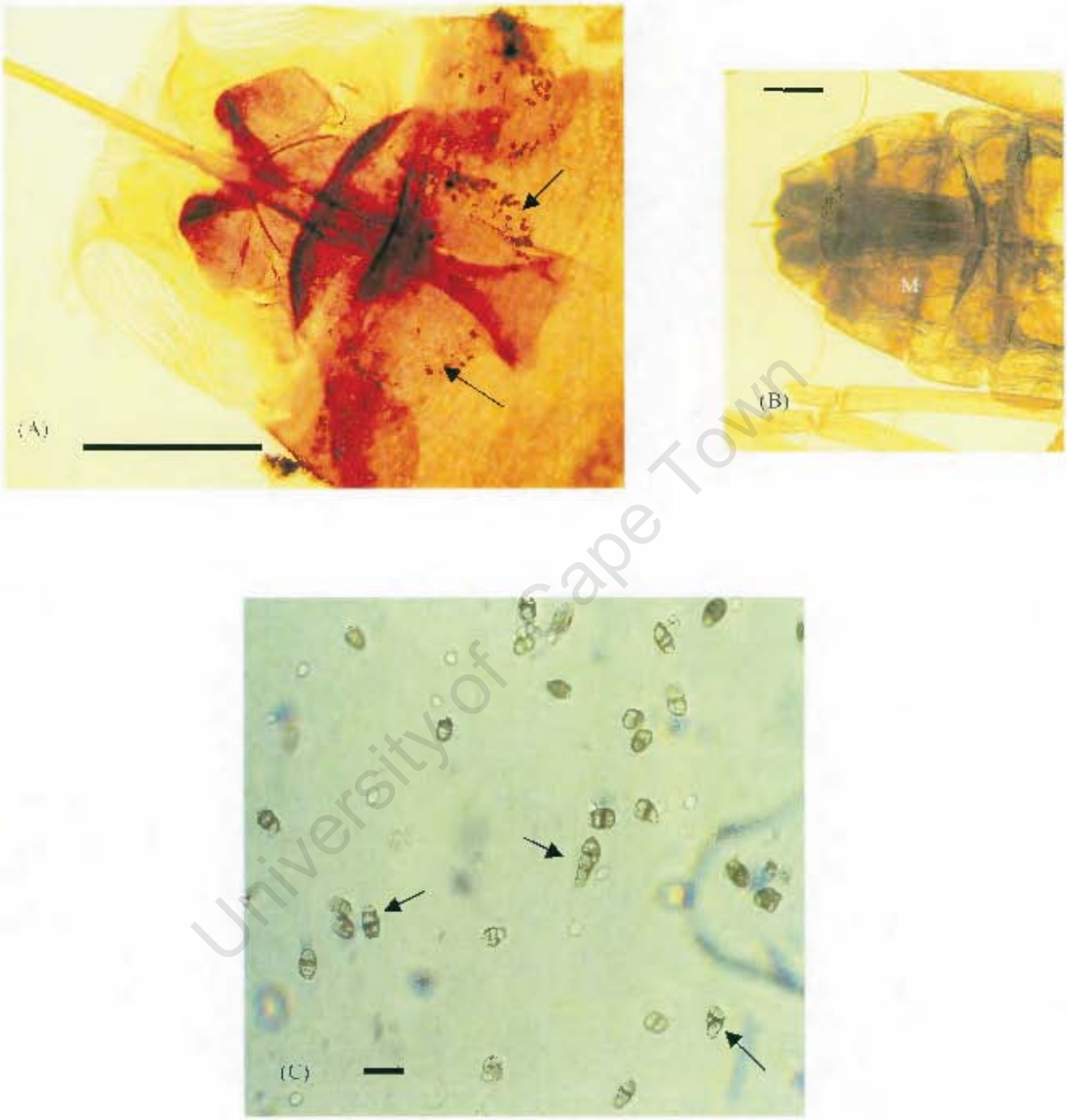


Figure 4.2. Cleared abdomens of *Asphondylia* sp. (Glabrous Bud Galler) (3113) (a) spores present in mycangium of a wild caught fly indicated by arrows (b) barren mycangium of a laboratory-reared fly, M = mycangium (c) spores of *Camarosporium* sp. isolated from *Asphondylia* sp. (Pubescent Bud Galler) (3002). Arrows point to multiseptate spores typical of *Camarosporium*. (a, b) scale bar = 200µm, (c) scale bar = 20µm.

Discussion

Asphondylia is a large cosmopolitan genus with around 260 described species (Gagné 1994), with six described species from Australia (Kolesik *et al.* 1997) and five described species from South Africa (Crosskey 1980). Many *Asphondylia* species are pests of crops causing galls on shoots, buds, flowers or fruits. *Asphondylia* has radiated on *Acacia* in Australia with at least eight species occurring on buds and ovules of 17 primary hosts (Chapter 2). *Asphondylia* appears less diverse in South Africa, with two species recorded from the flower-heads of *A. karroo*, but two other species occur on related *Acacia* from Kenya (Gagné & Marohasy 1993).

Asphondylia species are generally associated with fungi in galls and considerable debate has occurred on the nature of the cecidomyiid-fungus relationship. In Australian and South African *Asphondylia,* galls usually contain communities of fungi with up to 11 taxa present. The presence of fungal communities in European 'ambrosia galls' prompted Mani (1964) to suggest true symbiosis was absent as no primary symbiont could be identified. Others concluded that fungal co-associates were inquilines and their presence in galls was purely incidental (Ross 1932). Compelling evidence for Baccarini's (1893) original proposal for symbiosis is supported by observations of conidia being deposited with eggs by *Asphondylia* on *Symplocos* (van Leeuwin 1929), the isolation of a proposed symbiont from *Asphondylia* (= *Ischnonyx*) galls on *Prunus* (Goidanich 1941) and evidence of symbiosis in *Lasioptera* on *Eryngium* (Meyer 1952). The discovery of spore-containing mycangia on the abdomens or ovipositors of female cecidomyiids associated with fungi provided strong support for mutualism between *Asphondyliini* (and others) and a range of coelomycete fungi (Borkent & Bissett 1985, Bissett & Borkent 1988). However, the suggestion that spores in mycangia were the same as those produced by fungi in galls was not conclusive as fungi within mycangia were not identified by isolation and *in-vitro* culturing.

Asphondylia galls on Australian *Acacia* invariably occur with fungi that proliferate in the early stages of gall development. In buds, fungal mycelia ramify and surround enlarged stamens that are eventually consumed by the developing insect or are compacted within melanised hyphae that surround the inner surface of the gall. Intra-cellular penetration seems to be absent. In fruit galls, mycelia surround the developing seed causing its abortion and eventual destruction. Adult *Asphondylia* emerge through the wall of the gall leaving their exuvia in the emergence hole cut by the pupa. In bud galls from *Acacia*, fungal fruiting bodies were never observed, but on few occasions, erumpent pycnidia of *Camarosporium* sp. were found on the exterior surface of fruit galls from *A. mearnsii* at the time of emergence of *Asphondylia*. *Camarosporium* sp. was the most abundant and widespread fungus isolated

from *Asphondylia* galls from Australian *Acacia* species and in several cases the only present. Spores of *Camarosporium* sp. predominate in mycangia of wild *Asphondylia* from *Acacia* and have the same spore characteristics as those grown from *Acacia* galls. This evidence suggests that *Camarosporium* sp. is the primary fungus present in bud and fruit galls from Australian *Acacia* and is actively collected by female *Asphondylia* and utilized to foster larval development.

The inadvertent introduction of plant pathogens, such as *Uromycladium* spp., with insects used for the biological control of Australian acacias in South Africa is a concern shared by the biological control fraternity and commercial forestry industries in South Africa. Insects can act as vectors of plant pathogen spores (Favaro & Battisti 1993, James *et al.* 1995, Pakaluk & Anagnostakis 1997) and direct-releases of insects have a greater risk of accidental introductions of pathogens than insects that are reared through one or more generations in a quarantine laboratory prior to release in the field.

Laboratory-reared *Asphondylia* have a low diversity and incidence of phoretic fungi primarily because of ecdysis within the gall wall and the scarcity of sporulating structures on the buds and fruits of most Australian *Acacia*. If direct release of *Asphondylia* adults were considered in a biological control program for Australian *Acacia,* surface sterilization of *Asphondylia* galls prior to adult emergence would eliminate the risk of accidental transmission of potential pathogens such as *Uromycladium* on *A. mearnsii*, a potentially serious disease in South Africa.

Camarosporium is a cosmopolitan genus of mainly saprophytic species, but it also includes pathogens of economic crops such as olive (Prota 1995), pistachio (Michailides *et al.* 1998), black locust (Halasz *et al.* 2001) and Australian *Acacia* species (Shivas 1989). Although *Camarosporium* species can be endophytic (Fisher & Petrini 1987, Suryanarayanan & Kumaresan 2000), the occurrence of *Camarosporium* sp. in *Asphondylia* galls as an endophyte within *A. mearnsii* is highly unlikely. *Camarosporium* sp. was isolated from surface-sterilized buds, but incidence levels were low. Later inspections of sites revealed that *Asphondylia* galls were present, indicating sampled buds may have already contained *Camarosporium* sp. conidia deposited by female flies rather than the fungus occurring as an endophyte. The absence of *Camarosporium* sp. in buds of *A. mearnsii* from most Australian sites indicates this fungus is not an endophyte and the proliferation of *Camarosporium* sp. following the development of insect-induced galls (Wilson 1995) is not a valid mechanism of transmission for this fungus.

The occurrence of *Camarosporium* sp. in *Asphondylia* galls from eastern and Western Australia suggests *Camarosporium* is ubiquitous and readily available to female *Asphondylia*.

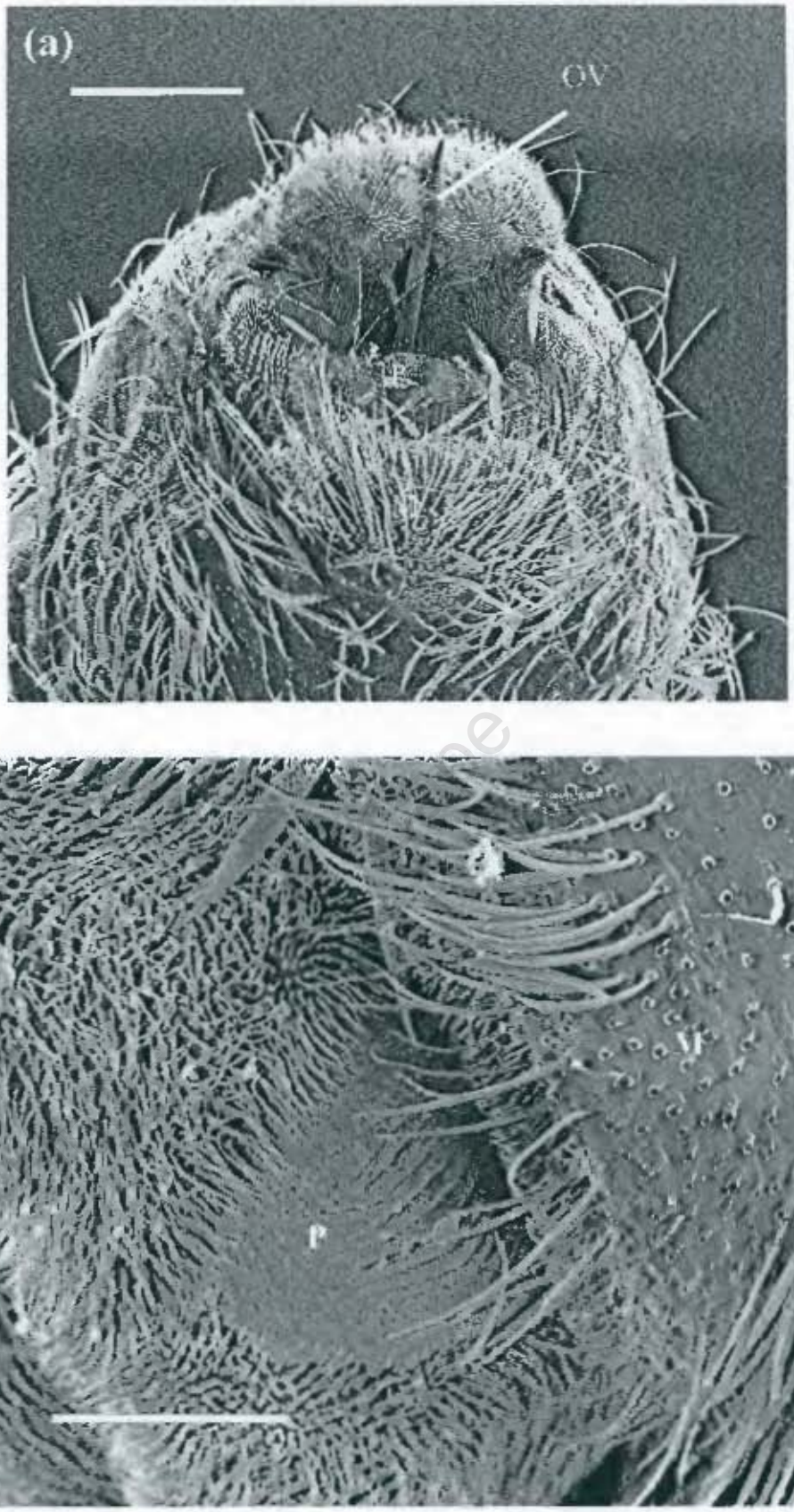


Figure 4.3. Scanning electron micrograph of the abdominal sternite and mycangium of *Asphondylia* sp. from *A. decurrens* (3171). (a) ventral view of abdomen (b) enlarged view of entrance to mycangium. OV = ovipositor, M = mycangium, P = posterior plate. (a) Scale bar = 200 µm, (b) Scale bar =100 µm

However, the source of *Camarosporium* sp. spores to *Asphondylia* is obscure. Although pycnidia were found on fruits of *A. mearnsii* these were rare and not observed on other *Acacia* species or genera with *Asphondylia* galls. It is possible that infected host organs fall to ground after adult emergence and sporulation occurs in the detritus layer beneath the host plant where spores are then collected by foraging females. Sporulation of *Macrophoma gallicola* Sacc., the fungal associate of *Asteromia carbonifera* (O.S.) (Lasiopterini) galls from *Solidago,* has been observed on an infected leaf that had fallen in the previous autumn (Bissett & Borkent 1988).

The method of spore collection by *Asphondylia* is unresolved, but may occur by scooping spores into the mycangium (Borkent & Bissett 1985). The furrowed and hairless pad at the entrance to the mycangium, together with the obtuse apex renders such a mechanism feasible, but cannot readily explain how female *Asphondylia* remove conidia from the mycangium and insert these onto or into host tissue. Future studies are needed to clarify the method of spore transfer to help resolve if the presence of spores in mycangia is incidental (Gagné 1989) or a part of a highly refined mutualistic relationship. Bissett & Borkent (1988) propose that *Asphondylia* simultaneously deposits conidia of the fungal symbiont and eggs internally within the host during oviposition. Fecal transfer of spores (Haridass 1987) remains a possibility and also requires investigation, but as spores wiped onto the buds of *A. mearnsii* in laboratory studies failed to induce gall formation, this method of transfer seems unlikely. This together with the failure of spore-free laboratory-reared *Asphondylia* to induce galls in sleeved cages in the field (Chapter 3, see Materials & Methods), indicates that the simultaneous presence of *Camarosporium* and the immature stages of *Asphondylia* may be required to initiate gall development.

Asphondylia ricini Mani occurs in male flowers of *Ricinus communis* L. in South Africa where both the cecidomyiid and host plant are naturalized. *Ricinus communis* is native to tropical east Africa, but occurs as a weed and commercial crop in many parts of the world, particularly in the tropics. The fungal co-associate in *A. ricini* galls is *Macrophoma* sp. and is more closely allied with northern hemisphere taxa than South African cecidomyiid-fungi associations. *Asphondylia ricini* could have migrated to South Africa following naturalized *R. communis* through southern Africa transporting its original fungal co-associate in mycangia. Alternatively, the insect may have adopted a new, but compatible fungal associate, or the *A. ricini* symbiont is not restricted to this insect and is widespread and readily available. The recent arrival of the palaearctic *A. sarothamni* and *A. cytisi* on *Sarothamus* in North America (R. Gagné pers. comm. 2002) demonstrates that *Asphondylia* are able to establish in novel environments. Whether these insects were introduced with their fungal co-associate

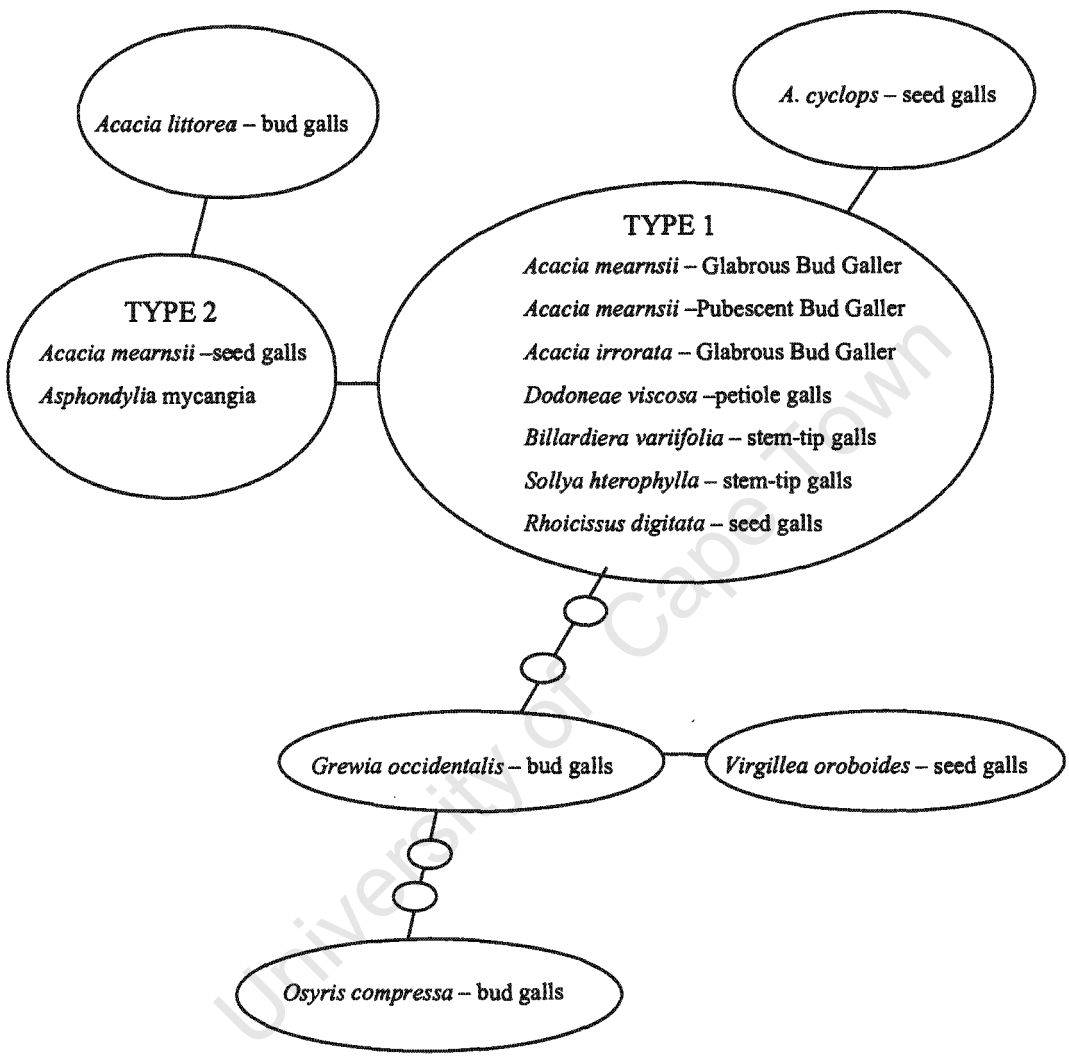


Figure 4.4. Unrooted statistical parsimony network for isolates of *Camarosporium* isolated from *Asphondylia* galls or mycangia from Australia and South Africa. The smaller circles connecting haplotypes represent one indel difference. All connections in the network have a >95% probability of parsimony.

(*Macrophoma* Simova & Komnenovic 1979) is unknown, but as pycnidia have been observed on galls (Neger 1910), both insect and fungus could have naturalized by the transport of gall material from Europe to America.

The association of fungi in cecidomyiid galls from the Holarctic has been the primary focus of biologists since this relationship was first postulated by Baccarini (1893). A range of *Macrophoma*-like fungi with aseptate to transversely multiseptate conidia dominate as fungal associates (Bissett & Borkent 1988). The only previous account of *Camarosporium* from a cecidomyiid is from the predatory *Prolasioptera berlesiana* (Paoli) that inoculates the olive pathogen *C. dalmaticum* during oviposition into wounds caused by the tephritid *Dacus oleae* (Gmel.) (de Laurentiis 1993). In Australia and South Africa, *Camarosporium* is widespread and dominates in *Asphondylia* galls from a diverse range of host families, suggesting a possible Gondwanan ancestry for this association. If this is correct, then *Asphondylia* from the temperate neotropics, which shares strong biotic affinities with Australia and southern Africa, should also be associated with *Camarosporium*. The *Asphondylia-Camarosporium* association is possibly unique to the southern hemisphere and is likely to have evolved independently from similar associations in the northern hemisphere that are dominated by *Macrophoma*-like coelomycetes as fungal associates.

The taxonomic status of *Camarosporium* isolates from Australian and South African *Asphondylia* galls could not be determined, although conidia structure and size were similar, suggesting a close or identical relationship in the taxa involved. One South African *Camarosporium* isolate from the liana *Rhoicissus digitata* (Vitaceae) (3242) had identical DNA sequences in the ITS region of the rRNA cistron to *Camarosporium* isolated from Australian *Asphondylia* galls, implying that the same species and genotype is present in both continents, but apparently with a restricted distribution in southern Africa. If this is the case, Australian *Asphondylia* could be released free of Australian fungal material and provided with South African isolates of *Camarosporium* for gall development. However, until the mechanism of spore collection by female *Asphondylia* is resolved, matching South African *Camarosporium* isolates with Australian midges to initiate establishment of these insects in the field may prove difficult.

Asphondylia and fungal co-associate compatibility is an important issue in the development of these insects as biological control agents and may have been a contributing factor for the failure of *A. opuntiae* Felt to establish on the cacti *Opuntia inermis* DeCandolle and *O. stricta* (Haw.) Haw. in Australia, despite the release of large numbers of insects (Hamlin 1924, Mann 1969). These considerations may be important for use of *Asphondylia* as biological control agents for other weeds such as *Chromolaena odorata, Ligustrum* spp.,

Prosopis spp., *Lantana camara, Ulex europeus, Sida acuta, S. rhombifolia* and *Cytisus scoparius*, particularly if introductions are to occur across the equator, and an obligate relationship exists between the gall midge and the fungus.

CHAPTER 5

Parasitoids of Cecidomyiidae associated with Australian and South African *Acacia*: implications for biological control of invasive *Acacia*.

Abstract

Australian and South African gall-forming Cecidomyiidae are attacked by a diverse range of ecto- and endoparasitoids. While host specialization occurs within parasitoids the majority appear to be generalist feeders utilizing a broad range of cecidomyiid hosts. In Australia, the Tiny Floret Galler is utilized by six hymenopteran parasitoid species, but predominantly by ?*Synopeas* sp. (Platygastridae). In Western Australia, parasitoid loads of the Tiny Floret Galler are lower than in eastern Australia and parasitoid diversity is smaller, with only two endoparasitoids, ?*Synopeas* sp. and ?*Conostigmus* sp., recorded. The life cycle of the Tiny Floret Galler and its principal parasitoid ?*Synopeas* sp. are closely synchronized with each other and to the flowering phenology of *A. mearnsii,* irrespective of regional climatic variation in southern Australia. Although the Platygastridae are in need of taxonomic revision, *?Synopeas* sp. from the Tiny Floret Galler appears to be a generalist parasitoid, utilising a range of cecidomyiid species that occur on Australian acacias. Parasitoids utilizing taxonomically related cecidomyiids from African *Acacia* or cecidomyiids with similar gall structure are likely to appear first as parasitoids of Australian cecidomyiids in South Africa. The South African ?*Synopeas* spp. (two species), Platygastridae (two genera, undetermined.), *Systasis* sp., *Gastrancistrus* sp., *Elasmus* sp., *Eupelmus* sp., *Aprostocetus* spp., *Eurytoma* sp., *Ormyrus* sp. and *Pediobius* sp. are likely South African parasitoid candidates of the Tiny Floret Galler and *D. dielsi,* if released in South Africa. Sugar-based insecticide baits failed to provide adequate selective suppression of hymenopteran parasitoids in the presence of cecidomyiids. Trials with protein-based baits are suggested.

Introduction

The ability to induce galls is a highly successful life history strategy for phytophagous insects (Price *et al.* 1987, Hawkins 1988). However, reproductive success is affected by a number of factors, including the impact of natural enemies (Fernandes & Price 1991). Insect parasitoids do not uniformly exploit different insect taxa because some types of insects are more susceptible to parasitism than others (Shaw & Askew 1979, Askew & Shaw 1986, Hawkins & Lawton 1987). Endophagous herbivores are reputedly more prone to parasitism because of their immobility and discoverability compared with ectophagous species (Askew & Shaw

1986). Well-concealed endophages generally support fewer parasitoid taxa than weakly concealed species (Hawkins 1990) because they are physically protected by plant tissue (Gross 1991) and thus provide parasitoids with fewer clues to their location. Gall-formation may be an adaptive response to avoidance of natural enemies, but evidence for the enemy avoidance hypothesis is equivocal and requires explicit testing (Price *et al.* 1987, Stone & Schönrogge 2003).

Cecidomyiidae gall-formers tend to be susceptible to attack by parasitoids with parasitoid-inquiline induced mortality commonly recorded at 50% or higher (Hawkins 1988) and reaching levels that can limit population densities of the host insects (Briggs & Latto 1996). In Australia, gall-forming Cecidomyiidae on *Acacia* species often experience high levels of parasitism from hymenoptera, which appear to cause populations to become spatially heterogeneous, often sparse, and occasionally may contribute to localized extinctions (Adair pers. observ.). Attack by parasitoids has compromised the effectiveness of cecidomyiids released for the biological of weeds in several countries (Goeden & Louda 1976, McFadyen 1985, Wehling & Piper 1988, Carlson & Mundal 1990), including perhaps a recently introduced bud-galling *Dasineura* sp. present on *Leptospermum laevigatum* (Myrtaceae) in South Africa. Other orders of gall-forming agents have suffered similar fates (Dodd 1961), but some, such as *Trichilogaster* on *Acacia* in South Africa, are not limited adversely by parasitism (Mangoni & Hoffmann 1995).

As the acquisition of generalist natural enemies by newly introduced insect species is common (Cornell & Hawkins 1993), there is a high probability that Australian cecidomyiids introduced into South Africa for control of acacias will be utilized by native natural enemies. Although parasitism is a factor considered important in the failure of some classical biological control agents to elicit effective suppression of their targeted host plant species (e.g. Dodd 1961, Julien & Griffiths 1998, Pratt *et al.* 2000), other constraints such as low reproductive potential of the agent, poor agent-host synchronisation (Dymock 1987, Schops *et al.* 1996) and weak host-damage impacts have been implicated in the failure of some biological control agents (Julien & Griffiths 1998). These limitations are generally only recognised in the post-release and evaluation phase of a weed control program as adequate assessment of these parameters rarely occurs prior to release.

In a situation where parasitoids could be substantially reduced, agent-host interactions, particularly the impact of the agent on its host, could be evaluated in the country of origin prior to detailed evaluative studies in quarantine. The early identification of intrinsic agent-host relationships could expedite the selection of good biological control agents and the

rejection of those that have a low probability of success, thereby reducing costs and possibly increasing the success rates of biological control programs.

Selective suppression of parasitoids using insecticides has potential to induce outbreaks of cecidomyiids in their natural habitat. Population densities of the cecidomyiid *Rhopalomyia californica* Felt on *Baccharis halimifolia* L. in North America increased dramatically after the distribution of insecticide baits for fruit flies that also coincidentally suppressed the cecidomyiid's hymenopteran parasitoids (Ehler *et al.* 1984, Ehler 1989).

This study compares the parasitoid fauna of cecidomyiids from the reproductive structures of acacias in Australia with those reared from galls of South African plants, with an emphasis on African acacias. Host utilisation trends by cecidomyiid parasitoids in both continents, together with a comparison of gall morphologies, are used to predict parasitism trends for three cecidomyiids (Tiny Floret Galler, Glabrous Bud Galler, *D. dielsi*) under consideration as biological control candidates for *A. mearnsii* and *A. cyclops*. Preliminary laboratory-based evaluation of a synthetic pyrethroid and an organophosphate for the selective control of parasitoids of the Tiny Floret Galler and the Pubescent Bud Galler is undertaken. The results are evaluated for possible application in field-based experiments designed to determine the impact of Australian cecidomyiids on their hosts within environments where low-densities of parasitoids have been artificially induced.

Materials and Methods

Australian and South African parasitoids of gall-forming Cecidomyiidae

Cecidomyiid galls on *Acacia* species were collected in southern Australia and South Africa. In addition, cecidomyiid galls from other host genera in South Africa were also collected. Ungalled material was removed and galls retained in ventilated plastic containers capped with fine gauze. Cecidomyiid larvae that emerged from the host tissue to complete development in soil were provided with clean sandy-loam. Emergence containers were held at room conditions and inspected regularly for adult parasitoids or cecidomyiids, which were collected and either preserved in 70% ethanol or stored as dried specimens. Parasitoids were sorted and voucher specimens were mounted on glass microscope slides following the techniques of Prinsloo (1980) and lodged with the South African National Insect Collection (Pretoria). O. Neser (ARC-Plant Protection Research Institute, Biosystematic Division) identified voucher specimens into families and genera. Collections were sorted into morphospecies where possible.

Although the mode of feeding of parasitoid larvae was recorded, where possible, it was not always possible to distinguish primary parasitoids from inquilines. All accession collections with detailed locality data were recorded in an Access database (appendix).

Parasitoid loads and mortality of Australian cecidomyiids on A. mearnsii

Parasitoid loads of *Dasineura* spp. (Tiny Floret Galler, Common Fluted Galler) and *Asphondylia* spp. (Glabrous Bud Galler and Pubescent Bud Galler) were determined. Galls were sampled at or near completion of larval development to obtain maximum levels of parasitoid activity. In eastern and Western Australia, sites with the Tiny Floret Galler were sampled to compare parasitoid levels within the insect's natural and introduced range in southern Australia. Ten mature galls were selected haphazardly from each of five trees and five floret galls were removed from each gall for dissection. Floret galls were cut with a scalpel to expose each chamber and the developmental status of cecidomyiids and parasitoids was recorded. At gall maturity, endoparasitoids (kionobionts) were readily recognized by the enlarged and discoloured appearance of the host cecidomyiid, or by the presence of hymenopteran cocoons within the interior of the cecidomyiid host. Ectoparasitoids (idiobionts) were recognised by the presence of developmental stages on the exterior of the host and, after emergence, by the chewed remains of the cecidomyiid. As Tiny Floret Galler larvae emerge from galls to pupate in soil, sampling immediately prior to emergence of larvae (late June- mid July), and of cocoons prior to adult emergence (September-October), was necessary to gauge parasitoid loads. Cocoons of the Tiny Floret Galler were sampled by harvesting mature galls and allowing larvae to emerge in containers with moist peat moss. This method was practical for collecting large number of insects across a range of sites in Australia, but would have excluded parasitoids that may have attacked Tiny Floret Galler larvae and pupae in the soil. Twenty to 100 cocoons were haphazardly selected shortly before adult emergence, dissected and examined for parasitoids. Cecidomyiid immatures that were dead within chambers and vacant chambers were also recorded. Sites were sampled over three seasons to detect seasonal variation in parasitoid levels.

The determination of parasitism impact by measuring percentage attack rates provides a simplistic static view of parasitism ecology. Although full population dynamic studies would be more suitable, these were beyond the scope of this study.

Fourteen sites were sampled to determine parasitoid loads and mortality of the Tiny Floret Galler: eight in Western Australia (Mt Barker 34^0.23'S, 117^0.31'E; Jarrahdale 32^0.18'S, 116^0.01'E; Albany 34^0.58'S, 117^0.53'E; Augusta 34^0.18'S, 115^0.09'E; Pemberton 34^0.26', 116^0.02'E; Denmark 34^0.59'S, 117^0.13'E; Dwellingup 32^0.47'S; 116^0.03'E, William Bay

34^0.59'S; 117^0.13'E) and six in eastern Australia (Wollongong 34^0.23'S, 150^0.53'E; Waramanga 35^0.20'S, 149^0.04'E; Casula 33^0.56'S, 150^0.53'E; Somerville 38^0.33'S, 145^0.18'E; Brimbank 37^0.43'S, 144^0.49'E; Frankston 38^0.12'S, 145^0.16'E). Four sites in eastern Australia were sampled for the Glabrous Bud Galler (Mittagong 34^0.26'S, 150^0.28'E; Wollongong, Casula, Wartook 37^0.03'S, 142^0.21'E) and three for the Pubescent Bud Galler (Waramanga, Wartook, Pearcedale 38^0.10'S, 145^0.13'E). Most sites were sampled in mid September when galls contained either III larvae or pupae. Three sites were sampled for Common Fluted Galler (Frankston, Burrumbeet 37^0.29'S, 143^0.40'E, Stirling 35^0.00'S, 138^0.42'E). Galls were sampled between early July and mid August when the chambers contained III instar larvae or pupae.

Emergence patterns of three cecidomyiids and their parasitoids

The emergence patterns of the Common Fluted Galler, Tiny Floret Galler and Glabrous Bud Galler and their parasitoids was monitored at six sites in southern Australia (Jarrahdale, Stirling, Frankston, Canberra, Casula and Wollongong) over 5-7 months between June 2000 and January 2001. At all sites, *A. mearnsii* was used for emergence studies, but at Stirling (South Australia), *A. melanoxylon* were also monitored because it is a co-host for the Common Fluted Galler.

Yellow cardboard traps (16 cm x 10.5 cm) coated on two sides with sticky rain-resistant glue were hung 1-2 m above the ground in the lower canopy of mature *A. mearnsii* trees. A single trap was hung in each of six trees at each site. Traps remained for two weeks before being removed and replaced. Used traps were placed in transparent plastic bags, labelled and stored at 0^0C until inspected. The sticky surface of each trap ($130cm^2$) was examined using a stereomicroscope for adults of the Common Fluted Galler, Tiny Floret Galler and Glabrous Bud Galler, which could be distinguished from each other by comparing wing length or wing venation patterns.

The Tiny Floret Galler was the only *Acacia* cecidomyiid present at Jarrahdale, Frankston, and Canberra. At Stirling, only the Common Fluted Galler was present, while at Casula the Tiny Floret Galler and the Glabrous Bud Galler were present. At Wollongong, five *Dasineura* species were present, and four of these occurred at high densities (table 3.2a). Platygastridae parasitoids that matched voucher specimens reared from the Tiny Floret Galler and Common Fluted Galler in Australia and *Ormyromorpha* spp. (Pteromalidae) reared from *Asphondylia* spp. were also counted. Other parasitoid species occur on cecidomyiids of *Acacia* and some of these may have been present on the traps, but they were not included in the counts because they could not be readily identified.

On each sampling occasion, the developmental state of the reproductive organs on each host tree was recorded by visually distinguishing seven phenological stages (bud initials, early bud, late bud, flower, flat fruit, fat fruit, mature fruit) and assigning each to abundance categories (0%, <1%, 1-10%, 10-40%, 40-80%, >80%) based on the percentage of the current season's crop in each stage.

Selective suppression of parasitoids

Selective insecticide baiting (Ehler *et al.* 1984) of gall-forming cecidomyiid parasitoids was tested using ?*Synopeas* (Platygastridae) reared from Tiny Floret Galler galls on *A. mearnsii,* which were collected in Western Australia at Jarrahdale, Dwellingup and Denmark, and parasitoids reared from Pubescent Bud Galler collected at Stawell, Victoria, on *A. baileyana*. Newly-emerged cecidomyiids and their parasitoids were aspirated directly into clear plastic cylinders (12 cm x 8.2 cm) and capped with ventilated lids. In each container, a stem of *A. mearnsii* with two leaves trimmed to 5-7 pairs of pinnae was held with the stem base in a small vial of water. A small droplet of insecticide solution (5-10μl) was applied to the rachis between a pair of pinnules on each leaf. Insecticides were mixed with undiluted honey at 0.1 ml of insecticide per 10ml of honey. Insecticides used were the organophosphate fenthion at 100 g l^{-1} a.i. and the synthetic pyrethroid cypermethrin at 20 g l^{-1} a.i. To test whether the Tiny Floret Galler and ?*Synopeas* were killed by consumption of insecticide-laced honey or by other modes of uptake, the same insecticides and rates were also applied in droplets of water that were kept separate from droplets of unlaced honey. Control treatments contained droplets of unlaced honey only.

Cylinders were maintained in a quarantine laboratory under room conditions with a temperature range of 15-20^{0}C and with natural lighting. In all treatments, 15-20 individuals of each cecidomyiid or its parasitoid were added separately to each cylinder. The numbers of dead insects were counted daily. There were five replicates for each treatment. Tests were maintained for 10 days.

Mortality rates were analysed by first attempting to find a model that fitted cumulative mortality for each treatment. No single model fitted all data, including the logistic dose response curve. The LT_{50} (time to 50% mortality) for each replicate was calculated by determining the linear equation between the two values either side of the LT_{50} and solving the equation for y = 50%. After removal of outliers that had no significant influence on main effects, data were found to have homogeneous variance and the residuals were normally distributed (Shapiro-Wilk test for non-normality). The calculated LT_{50} values were subjected to ANOVA using GLM and means were separated using pair-wise comparisons and the post

hoc Bonferroni test. Analyses were performed using Systat Version 10 and SAS Version 8.2. Mortality rates (LT_{50}) from experiments with the Pubescent Bud Galler and its parasitoids were log_{10} transformed to meet the assumptions of ANOVA.

Results

Parasitoids of gall-forming Cecidomyiidae from South Africa

Twelve families of micro-hymenopteran parasitoids were reared from galls of South African Cecidomyiidae, with the Chalcidoidea families of Eulophidae, Pteromalidae, Torymidae, Eupelmidae, Eurytomidae and the Platygasteroidea family Platygastridae containing the most frequent and genera-rich fauna (table 5.1).

Most parasitoid taxa from South African cecidomyiids utilized a broad range of cecidomyiid hosts from an equally broad range of host plants, indicating a 'generalist' feeding pattern. *Eurytoma* sp. (Eurytomidae) and the Eulophidae genera *Aprostocetus* and *Omphale* had the highest number of cecidomyiid hosts with 10, nine and six species, respectively (table 5.1).

A small proportion of hymenopterans reared from South African galls were restricted to cecidomyiids from African Mimosaceae. Seventeen cecidomyiid species forming galls on African *Acacia* and *Dichrostachys cinerea* (Mimosaceae) were parasitised by 21 species of Hymenoptera from seven families, with 19 species from *Acacia* and four from *D. cinerea* (table 5.1). Two parasitoid taxa (*Ormyrus* sp., *Torymoides* sp.) from inflorescence galls of *D. cinerea* were also reared from galls on African acacias. The parasitoids *Neanastatus* sp. (Eupelmidae), *Ormyrus* sp. (Ormyridae), nr *Omphale* sp. (Eulophidae), *Sycophila* sp. (Eurytomidae) and *Elasmus* sp. (Elasmidae) were only reared from galls on African *Acacia* species and predominantly from cecidomyiid galls on nodes (table 5.1). Similarly, the pteromalid *Systasis* sp. was predominantly reared from galls on the stem nodes and pinnae of African *Acacia*. Nineteen parasitoid taxa reared from inflorescences of African *Acacia* species with four species from bud galls induced by *Asphondylia* and 16 parasitoid taxa from galls associated with the receptacle or ovaries induced by *Acacidiplosis, Aposchizomyia* and *Contarinia*. *Aprostocetus* sp. (Eulophidae) occurred on cecidomyiid galls on buds and ovaries. The ovary-galling cecidomyiid *Aposchizomyia* sp. from *A. nilotica* and the node-galling *Acacidiplosis* from *A. tortilis* each had six parasitoid taxa, the highest number reared from any of the galls of South African Cecidomyiidae.

Cecidomyiid galls on the inflorescences of Australian *Acacia* are mostly lignified and form woody gall structures. Woody cecidomyiid galls occurred on three species of African *Acacia, A. luederitzii, A. mellifera* and *A. nigrescens* with the galls of *Contarinia* sp. (Lumpy

Table 5.1. Census of parasitoids reared from cecidomyiid galls in South Africa

Host plant	Host insect	Gall name	Host organ	Gross tissue type	Parasitoids (accession number)
**Achyranthes aspera*	*Schizomyia* sp. *Contarinia* sp.	inflorescence galls	floret	?	Eulophidae: *Aprostocetus* sp. (3219, 3078)
Acacia karroo	*?Lobesia* sp.[1]	pinnae galls	pinnae	slightly thicken overlapping pinnae	Pteromalidae: *Systasis* sp. (3044, 2898)
Acacia karroo	*Acacidiplosis* sp.	node galler	node	semi-soft, thin-walled	Pteromalidae: *Systasis* sp. (2764) Eupelmidae: *Eupelmus* sp. 2 (2764), *Neanastatus* sp. (2764) Elasmidae: *Elasmus* sp. 1 (2764)
Acacia erioloba	*Contarinia* sp.	receptacle galler	receptacle	slightly thickened receptacle tissue	Platygasteridae: *?Synopeas* sp. (3217)
Acacia karroo	*Acacidiplosis* sp.	lumpy node galler	node	semi-woody, thick-walled, highly lobed	Platygastridae: indet (2851)
Acacia karroo	*Asphondylia* sp.	bud galler	bud	soft tissue, thin walled	Eulophidae: *Aprostocetus* sp. (3058), *Entedon* sp. (3058) , *?Baryscapus* sp. (3058) Pteromalidae: nr *Pseudocatolaccus* sp. (3058)
Acacia luederitzii	*Asphondylia* sp.	bead galler	ovary	woody, thick walled	Elasmidae: *Elasmus* sp. 2 (3071) Eurytomidae: *Eurytoma* sp. (3071) Ormyridae: *Ormyrus* sp. (3071)
Acacia nilotica	*Asphondylia* sp.	?bead galler			Ormyridae: *Ormyrus* sp. (2854)
Acacia mellifera	*Contarinia* sp.	lumpy floret galler	ovary	woody, thick walled	Eupelmidae: *Eupelmus* sp. 3 (3216, 3265), *Euplemus* sp. 2 (3265) Platygastridae: indet (3262) Pteromalidae: *Systasis* sp. (3262)
Acacia mellifera	?	oyster-shell galler	ovary	woody, thick walled	Pteromalidae: *Systasis* sp. (3265), *Gastrancistrus* sp. (3265)
Acacia nigrescens	?	knobby hair tuft galler	ovary, pinnae	woody, shallow chambers in dense indumentum	Torymidae: *?Pseudotorymus* sp. (3227, 3092) Eulophidae: *Aprostocetus* sp. (3092)
Acacia nilotica	*Aposchizomyia* sp.	minute floret galler	ovary, bud, receptacle	semi-woody, thin walled	Eulophidae: *nr Omphale* sp. (2849)., Tetrastichinae (2859) Eurytomidae: *Eurytoma* sp. (2849) Platygastridae: indet (2859) Eupelmidae: *Eupelmus* sp. 3 (2859, 2853) Torymidae: *?Torymoides* sp. (3095, 3229, 2859)
Acacia exuvialis					Eupelmidae: *Euplemus* sp. 1 (3091) Torymidae: *?Torymoides* sp. (3090)
Acacia nilotica	?	artichoke galler	node	semi-woody, thin walled	Eulophidae: nr *Omphale* sp. (2855, 2860) Eurytomidae: *Eurytoma* sp. (2855)
Acacia nilotica	*?Aposhizomyia acuta*	petiole/stem galler	stem, petiole	woody	Eulophidae: nr *Omphale* sp. (2861) Eurytomidae: *Eurytoma* sp. (2861)
Acacia tortilis	*Acacidiplosis* sp.	bracteose node galler	node	semi-soft, thin walled, bracteose	Eulophidae: nr *Omphale* sp. (3065) Eurytomidae: *Eurytoma* sp. (3065) Eupelmidae: *Neanastatus* sp. (3065)
Acacia gerradii					Eulopidae: nr *Omphale* sp. (2857), Eurytomidae: *Sycophila* sp. (2857)

* Introduced species

Table 5.1. Census of parasitoids reared from cecidomyiid galls in South Africa (continued)

Host plant	Host insect	Gall name	Host organ	Gross tissue type	Parasitoids (accession number)
Acacia tortilis	*Acacidiplosis* sp.	durian galler	node	semi-woody, thin walled.	Eulophidae: *nr Omphale* sp. (3061, 2856) Eurytomidae: *Sycophila* sp. (2858) Eupelmidae: *Neanastatus* sp. (3061) Platygastridae: indet (3061, 2858, 3062) Ormyridae: *Ormyrus* sp. (3063) Torymidae: *Pseudotorymus* sp. (2856)
Acacia tortilis	?	tuft galler	node	semi-woody thin walled	Eurytomidae: *Sycophila* sp. (2852)
Acacia tortilis	?	pinebud galler	node	semi-woody, thin walled	Eupelmidae: *Eupelmus* sp. 1 (3096) Torymidae: *Torymoides* sp. (3096) Pteromalidae: *Systasis* sp. (3096)
Asparagus sp.	*Contarinia* sp.	shoot galler	axillary shoot	thin walled, imbricate bracts	Pteromalidae: nr *Spathopus* sp. (3031)
Berzelia lanuginosa	?	shoot tip galler	shoot apex	semi-soft, thin walled,	Platygastridae: indet (3269)
Carpobrotus ?edulis.	*Lasioptera* sp.	leaf galler	leaf	normal leaf tissue	Platygastridae: indet (3083)
Centella deformans	*Schizomyia* sp.	shoot galler	?	semi-woody,	Ichneumonidae: indet (3222) Eulophidae: *Aprostocetus* sp. (3222) Pteromalidae: *Macroglenes* sp. (3046)
Conyza scabrida	*Lasioptera* sp.	stem galler	upper stem	semi-woody, thick walled	Eupelmidae: *Eupelmus* sp. C (3176)
Chrysanthemoides incana	*?Cecidomyia deformans*	shoot galler	shoot apex	semi-woody, thick walled	Platygastridae: ?*Synopeas* sp.(3239) Torymidae: *Torymus* sp. (3239), *Pseudotorymus* sp. (3239)
Chrysanthemoides monilifera	*Lasioptera* sp.	peduncle galler	peduncle	woody, thick walled	Eurytomidae: *Eurytoma* sp. (3050) Eupelmidae: *Eupelmus* sp. 1 (3050, 3169), *Eupelmus* sp. 2 (3169, 3050) Platygastridae: indet (3169)
Cliffortia sp.	*?Rhopalomyia* sp.	shoot galls	shoot apex	semi-soft, thick imbricate layer of bracts	Platygastridae: indet (3036) Pteromalidae: indet (3036)
Convolulus farinosus	*Schizomyia* sp	fruit galler	ovary	semi-soft, thin walled	Eulophidae: *Aprostocetus* sp. (3223) Eurytomidae: *Eurytoma* sp. (3223) Eupelmidae: *Eupelmus* sp. 1 (3223)
Cynanchum obtusifolium	?	bud galler	bud	soft, thin walled	Eulophidae: *Bruchophagus* sp. (3175) Pteromalidae: indet (3175)
Dichrostachys cinerea	?	floret galler - round	ovary	semi-soft, thin walled	Ormyridae: *Ormyrus* sp. (2848, 2846) Bethylidae: indet (3069) Eulophidae: *Pediobius* sp. (3069) Torymidae: *Torymoides* sp. (3069)
Diospyros glabra	*Asphondylia* sp.	seed galler	fruit	semi-woody, thick walled	Eupelmidae: *Eupelmus* sp. 4 (3243/a), *Eupelmus* sp. 3 (3243/a), *Neanastatus* sp. (3243/a) Pteromalidae: indet (3243/a)
Dipogon lignosus	*Schizomyia* sp.	node galler	bud	semi-woody, thick walled	Eulophidae: *Sigmophora* sp. (3280) Eurytomidae: *Bruchophagus* sp. (3280)
Elytropappus scaber	?	shoot tip	shoot apex	semi-soft, thick imbricate layer of bracts	Pteromalidae: *Mesopolobus* sp. (3224)

Table 5.1. Census of parasitoids reared from cecidomyiid galls in South Africa (continued)

Host plant	Host insect	Gall name	Host organ	Gross tissue type	Parasitoids (collection number)
Erica sp.	?	shoot galler – red lump	stem	semi-woody, thin walled	Torymidae: *Torymoides* sp. (3052)
					Eupelmidae: *Eupelmus* sp.1 (3052)
Eriocephalus sp.	Lasiopteridi - *?de novo genus*	shoot galler	shoot apex	semi-soft, thick imbricate layer of bracts	Eupelmidae: *Eupelmus* sp. 1 (3162)
					Eulophidae: *Tetrastichinae* (3162), Entedoninae (3215)
					Torymidae: *?Torymoides* sp. (3162), *Pseudotorymus* sp. (3162)
					Platygastridae: indet (3215, 3162)
Helichrysum cymosum	?	shoot galler	shoot apex	thick rosette of leafy bracts	Cecidomyiidae: *Lestodiplosis* sp. (3166)
					Eulophidae: *Aprostocetus* sp. (3166)
					Eupelmidae: *Eupelmus* sp. 1 (3166)
					Torymidae: *Torymus* sp. (3166)
Polygala myrtifolia	*Lasioptera* sp.	twig galler	stem	woody	Eulophidae: *Aprostocetus* sp. (AcUCT 599)
Psoralea aphylla	*Asphondylia* sp.	pod galler	ovary	semi-soft, thin walled	Eulophidae: *Aprostocetus* sp. (3045), *Pediobius* sp. (3045),
					Eurytomidae: *Eurytoma* sp. (3045)
					Pteromalidae: nr *Pseudocatolaccus* sp. (3045)
Psoralea oligophylla	?	bud galler	bud, flower	semi-woody, thick walled	Eulophidae: *Aprostocetus* sp. (3173),
					Eurytomidae: *Bruchophagus* sp. (3173),
Rhoicissus tomentosa	*Asphondylia* sp.	leafy node galler	flower bud	semi-soft tissue, dense bract layer	Cecidomyiidae: *Lestodiplosis* sp. (3240)
Rhynchosia caribaea	*Schizomyia* sp.	bud/flower galler	bud, flower	semi-woody, thick walled	Eulophidae: *Aprostocetus* sp. (2863, 3181)
Salvia chamelaeagnea	*Lasioptera ?salviae*	leaf bunch galler	shoot apex	semi-soft, thick walled, rosette of leafy bracts	Eulophidae: *?Aprostocetus* sp. (3282)
					Eurytomidae: *Eurytoma* sp. (3167)
					Torymidae: *Pseudotorymus* sp. (3167)
					Platygastridae: indet (3282)
Senecio tamoides		flower galler	flowers	woody, thick walled	Eulophidae: *?Aprostocetus* sp. (3235)
					Eurytomidae: *Bruchophagus* sp. (3235)
Stoebe buchelii	*Lasioptera* sp.	shoot galler	shoot apex	semi-soft, dense imbricate layer of bracts	Pteromalidae: indet (3033)
Stoebe sp.	*Dasineura* sp.	shoot galls	shoot apex	semi-soft, dense imbricate layer of bracts	Encyrtidae: indet (3037)
Tarchonanthus camphoratus	*Afrodiplosis tarchonanthi*	stem galler	stem	woody, thick walled	Eurytomidae: *Eurytoma* sp. (3168)
					Eupelmidae: *Eupelmus* sp. 1 (3168, 3076)
Zygophyllum morgsana	*nr Dasineura* sp.	flower distorter	bud, flower	soft tissue, thin walled	Platygastridae: *?Synopeas* sp. (3079)
					Pteromalidae: *Systasis* sp. (3079)

1. based on comparison with illustrated and description of galls in Gagne & Marohasy 1993

Floret Galler) on *A. mellifera* closely matching, morphologically, the woody galls of *Dasineura* on Australian *Acacia* species. Thick-walled, semi-woody galls also occurred on the peduncles of *Chrysanthemoides monilifera* (DC.) Norl. (Asteraceae) induced by *Lasioptera* sp., on the twigs of *Tarchonanthus camphoratus* L. (Asteraceae) induced by *Afrodiplosis tarchonanthi* Felt, on the buds and flowers of *Rhynchosia caribaea* (Fabaceae) by a *Schizomyia* sp., and on the twigs of *Polygala myrtifolia* L. (Polygalaceae) induced by a *Lasioptera* sp. Parasitoid genera occurring on woody galls of *Acacia* were: *Elasmus, Eurytoma, Ormyrus, Eupelmus, ?Pseudotorymus, Aprostocetus*; and on non-*Acacia*: *Eurytoma, Eupelmus, Platygastridae, Torymoides, Aprostocetus* and *Bruchophagus* (table 5.1).

One *Asphondylia* species (3057) was found on the buds of *A. karroo* in the Western Cape, where four parasitoids were reared, with only the eulophids *Entedon* sp. and ?*Baryscapus* sp. recovered from this cecidomyiid. The parasitoids nr *Pseudocatolaccus* sp. and *Aprostocetus* sp. were also reared from *Asphondylia* galls developing on the fruits of *Psoralea aphylla* (Fabaceae) (table 5.1).

Parasitoids of gall-forming Cecidomyiidae from inflorescences of Australian Acacia

Australian Cecidomyiidae that gall inflorescences of *Acacia* were parasitised by six families and 22 genera of micro-hymenoptera (table 5.2). The most species-diverse hymenopteran families reared from cecidomyiid galls from Australian acacias were the *Eulophidae* with 10 taxa, the *Pteromalidae* with eight taxa and the *Torymidae* with six taxa. Two host-utilization patterns were found for hymenopteran parasitoids reared from *Acacia* galls in Australia with one group of stenophagous parasitoids confined to specific cecidomyiid genera, and another polyphagous group utilizing cecidomyiids from *Dasineura* and *Asphondylia* and across a range of gall types.

Endoparasitic Platygastridae were confined to *Dasineura*, predominantly in the Fluted Galler group of Australian cecidomyiids. A ?*Synopeas* sp. was the most abundant parasitoid of the Tiny Floret Galler, in both eastern and Western Australia (table 5.7). Similarly, four *Aprostocetus* spp. were confined to *Dasineura* and almost restricted to the Fluted Galler group. A pteromalid *Systasis* sp. 1 and a torymid *Torymoides* sp. 2 were also restricted to *Dasineura* from several gall-type groups (table 5.2). The Fluted Galler group of Australian cecidomyiids had the highest number of parasitoid species (19) with *D. dielsi* (Small Fluted Galler) from *A. cyclops* having the highest diversity of parasitoids for an individual cecidomyiid species with nine parasitoid taxa reared from four gall accessions from Western and South Australia.

Table 5.2. Census of parasitoids reared from galls of Australian Cecidomyiidae from *Acacia*

Parasitoid family	Genus	Host insect	Gall group[1]	Host *Acacia* and accession number of parasitoid	Feeding Strategy[2]
Pteromalidae	*Ormyromorpha* sp.1	*Asphondylia*	seed	*A. retinoides* 2578/5, *A. meissneri* 2882/1	
	"		bud	*A. ephredoides* 2838/2, *A. ramulosa* 2894	
	"	*Dasineura*	fluted	A. maidenii 2724/1, A. pycnantha 2576/5	
	Ormyromorpha sp.2	*Asphondylia*	GBG	*A. mearnsii* 3118(c)	Ecto
	"		bud	*A. parvipinnula* 2647/1, *A. paradoxa* 2666/2, *A. mearnsii* 2740/2, *A. floribunda* 2635/3, *A. parvipinnula* 2636, *A. sophorae* 2638, 2877, *A. lunata* 2813/3, *A. irrorata* 2783/1, *A. parvipinnula* 2648, *A. dealbata* 2653	
	"		seed	*A. deanei* 2775	
	"	*Dasineura*	pouch	*A. verticillata* 2570/2	
	Gastrancistrus sp.	*Asphondylia*	bud	*A. schinoides* 2651	Ecto
	"		seed	*A. mearnsii* 3126	
	"	*Dasineura*	fluted	*A. pycnantha* 2576/3, *A. meanrsii* 2739/1, 2659, 2600, 2685/2, 2739/1, 3019/a; 3099/1, 3258	
	"			*A. melanoxylon* 2604/3, *A. maidenii* 2574/1, 2724/3; *A. pendula* 2710/3, 2710/b; *A. cyclops* 2571/2, 3023/1, *A. sophorae* 2606, *A. mearnsii* 3127(a)	
	"		inflated	*A. dealbata* 2661, *A. baileyana* 2624/1, *A. oshanesii* 2729/1, *A. oshanesii* 3155	
	"		pouch	*A. cochlearis* 2751/1	
	"		floret	*A. deanei* 2800	
	Systasis sp.1	*Dasineura*	pouch	*A. verticillata* 2570/1, *A. littorea* 2583/3, 2582; *A. truncata* 2631, *A. cochlearis* 2751/4	
	"		inflated	*A. oshanesii* 2729	
	"		floret	*A. irrorata* 2721, *A. deanei* 2888/1, 2881/5	
	"		fluted	*A. cyclops* 2883/3	
	"		hollow	*A. leucoclada* 2809	
	Systasis sp. 2	*A.sphondylia*	bud	*A. baileyana* 2639/3, 2641; *A. dealbata* 2634/3, *A. mearnsii* 2620, 3118(a); *A. maidenii* 2755	
	"		seed	*A. irrorata* 2783/2	
	"	*Dasineura*	fluted	*A. cyclops* 2598/2, 2883/3, *A. retinoides* 2610	
	Isoplatoides sp.	*Asphondylia*	seed	*A. sophorae* 2896/2	
	nr Perilampomyia sp.	*Asphondylia*	bud	*A. baileyana* 2639/1	?Ecto
	Chromeurytoma sp.	*Asphondylia*	bud	*A. parvipinnula* 2647/4	
	Macroglenes sp.	*Dasineura*	floret	*A. mearnsii* 3208	
Torymidae	*Torymoides* sp. 1	*Dasineura*	fluted	*A. pycnantha* 2576/2, *A. melanoxylon* 2604/2	
	Torymoides sp. 2	*Dasineura*	fluted	*A. stricta* 2626/1, *A. melanoxylon* 2604/2, *A. pycnantha* 2576/2,	
	Torymoides sp. 3	*Asphondylia*	bud	*A. lunata* 2813/2, *A. stricta* 2872, *A. ephredoides* 2838/3, *A. mearnsii* 2740, *A. decurrens* 2784/2	Ecto
	"		seed	*A. sophorae* 2649, 2896/2; *A. retinoides* 2578/4, *A. decurrens* 2664/2, *A. meissneri* 2882/2, *A. ligulata* 3020	
	Torymoides sp. 4	*Asphondylia*	bud	*A. baileyana* 2756/3	Ecto
	"	*Dasineura*	fluted	*A. maidenii* 2724/2, *A. sophorae* 2606, *A. cyclops* 3023/2	
	Torymoides sp. 5	*Asphondylia*	GBG	*A. mearnsii* 3118(d)	Ecto
	"	*Dasineura*	fluted	*A. mearnsii* 3019(d), 2658/2, *A. pycnantha* 2576/2, *A melanoxylon* 2604/2, *A. meanrsii* 3127(d)(f), *A. melanoxylon* 3109, 2708/3 *A. stricta* 2626	
	Megastigmus sp.	*Asphondylia*	seed	*A. meissneri* 2882/5, *A. sophorae* 2896/3, *A. irrorata* 2783/3, *A. retinoides* 2578/6, *A. decurrens* 2664/1	Ecto
	"		bud	*A. urophylla* 3114/b	
	"	*Dasineura*	fluted	*A. maidenii* 2724/4, *A. cyclops* 2883/1, 2571/1, 2883/2, 3023/5; *A. stricta* 2626/2, *A. mucronata* 2903, *A. melanoxylon* 2708/1, 3108, 3127/c, *A. pycnantha* 2576/1, *A. schinoides* 2652, *A. melanoxylon* 2708/1, 3108, 2604/1, *A. mearnsii* 3127(c)	
	"		floret	*A. deanei* 2881/4, 2881/2	
	"		pouch	*A. cochlearis* 2751/3	

1 Gall type categorization following Table 2.2. 2 Feeding strategy of parasitoid :Ecto=ectoparasitoid, Endo=endoparasitoid

Table 5.2 Census of parasitoids reared from galls of Australian Cecidomyiidae from *Acacia* (continued)

Parasitoid family	Genus	Host insect	Gall type	Host *Acacia* and accession number of parasitoid	Feeding Strategy
Eurytomidae	*Eurytoma* sp. 1	*Asphondylia*	bud	*A. melanoxylon* 2742/2, *A. meissneri* 2882/4, *A. drummondii* 2896/1, *A. myrtifolia* 3102, *A. mearnsii* 3111, 2740/3	
	Eurytoma sp. 2	*Asphondylia*	bud	*A. parvipinnula* 2647/3, *A. mearnsii* 2874, 2640/1, 2640/2, 2620/1, 3111; *A. ulicifolia* 2772/3, *A. alata* 2876, *A. deanei* 2637, 2757; *A. dealbata* 2637, 2757, 2634/1, 2634/2, *A. drummondii* 2829, *A. verticillata* 3107/2, *A. retinoides* 2578/1, 2578/2, *A. sophorae* 2896/1	Ecto
	"		seed		
	Eurytoma sp. 3	*Dasineura*	groove	*A. genistifolia* 3191	
Eulophidae	*Sigmophora* sp.	*Asphondylia*	bud	*A. melanoxylon* 2742/1, *A. decora* 2731, *A. fimbriata* 2873/2, *A. deanei* 2726/1, 2784/1; *A. floribunda* 2635/1, 2635/2; *A. baileyana* 2639/2, 2756/1, 2756/2, 2670; *A. verticillata* 2650/1, 2650/2, 3107/1; *A. salicina* 2621/2, 2621/1, *A. lunata* 2813/1, *A. cochlearis* 2613, *A. gladiformis* 2718, *A. mearnsii* 2620/3, 2740/1; *A. ephredoides* 2838, *A. paradoxa* 2666, *A. polybotrya* 2722/2, 2722/4; *A. parvipinnula* 2647/2, *A. buxifolia* 2813/1	Ecto
	"		seed	*A. retinoides* 2578/3, *A. meissneri* 2882/3	
~~Eulophidae~~	?*Aprostocetus* sp.1	*Dasineura*	inflated	*A. baileyana* 2624/2	?Ecto
	"		fluted	*A. cyclops* 3023, *A. pendula* 2710b/2, 2710/2	
	"		floret	*A. deanei* 2881/3	
	?*Aprostocetus* sp.2	*Dasineura*	fluted	*A. mearnsii* 2739/2, 2659, 3019(c); *A. melanoxylon* 2604/4, *A. pycnantha* 2576/4	
				A. mearnsii 3127	
	?*Aprostocetus* sp.3	*Dasineura*	fluted	*A. pendula* 2710/1, *A. melanoxylon* 2708/5, *A. oldfieldii* 3212	
	?*Aprostocetus* sp.4	*Dasineura*	fluted	*A. pendula* 2710/4	
	?*Aprostocetus* sp.5	*Asphondylia*	bud	*A. polybotrya* 2722/1, *A. irrorata* 3199	
	Diaulomorpha sp.	*Asphondylia*	seed	*A. meissneri* 2882/6	
	Euderus sp.	*Dasineura*	fluted	*A. cyclops* 3023/6e-f	
	Elachertus sp.	*Dasineura*	fluted	*A. cyclops* 3023/6a	
	?*Goethella* sp.	*Asphondylia*	bud	*A. polybotrya* 2722/1, *A. deanei* 2726/2, *A. ephedroides* 2838/5, *A. ramulosa* 2894/2, *A. dodinaeifolia* 3101, *A. melanoxylon* 3104, 3106	Ecto
Elasmidae	*Elasmus* sp.	*Dasineura*	fluted	*A. cyclops* 3023/6b	
Platygastridae	?*Synopeas* sp.1	*Dasineura*	inflated	*A. dealbata* 2736, 3100; *A. baileyana* 2602	Endo
	"		fluted	*A. melanoxylon* 2662, *A. implexa* 2572, *A. sophorae* hybrid 2738, *A. mearnsii* 3127(d), 3127(b), *A. cyclops* 2598/1, 2883/4; *A. mearnsii* 2658/1, 3019(b), 3099/2; *A. pycnantha* 2576, *A. retinoides* 3103	
	"		floret	*A. mearnsii* 3251	
	"		pouch	*A. cochlearis* 2751/1	
	?*Synopeas* sp.2	*Dasineura*	fluted	*A. maidenii* 2574/1	
	?*Platygaster* spp.	*Dasineura*	pouch	*A. verticillata* 2570/3, *A. littorea* 2582	
	"		inflated	*A. baileyana* 2602	
	?*Inostemma* sp.	*Dasineura*	fluted	*A. cyclops* 2883/4	
	Indet	*Dasineura*	inflated	*A. decurrens* 2884/2	
Ceraphronidae	?*Conostigmus* sp.	*Dasineura*	floret	*A. mearnsii* 2901	Endo
Encyrtidae	*Paralitomastix* sp.	*Dasineura*	inflated	*A. decurrens* 2884/1	
	Homalotylus sp.	*Dasineura*	fluted	*A. cyclops* 3023/4	

Several parasitoids were restricted, or almost so, to *Asphondylia* from inflorescences of Australian *Acacia*, including *Ormyromorpha* sp.1 (Pteromalidae), *Torymoides* sp. 3 (Torymidae), *Eurytoma* sp. (Eurytomidae), and particularly *Sigmophora* sp. (Eulophidae). The latter was common throughout southern Australia from a broad range of *Acacia* hosts with *Asphondylia* bud and seed galls. The highest diversity of parasitoids reared from *Asphondylia* was from the Pubescent Bud Galler with 11 species (table 5.2).

Emergence patterns of three Australian cecidomyiids and their parasitoids

Asphondylia

Bud galls of *Asphondylia* spp. from *A. mearnsii* in eastern Australia were primarily attacked by ectoparasitic Hymenoptera (table 5.3, 5.4), predominantly by species of *Sigmophora, Ormyromorpha, Systasis, Torymoides* and *Eurytoma* (table 5.2). Levels of attack varied across seasons, with the highest parasitoid induced mortality (35%) occurring at Wollongong, New South Wales. Endoparasitoids were relatively rare in Australian *Asphondylia* and mostly encountered as adults within or emerging from *Asphondylia* pupae. Mortality of *Asphondylia* larvae from unknown causes ranged from 0-32% with attack from an unidentified gall-chewing predator occurring at relatively low levels in most seasons and sites (table 5.3, 5.4).

Dasineura

At all sampling sites in Eastern Australia, Tiny Floret Gallers were parasitised primarily by endoparasitoids that caused 2-50% morality of larvae and pupae (table 5.5), but were only detectable by dissection of cocoons. The Platygastridae genera ?*Synopeas*, ?*Platygaster* and ?*Inostemma* were reared as endoparasitoids from Tiny Floret Galler cocoons (table 5.7). Ectoparasitoids were mostly present on larvae developing after emergence from floret galls and at all sites, except Somerville (Victoria) and Waramanga (ACT) caused lower levels of mortality (2.5-39%) than the endoparasitoids at the same sites (table 5.5). Mortality of Tiny Floret Galler larvae from unidentified causes showed little variation between sites in eastern Australia and between seasons.

In Western Australia, no ectoparasitoids were found on the Tiny Floret Galler, but endoparasitoid-induced mortality of 4-50%, predominantly by ?*Synopeas* sp., occurred in final instar larvae after emergence from the gall chamber (table 5.5). In Western Australia in 2002, the total mortality of Tiny Floret Galler immatures was 50.9%, which was 10% lower than the mean mortality for sites in eastern Australia (60.7%), due mainly to the absence of ectoparasitoids and lower mortality from unknown causes, particularly after emergence from the galls (table 5.5).

In the Common Fluted Galler, ectoparasitoid and endoparasitoid levels varied by up to 50% between seasons, but mortality resulting from other causes was similar. The Common Fluted Galler at Frankston had a progressive increase in both endoparasitoid and ectoparasitoid attack over three successive seasons, while other causes of mortality remained relatively constant (table 5.6).

The efficacy of yellow sticky traps in catching cecidomyiids and parasitoids in proportion to their abundance was not determined prior to commencement of this study. Biased sampling of either group could have skewed abundance records, but temporal emergence trends are unlikely to be affected.

Emergence of adult Tiny Floret Galler was closely synchronized with the flowering of *A. mearnsii* within a range of climate zones in southern Australia (figure 5.1). Tiny Floret Galler emergence occurred slightly before, or at the onset, of flowering of *A. mearnsii* and peaked in the mid-flowering period, then declined with the end of flowering. Peak emergence of the Tiny Floret Galler and flowering of *A. mearnsii* varied between sites, and commenced earliest at the low elevations sites of Jarrahdale (Western Australia), with a warm-temperate climate and Wollongong (New South Wales), with a Mediterranean-type climate, in late September (figure 5.1a,b). At the cool-temperate sites of Frankston (Victoria) and Casula (New South Wales), Tiny Floret Galler emergence and flowering of *A. mearnsii* peaked in early November (figure 5.1d,e). At Canberra, a cool-temperate site at high altitude where mean winter temperature are lower than the other sites, two Tiny Floret Galler emergence peaks occurred, the first in late September when *A. mearnsii* was in bud, followed by a second peak in early December when *A. mearnsii* was in flower (figure 5.1c). The flowering period of *A. mearnsii* was 12-14 weeks at Jarrahdale and Wollongong, and 5-8 weeks at other sites. At Wollongong, five *Dasineura* spp. were present and although Tiny Floret Galler adults could be distinguished on sticky traps, the Platygastridae parasitoids recorded from this site could have been associated with at least four of the *Dasineura* spp. (Inflated Galler, Elongate Fluted Galler, *D. acaciaelongifoliae* and the Tiny Floret Galler) (table 5.2).

The emergence pattern of adult ?*Synopeas* sp. (Platygastridae), the principal endoparasitoid of the Tiny Floret Galler, was very similar to its host at all study sites in southern Australia, but the number of trapped individuals was higher than those of the Tiny Floret Galler, particularly at Casula (NSW) where in peak emergence a mean (± SE) of 3689 ± 1336 ?*Synopeas* were recorded from 130 cm^2 of sticky trap over a fortnight's sampling period (figure 5.1e).

The Common Fluted Galler utilized *A. mearnsii* and *A. melanoxylon* as hosts at Stirling, South Australia and was well synchronized with the flowering phenology of both

Table 5.3 Mean percentage parasitism levels (± SE) per tree of *Asphondylia* sp. (Glabrous Bud Galler) from *A. mearnsii*. † Chambers where cecidomyiid immatures were found dead. ‡ Vacant chambers where immatures were undetectable and where galls had been attacked by external predators, the latter in parenthesis.

	2000				2001				2002			
Site	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡
Mittagong (NSW)	-	-	-	-	26.1 ± 4.1	2.8 ± 1.7	6.9 ± 2.1	1.2 ± 0.7 (1.6 ± 1.6)	19.2 ± 5.8	0	5.6 ± 1.8	11.2 ± 5.2 (0)
Wollongong (NSW)	0	0	0	5.0 ± 5.0	35.2 ± 20.1	4.1 ± 1.7	14.4 ± 3.8	3.3 ± 1.6 (4.4 ± 2.2)	-	-	-	-
Casula (NSW)	0.8 ± 0.8	0	0	2.4 ± 1.3	13.4 ± 2.7	0	17.8 ± 3.3	0 (9.2 ± 2.9)	2.0 ± 1.2	0	7.5 ± 2.0	4.5 ± 1.8 (11.5 ± 4.5)
Wartook (VIC)	-	-	-	-	13.8 ± 4.2	0	22.3 ± 4.5	0 (0)	-	-	-	-

Table 5.4 Mean percentage parasitism levels (± SE) of *Asphondylia* sp. (Pubescent Bud Galler) from *A. mearnsii*. † Chambers where cecidomyiid immatures were found dead. ‡ Vacant chambers where immatures were undetectable and where galls had been attacked by external predators, the latter in parenthesis.

	1998				2000				2001			
Site	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡
Waramanga (ACT)	-	-	-	-	20.8 ± 4.0	0	0	12.9 ±2.3 (0)	-	-	-	
Wartook (VIC)	-	-	-	-	-	-	-	-	14.7 ± 4.9	1.8 ± 1.3	27.3 ± 4.6	7.5 ± 4.2 (1.6 ± 1.0)
Pearcedale[1] (VIC)	12.6 ± 6.3	0	0	0 (0)	-	-	-	-	1.0 ± 1.0	0	2.5 ± 1.1	5.6 ± 2.5 (1.0 ± 0.7)

1 Collected from *A. baileyana*

Table 5.5 Mean percentage parasitism levels (± SE) of *Dasineura* sp. (Tiny Floret Galler) from *A. mearnsii* from (a) eastern Australia and (b) Western Australia. (a) eastern Australia

(a)

		2000				2001				2002			
Site		Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead †	Other‡	Ectoparasitoid	Endoparasitoid	Dead †	Other‡
Wollongong	*	0.2 ± 0.2	0	11.1 ± 1.8	4.1 ± 1.1	-	-	-	-	0	0	7.2 ± 1.8	1.3 ± 0.9
(NSW)	**	-	-	-	-					2.5 ± 1.4	11.2 ± 5.5	9.1 ± 2.2	0
Waramanga	*	10.4 ± 2.5	0	6.0 ± 1.5	3.7 ± 1.4	-	-	-	-	14.9 ± 3.7	0	6.0 ± 1.8	1.4 ± 1.0
(ACT)	**	-	-	-	-					25.9 ± 4.4	35.2 ± 5.7	0	0
Casula	*	0	0	10.8 ± 1.8	2.3 ± 0.7	-	-	-	-	0	0	6.5 ± 2.1	2.1 ± 1.7
(NSW)	**	5.0 ± 1.4	40.0 ± 0.2	8.0 ± 0.0	0					3.0 ± 2.0	8.8 ± 4.0	6.0 ± 1.9	0
Somerville	*	-	-	-	-	-	-	-	-	1.1 ± 0.7	0	10.5 ± 3.7	1.6 ± 0.8
(VIC)	**	-	-	-	-					11.4 ± 4.0	8.0 ± 3.0	21.2 ± 8.4	0
Brimbank	*	0.9 ± 0.5	0	7.9 ± 1.9	1.2 ± 0.7	-	-	-	-	2.3 ± 1.1	0	16.6 ± 4.7	3.1 ± 1.2
(VIC)	**	-	-	-	-					6.0 ± 2.9	35.0 ± 7.0	11.5 ± 3.8	0
Frankston	*	0.9 ± 0.5	0	8.6 ± 1.4	3.8 ± 1.1	-	-	-	-	3.8 ± 1.4	0	14.2 ± 2.9	0
(VIC)	**	-	-	-	-	0	21.4 ± 5.1	6.0 ± 2.3	0	10.6 ± 7.9	44.9 ± 15.4	21.8 ± 5.8	0

* Third instar larvae sampled from gall chambers (mean per tree), ** larvae and pupae sampled from cocoons after emergence of instars from gall developmental chambers (mean per site)

(b)

Site		2000				2001				2002			
		Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead †	Other‡	Ectoparasitoid	Endoparasitoid	Dead †	Other‡
Mt Barker	*	-	-	-	-	-	-	-	-	0	0	11.6 ± 7.5	1.5 ± 1.5
	**	-	-	-	-	0	7.7 ± 4.2	1.1 ± 0.6	0	0	41.6 ± 4.4	4.4 ± 2.4	0
Jarrahdale	*	0	0	11.0 ± 1.6	3.8 ± 0.8	-	-	-	-	0	0	10.4 ± 2.2	1.5 ± 0.8
	**	0	12.0 ± 1.1	-	-	0	38.5 ± 4.1	1.0 ± 0.6	0	0	30.0 ± 7.9	6.0 ± 1.9	0
Albany	*	-	-	-	-	-	-	-	-	0	0	6.9 ± 2.8	5.1 ± 1.5
	**	-	-	-	-	0	6.6 ± 1.3	2.6 ± 1.3	0	0	16.6 ± 4.4	1.66 ± 1.66	0
Augusta	**	-	-	-	-	0	4	0	0	-	-	-	-
Pemberton	*	-	-	-	-	-	-	-	-	0	0	3.5 ± 1.4	3.0 ± 1.4
	**	-	-	-	-	0	36.0	0	0	0	48.7 ± 10.6	8.7 ± 4.3	0
Denmark	*	-	-	-	-	-	-	-	-	0	0	4.1 ± 1.4	2.3 ± 1.1
	**	-	-	-	-	0	32.0 ± 4.2	2.2 ± 1.3	0	0	44.2 ± 14.0	0	0
Dwellingup	*	-	-	-	-	-	-	-	-	0	0	6.4 ± 1.5	3.0 ± 1.0
	**	-	-	-	-	0	67.0 ± 3.6	1.5 ± 0.7	0	0	37.5 ± 5.7	0	0
William Bay	*	-	-	-	-	-	-	-	-	0	0	5.7 ± 1.5	0.8 ± 0.6
	**	-	-	-	-	-	-	-	-	0	49.6 ± 9.6	2.0 ± 2.0	0

Table 5.6. Mean percentage parasitism levels (± SE) per tree of *Dasineura sp.* (Common Fluted Galler) from *A. mearnsii.* † Chambers where cecidomyiid immatures were found dead. ‡ Vacant chambers where immatures were undetectable and where galls had been attacked by external predators, the latter in parenthesis.

Site	2000				2001				2002			
	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡	Ectoparasitoid	Endoparasitoid	Dead†	Other‡
Frankston (VIC)	7.2 ± 1.4	2.6 ± 0.8	17.6 ± 7.3	11.9 ± 5.3	7.8 ± 2.2	15.7 ± 3.4	17.4 ± 3.9	9.7 ± 2.9	25.6 ± 5.6	35.5 ± 6.0	9.5 ± 3.4	5.6 ± 2.2
Burrumbeet (VIC)	7.9 ± 2.6	4.8 ± 1.4	10.1 ± 5.1	3.4 ±1.1	45. 1 ± 3.0	4.2 ± 1.7	17.1 ± 2.6	5.9 ± 2.2	-	-	-	-
Stirling (SA.)	3.4 ± 1.0	0.22 ± 0.2	6.0 ± 1.2	9.7 ± 2.1	-	-	-	-	-	-	-	-

Table 5.7. Parasitoids reared from *Dasineura* sp. (Tiny Floret Galler) galls in eastern and Western Australia. Numbers in parenthesis are accession numbers.

Location	Site	Endoparasitoid	Ectoparasitoid
Western Australia	Jarrahdale	Platygastridae: ?*Synopeas* sp. 1 (3251, 3285)	
	Dwellingup	Platygastridae: ?*Synopeas* sp. 1 (3288)	
	Albany	Ceraphronidae: ?*Conostigmus* sp. (2901)	
		Platygastridae: ?*Synopeas* sp. 1 (3286)	
	Mt Barker	Platygastridae: ?*Synopeas* sp. 1 (3284)	
	Pemberton	Ceraphronidae: ?*Conostigmus* sp.	
		Platygastridae: ?*Synopeas* sp. 1 (3283)	
	William Bay	Platygastridae: ?*Synopeas* sp. 1 (3287)	
Eastern Australia	Wollongong (NSW)	Platygastridae: ?*Synopeas* sp. 1 (3290)	Pteromalidae: *Gastrancistrus* sp., *Systasis* sp. 1 (3290)
	Casula (NSW)	Platygastridae: ?*Synopeas* sp. 1 (3289)	Pteromalidae: *Systasis* sp. 1 (3289)
	Frankston (VIC)	Platygastridae: ?*Synopeas* sp. 1, genus indet. (3293)	Ptermalidae: *Systasis* sp. 1 (3293)
	Brimbank (VIC)	Platygastridae: ?*Synopeas* sp. 1, genus indet. (3291)	Eulophidae: *Aprostocetus* sp. 2 (3291)
			Pteromalidae: *Gastrancistrus* sp., *Systasis* sp. 1 (3291)
	Somerville (VIC)	Platygasteridae: genus indet. (3292)	Pteromalidae: *Gastrancistrus* sp. (3292)
			Pteromalidae: indet , *Systasis* sp. 1 (3292)

Acacia species (figure 5.1g,h). Emergence of Common Fluted Gallers first occurred from galls on *A. mearnsii* during August-September when *A. melanoxylon* was in flower, followed by a second emergence of lower magnitude from galls on *A. melanoxylon* during November-December when *A. mearnsii* was in flower, but not *A. melanoxylon* (figure 5.1g,h). The main parasitoid of the Common Fluted Galler, ?*Synopeas* sp., emerged in greatest numbers with the emergence of the Common Fluted Galler from galls on *A. mearnsii* during November-December.

Adult Glabrous Bud Gallers emerged from *A. mearnsii* over August-September with peak emergence 2-4 weeks before flowering of *A. mearnsii* commenced. The ectoparasitoid *Ormyromorpha* sp. (Pteromalidae) had a similar emergence pattern to the Glabrous Bud Galler (figure 5.1f).

Selective suppression of parasitoids

The LT_{50} response of the Tiny Floret Galler, the Pubescent Bud Galler, and their parasitoids to insecticide treatments showed highly significant main effects and interaction terms (table 5.8). Consequently, data analyses were performed for each cecidomyiid and their parasitoids separately.

When mixed with honey, the organophosphate fenthion and the synthetic pyrethroid cypermethrin caused faster rates of mortality to both the Tiny Floret Galler and Pubescent Bud Galler and their parasitoids compared to untreated honey controls with all differences being significant ($P<0.01$), except for the Pubescent Bud Galler where mortality caused by cypermethrin, although lower than controls, was not significantly different (table 5.9).

When insecticides were applied in water, the LT_{50} from cypermethrin was not significantly different ($P>0.05$) to controls for the Tiny Floret Galler, but water-applied fenthion and cypermethrin treatments caused faster LT_{50} mortality in the Tiny Floret Galler parasitoids compared to the controls ($P<0.01$) (table 5.9).

When applied with honey, the Tiny Floret Galler's LT_{50} mortality was significantly slower for cypermethrin than for fenthion ($P = 0.049$), but no detectable differences were found between the two insecticides for Tiny Floret Galler parasitoids. Fenthion caused faster LT_{50} mortality in the Pubescent Bud Galler than cypermethrin ($P = 0.046$), but there was no significant difference between insecticide types on mortality rates of Pubescent Bud Galler parasitoids (table 5.9).

Discussion

The susceptibility of herbivorous insects to attack by parasitoids and the number and type of parasitoid species may be influenced by the herbivores' feeding niche (Askew & Shaw 1986, Hawkins & Lawton 1987, Gross & Price 1988, Price & Pschorn-Walcher 1988, Hawkins

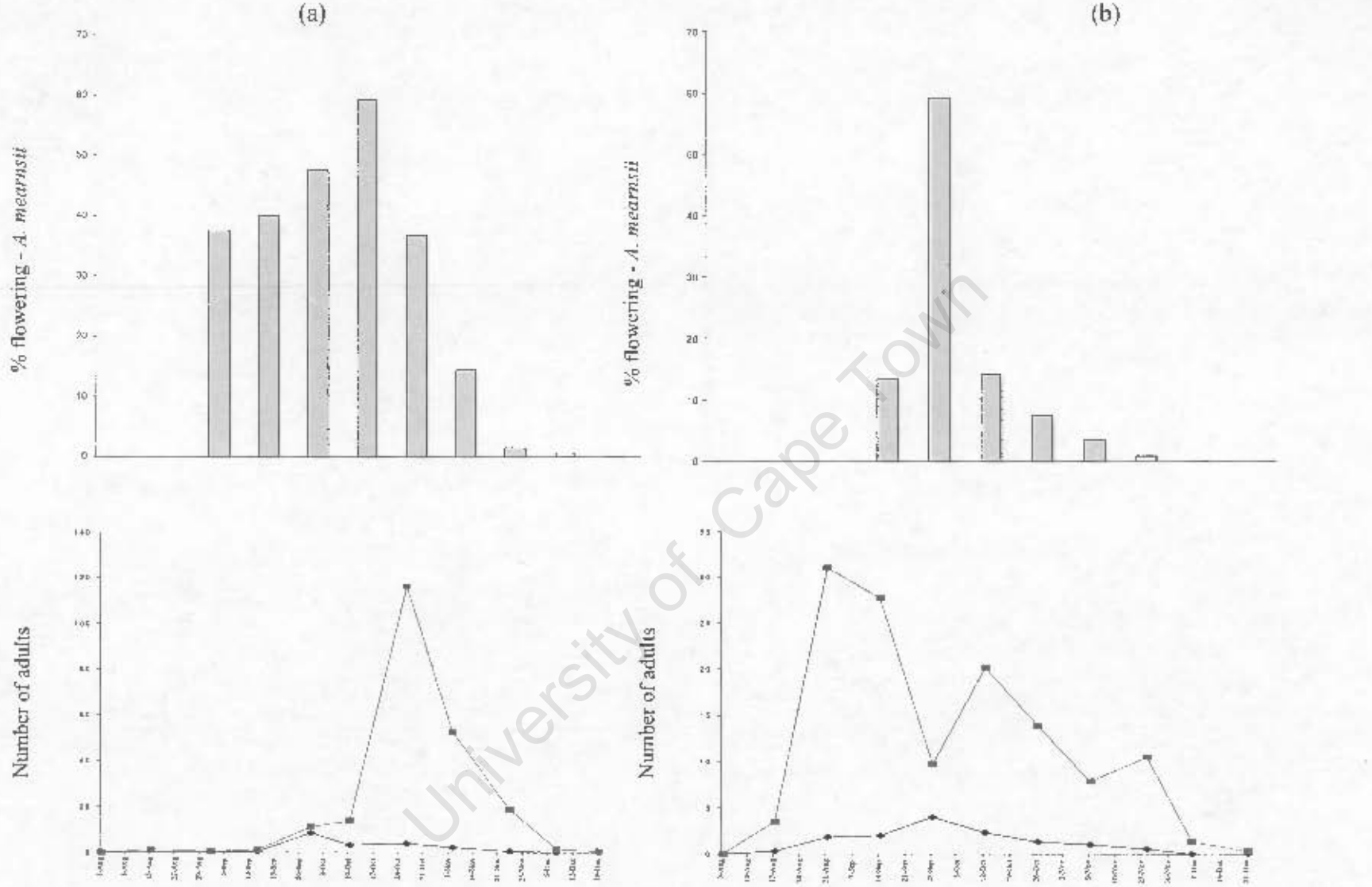


Figure 5.1(a-f). Emergence patterns of Cecidomyiidae from *Acacia:* (a-e) *Dasineura* sp. (Tiny Floret Galler) (♦) and Platygastridae parasitoids (■) in relation to flowering of *A. mearnsii* at (a) Jarrahdale, Western Australia, (b) Wollongong, New South Wales, (c) Canberra, ACT, (d) Frankston, Victoria, (e) Casula, New South Wales; (f) emergence pattern of *Asphondylia* sp. (Glabrous Bud Galler) (♦) and parasitoids (■) at Casula, New South Wales; (g-h) emergence patterns of *Dasineura* sp (Common Fluted Galler) (♦) and Platygastridae parasitoids (■) from (g) *A. mearnsii* and (h) *A. melanoxylon* at Stirling, South Australia. Insect data are the mean number/130cm^2 of sticky trap.

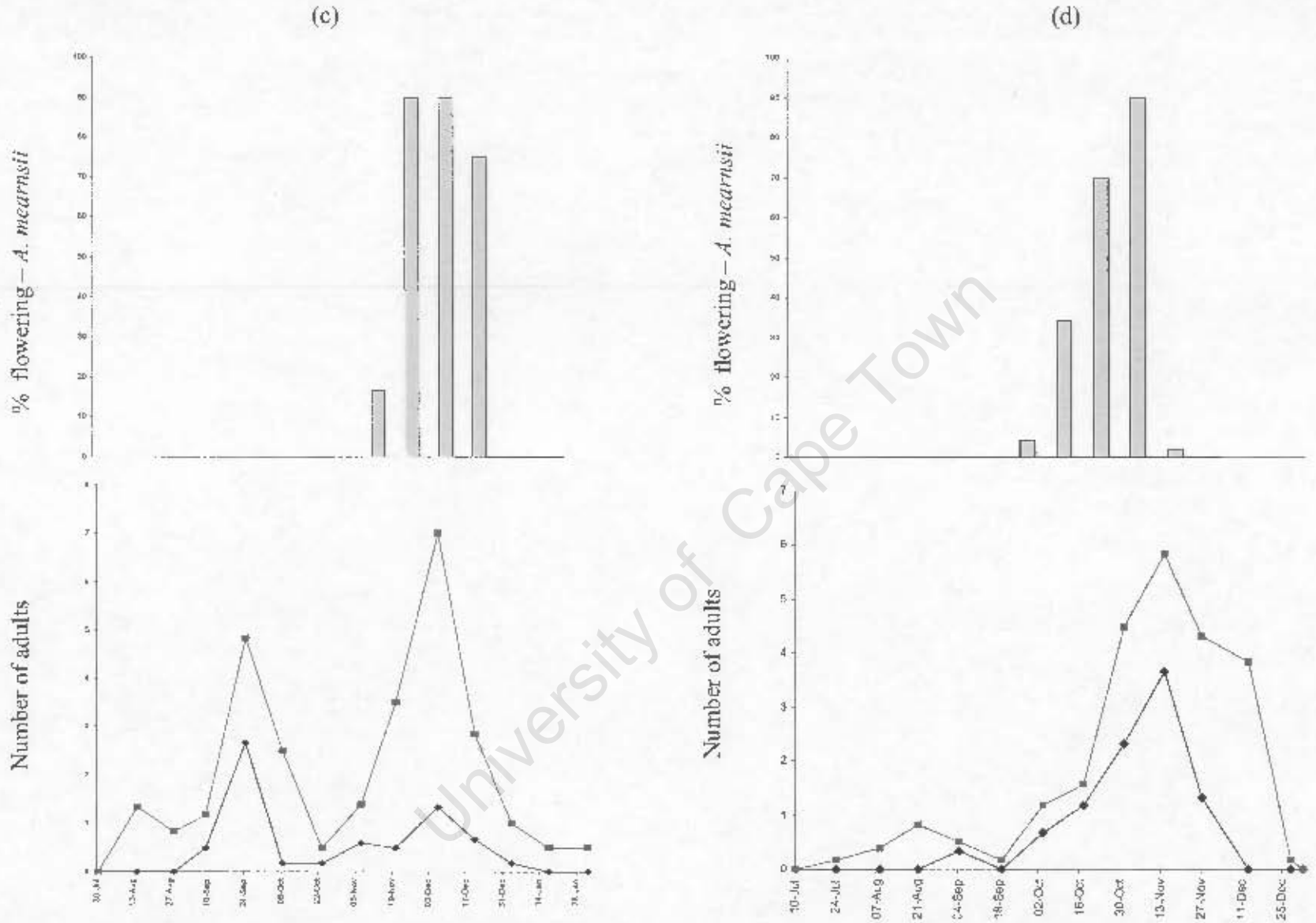


Figure 5.1(a-f). Emergence patterns of Cecidomyiidae from *Acacia*: (a-e) *Dasineura* sp. (Tiny Floret Galler) (♦) and Platygastridae parasitoids (■) in relation to flowering of *A. mearnsii* at (a) Jarrahdale, Western Australia, (b) Wollongong, New South Wales, (c) Canberra, ACT, (d) Frankston, Victoria, (e) Casula, New South Wales; (f) emergence pattern of *Asphondylia* sp. (Glabrous Bud Galler) (♦) and parasitoids (■) at Casula, New South Wales; (g-h) emergence patterns of *Dasineura* sp (Common Fluted Galler) (♦) and Platygastridae parasitoids (■) from (g) *A. mearnsii* and (h) *A. melanoxylon* at Stirling, South Australia. Insect data are the mean number/130cm^2 of sticky trap.

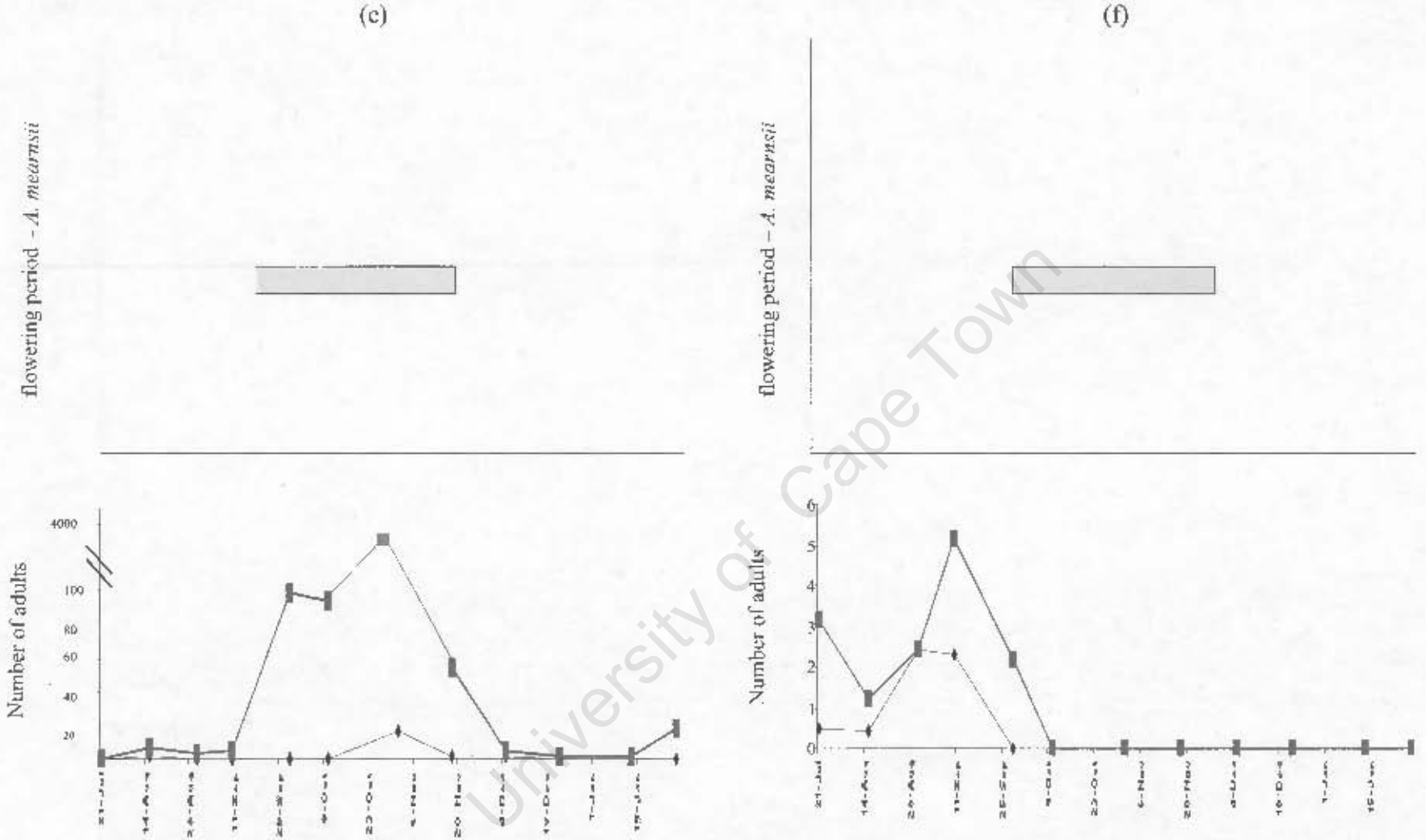


Figure 5.1(a-f). Emergence patterns of Cecidomyiidae from *Acacia:* (a-c) *Dasineura* sp. (Tiny Floret Galler) (♦) and Platygastridae parasitoids (■) in relation to flowering of *A. mearnsii* at (a) Jarrahdale, Western Australia, (b) Wollongong, New South Wales, (c) Canberra, ACT, (d) Frankston, Victoria, (e) Casula, New South Wales; (f) emergence pattern of *Asphondylia* sp. (Glabrous Bud Galler) (♦) and parasitoids (■) at Casula, New South Wales; (g-h) emergence patterns of *Dasineura* sp (Common Fluted Galler) (♦) and Platygastridae parasitoids (■) from (g) *A. mearnsii* and (h) *A. melanoxylon* at Stirling, South Australia. Insect data are the mean number/130cm^2 of sticky trap.

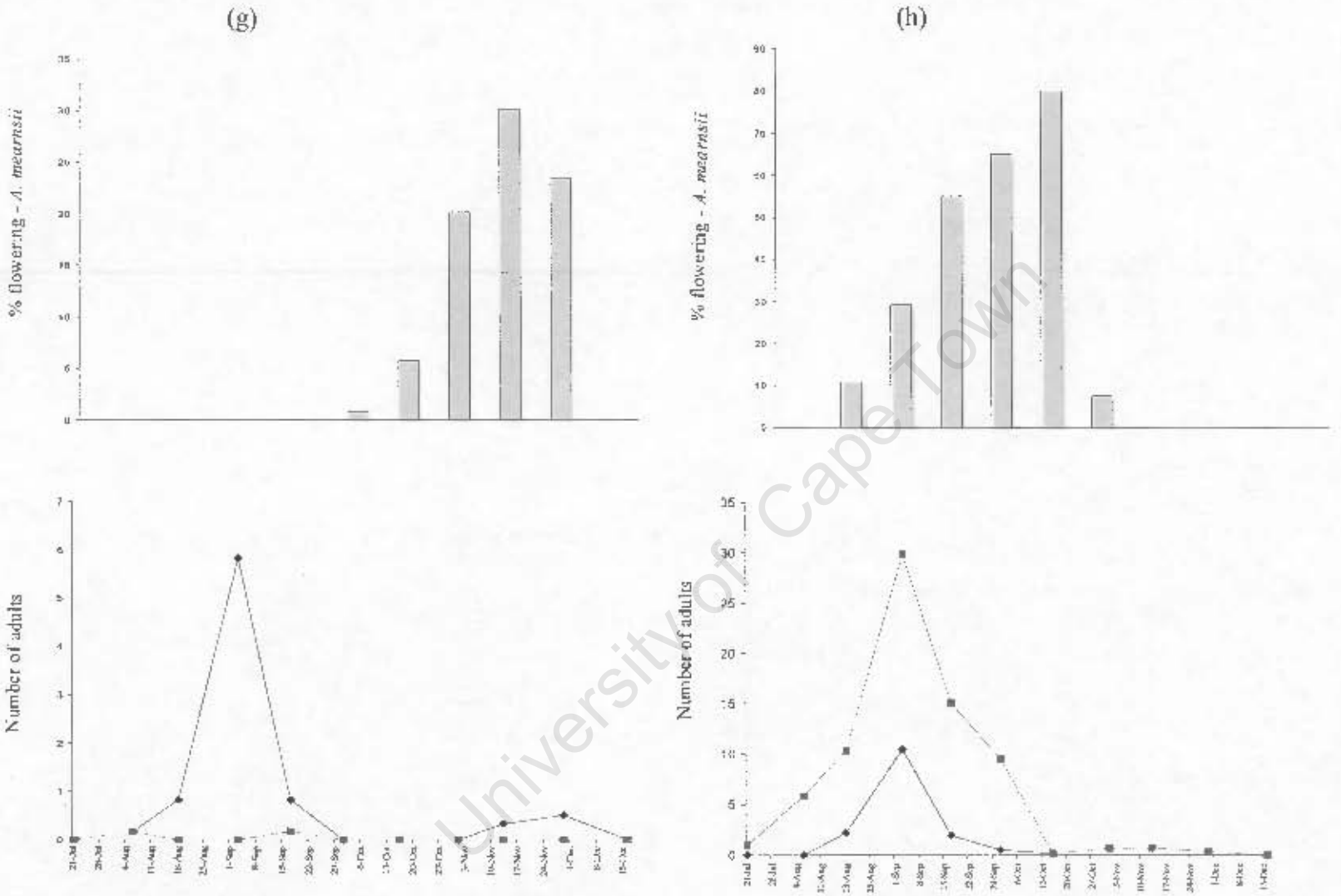


Figure 5.1(a-f). Emergence patterns of Cecidomyiidae from *Acacia:* (a-e) *Dasineura* sp. (Tiny Floret Galler) (♦) and Platygastridae parasitoids (■) in relation to flowering of *A. mearnsii* at (a) Jarrahdale, Western Australia, (b) Wollongong, New South Wales, (c) Canberra, ACT, (d) Frankston, Victoria, (e) Casula, New South Wales; (f) emergence pattern of *Asphondylia* sp. (Glabrous Bud Galler) (♦) and parasitoids (■) at Casula, New South Wales; (g-h) emergence patterns of *Dasineura* sp (Common Fluted Galler) (♦) and Platygastridae parasitoids (■) from (g) *A. mearnsii* and (h) *A. melanoxylon* at Stirling, South Australia. Insect data are the mean number/130cm^2 of sticky trap.

1988, Gross 1991, Hill & Hulley 1995). Endophagous hosts support parasitoid complexes containing larger proportions of generalists, whereas external feeders support relatively more specialist parasitoids (Hawkins 1990, Hawkins *et al.* 1992, Cornell & Hawkins 1993). Weakly concealed endophages have minimal protection from attack (Hawkins 1988), while well-concealed endophages generally support low numbers of parasitoids (Hawkins 1990, Hawkins & Gross 1992) as they are physically protected by plant tissue (Gross 1991) and provide few clues to their location. In contrast, well-concealed endophages in South Africa support a relatively rich fauna of generalist parasitoids (Hill & Hulley 1995).

Gall-forming insects generally have rich parasitoid faunas (Askew 1980, Hawkins & Goeden 1984). Several factors may influence the species complement of gall communities including: season for gall induction, position of galls on the host and gall size and structure (Askew 1961, Brooks & Shorthouse 1997). A high proportion of parasitoids of gall-forming insects are idiobionts (Hawkins 1990), which tend to have a wide host range (Cornell & Hawkins 1993). In Australian gall-forming Cecidomyiidae, most, if not all recorded parasitoids appear to be idiobionts with parasitoids paralysing their host in their early stages of development. This certainly appears to be the case with the endoparasitoids from the Platygastridae, where the larva host dies only on the late stages of larval development or as pupae. There was a higher species richness of ectoparasitoids than endoparasitoids in gall midges of acacias, although numerically, the endoparasitiods appear to have the greatest impact. More detailed field studies are required to clarify these observations. No idiobiont endoparasitoids were noted for gall-forming midges of acacias. Endoparasitoids were restricted to the Platygastridae and all appeared to be kionobionts. Again, further observations and data are required to confirm these observations.

Classical biological control agents often acquire native parasitoids in their country of introduction within several years (Hill & Hulley 1995), but the full complement of parasitoids may be gradual and over a much longer time, perhaps up to 150 years as some native parasitoids may need to adjust their behaviour and ecology to exploit novel hosts (Cornell & Hawkins 1993). Parasitoid accumulation patterns on introduced herbivores are explicable in part to intrinsic biological characters of hosts, time since host introduction, and host spectrum of native parasitoids (Hawkins & Gross 1992). Gall-forming Cecidomyiidae introduced as biological control agents can suffer high mortality from indigenous parasitoids, which can compromise their efficacy (Goeden & Louda 1976, McFadyen 1985, Wehling & Piper 1988, Carlson & Mundal 1990). In some cases, parasitoids of cecidomyiids may consume their host and then complete development by feeding on plant tissue (Slobodchikoff 1967, Askew 1971).

Several Australian gall-forming cecidomyiids are under consideration as biological control agents of the seeds of *A. mearnsii* and *A. cyclops* in South Africa (Adair *et al.* 2000). If released, attack from indigenous South African parasitoids is likely to follow the general trend for herbivores on new plants: rapid accumulation, lower richness and a higher proportion of generalists than specialists than what occurs on similar native hosts (Cornell & Hawkins 1993). In addition, the composition of the acquired parasitoid fauna may follow patterns linked to the degree of taxonomic and ecological relatedness of the introduced insects to endemic cecidomyiids, and similarities in host plants utilized and gall morphology and structure of cecidomyiids between the two continents.

Australian and South African cecidomyiids of *Acacia* have few taxonomic and ecological similarities. The Australian cecidomyiid fauna consists entirely of *Dasineura* and *Asphondylia,* while those from related host structures on South African *Acacia* are *Acacidiplosis, Aposchizomyia, Contarinia* and *Asphondylia.* Australian cecidomyiid galls from *Acacia* are mostly woody and insects emerge through ostioles that form in the early stages of gall development. African cecidomyiid galls from *Acacia* are semi-woody galls nearly always lacking pre-formed ostioles. The cecidomyiid fauna from *Acacia* in South Africa and Australia are dominated by species with univoltine life histories. Gall morphology influences parasitoid composition and abundance (Dixon *et al.* 1998) where gall hardness and diameter may impede attack by certain parasitoids in cynipid galls (Washburn & Cornell 1979, Abe 1992) and other insects (Weis *et al.* 1985, Craig *et al.* 1990, Craig 1994). In *Carduus nutans* L., the mass of gall tissue surrounding the weevil *Rhinocyllus conicus* offered protection from parasitoids (Harris & Shorthouse 1996). Similar trends occur in larger galls of *Trichilogaster acaciaelongifoliae* on *A. longifolia* (Mangoni & Hoffmann 1995). Divergence in gall morphology amongst co-existing inducer species has most likely been driven by parasitoid attack (Askew 1961, Price 1980, Cornell 1983).

The Tiny Floret Galler from *A. mearnsii* and *D. dielsi* from *A. cyclops,* potential biological control candidates for these hosts in South Africa, respectively, are parasitised predominantly by the endoparasitoid ?*Synopeas* sp. (Platygastridae) (3251), which is found throughout the range of these cecidomyiids in southern Australia. ?*Synopeas* sp. emerges in close synchronisation with the Tiny Floret Galler and the flowering of *A. mearnsii* over a range of climate-types in southern Australia. A similar analogue of this Australian tri-trophic relationship occurs in South Africa with the ovary-galling midges *Contarinia* spp. (Lumpy Node Galler, Oyster-shell Galler) on *A. mellifera*, which induce woody polythalamous galls after oviposition in open florets.

Similarities in the parasitoid fauna of *Contarinia* spp. from *A. mellifera* and the Tiny Floret Galler and *D. dielsi* in Australia, with all cecidomyiids sharing an endoparasitic Platygastridae (indet.) and the pteromalids, *Systasis* and *Gastrancistrus*, indicate that the South African species of these genera are likely candidates for developing new host associations on the Tiny Floret Galler and *D. dielsi,* if released in South Africa. Furthermore, although *A. mellifera* occurs in dry savanna regions of summer-rainfall areas of South Africa, and rarely occurs sympatrically with *A. mearnsii*, the same or closely-related Platygastridae occurs on node galls of *A. karroo* and receptacle galls of *A. erioloba* as well on other hosts including, *Salvia, Chrysanthemoides, Berzelia* and *Eriocephalus*. These native host plants and many of their cecidomyiid gall-inducers are common in the temperate distribution of *A. mearnsii* in South Africa, which may facilitate hosts shifts of Platygastridae from South African cecidomyiids to those introduced for biological control of *A. mearnsii* or *A. cyclops*.

South African parasitoids utilising cecidomyiids on African *Acacia* are known to occur in woody to semi-woody cecidomyiid galls from other host plants and are thus candidates as parasitoids for the Tiny Floret Galler and *D. dielsi*. Apart from the Platygastridae described above, additional taxa are: *Elasmus* spp. (Elasmidae), *Eupelmus* spp (two species) (Eupelmidae), *Aprostocetus* spp. (Eulophidae), *Eurytoma* sp. (Eurytomidae), *Ormyrus* sp. (Ormyridae) and *Pediobius* sp. (Eulophidae). The generalist parasitoids Platygastridae (indet.), *Eupelmus* spp. and *Aprostocetus* spp. which have broad climatic tolerance in South Africa (table 5.1) are most likely to appear first on *Dasineura* released as biological control agents for Australian *Acacia*. They are likely to be followed by *Ormyrus* sp., *Elasmus* sp., and *Pediobius* sp., which appear to have more specialized host utilisation patterns (table 5.1). These predictions are further supported by the occurrence of Platygastridae (indet.), *Elasmus* sp., *Aprostocetus* sp. on other cecidomyiids *Dasineura* sp. and *Zeuxidiplosis giardi* Kieffer introduced into South Africa for the biological control of *Leptospermum laevigatum* (Sol. ex Gaertn.) and *Hypericum perforatum* L., respectively (O. Neser pers. comm. 2002).

The univoltine Glabrous Bud Galler, also a candidate biological control agent for *A. mearnsii* (Adair *et al.* 2000), has thin-walled galls and is likely to be susceptible to parasitism by *Aprostocetus* sp., *Entedon* sp. (Eulophidae), ?*Baryscapus* sp. (Eulophidae), and nr. *Pseudocatolaccus* sp. (Pteromalidae), which occur on indigenous *Asphondylia* in South Africa with similar gall and biological attributes (table 5.1).

Table 5.8. Results from a GLM analysis on the insect mortality in response to five insecticide treatments for Tiny Floret Galler (TFG) and its parasitoids using untransformed LD_{50} values, and Pubescent Bud Galler (PBG) and its parasitoids using log_{10} transformed LT_{50} values.

Experiment	Factor	df	F ratio	P
TFG & parasitoids	insect	1	83.65	0.000
	insecticide	4	204.28	0.000
	insect x insecticide	4	87.19	0.000
	error	36		
PBG & parasitoids	insect	1	0.195	0.663
	insecticide	2	80.461	0.000
	insect x insecticide	2	18.309	0.000
	error	21		

Table 5. 9. Mean LT_{50} as days (± SD) for two Australian cecidomyiids, Tiny Floret Galler (TFG), Pubescent Bud Galler (PBG), and their parasitoids after treatment with insecticides. Values in columns followed by the same letter are not significantly different (P>0.05).

Treatment	TFG	TFG parasitoids	PBG	PBG parasitoids
control	2.40 ± 0.4 a	7.78 ± 0.6 a	2.21 ± 0.4 a	6.06 ± 1.5 a
cypermethrin + honey	1.36 ± 0.6 b	0.53 ± 0.03 bc	1.40 ± 0.6 a	0.65 ± 0.2 b
cypermethrin + water	2.57 ± 0.5 a	3.36 ± 0.8 e	-	-
fenthion + honey	0.50 ± 0.01 c	0.56 ± 0.05 bd	0.68 ± 0.2 b	0.61 ± 0.2 c
fenthion + water	1.18 ± 0.4 bc	1.18 ± 0.4 cd	-	-

The predatory mite *Pyemotes* spp. (Acari: Pyemotidae) cause mortality of gall-forming cecidomyiids and have been considered as biological control agents for midges that damage plants of economic importance (Shazli & Mostafa 1980, Moser *et al.* 1986, Weatherby *et al.* 1989). In Australia, a *Pyemotes* sp. can cause sporadic mortality on *Dasineura* sp. and is likely to be responsible for a considerable proportion of larvae recorded as dead in galls of the Tiny Floret Galler in eastern and Western Australia. Although *Pyemotes* sp. adults or juveniles were not regularly seen, many cecidomyiid larvae found dead in galls were intact, but devoid of body fluids, symptoms that usually occur after attack by *Pyemotes* sp. Although other haemolymph-sucking predators may also cause these symptoms, apart from the thrip *Rhopalothripoides froggattii*, which may be facultatively entomophagous, these were never observed on or in *Dasineura* galls in Australia. *Pyemotes* sp. has been observed in bud-galls of an undescribed *Dasineura* on *L. laevigatum* in South Africa (S. Neser pers. comm. 2002) and may contribute to mortality of *Dasineura* spp., if released as biological control agents of *Acacia* in South Africa. The open ostioles of the Tiny Floret Galler in the late instar stage, may render this cecidomyiid susceptible to attack by *Pyemotes* sp. However, the dense indumentum and membranous hymen in the ostioles of *D. dielsi* floret galls are likely to reduce invasion by *Pyemotes* sp. and may well be an adaptation to avoid this form of predation.

While the composition of South African parasitoids and predators likely to attack Australian gall-forming cecidomyiids released as biological control agents for acacias can be predicted, although at an uncertain level of accuracy, their impact on the host cecidomyiids cannot. This can only be determined by detailed post-release evaluation, an important but often neglected, component of classical biological control programs.

In-situ baiting with insecticides that selectively reduce parasitoids of phytophagous insects in the country of origin has the potential to induce outbreak populations that may mimic low parasitoid environments in countries where these insects may be used as biological control agents. The suppression of parasitoids of *Rhopalomyia* in California with protein-based baits and the increase in galling levels on *Baccharis halimifolia* indicates the potential of this technique (Ehler *et al.* 1984). Although this study showed sugar-based insecticides can selectively increase mortality of Pubescent Bud Galler parasitoids, these were ineffective for the Tiny Floret Galler, where both adult cecidomyiids and hymenopteran parasitoids fed on sugar-based baits. Further investigation using protein-based baits may yield more encouraging results that could justify evaluation trials under field conditions.

Biological control agents can remain effective despite attack by parasitoids in their introduced range. In South Africa, parasitoids destroy up to 48% of *Trichilogaster*

acaciaelongifoliae on *A. longifolia* (McGeoch & Wossler 2000), although overall parasitism is likely to be lower (J.H. Hoffmann pers. comm. 2003), and this bud-galling hymenopteran remains a highly effective biological control agent. In Western Australia, 51% of Tiny Floret Galler galls were parasitized, mainly by ?*Synopeas*, yet regular, high-density galling occurs on *A. mearnsii*, which reduces seed production.

Comparison of parasitoid attack of the Tiny Floret Galler between populations in eastern Australia, where the insect is native, and in Western Australia, suggest a small reduction in cecidomyiid mortality may contribute to regular and substantial increases in gall densities on *A. mearnsii*. Outbreak populations of the Tiny Floret Galler in Western Australia even in the presence of well-synchronized and abundant parasitoids indicate that this insect should not be rejected as a potential biological control agent of *A. mearnsii* in South Africa, even though attack from endemic South African parasitoids is likely. Similarly, there is a strong probability that endemic parasitoids will utilise *D. dielsi* as a host, if this insect is released as a biological control agent for *A. cyclops* in South Africa. Post-release evaluation of parasitoid and predator composition and impact on Australian gall-forming cecidomyiids released as biological control agents for Australian acacias could substantiate the predictions made in this study and determine if the approach used has value for other biological control programs.

CHAPTER 6

The impact of resource loading by gall-forming Cecidomyiidae on *Acacia mearnsii* and *A. cyclops*

Abstract

Australian gall-forming cecidomyiids under consideration as potential biological control agents for commercially useful acacias in South Africa need to be evaluated for their impact on the vegetative growth of the host species. *Acacia mearnsii* and to a lesser extent *A. cyclops* are of commercial importance in South Africa where they are grown as silvicultural crops (*A. mearnsii*) or utilised as a domestic-fuel source, particularly by resource-poor, rural communities. Gall-forming insects that create resource sinks with detrimental impacts on the vegetative growth of commercially important acacias are incompatible with forestry industries in South Africa. The impact of gall-formation was evaluated for six cecidomyiids from *A. mearnsii* and *Dasineura dielsi* from *A. cyclops*. The Tiny Floret Galler is considered to have the greatest potential as a biological control agent for *A. mearnsii*. Flower-head galls of the Tiny Floret Galler had a calorific value less than mature fruits of *A. mearnsii*. Biomass accumulation patterns of the Tiny Floret Galler were similar to those of *A. mearnsii* fruits in the early-mid stages of development, but *A. mearnsii* fruits exceeded gall biomass by a factor of three at fruit maturity. Galls of the Tiny Floret Galler have a shorter development period than *A. mearnsii* fruits. In populations of *A. mearnsii* in Western Australia where the Tiny Floret Galler occurs in outbreak densities, the mean dry biomass of galls on branches (4-6 mm diameter) over three successive seasons from 1999-2002 was 1.4-2.7 gm. Dry biomass of Tiny Floret Galler galls in Australia was similar to or less than the dry biomass of *A. mearnsii* fruits sampled from branches in Western Cape, KwaZulu-Natal, Mpumalanga and Gauteng, South Africa. There were no significant differences in dry fruit biomass of trees in the Western Cape, where *A. mearnsii* is widely naturalised, and in KwaZulu-Natal and Mpumalanga, where *A. mearnsii* is grown extensively in commercial forestry plantations. Stem growth and leaf production was negatively correlated with dry biomass of fruits on trees in South Africa suggesting that fruit production may create a resource sink on the host tree. As the mean gall biomass of the Tiny Floret Galler in outbreak densities in Western Australia did not exceed the mean biomass of *A. mearnsii* fruit in South Africa, the resource sink impact of the Tiny Floret Galler on the vegetative growth of *A. mearnsii* is expected to be similar to or less than that experienced by trees undergoing fruit production. There was no

evidence to support the rejection of the Tiny Floret Galler as a potential biological control agent for *A. mearnsii* in South Africa on the basis of possible deleterious impacts on vegetative growth of *A. mearnsii* in commercial forestry plantations. Site-based population growth models predict that the Tiny Floret Galler may achieve maximum gall densities within 8-12 years. The implications to the wattle industry are discussed if the Tiny Floret Galler was released as a biological control agent in South Africa.

Introduction

Nutrients as well as carbon and energy can be concentrated in galls (Palct & Hassler 1967, Jankiewiez *et al.* 1970, Hartnett & Abrahamson 1979, Stinner & Abrahamson 1979, Shannon & Brewer 1980, McCrea *et al.* 1985, Abrahamson & McCrea 1986, Larson & Whitham 1991, Paquette *et al.* 1993, Bagatto *et al.* 1996) and benefit the primary gall-forming agent and associated biota, and can reduce growth and reproduction of the host (Fay & Hartnett 1991). Higher nutritional status of galls compared to ungalled tissue is a general pattern and may favour development of the cecidogenic agent (Weis & Kapelinski 1984, Price *et al.* 1987), but there are exceptions (Hinz & Muller-Scharer 2000).

Gall-formation is initiated on young, undifferentiated plant tissues where assimilates can be redirected and tissues can be manipulated by the cecidogenic agent. Salivary secretions from larvae induce a proliferation of parenchyma or nutritive cells on the wall of the gall chamber and serves as a food source for insect development (Meyer & Maresquelle 1983, Bronner 1992). Galls develop characteristic morphologies that are mostly species specific and reflect an extension of the agent's genotype (Dawkins 1983, Stern 1995, Crespi *et al.* 1997), but little is known of the biochemical processes responsible for this process.

The impact of gall inducers on their hosts is affected by three interacting variables: vulnerability to parasitism and moisture stress; their power to act as a metabolic sink; and their ability to exploit nutritional resources (Harris & Shorthouse 1996). All three factors are therefore relevant in consideration of gall-forming organisms as biological control agents for weeds. Although gall formers were initially rated poorly as potential biological control agents (Harris 1973), despite high levels of host specificity (Mani 1964, Dixon 1983, Ananthakrishnan 1986), successful suppression of several *Acacia* species in South Africa (Morris 1999, Dennill *et al.* 1999) and Compositae in North America (Harris & Shorthouse 1996) have enhanced the guild's reputation as a source of potential biocontrol agents.

Damaging gall-forming biological control agents create vascular connections with their host and divert assimilates from other developmental processes (Harris & Shorthouse 1996) thereby inducing a partitioning of plant resources (Larson & Whitham 1991, Hartley

and Lawton 1992, Paquette *et al.* 1993). Cecidogenic agents that develop on reproductive organs create a resource-sink when they are well synchronized with the host's phenology and produce a greater dry mass and energy drain to galls than from corresponding plant structures (Dennill 1988, 1990). In addition, the impact is enhanced when gall formation spans the entire reproductive period and, or, growth phase of the host (Harris 1973, Goeden 1983, Dennill 1985, Hokkanen 1985, Wapshere 1985) and the dry mass of galls is greater during the early part of the reproductive season (Hartnett & Abrahamson 1979, Harris 1980, Dennill 1985, Maiteki & Lamb 1985). Many of these attributes are evident in the biological control programs aimed at suppressing non-commercial Australian acacias in South Africa with gall-forming insects and fungi (Dennill 1985, 1988, 1990; Dennill *et al.* 1999; Hoffmann *et al.* 2002).

Acacia longifolia is an invasive Australian shrub in the southern Cape of South Africa and has been successfully suppressed using a gall-forming biological control agent *Trichilogaster acaciaelongifoliae* (Pteromalidae) (Dennill *et al.* 1999). Reproduction in *A. longifolia* is a debilitating event (Dennill 1987b, 1990) with fruit formation strongly inversely related to vegetative growth (Dennill 1987b). When *T. acaciaelongifoliae* induces bud galls with 3-23 times more biomass than normal ungalled tissue, reproductive output is severely impaired, vegetative growth is reduced by 53%, and trees may collapse due to the physical stresses caused by high gall biomass (Dennill 1985, Dennill 1990). Gall development occurs prior to the normal abscission of developing inflorescences and a 'forced commitment' (Dennill 1988) to gall production occurs where the host produces more galls per branch than its normal quota of infructescences (Dennill 1988). A similar process occurs on *A. pycnantha* in South Africa when galled by *Trichilogaster* sp. (Hoffmann *et al.* 2002).

Although the debilitating impacts caused by forced commitment to gall formation have been fundamental to the successful suppression of invasive Australian acacias in South Africa, they are obviously not appropriate for those species that are grown as commercial forests in Africa. *Acacia mearnsii* is a timber, tannin and pulp crop in southern Africa and is commercially valuable, but severe environmental impacts occur from naturalized stands that have spread over 2.5 million ha in South Africa alone (de Wit *et al.* 2001), necessitating the use of seed-reducing biological control agents to suppress and reduce the rate of spread of wild populations (Neser & Annecke 1973, Adair 2002a).

Gall-forming cecidomyiids have potential as seed-reducing biological control agents for *A. mearnsii* providing there is no negative impact on vegetative growth of the host or the impact does not exceed that normally associated with fruit production. This is in contrast with Harris' (1981) host stress objectives and the process of 'forced commitment' (Dennill 1988)

that has successfully suppressed other Australian *Acacia* in South Africa. Cecidomyiids that produce galls with low biomass and calorific status, induce a short-term resource sink, or feed asynchronously with the main shoot growth period of the host are likely to cause the least disruption to vegetative growth of the host plant.

Most acacias produce an abundance of flowers each season, but only a small proportion develop into fruits (New 1984, Coe & Coe 1987, Tybirk 1993, Grant *et al.* 1994). Therefore, for cecidomyiids that develop on buds and flowers to be useful as biological control agents they must have close synchronisation of adult emergence with host-organ production and a capacity to affect a very high proportion of those organs.

This chapter evaluates the capacity of gall-forming cecidomyiids from the reproductive structures of *A. mearnsii* and *A. cyclops* to form resource sinks and examines their possible contribution to a conflict of interest with commercial forestry in South Africa.

Materials and Methods

Comparison of flower production of A. mearnsii *in Australia and South Africa*

Flower production of *A. mearnsii* in Western Australia and South Africa was compared to determine if Western Australian sites that are heavily galled by the Tiny Floret Galler could be used to extrapolate likely gall biomass loads in South Africa, if this insect were released as a biological control agent. Gall loads were assumed to be influenced by flower production, with high flower production contributing to high gall loads when insect abundance was also high. If flower production of *A. mearnsii* were not similar in Western Australian to climatically matched areas of South Africa, then gall load predictions would not be valid. The Tiny Floret Galler was selected for detailed study as a potential biological control candidate because it forms small galls and has the capacity to develop dense populations.

Using similar sized *A. mearnsii* trees from climatically matched areas, five randomly selected branches with stem diameters of 4-5 mm were collected from 25 trees at each of three sites in Western Australia (Albany 34^0.58'S, 117^0.53'E, Denmark 34^0.59'S, 117^0.13'E, William Bay 34^0.59'S, 117^0.13'E). Using Climex Version 1.1, five sites in South Africa with a climate match index greater than 0.75 to the Australian sample sites (Heldervue 34^0.02'S, 18^0.49'E; Villiersdorp 34^0.01'S, 19^0.15'E; Berg River 33^0.52'S, 19^0.02'E; Groot Drakenstein 33^0.55'S, 18^0.55'E; Stellenbosch 33^0.58'S, 18^0.46'E) were selected and 31 *A. mearnsii* trees were sampled using the methods described above. The number of inflorescences on each branch was recorded together with the number of flower-heads, either in bud or flower. All branch samples were collected between 20 September and 3 October 2002 when trees were mostly in late bud.

Site data within each country were combined and where variances were homogeneous, a two-sample student's t-test ($\propto$ = 0.05) was used to detect differences between means. Where variances were heterogeneous, an unequal variance t-test was used.

Calorimetry

Cecidomyiid galls that develop on the inflorescences of Australian acacias prevent fruit development from the bud or flower that is utilised by the insect. The biomass and energy content of galls relative to acacia fruits provides a measure of the potential for 'resource-loading' on the host plant, if galls were to replace fruit formation in a biological control program. Galling insects that induce galls with a higher energy or biomass content than fruits are unlikely to make suitable biological control agents for acacias of commercial importance in South Africa. This experiment compared the energy content of Australian cecidomyiid galls with the energy content of fruits of their respective host *Acacia*.

The energy content (Kj/g dry mass) of mature galls containing fully developed larvae from six cecidomyiid species was determined using an Auto Bomb Calorimeter. The cecidomyiid galls sampled were the Tiny Floret Galler, Common Fluted Galler, Elongate Fluted Galler, Glabrous Bud Galler, Pubescent Bud Galler, Egg Galler from *A. mearnsii* and *D. dielsi* from *A. cyclops*. The energy contents of mature fruit of *A. mearnsii* and *A. cyclops* were also determined. Galls and fruits were oven dried at 80^0C for five days then ground to a powder using an electric food processor. Samples of around 0.5 g of ground gall or fruit material was used for each calorific determination. There were at least three replicates for each site collection, except for the Egg Galler, where because of the small gall size and difficulty in finding sites, only one replicate with three subsamples was used for the calorific determination. *Acacia mearnsii* fruit were collected in South Africa from Stellenbosch (33^0.56'S, 18^0.50'E), Villiersdorp (33^0.58'S, 19^0.08'E), Paulpietersberg (27^0.19'S, 30^0.53'E) Piet Retief (26^0.54'S, 30^0.42'E), Vryheid (27^0.50'S, 30^0.40'E), Grabouw (34^0.11'S, 19^0.05'E) and Heldervue (34^0.02'S, 18^0.49'E). Gall samples of the Tiny Floret Galler were collected in Australia at Denmark (34^0.59'S, 117^0.13'E), Jarrahdale (32^0.20'S, 116^0.03'E), Albany (34^0.58'S, 117^0.53'E), and Brimbank (37^0.43'S, 144^0.49'E). Mature Common Fluted Galler galls were collected at Lake Burrumbeet (37^0.29'S, 143^0.40'E) and Stirling (35^0.00'S, 138^0.42'E); Elongate Fluted Galler galls were collected at Lake Burrumbeet and Brimbank; the Egg Galler was collected at Rowville (37^0.55'S, 145^0.14'E); Glabrous Bud Galler galls were collected at Wartook (37^0.03'S, 142^0.21'E), Pubescent Bud Galler galls were collected at Stawell (36^0.59'S, 142^0.39'E) from *A. mearnsii,* and *D. dielsi* galls were collected from Moonta (34^0.03'S, 137^0.33'E) as well as from two South African sites: Strand (34^0.06'S,

18^0.48'E) and Somerset West (34^0.03'S, 18^0.51'E). In South Africa, *A. cyclops* fruit was collected at Strand and Langebaanweg (32^0.58'S, 18^0.05'E).

A two-sample student's t-test for samples with unequal variance was used to test differences in calorific values of galls for each of the seven cecidomyiids with fruits of each insect's host plant.

Phenology of the Tiny Floret Gall and A. mearnsii *fruit*

The temporal development pattern of gall biomass in relation to biomass production of the host's organs that are utilised by the galling agent can influence growth patterns of the host species (Dennil 1987b). Organisms that induce high gall biomass production earlier in the growth season relative to normal development of the host are likely to be more disruptive to growth patterns than those with later biomass development (Dennill 1987b, Dennill 1988). In this experiment, the biomass increment pattern of the Tiny Floret Galler is compared with fruit biomass development of *A. mearnsii*. Time and logistic constraints prevented additional cecidomyiids from being included in this study.

The increment in biomass of Tiny Floret Galler galls and fruits of *A. mearnsii* was determined at Jarrahdale, Western Australia between November 2001 and October 2002. Each month after flowering, haphazardly selected infructescences with Tiny Floret Galler galls or fruits were collected from five trees. On each infructescence, the number of flowers producing galls or fruits was recorded. Galls and fruits were dried separately at 80^0C for five days before weighing. Flowers producing a combination of galls and fruits were discarded from the sampling process.

Gall and fruit loads

In this study, three separate, but related, experiments were undertaken to determine the potential impact of gall-forming cecidomyiids on *A. mearnsii* and *A. cyclops*. The emphasis was on the Tiny Floret Galler as it was considered a strong candidate for biological control of *A. mearnsii* in South Africa. The first experiment compares the dry biomass of galls of Australian cecidomyiids from *A. mearnsii* and *A. cyclops* with? biomass of fruits produced on flower-heads. Cecidomyiids with low gall biomass in relation to fruit biomass would make more suitable biological control agents for *A. mearnsii* in South Africa than those with high gall biomass. In the second experiment, gall biomass loads of the Tiny Floret Galler were measured on trees in Western Australia where outbreak population densities of this midge were discovered in 1998. Gall loads from Western Australian trees where collected as an indicator of possible gall loads that may occur in South Africa, if this insect was as a

biological control agent. In the third experiment, fruit biomass loads of *A. mearnsii* trees in South Africa were measured over three seasons. Fruit biomass loads of *A. mearnsii* in South Africa are compared to gall loads induced by the Tiny Floret Galler in Australia to assess the potential 'resource-loading' impact of the Tiny Floret Galler.

Experiment 1

The relationship between floret gall number per galled flower-head and dry biomass, and the number of chambers per floret gall and dry biomass was determined for galls of five cecidomyiids from *A. mearnsii* (Tiny Floret Galler, Common Fluted Galler, Elongate Fluted Galler, Glabrous Bud Galler, Pubescent Bud Galler) and *D. dielsi* from *A. cyclops* from mature galls at one or more locations in Australia. Biomasses were determined after drying gall samples at 80^0C in paper envelopes for five days. In the case of the Tiny Floret Galler, the number of chambers in each floret gall was determined by transversely cutting the proximal end of each gall. In all other floret galls, the number chambers were determined by counting ostioles.

The relationship between the total number of ovules of *A. mearnsii* per flower-head and dry biomass was also determined. Pods were collected from five sites in South Africa: Stellenbosch, Grabouw, Paulpietersberg, Piet Retief and Vryheid. Total ovule number per flower-head rather than pod number per flower-head was considered a more sensitive method of exploring the relationship between biomass and pod production.

Regression models were fitted to fruit and gall biomass against the number of each and 95% confidence intervals calculated for the regression coefficient for each linear model.

Experiment 2

In Australia, the number and dry biomass of galls produced per branch was determined for the Tiny Floret Galler on *A. mearnsii* in June-July of 2000, 2001 and 2002. At six sites in Western Australia (Jarrahdale, Albany, Denmark, Mt Barker, Williams Bay and Augusta) where the Tiny Floret Galler was abundant, outer canopy branches of *A. mearnsii* with a basal stem diameter of 4-6 mm diameter were haphazardly selected within 1-4.5 m from ground level. Branches higher than 2 m were cut with an extendable tree-pruner. Ten branches were haphazardly taken from each tree. All or most *A. mearnsii* trees at each site were sampled. Tree numbers at each site varied from one (Augusta) to 59 (Denmark). The sample size of trees in Australia increased between 2000 and 2003 as new sites of *A. mearnsii* were located in south-west Western Australia. The number of infructescences on each branch and the number of Tiny Floret Galler galls and fruits per flower-head on each infructescence were recorded. Tiny Floret Galler galls were removed at their junction with the flower-head

peduncle and dried at 80^0C for five days in paper envelopes before weighing. The stem diameter and length of each branch was measured.

Experiment 3

In South Africa, the dry biomass of mature *A. mearnsii* fruits was determined from naturalized trees in the Western Cape, and naturalized and plantation trees in KwaZulu-Natal and Mpumalanga. In October-November, 10 outer canopy branches were haphazardly sampled from each of six trees per site following the techniques described above. There were 39 sites sampled in 2000, 46 in 2001 and 32 in 2002. The number of infructescences and the number of fruits per flower-head from each infructescence were counted as above. Fruits were dried at 80^0C for 5 days before weighing.

The total dry biomass of fruits and galls from trees in Australia, and fruit biomass from trees in South Africa for each sample season were determined with 95% confidence intervals. An ANOVA was used to determine differences in dry biomass across seasons after gall biomass was adjusted for calorific content in relation to mature fruits. Calorific adjustment was made by multiplying mean gall biomass for each season by 0.917, the difference in calorific value between galls and fruits (see *Calorimetry*). Total biomass values were square root transformed to improve normality. A Tukey's HSD test was used for unplanned pair-wise comparison of means.

A student's t-test was used to detect differences between fruit biomass produced on trees sampled in northern South Africa (KwaZulu-Natal & Mpumalanga), where *A. mearnsii* is grown commercially, with those in the Western Cape where *A. mearnsii* is a serious invader.

Correlations between fruit loads and vegetative growth of A. mearnsii

Gall-forming biological control agents have the potential to affect stem growth rates of their hosts by altering the allocation of host resources (see introduction for references). However, the impact on gall-forming cecidomyiids on stem growth of *A. mearnsii* could not be directly assessed in Australia due to logistic constraints. Fruit formation involves host resource commitment and as *Dasineura* galls and fruits both originate from flowers, and have similar calorific values (determined in this study) the impact of fruit loads on stem growth of *A. mearnsii* was measured in South Africa and used to indirectly assess the effect of galling by the Tiny Floret Galler. The Tiny Floret Galler was the focus of this study as it was recognised as a strong candidate for biological control of *A. mearnsii* in South Africa.

At Stellenbosch (33^0.56'S, 18^0.50'E) and Theewaterskloof Dam (33^0.58'S, 19^0.08'E), branches of *A. mearnsii* with basal stem diameters between 2-8 mm were haphazardly

selected from the outer canopy of sexually mature trees. In 2000, 32 branches from nine trees were sampled at Stellenbosch and a single branch from each of 44 trees was sampled at Theewaterskloof, while in 2001 there were 110 branches from 12 trees at Stellenbosch and 180 branches from 53 trees at Theewaterskloof. At the commencement of sampling in January-February, stem diameter, branch length, number of leaf nodes and fruits were measured. Branches were tagged with aluminium labels tied with plastic-coated wire. Fruit numbers were recounted in May-June and all branch measurements were repeated in November, shortly before fruit dehiscence. Branches were then harvested and the number and dry biomass of fruit was determined.

Differences in total stem length and leaf number over the monitoring period were calculated and partial correlations determined between fruit number and dry fruit biomass per branch after adjustment for initial stem diameter. Fruit biomass and fruit number were transformed with a cube root function to improve normality.

Correlation between gall numbers of *Tiny Floret Galler* and *fruit production*

The relationship between gall numbers of the Tiny Floret Galler and fruit production of *A. mearnsii* was determined at three sites in Western Australia (Jarrahdale, Dwellingup, Denmark) where Tiny Floret Galler population densities were high. In September 2001, fully developed inflorescences in late bud were tagged using small plastic strips tied to the branch immediately below each inflorescence. The number of flower-heads (buds, flowers or peduncle scars) on each inflorescence was recorded. Inflorescences with a large proportion of flower-heads with open florets or shed flower-heads at the start of the experiment were rejected as accurate flower-head counts were not possible. A total of 1496 inflorescences were tagged from 73 trees, with up to 25 inflorescences tagged per tree.

During March 2002, tagged infructescences were collected and the number of Tiny Floret Galls and *A. mearnsii* fruits were recorded. The percentage of flower-heads galled was arcsine transformed to improve normality. A square root transformation was used for the number of fruits per infructescence. A correlation coefficient for percentage of flower-heads galled and the number of fruits produced was then determined to assess whether high gall densities are associated with lower fruit numbers. Data from seasonally high fruiting sites (Dwellingup & Denmark) and a low fruiting site (Jarrahdale) were examined separately to explore the efficacy of the Tiny Floret Galler under different levels of fruit production.

In a separate experiment, the impact of the Tiny Floret Galler on fruit development when fruits and galls occur simultaneously on the same flower-head was determined by haphazardly sampling branches of 5-6 mm diameter from trees at Denmark, Western

Australia in September 2002. All infructescences were removed and the number of floret galls and fruits was recorded per flower-head. A flower-head consists of an aggregation of florets (flowers) on a single receptacle. An inflorescence consists of flowers-heads arranged in a raceme or panicle and is subtended by a leaf. Each floret is capable of producing a fruit or a gall. On each flower-head, the condition of fruit was recorded as either normal (green) or aborted (shrivelled, dry, brown) and the number of ovules (seeds) in green fruits was recorded. Ovule number in aborted fruits could not be readily determined. It was assumed green fruits would develop normally and reach maturity in October-November. Ovule, floret gall and fruit number were $\log_{10}$ transformed to improve normality. A partial correlation coefficient was determined between floret gall number per flower-head and the number of aborted fruits after adjustment for the number of green ovules.

Population growth of the Tiny Floret Galler

Gall-forming Cecidomyiidae under consideration for biological control agents of *A. mearnsii* in South Africa are expected to affect seed production in commercial silviculture plantations, if approved for release. The rate of increase in gall densities is one of a number factors that could influence seed fall in plantations and the crop regeneration procedures used after trees are harvested. The rate of gall density increase for the Tiny Floret Galler was determined in Western Australia where the insect was previously absent. The experiment was undertaken in Australia, as this insect has not yet been approved for release as a biological control agent in South Africa. Uninfected trees were used as changes in gall densities over time were more likely to be apparent and measurable than infected trees where the insect may have formed stable population densities.

The rate of population build-up of the Tiny Floret Galler was empirically determined using sites of *A. mearnsii* in Western Australia where the Tiny Floret Galler either was absent or newly arrived. These sites were selected based on observations between 1998-2001. Soil samples containing around 1,100 cocoons with unparasitized larvae of the Tiny Floret Galler were collected at Jarrahdale (32^0.18'S, 116^0.01'E) in September 2001 were spread in the litter layer beneath the canopy of mature *A. mearnsii* trees at Collie (33^0.21'S, 116^0.08'E), Mundijong (32^0.14'S, 116^0.00'E), Keysbrook (32^0.28'S, 115^0.58'E) and Rockingham (32^0.16'S, 115^0.50'E). At Collie and Keysbrook there was an isolated *A. mearnsii* tree; at Mundijong and Rockingham, there were nine and four trees, respectively. In June-July 2001, 2002 and March 2003, trees were sampled for the Tiny Floret Galler by haphazardly selecting branches from the outer canopy. The number of infructescences, flower-head galls and fruits per infructescence were recorded. Exponential growth functions were fitted to the mean

number of galls per site over the sampling period using TableCurve® Version 5.01 and gall levels per branch predicted for eight and 12 years population growth of the Tiny Floret Galler.

Data analysis

All data were examined for normality and homogeneity of variances and appropriately transformed, if required, before undertaking analyses. All statistical tests were performed using Systat Version 10, unless otherwise stated. Partial correlations were determined by applying the formulae of Snedecor & Cochran (1967).

Results

Comparison of flower production of A. mearnsii *in Australia and South Africa*

In climatically matched sites (Climex Version 1.1, MI = 0.75) between Australia and South Africa, there were no significant differences ($P>0.05$) between the number of inflorescences produced on branches (4-6 mm diameter) of *A. mearnsii* and the number of buds produced on inflorescences (table 6.1).

Calorimetry

The calorific values of galls from two *Asphondylia* spp. (Glabrous Bud Galler, Pubescent Bud Galler) and two *Dasineura* spp. (Common Fluted Galler, Elongate Fluted Galler) from *A. mearnsii* were not significantly different to that of mature fruits (table 6.2). Galls of the Tiny Floret Galler had a mean calorific value of 18.53 Kj/g and although the difference between the mean calorific value of *A. mearnsii* fruits (20.19 Kj/g) and galls of the Tiny Floret Galler was small (0.917), this difference was statistically significant ($P = 0.047$) The calorific value of galls produced by the Egg Galler were also significantly less than mature fruits of *A. mearnsii* ($P = 0.013$).

The calorific value of *D. dielsi* galls was slightly, but significantly lower ($P = 0.001$) than the calorific value obtained from the fruits of its host, *A. cyclops* (table 6.2).

Phenology of Tiny Floret Galler galls and A. mearnsii *fruit*

The rate of biomass accumulation of Tiny Floret Galler galls and fruit of *A. mearnsii* showed three main development phases (figure 6.1). In the first phase (November-mid March), galls and *A. mearnsii* fruit biomass accrual was slight with fruit and gall biomass similar to each other. The second phase consisted of accelerated rates of biomass accrual by both galls and fruits, with gall biomass exceeding fruits until gall maturity and emergence of

larvae in June-July. In the third phase, there was a steep rate of increase in fruit biomass from mid July to September when fruits reached maximum development and had a mean biomass 3.5 greater than the biomass of galls.

Gall and fruit loads

Experiment 1

There was a positive linear correlation between the total number of *A. mearnsii* ovules per flower-head and dry biomass (r^2 = 0.87). There was also a positive linear increase between the number of floret galls per flower-head of the Elongate Floret Galler, Tiny Floret Galler, Glabrous Bud Galler, Pubescent Bud Galler, Egg Galler and gall dry biomass (figures 6.2a-c). A non-linear exponential curve best fitted the relationship between the number of floret galls of the Common Floret Galler and gall dry biomass (r^2 = 0.71) (figure 6.2b).

The slopes of the linear correlations between the number of galls of the Elongate Floret Galler and *A. mearnsii* fruits with their dry biomass were considerably greater than the slopes for the Tiny Floret Galler, Egg Galler, Glabrous Bud Galler and Pubescent Bud Galler, which showed only marginal differences (figure 6.2d). The dry biomass of the Tiny Floret Galler flower-head galls in eastern Australia (Brimbank, Victoria) ($y = 0.0062x+0.0035$, r^2 = 0.86, n = 59, y = dry biomass, x = number), where the cecidomyiid is indigenous, was slightly higher than those from a naturalized population at Jarrahdale, Western Australia ($y = 0.0054x+0.0008$, $r^2 = 0.91$, n = 99) (figure 6.3). The difference in slopes between the two Tiny Floret Galler populations was statistically significant ($t = 2.5$, $P<0.02$), but has no biological relevance as the differences were slight. Gall biomass from eastern and Western Australian sites were combined to produce a single figure (figure 6.2b). The dry biomass of *D. dielsi* galls per flower-head was significantly lower ($P<0.001$) than that of mature fruit of *A. cyclops* expressed as the number of ovules per flower-head. (figure 6.5). The dry biomass of the Tiny Floret Galler, Elongate Floret Galler, Common Floret Galler and *D. dielsi* galls increased with the number of chambers present (figure 6.3, 6.6). Dry biomass of Tiny Floret Galler galls from eastern and Western Australia increased with the number of chambers per floret gall with no clear differences between eastern and Western Australian populations (figure 6.4).

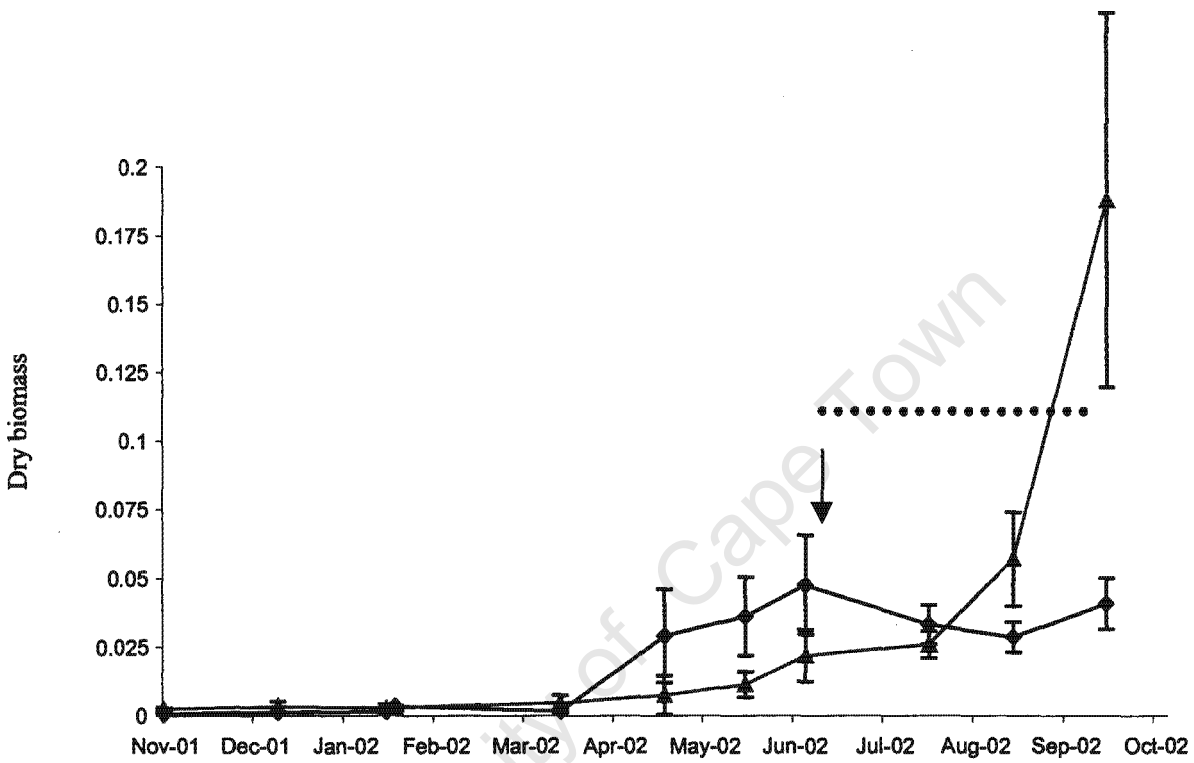


Figure 6.1. Mean (± SD) dry biomass (g) of (*Dasineura* sp.) Tiny Floret Galler galls (♦) and *A. mearnsii* pods (▲) between November 2001 and October 2002. The arrow shows commencement of Tiny Floret Galler emergence. After emergence of the Tiny Floret Galler, galls die but pod formation continues and requires host resource allocation. The dotted line is the period of 'commitment release' because of galling by the Tiny Floret Galler.

Experiments 2 & 3

When data from South African sites used to measure fruit biomass of *A. mearnsii* were separated into sites from the Western Cape, where major *A. mearnsii* infestations occur, and sites from KwaZulu-Natal and Mpumalanga, where most commercial wattle plantations are located, no differences in mean dry biomass of fruit between the two regions were detectable over three successive seasons ($P>0.05$) (table 6.3).

Branches of *A. mearnsii* sampled for Tiny Floret Galler gall loads in Australia and fruit loads in South Africa over three successive seasons had similar mean basal diameters (5.6-5.9 mm) and length (42-47 cm) (table 6.4) validating the comparison of gall and fruit biomass from samples collected between the two continents.

In Australia, the mean number of infructescences produced per branch per tree of *A. mearnsii* was approximately three times greater than *A. mearnsii* trees in South Africa over the 2000 and 2001 seasons (table 6.5), when fruit production levels in South Africa were lowest (figure 6.7). In 2002, mean infructescence numbers from *A. mearnsii* trees in Australia and South Africa were similar (table 6.5). The number of gall points per infructescence in Australia was significantly higher than the number of fruits points per infructescence in South Africa for the 2001 and 2002 seasons, but not for 2000. The significance values for each of the seasons were 2000 ($t = -1.158$, $P = 0.247$), 2001 ($t = -20.328$, $P = 0.000$) and 2002 ($t = 7.540$, $P = 0.000$).

Tiny Floret Galler galls mature 3-4 months earlier than *A. mearnsii* fruits (figure 6.1) and are gradually shed from the tree after emergence of larvae in June-July. Fruits mature in October–December. The dry biomass of mature fruit on branches destructively sampled for gall loads in Australia in June-July was calculated by extrapolation using fruit number on Australian branches and the equation $y = 0.29x+0.32$, $r^2 = 0.87$, where y = dry biomass and x = fruit number (figure 6.2a) calculated for fruit biomass and fruit number. Also taken into account for calculation of fruit biomass on Australian branches is natural fruit shedding rates between June-October at 29.3% (see *Impact of fruit loads on vegetative growth of* A. mearnsii).

Over three successive seasons (2000-2003) there were significant differences between mean biomass of Tiny Floret Galler galls in Australia and fruits of *A. mearnsii* in South Africa ($F_{5,779} = 3\ 2.07$, $P<0.001$). The total gall and fruit dry biomass produced on *A. mearnsii* trees in Western Australia was similar to (seasons 2000 & 2001) or significantly less (season 2002) ($P<0.05$) than the biomass of fruits produced on *A. mearnsii* in South Africa, after adjustment for the relative calorific value of galls and fruit shedding (figure 6.7).

Correlation between fruit loads and vegetative growth of A. mearnsii

There were statistically significant negative partial correlations between fruit production of *A. mearnsii* at Stellenbosch and Theewaterskloof and changes in stem length and leaf number, after adjustment for branch diameter over the growing seasons of 2000 and 2001 (table 6.6). However, data were highly variable with only 9-21% of the variation explained.

Between May-June and November in 2000 and 2001, between 20.8% and 33.6% of fruits were shed from infructescences of *A. mearnsii* at Stellenbosch and Theewaterskloof Dam. The mean percentage (± SD) fruit shed across both sites and years was 29.3 ± 5.7%.

Correlation between gall numbers of Tiny Floret Galler and *fruit production*

In Western Australia, there was a significant negative correlation (r^2 = -0.46, P<0.001) between flower-heads galled by the Tiny Floret Galler and the number of fruits produced *A. mearnsii* (figure 6.8). In 2002, this correlation was apparent at Dwellingup and Denmark, but not at Jarrahdale (figure 6.9).

On *A. mearnsii* flower-heads developing 1-22 floret galls, there was no significant partial correlation between floret gall number and the number of aborted fruits after adjustment for the number of green ovules present (r^2 = -0.056, n = 283). However, a simple correlation showed the total number of green ovules per flower-head was negatively correlated with floret gall number (r^2 = -0.255, P<0.001).

Population growth of Tiny Floret Galler

At four sites in Western Australia where *A. mearnsii* occurred in small, isolated populations or as single trees and the Tiny Floret Galler was either absent or newly arrived (Adair pers. obser.), the mean number of galls increased progressively over three seasons after cocoons were placed out at the sites (figure 6.10). Population growth was highest at Mundijong and Keysbrook where the incidence of galls increased by a factor of 15 and 3.8 after two seasons, respectively. Slower growth occurred at Collie and Rockingham. Flowering levels, as indicated by the number of infructescences per branch, were similar over the three monitoring seasons for all sites, except at Mundijong where there was an increase in the number of infructescences in 2003 (table 6.7).

Table 6.1. Mean number (± standard error) of flower-head buds per inflorescence, inflorescences per branch and sample branch diameter of *A. mearnsii* from Australia and South Africa. *n* = number of trees. Values in each column followed by the same letter are not statistically different ($p<0.05$).

	n	Sample branch diameter (mm)	Number of inflorescences (branch^{-1})	Number of flower-head buds (inflorescence^{-1})
South Africa	32	5.33 ± 0.03 a	8.13 ± 0.49 a	31.67 ± 2.4 a
Australia	25	4.64 ± 0.02 a	9.29 ± 0.43 a	33.11 ± 2.7 a

Table 6.2 Mean (± SE) calorific value (Kj/g) for galls of six cecidomyiid species from *A. mearnsii*, and *D. dielsi* from *A. cyclops*, and mature pods from each host plant. TFG = Tiny Floret Galler, CFG = Common Fluted Galler, EFG = Elongate Floret Galler, EG = Egg Galler, GBG = Glabrous Bud Galler, PBG = Pubescent Bud Galler. *n* = number of sites sampled.

Host *Acacia*	Source	*n*	Cecidomyiid code	Calorific value (KJ/g)	Students *t*-test[a]
A. mearnsii	Mature pods	8	-	20.19 ± 0.61	
	Galls - *Dasineura* sp. 1	5	TFG	18.53 ± 0.28	*
	Galls - *Dasineura* sp. 2	2	CFG	19.42 ± 0.12	ns
	Galls - *Dasineura* sp 3	2	EFG	19.70 ± 0.19	ns
	Galls - *Dasineura* sp. 4	1	EG	18.15 ± 0.04	*
	Galls - *Asphondylia* sp. 1	1	GBG	20.50 ± 0.02	ns
	Galls - *Asphondylia* sp. 2	2	PBG	21.00 ± 0.27	ns
A. cyclops	Mature pods	2	-	19.53 ± 0.03	
	Galls - *Dasineura dielsi*	3	-	18.07 ± 0.08	*

a = Comparison of the calorific value of cecidomyiid galls with mature pods of the insects host. Gall values with an * are statistically different from host fruit calorific values ($P<0.05$). ns = not statistically different. Three sub-samples for each of EG and GBG were used for the students t-test.

Table 6.3 Mean (± SD) dry biomass of fruit per tree (g) of *A. mearnsii* from sites in Western Cape and KwaZulu-Natal/Mpumlanga in South Africa over three successive seasons. Means in columns with the same letter are not statistically different ($P>0.05$). *n* = number of trees sampled.

Province	2000	2001	2002
Western Cape	2.01 ± 2.69 a	3.30 ± 3.13 a	7.32 ± 13.30 a
	n=146	*n*=127	*n*=102
KwaZulu Natal & Mpumalanga	2.68 ± 3.27 a	2.86 ± 2.56 a	5.68 ± 6.85 a
	n=62	*n*=129	*n*=95

Table 6.4 Mean (± SD) diameter (mm) and length (cm) of branches per tree of *A. mearnsii* used to determine pod and gall weights and infructesence characteristics in Australia and South Africa. *n* = number of trees.

Year	Country	*n*	Stem diameter	Length
2000	Australia	17	5.60 ± 0.6	42.23 ± 7.7
	South Africa	207	5.87 ± 0.4	46.57 ± 5.3
2001	Australia	40	5.98 ± 0.4	41.67 ± 6.6
	South Africa	256	5.58 ± 1.3	44.83 ± 12.0
2002	Australia	81	5.76 ± 0.6	46.85 ± 9.7
	South Africa	195	5.65 ± 0.3	45.50 ± 5.7

Table 6.5. Infructesence characteristics of *A. mearnsii* in South Africa, and Australia in the presence of high populations of *Dasineura* sp. (Tiny Floret Galler). Data are means per tree ± 95% confidence intervals. *n* = number of trees.

		Australia		South Africa	
Year	Variable	*n*		*n*	
2000	Infructescences/branch	17	7.57 ± 0.97	330	2.48 ± 0.29
	Gall points/infructesence		4.58 ± 0.46		-
	Fruit points/infructesence		0.51 ± 0.21		3.69 ± 0.23
2001	Infructesences/branch	40	11.59 ± 1.65	256	4.20 ± 0.39
	Gall points/infructesence		7.15 ± 3.29		-
	Fruit points/infructesence		0.60 ± 0.26		3.30 ± 0.21
2002	Infructesences/branch	81	6.10 ± 1.76	195	6.37 ± 0.49
	Gall points/infructesence		4.81 ± 1.46		-
	Fruit points/infructesence		2.26 ± 0.27		3.61 ± 0.09

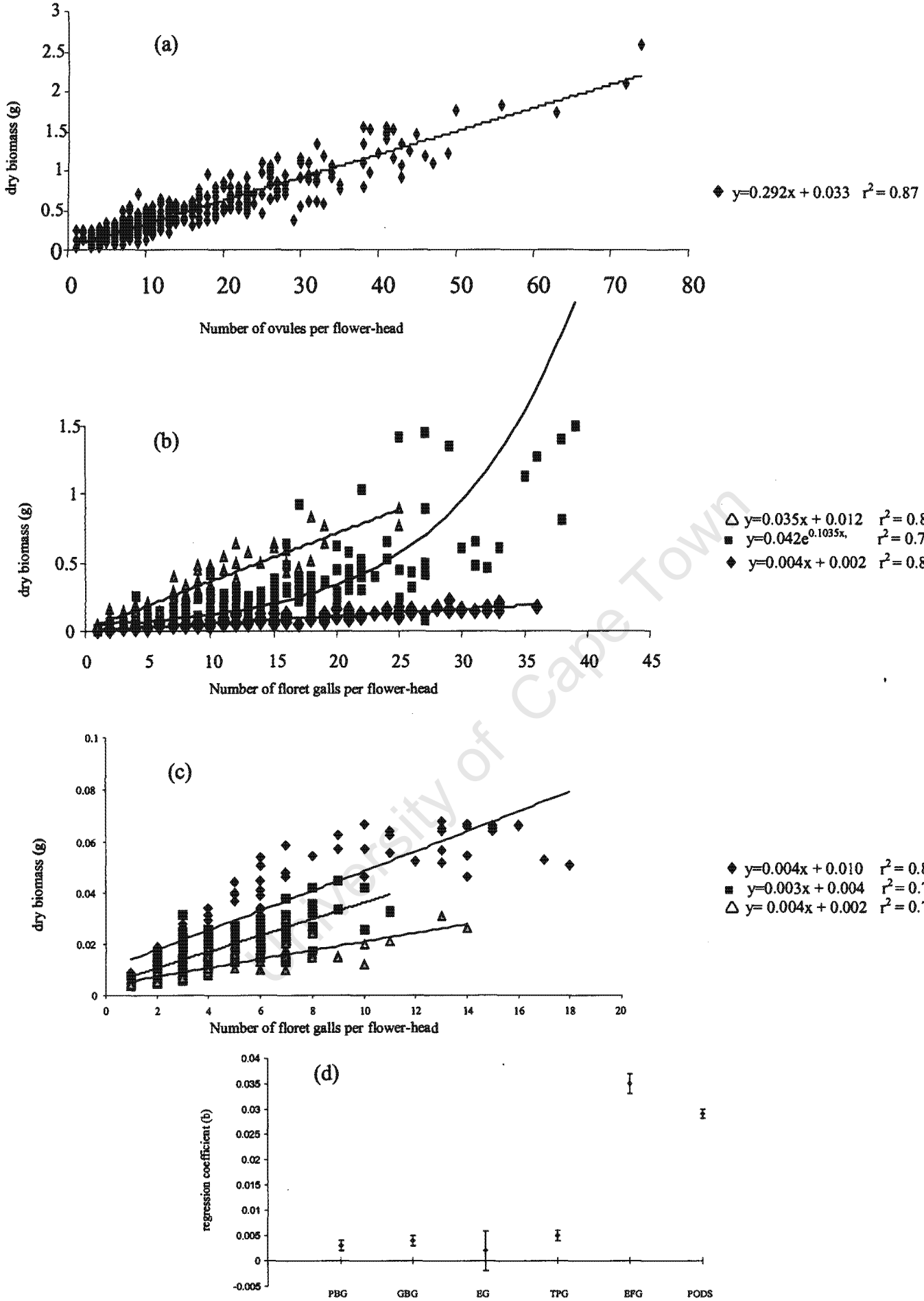


Figure 6.2 (a-d). Dry biomass (g) of pods and galls of six cecidomyiids from *A. mearnsii*. (a) *A. mearnsii* fruits (b) galls of Elongate Fluted Galler (EFG) (△) , Common Fluted Galler (CFG) (■) and Tiny Floret Galler (TFG) (♦) (c) galls of Glabrous Bud Galler (GBG) (♦), Pubescent Bud Galler (PBG) (■) and Egg Galler (△) (d) linear regression coefficients (slopes) and 95% confidence intervals (bars) for five cecidomyiids and fruits.

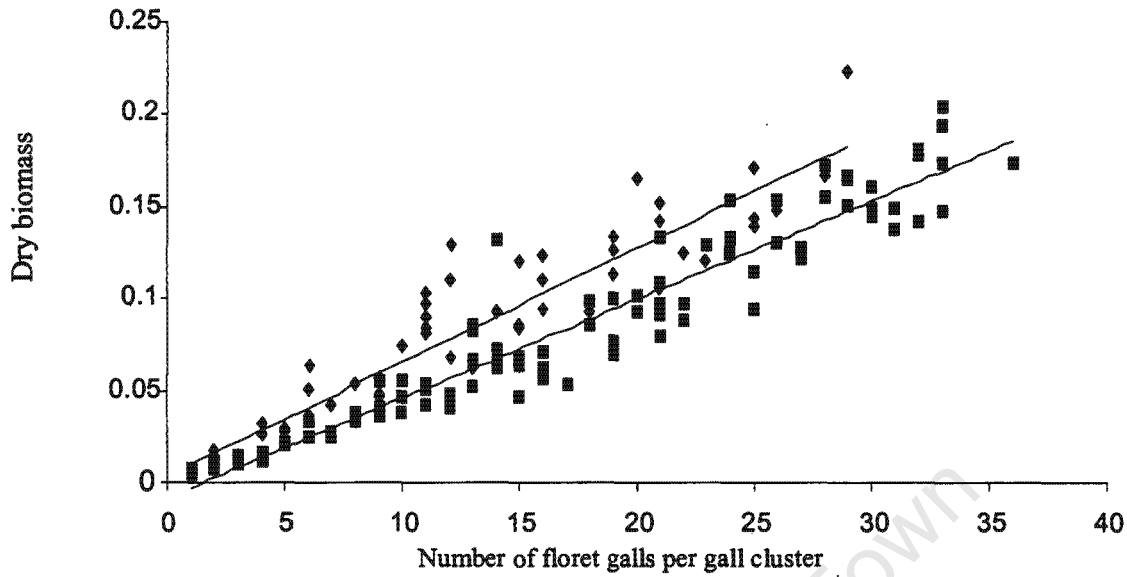


Figure 6.3 Mean dry biomass (g) of Tiny Floret Galler gall clusters from *A. mearnsii* with different numbers of floret galls at (♦) Brimbank (Eastern Australia) and (■) Jarrahdale (Western Australia). All galls were from *A. mearnsii*

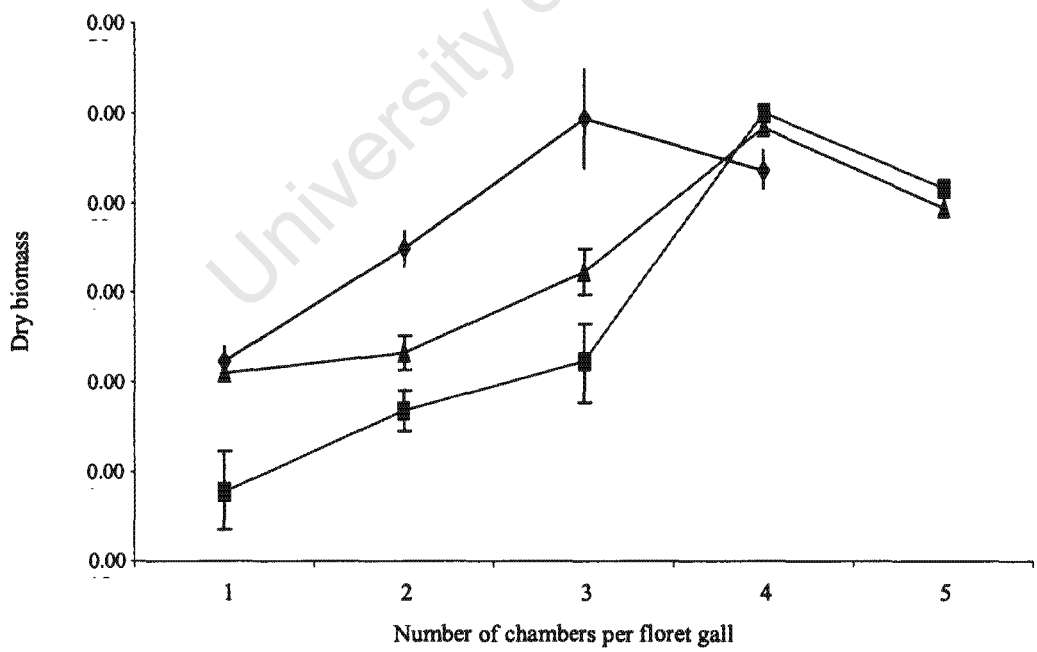


Figure 6.4 Mean dry biomass (g) of floret galls of the Tiny Floret Galler with different numbers of chambers from (♦) Brimbank (eastern Australia), (■) Casula (eastern Australia) and (▲) Jarrahdale (Western Australia). Several standard error bars have been deleted to improve clarity. All galls were from *A. mearnsii*

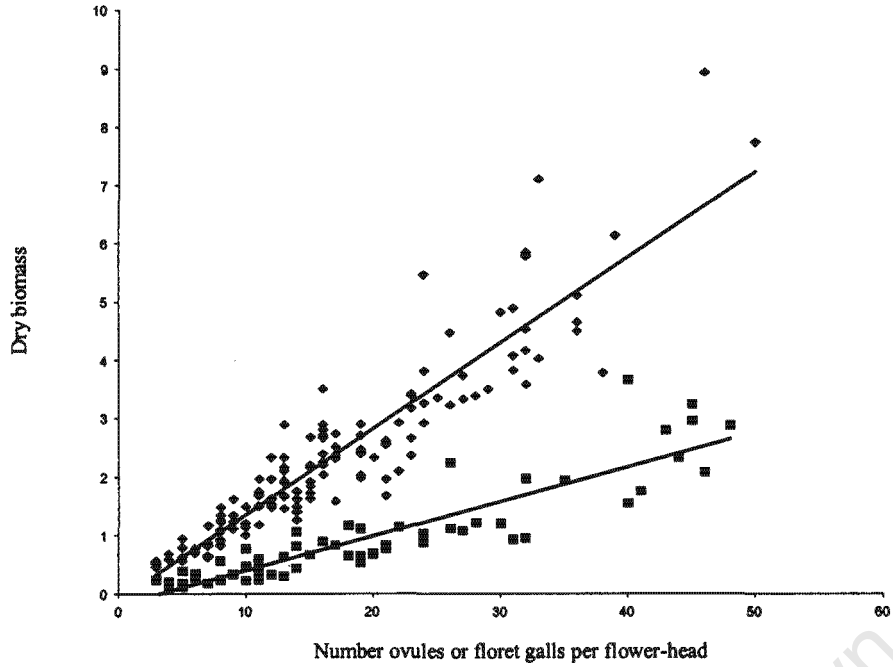


Figure 6.5 Dry biomass (g) of mature *A. cyclops* pods (♦) counted as the total number of ovules per flower-head (y = 0.1474x - 0.1237, r^2 = 0.861, y = dry biomass, x = number of ovules) and (■) floret galls of *Dasineura dielsi* per flower-head (y = 0.592x - 0.1882, r^2 = 0.8039, y = dry biomass, x = number of floret galls)

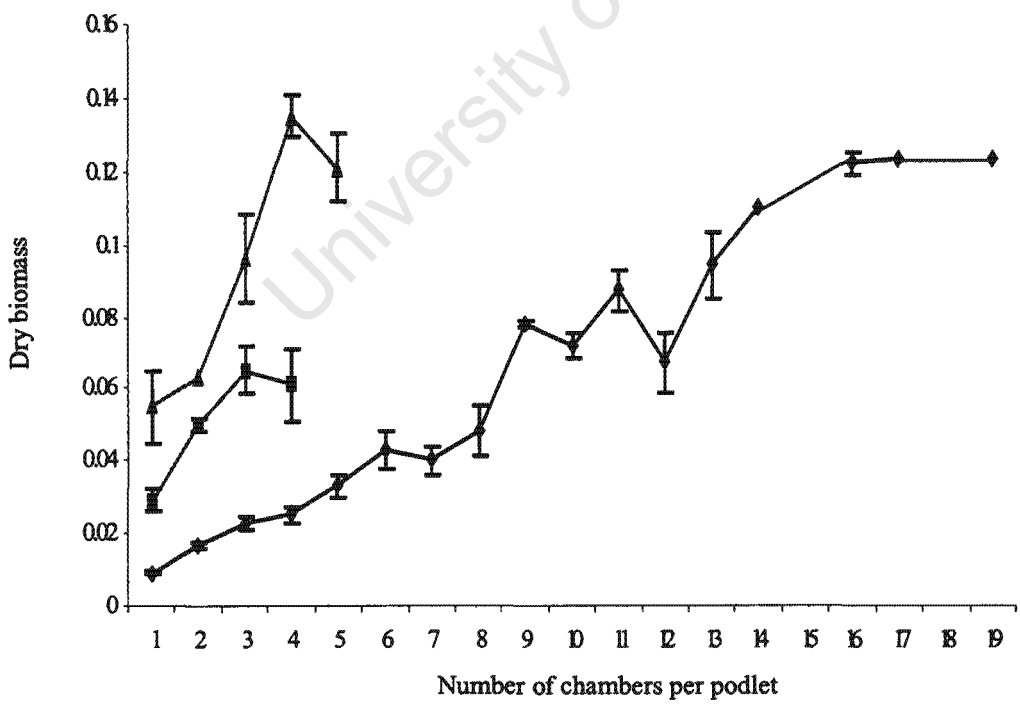


Figure 6.6 Mean dry biomass (g) of Elongate Fluted Galler (▲), Common Fluted Galler (♦) from *A. mearnsii* and *D. dielsi* (■) from *A. cyclops* with different numbers of chambers per floret gall. Bars are standard errors of the means.

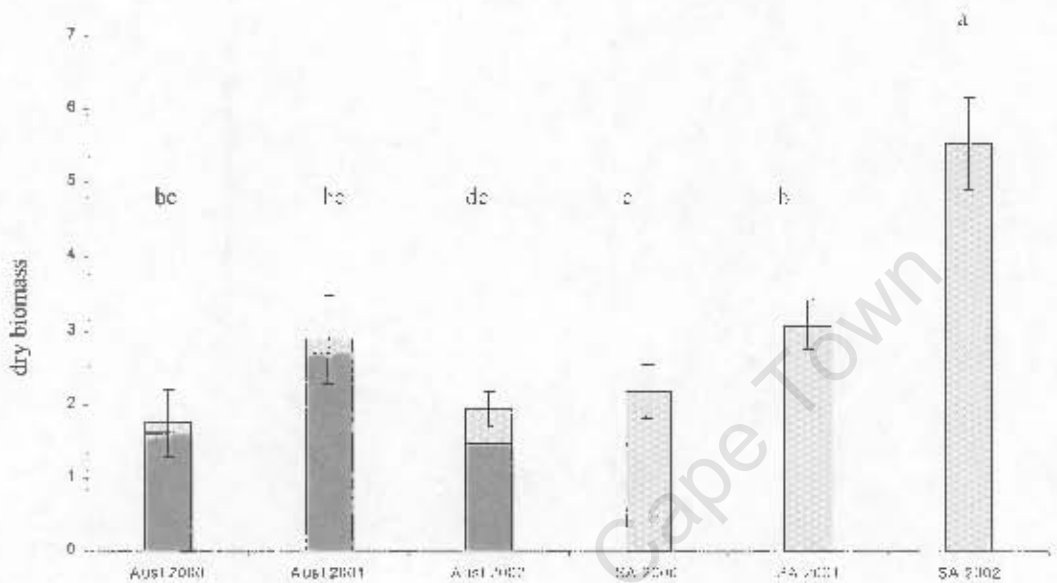


Figure 6.7 Dry biomass (g) of fruits (stiple) and galls of *Dasineura* sp. (Tiny Floret Galler) (cross-hatch) from branches of *A. mearnsii* in Australia (Aust) and South Africa (SA) from three successive fruiting seasons between 2000-2002. Bars are 95% confidence intervals of the mean weight. Gall weights are adjusted to compensate for the lower calorific value (0.917) of dry galls compared to dry mature pods. Pod weights on Australian trees were estimated by counting pod numbers in June/July, then allowing for a 29.3% pod shedding up to maturity. A predicted pod mass was determined from the regression equation $y = 0.29x + 0.32$, where x = pod number, y = pod weight. Columns with the same letter are not statistically different (Tukey's HSD test, $P=<0.05$).

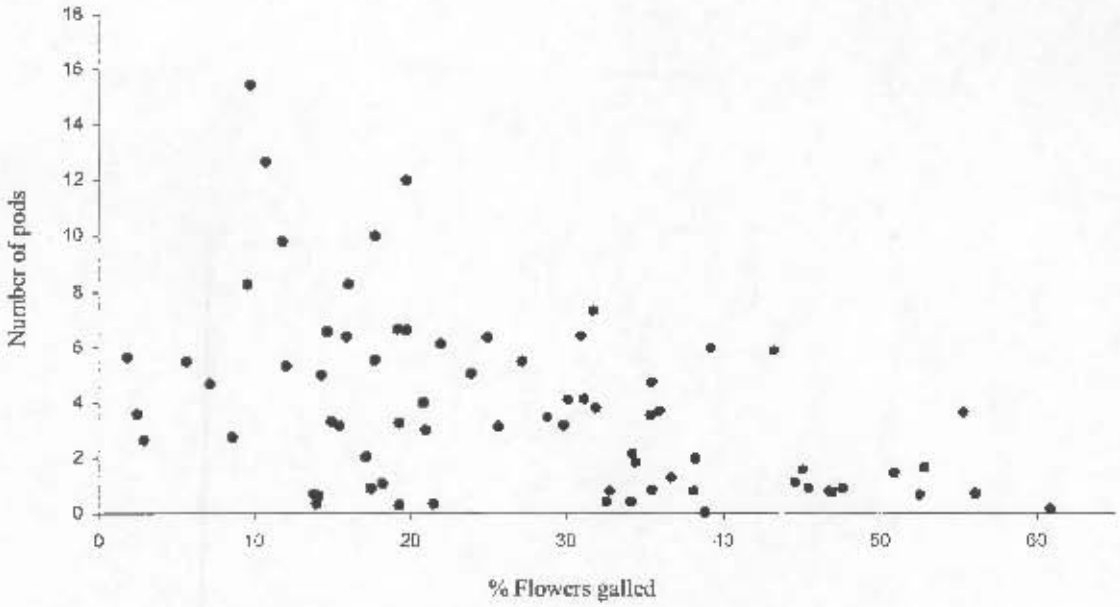


Figure 6.8 Number of *A. mearnsii* pods at different levels of galling by Tiny Floret Galler in Western Australia.

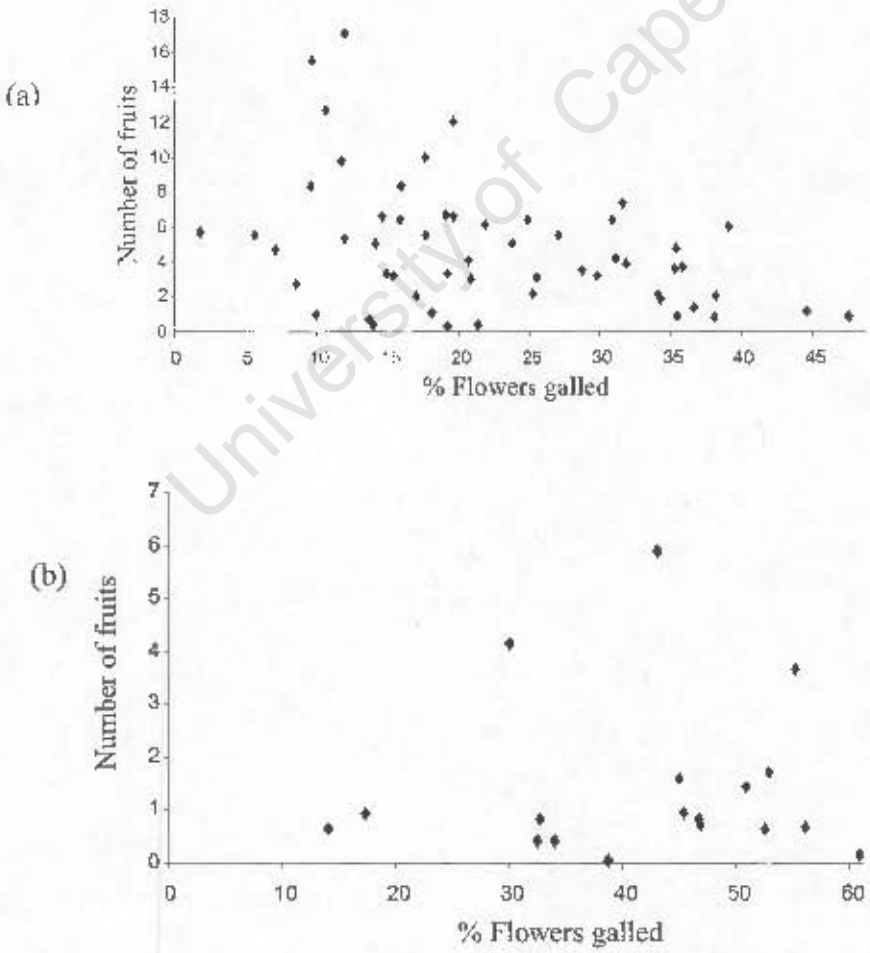


Figure 6.9 Number of *A. mearnsii* fruits at different levels of galling the Tiny Floret Galler at (a) Dwellingup and Denmark and (b) Jarrahdale

Discussion

Acacia mearnsii is an adventive in Western Australia, introduced for amenity planting's in horticulture and particularly for shelterbelts in rural areas. Limited naturalisation has confined populations to the higher rainfall areas of south-west Western Australia. *Acacia mearnsii* has low levels of herbivory in Western Australia, despite occurring in a region that has the highest diversity of *Acacia* species (Hnatiuk & Maslin 1988). Herbivores occurring on indigenous Western Australian *Acacia* species appear to have little or no impact on *A. mearnsii* trees and pre-dispersal seed crops. Similar levels of flower production by *A. mearnsii* in Western Australia and South Africa are probably the result of the low herbivory and provide a basis for comparison of impacts caused by the Tiny Floret Galler and fruit production of *A. mearnsii* in South Africa.

The galls of *Dasineura* occurring on flowers of *A. mearnsii* are closely-allied histologically to fruits and develop by proplasmic growth caused predominantly by hyperplasy of ovary tissue. In most *Dasineura* galls the loculus with undeveloped ovules are evident in the distal region of floret galls. Similarities in the calorific value of *Dasineura* galls and fruits of *A. mearnsii* indicate that galls are nourished with a normal nutrient supply from the host (Harris & Shorthouse 1996) and reflect close developmental and physiological affinities between the two organ types. Highly, lignified galls can form powerful resource sinks on host plants as assimilates are diverted from normal growth (Harris & Shorthouse 1996) and galls are supplied with phloem sap with a high carbon: nitrogen ratio (Pate 1980). The slightly lower calorific value of Tiny Floret Galler galls compared to fruit of *A. mearnsii* may be caused by relatively low levels of lignification and a high proportion of parenchymous tissue. Despite the presence of fungal mycelium in *Asphondylia* galls and a high insect to plant tissue ratio, the calorific content of Glabrous Bud Galler and Pubescent Bud Galler galls were very similar to fruits of *A. mearnsii*.

The biomass of cecidomyiid galls on reproductive organs of Australian *Acacia* is directly proportional to the number of ovaries galled in a flower, and for gregarious species, the number of larvae present in each floret gall. Regulation of the number of larvae occurring in *Dasineura* floret galls is apparent in the Tiny Floret Galler, Elongate Fluted Galler and Egg Galler from *A. mearnsii* and *D. dielsi* from *A. cyclops* where the number of larvae per floret gall rarely exceeds five (table 2.5). Even in outbreak densities such as Tiny Floret Galler populations in Western Australia, *Dasineura* larvae are not known to be cannibalistic, therefore oviposition is likely to govern the number of larvae developing in floret galls. Oviposition regulation may occur through sensory perception of the egg deposition history of florets, possibly through receptors on the ovipositor, which is used to probe florets during egg

Table 6.6. Partial correlation coefficients between fruit number (2000) or fruit dry biomass (2001) and the change in total stem length and leaf number over 11 months when adjusted for basal stem diameter of tagged branches. Measurements were taken from branches of *A. meanrsii* at Stellenbosch and Theewaterskloof Dam, South Africa. n = number of branches per site. Statistical significance of partial correlation coefficients are NS = not significant, * = $p<0.05$, *** = $p<0.001$.

Year	2000		2001	
Site	Stellenbosch	Theewaterskloof	Stellenbosch	Theewaterskloof
	n=32	n=44	n=110	n=180
Change in leaf number	-	-	-0.459 ***	-0.308 ***
Change in stem length	-0.368 *	-0.150 NS	-0.344 ***	-0.314 ***

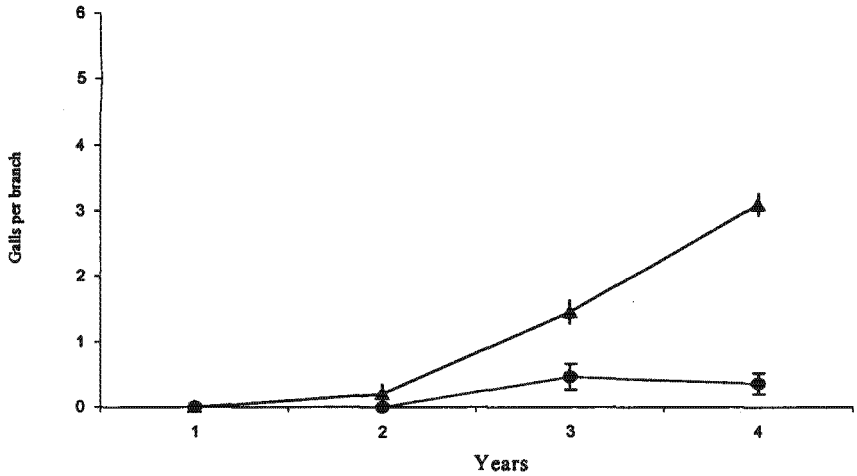
Table 6.7. Mean (± SD) number of infructescences (branch^{-1}) on *A. mearnsii* at four sites in Western Australia between 2001-2003. Sites were inoculated with Tiny Floret Galler in September 2000.

	Number of infructescences (branch^{-1})		
Site	2001	2002	2003
Collie	0	2.3 ± 3.6	2.9 ± 5.1
Keysbrook	6.9 ± 7.6	6.0 ± 4.7	6.6 ± 5.3
Mundijong	3.8 ± 6.5	4.6 ± 5.5	9.1 ± 7.8
Rockingham	1.2 ± 2.7	1.6 ± 3.0	0.9 ± 1.7

Table 6.8. Predicted mean number of Tiny Floret Galler flower-head galls (branch^{-1}) on *A. mearnsii* after 8 and 12 years using exponential equations applied to empirical population growth data (4 data points) from Mundijong and Keysbrook, Western Australia. Equations for each site represent the range of exponential growth curves with the best fit to empirical data. Eight years is the normal maximum number of flowering years of *A. mearnsii* in commercial plantations in South Africa.

Site	Function	R^2	Predicted number of galls (branch^{-1}) at 8 years	Predicted number of galls (branch^{-1}) at 12 years
Mundijong	$LnY=-4.87+3.00X^{0.5}$	0.97	36.2	249
	$LnY=6.27+-10.28/X^{0.5}$	0.99	13.8	27.1
Keysbrook	$Y^2=-0.61+0.226e^{x}$	0.235	25.6	189.2
	$Y=1.25+0.49X^3$	0.262	26.3 ?	85.9 ?

(a)



(b)

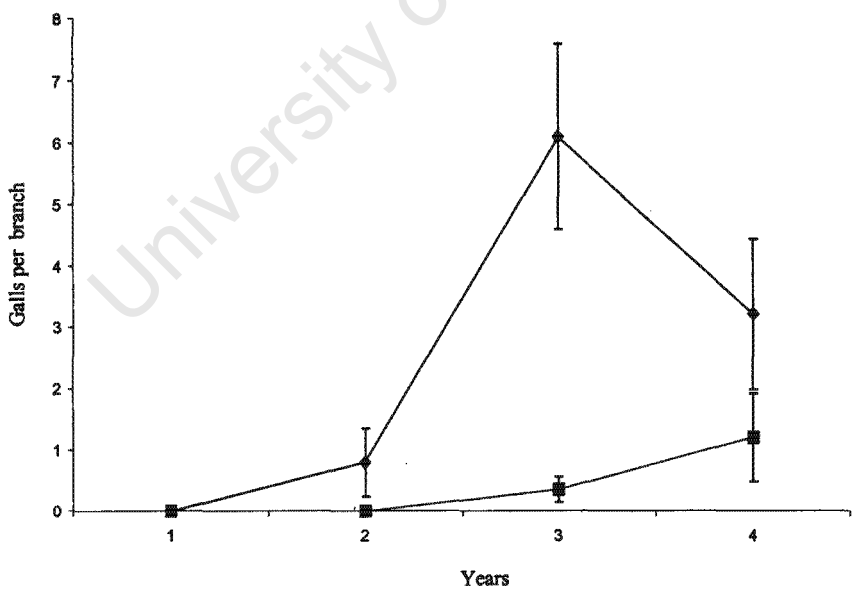


Figure 6.10. Mean number of Tiny Floret Galler flower-head galls branch^{-1} (± SE) on *A. mearnsii* after inoculation at four sites in Western Australia. (a) Mundijong (▲), Rockingham (●), (b) Collie (■), Keysbrook (♦).

laying. Regulation of oviposition density through deterrence in the presence of conspecifics or their pheromones is known in other phytophagous insects and may benefit the survival prospects of populations (Rothschild & Schoonhoven 1977, Roitberg & Prokopy 1987), or enhance adult fitness by reducing competition amongst larvae (Ehler & Kinsey 1990).

In cecidogenic insects, polythalamous gall development may reduce the impact of parasitoids by restricting attack to chambers near the gall surface (Manongi & Hoffmann 1995). A single *Dasineura* larva will induce gall-formation on the ovary of an *Acacia* floret, terminating any further development of the ovary. Unless there are strong adaptive advantages in the formation of polythalamous galls, cecidomyiids from *Acacia* galls with few larvae per floret gall (e.g. Tiny Floret Galler, Egg Galler, Glabrous Bud Galler, Pubescent Bud Galler, Groove Galler, *D. dielsi*) are likely to make more effective biological control agents for commercially utilized acacias than those with large numbers of larvae occurring in a single floret gall (e.g. Common Fluted Galler). This is particularly important where the overriding aim of the control program is to reduce seed production with minimal disruption to vegetative growth of the host, an important prerequisite for acceptable biological control of *A. mearnsii* seeds in South Africa. In this respect, the *Dasineura* spp., Tiny Floret Galler and Egg Galler, and the *Asphondylia* spp., Glabrous Bud Galler and Pubescent Bud Galler, are strong candidates for biological control of *A. mearnsii*. As large galls create a bigger energy sinks than small galls (Harris & Shorthouse 1996), biological control agents inducing galls with a large biomass are appropriate, and have been highly successful (Dennill 1988), on *Acacia* species where there are no conflicts of interest with commercial industries or domestic utilization.

The success of the *Trichilogaster* spp. as biological control agents on *A. longifolia* and A. *pycnantha* in South Africa is through the 'forced commitment' (Dennill 1988) of the host to gall formation. One of several key attributes of this process is early initiation of gall development compared to normal (ungalled) organs of the host (Hartnett & Abrahamson 1979). On Australian *Acacia*, *Dasineura* galls commence development in flowers in synchrony with *Acacia* fruits. In the case of the Tiny Floret Galler on *A. mearnsii*, gall maturity occurs several months earlier than fruits and galls have 3.5 times less dry biomass than mature fruits (figure 6.1). In contrast to gall formation by *Trichilogaster* spp. (Hymenoptera) that initiate gall formation on buds ahead of fruit formation on *A. longifolia* and *A. pycnantha*, Tiny Floret Galler gall development represents a 'commitment release' (figure 6.1) to host resource allocation, where gall mass accrual ceases earlier and has a lower final biomass than normal fruit development.

Acacia mearnsii, like many Australian acacias produces an abundance of mostly self-incompatible (Kenrick & Knox 1989, Moffet & Nixon 1974, Moncur *et al.* 1991), short-lived flowers. Most abscise soon after anthesis and only 0.6-2% develop into fruits (Moncur *et al.* 1991, New 1984). Oviposition by the Tiny Floret Galler and subsequent development of galls reduces rachis shedding in the absence of fruits with a single floret gall capable of causing sufficient physiological signal to prevent abscission. The higher infructescence retention rates on *A. mearnsii* in Western Australia compared to trees in South Africa are almost certainly due to this process.

Although galling of *A. mearnsii* flowers by cecidogenic insects has the potential to cause disruptive resource sinks, individual as well as total gall biomass is critical to the outcome of this process. Although high numbers of Tiny Floret Galler galls may form on *A. mearnsii* in Australia, gall biomass is low creating a total net resource commitment that is either similar to or less than *A. mearnsii* trees would naturally allocate to fruit production in a normal year (figure 6.7). However, fruit production by *A. mearnsii* varies seasonally in South Africa. In seasons of potentially high fruit production (e.g. 2002), Tiny Floret Galler gall formation may reduce total biomass allocated to fruit development improving the prospects for vegetative growth production of *A. mearnsii*. Conversely, as *A. mearnsii* trees that flower but produce few or no fruits, commitment to Tiny Floret Galler-induced biomass production is likely to reduce stem growth that would otherwise have occurred in the absence of this insect. In South Africa, this impact will be localised or restricted to individual trees as *A. mearnsii* normally flowers and produces large fruit loads in successive seasons with no evidence of 'masting', which is reported in other Australian *Acacia*, such as *A. melanoxylon* (New 1984). The negative correlation between fruit biomass and vegetative growth of *A. mearnsii*, or by extrapolation cecidomyiid gall mass and vegetative growth, suggests that attempts by the commercial wattle industry in South Africa to develop sterile cultivars of *A. mearnsii* (Dunlop & Beck 2002) could enhance the growth performance of *A. mearnsii,* if flower production were incapacitated or removed from the tree's genome.

Gall-forming cecidomyiids of *A. mearnsii* that induce gall biomass that far exceeds fruit production have the potential to cause resource sinks to the possible detriment of vegetative growth and tree health. The Elongate Fluted Galler rarely occurs in outbreak densities in Australia, but at Brimbank, Victoria, mean (±SD) dry biomass of galls per branch per tree was 13.4 ± 5.8 g, about 2-4 times the mean dry biomass of fruits from equivalent sized branches on trees in South Africa. A similar trend was evident with the Common Fluted Galler where at Lake Burrumbeet, Victoria, an outbreak population yielded a mean (± SD) dry biomass of 4.2 ± 3.3 g, twice the mean biomass production of the Tiny Floret Galler and

higher than fruit biomass production of *A. mearnsii* in South Africa in most seasons. Therefore, Australian cecidomyiids such as the Elongate Fluted Galler and the Common Fluted Galler are inappropriate as potential seed-reducing biological control for *A. mearnsii*, while this tree has commercial value in South Africa.

Female Tiny Floret Gallers oviposit on or around the ovary surface where 1-5 solitary larvae develop basal chambers in floret galls. An *A. mearnsii* flower-head has up to 45 florets (Raymond 1997), most of which are bisexual and capable of forming fruits or floret galls. Although Tiny Floret Galler galls are negatively correlated with fruit production of *A. mearnsii*, floret galls do not affect ovule development on flowers-heads where fruits and floret galls co-occur. This is in stark contrast to the non-linear relationship between galling intensity and fruit production of *Trichilogaster* galls where fruit production is eliminated on affected flowers and drastically reduced on other flowers located on galled branches (Dennill 1988).

The Tiny Floret Galler is restricted to a small group of *Botrycephalae Acacia* in eastern Australia. This cecidomyiid was not recorded on *A. mearnsii* in Western Australia in the 1970s where phytophagous surveys for seed-reducing insects were undertaken by South African entomologists (M. Van Den Berg unpublished records, S. Neser pers. comm. 2000). Most *A. mearnsii* in south-west Western Australia now have populations of the Tiny Floret Galler, including single trees or small groups that are isolated in dense bushland, indicating that the Tiny Floret Galler has well developed host location capabilities. This is also apparent in *D. dielsi,* recently released in South Africa for the biological control of *A. cyclops*, where most trees within 450 km of the prevailing SE wind stream were colonized within three years of naturalization (Chapter 8). Location and colonisation of isolated host plants offers potential for the rapid suppression of nascent foci (Moody & Mack 1998), and may reduce the risk of expansion of host plant populations.

Exponential trend lines providing the best fit to Tiny Floret Galler gall data from Mundijong and Keysbrook, predict mean gall levels will be between 14-36 galls per branch after eight years, but considerably higher after 12 years with 27-249 galls per branch (table 6.8). This compares with the maximum mean (± SD) gall numbers recorded in Australia on *A. mearnsii* were at Albany with 106 ± 64.4 galls per branch in 2001 and 37.8 ± 54.5 galls per branch in 2002. Another high-density site was at Augusta, WA where in 2001 there was a mean (± SD) of 51.9 ± 74.4 galls per branch. At Albany and Augusta, *A. mearnsii* trees were devoid of fruit in the presence of high population densities of Tiny Floret Galler.

Both *D. dielsi* and the Tiny Floret Galler increase rapidly once established, particularly *D. dielsi*, which is multivoltine. The Tiny Floret Galler is univoltine and once

established, saturation densities could develop within 8-12 years, but the rate of population growth is likely to be influenced by the size of the founder population, levels of parasitism, consistency of host flowering and size of the host plant population. These factors are of relevance in commercial *A. mearnsii* plantations in South Africa where seed fall is exploited for crop regeneration. In blocks of *A. mearnsii* undergoing crop regeneration, *A. mearnsii* seedlings commence flowering after two years and then seed production after three to four years. Trees are normally harvested by clear-felling after 8-10 years. The Tiny Floret Galler cannot reproduce in the absence of *A. mearnsii* flowers and if present in wattle plantations will become locally extinct within 12 months of harvesting. As seedlings reach sexual maturity, rapid colonization by the Tiny Floret Galler adults is likely to occur from contiguous blocks of infected trees, particularly along block margins. In isolated blocks of regenerating trees, Tiny Floret Galler invasion will be slower with full recruitment expected to occur within 12 flowering seasons from arrival of founder populations with a gradual decline in seed production over the normal rotation period of 10 years. However, seed production may not be adversely affected, if at all, until shortly before harvesting. Plantation-grown *A. mearnsii* produce large numbers of seed after 4 years from planting (Chapter 7) and wattle growers utilizing natural regeneration techniques to replace crops are unlikely to be affected by the presence of the Tiny Floret Galler. In seed orchards of *A. mearnsii*, or plantations where seed is harvested for line-sowing, trees may require treatment with insecticide for suppression of the Tiny Floret Galler, if population densities of the insect exceed tolerance thresholds. The efficacy of a range of insecticide treatments for the control of the Tiny Floret Galler on *A. mearnsii* is explored in Chapter 7.

CHAPTER 7

Control of seed-reducing Cecidomyiidae in commercial wattle plantations in South Africa: the role of insecticides, seed rain and soil-stored seed in crop regeneration and seed production.

Abstract

In laboratory tests, the Tiny Floret Galler is susceptible to the synthetic pyrethroid deltamethrin when applied to soil prior to emergence of larvae or before emergence of adults. Application of the insecticide before emergence of larvae results in lower adult emergence than applications shortly before ecdysis. Six treatments involving the systemic insecticides acephate, dimethoate, oxydemeton-s-methyl and imidacloprid applied to the soil or by stem-wiping failed to reduce gall densities of the Tiny Floret Galler at Denmark and Frankston in southern Australia. Foliar application of the synthetic pyrethroid cyfluthrin at 0.02 g l^{-1} a.i reduced gall densities of Tiny Floret Galler, indicating that this treatment has potential for further development as a field control technique. In South African wattle plantations, seed production of *Acacia mearnsii* increases with age, with seed-fall ranging from 127-995 seeds per 0.5m^2. After slash-burning, 26-50% of seeds remain in the soil profile after the main pulse of seed germination. The proposed release of the Tiny Floret Galler in South Africa is unlikely to cause any change in the management of plantations utilising natural regeneration techniques, but in areas where seed is harvested for line-sowing or seedling production, applications of insecticides may be required to maintain high yields in seed orchards. The cost nationally to the South African wattle industry for insecticide protection of seed-producing plantations is between R130,000-R200,000 $annum^{-1}$. Slight modifications to the location of seed-producing plantations and harvesting procedures could reduce this cost.

Introduction

Acacia mearnsii is commercially grown for timber and tannin production in managed plantations in South Africa, mainly in the summer-rainfall regions of KwaZulu-Natal and Mpumalanga. Trees are harvested after 8-12 years and the plantations are then regenerated using either soil-stored seed that has accumulated during the growth of the previous plantation (natural regeneration), plantation-collected seed sown into drill lines (line-sowing), or from hand-planted seedlings produced from seed orchards of improved tree genotypes. Sixty percent of the wattle industry in South Africa uses a combination of natural

regeneration (25%) and line-sowing (35%), the remainder is from improved tree stock (R. Dunlop, pers. comm. 2002).

The planned use of seed-reducing biological control agents to suppress the reproduction of wild, populations of *A. mearnsii* in South Africa has been opposed by commercial forestry representatives for several decades (Stubbings 1977, Luckhoff 1977, Pieterse & Boucher 1997a, de Wit *et al.* 2001). However, more recently the conflict has abated and in 1993 a seed-feeding weevil, *Melanterius maculatus*, was accepted for release as a biological control agent for *A. mearnsii.* Approval for release was granted after it was demonstrated that a similar *Melanterius* species could be suppressed using synthetic pyrethroid insecticides applied to foliage (Donnelly *et al.* 1992). Clearance for release of gall-forming cecidomyiids in South Africa also necessitated confirmation that these insects can be controlled in situations where seed of the host plants is required.

Insecticides used routinely in commercial *A. mearnsii* plantations to control foliage- and root-feeding pests (Nel *et al.* 1999) have the potential to suppress gall-forming Cecidomyiidae that are under consideration as biological control agents. Synthetic pyrethroids used to control bagworms (Eucosmidae, Lasiocampidae, Psychidae) and mirids (Miridae) on *A. mearnsii* have been effective in controlling the cecidomyiids *Contarinia* on lentil and sorghum (Gunning 1998, Kolesik *et al.* 1992), *Dasineura* on a range of crops (Wilson *et al.* 1988, Hard *et al.* 1988, Lane & Cooper 1989, Malik *et al.* 1996), and *Asphondylia* on capsicum and sesame (Hubaishan 1989, David *et al.* 1990).

Ephemeral, contact-based insecticides, however, may be of limited value in suppressing flower-ovipositing insects with long emergence periods and rapid development to sexual maturity in adults, typical characteristics of cecidomyiids developing within reproductive organs of *A. mearnsii.* Systemic insecticides with long residual periods could reduce application frequency (Fogal & Plowman 1989) compared to synthetic pyrethroids and may be translocated to galling sites, killing larval stages rather than adults. Implanted or injected systemic insecticides reduce non-target and environmental impacts and application rates are usually lower than foliage-applied treatments. Internal applications of insecticides have been effective in controlling some forest insects (Reardon & Haskett 1981, Fleischer *et al.* 1989, McClure 1992), including Diptera (West & Sunadram 1992), but results are highly variable and development restricted to conifers. Acephate implants reduced seed damage by the cecidomyiids *Contarinia oregonensis* Foote on *Pseudotsuga menziesii* (Mirb.) Franco (Stein *et al.* 1993) and *Dasineura* in cones of *Abies procera* Rehder (Overhulser & Tanaka 1983), but were ineffective against *Contarinia oregonensis* on *Pseudotsuga menziesii* (Mirb.) (Reardon *et al.* 1985, Stein *et al.* 1988, Stein *et al.* 1993), *Dasineura rachiphaga* Tripp on

Picea mariana (Mill.) B.S.P. (West & Sundaram 1992), *Resseliella skuhravyorum* Skrz. on *Larix* sp. and possibly *Kaltenbachiola strobi* Winn. in the cones of *Picea abies* (L.) Karst. (Roques *et al.* 1996). Injections of the systemic organophosphate oxydemeton-s-methyl reduced infestations of *Contarinia oregonensis* on *Pseudotsuga* (Stein & Markin 1986), while stem injection of *Picea glauca* with dicrotophos reduced cone damage by *Dasineura rachiphaga* (Fogal & Lopushanski 1989). Dimethoate injection was ineffective against *Resseliella skuhravyorum* on *Larix* in Romania (Olenici 1996), but gave some control of *Kaltenbachiola strobi* in cones of Norway Spruce (Dumcius 1989).

Cecidomyiids, including seed-feeding species, can also be suppressed with soil-applied insecticides. Soil application of insecticides reduces secondary infection by pathogenic fungi that can be associated with injection treatments. In commercial *A. mearnsii* plantations, the fungus *Ceratocystis fimbriata* Ell & Halst colonizes damaged tissue causing lethal gummosis and wilt symptoms (Morris *et al.* 1993) and could constrain the use of insecticide injection techniques. The root-absorbed carbamate carbofuran, as a granule formulation, reduces damage by the cecidomyiids *Paradiplosis tumifex* Gagné on *Abies balsamea* (L.) Miller (Benjamin *et al.* 1979), *Pinyonia edulicola* Gagné on *Pinus edulis* Engelm. (Baskin & Brewer 1983) and *Oresolia oryzea* Wood-Mason on *Oryza* (Harinkhere *et al.* 1993, Sontakke & Dash 2000). All are shoot-deforming cecidomyiids. Similarly, soil- and injection-applied imidacloprid decreases damage by the pine needle gall midge *Thecodiplosis japonensis* Uchida *et* Inouye on *Pinus* (Kim & Kim 1996) and is translocated in citrus by trunk-surface application (Mendel *et al.* 2000) indicating a potential pest control role in *A. mearnsii* plantations.

Suppression of biological control agents in commercial plantations of *A. mearnsii* may only be necessary if seed destruction reduces soil-seed accumulation below the threshold required for successful re-establishment following post-harvest burning for natural crop regeneration methods, or seed destruction tolerance levels are exceeded in *A. mearnsii* seed orchards. Minimal soil-seed load data for natural crop regeneration is not available for South Africa even though *A. mearnsii* has been cultivated commercially for 130 years. Similarly, annual seed-rain data is lacking, except for 18 year-old trees in Japan (Watanabe *et al.* 1981). Both data could form the basis of a simulation model that could help predict the impact of biological control agents on regeneration prospects and seed harvest potential of *A. mearnsii* in South Africa. Similarly, although *A. mearnsii* is recognized as a fire-adapted pioneer plant (Sherry 1971) and heat stimulates soil-stored seed to germinate (Pieterse & Boucher 1997b), the extent of soil-seed depletion following fire in wild or plantation stands of *A. mearnsii* has

not been measured in South Africa. Post-fire soil-seed dynamics would also contribute to modelling the impact of biological control agents on *A. mearnsii*

This chapter reports on studies that examined the impact of systemic and synthetic pyrethroid insecticide treatments on the Tiny Floret Galler in Australia and explores the suitability of these insecticides for use in commercial plantations in South Africa. Seed-rain in relation to age of *A. mearnsii* was measured over two seasons, together with the impact of post-harvest slash burning on seeds in the soil, to assist with modelling of the impact of biological control agents on *A. mearnsii*.

Materials and Methods

Suppression of the Tiny Floret Galler with a soil-applied synthetic pyrethroid

Experiment 1

This experiment was designed to determine if synthetic pyrethroids could be used as a soil applied treatment during emergence of Tiny Floret Galler larvae from galls. Larvae emerge from galls in late June-early July and pupate in the soil beneath the host tree.

In a quarantine laboratory at Stellenbosch, South Africa, plastic pots (top diameter = 12 cm, surface area = 113 cm^2) were filled with autoclaved sandy-loam and levelled. Leaf litter from beneath *A. mearnsii* was collected and large coarse items, such as stones and twigs, were removed before autoclaving. Sterile leaf litter was applied to completely cover the soil surface and aimed to replicate natural litter conditions. Aqueous solutions of the synthetic pyrethroid insecticide deltamethrin (Bitam® SC) were prepared at 0.01 g l^{-1} a.i, 0.02 gl^{-1} a.i. and 0.04 g l^{-1} a.i. and applied to the surface of pots using a hand-mister at 22.6 ml per pot. Control pots were sprayed with tap water. There were 5 –7 replicates per treatment and pots were arranged in a completely randomized design.

One-hundred mature Tiny Floret Galler galls collected at Denmark, Western Australia were added to the litter surface of each pot 1-3 hrs after insecticide application. Galls were lightly misted with water every 2-3 days to reduce dehydration. After six weeks, when the Tiny Floret Galler larvae should have emerged and pupated in the soil, galls were removed and a clear plastic bag was fitted tightly over each pot. Emerging Tiny Floret Galler adults and parasitoids were collected and counted daily until no further emergence occurred.

Experiment 2

A second experiment was established to assess the feasibility of applying insecticides to the soil after the Tiny Floret Galler larvae has emerged and buried themselves in the soil profile.

Plastic pots were prepared as above but without *A. mearnsii* leaf litter. Mature Tiny Floret Galler galls were collected at Dwellingup, Denmark and Jarrahdale in Western Australia and placed over a thick layer of pulverized garden peat in large plastic trays where they were misted with water every 2-7 days for 10 weeks. Larvae emerged from galls to pupate in the peat medium. Several days before emergence of adult Tiny Floret Gallers, samples of peat containing cocoons were spread evenly over the surface of the soil in the pots to a depth of 5-8 mm and then sprayed with deltamethrin at concentrations described above. There were five replicates for each treatment. Control pots were sprayed with tap water. Immediately following treatment, pots were fitted with clear plastic bags and emerging cecidomyiid adults and parasitoids were counted daily. Insecticide treatments were re-applied after 22 days.

Data were analysed by first performing a two-way ANOVA using the number of adults that emerged as the dependent variable and insect type (cecidomyiid and parasitoid) and insecticide treatment (rate of pyrethroid) as the factors. Data from experiments using post-emergence larval treatments were $\log_{10}$ transformed and data using pre-emergence larval treatments were cuberoot transformed to improve normality. After transformation, data were found to have homogenous variances and errors were normally distributed. Adult emergence data for cecidomyiids and parasitoids were combined when main effect interactions and the effect of insect type were not significant. A one-way ANOVA was then used to test the effect of insecticide rate and means were separated using the Bonferroni multiple comparison test.

Suppression of the Tiny Floret Galler with injected and trunk-applied systemic insecticides

At Denmark, Western Australia (34^0.59'S, 117^0.13'E), *A. mearnsii* trees were graded according to relative Tiny Floret Galler densities (high, high-medium, medium, medium-low, low, absent). Trees with single trunks and medium-low to high Tiny Floret Galler densities were selected and insecticide treatments applied between 9-10 August 2002, in a randomized block design following Tiny Floret Galler density. Individual insecticide treatments were acephate at 0.87 g a.i. applied as Acecap 97® implant cartridges, dimethoate at 270 g l^{-1} a.i. (Rogor®), imidacloprid at 200 g l^{-1} a.i. as a suspension concentrate (Confidor® SC 200) and imidacloprid at 200 g l^{-1} a.i. as a soluble concentrate (Confidor® SL 200).

Acephate cartridges were inserted at 10 cm intervals into 9.5 mm diameter holes bored to 35 mm in a spiral around the base of the main trunk. Holes were plugged with putty following treatment. Dimethoate was applied by inserting 8 mm diameter plastic tubing into 8.7 mm holes in a spiral at 10 cm intervals. Twenty millilitres of dimethoate was added to each tube and gravity-fed into the tree. Holes were sealed with silicone glue. Undiluted

imidacloprid formulations were painted onto the basal 1-1.5 m of the main truck at 1 ml per 10 cm of basal truck diameter using a paintbrush. There were seven replicates for each treatment.

In October 2002 when trees were in full flower, four trees from each insecticide treatment were randomly selected and 100-200 g fresh weight of inflorescences were collected, packed in plastic bags and frozen in preparation for determination of insecticide residues. High-pressure liquid chromatography with UV detection was used for imidacloprid, gas chromatography with pulsed flame photometric detection for dimethoate, and gas chromatography with nitrogen-phosphorus detection for acephate and oxydemeton-S-methyl. Oxydemeton-S-methyl was oxidized to di-oxydemeton-methyl with metachloroperbenzoic acid prior to injection into the gas chromatograph. In all analyses external standard quantitation was used.

In March 2003, 10 haphazardly selected branches with a stem diameter of 4-6 mm were removed from the outer canopy of treated trees and the number of infructescences, the number of Tiny Floret Galler galls and number of fruits per infructescence were recorded. A GLM was used to test the significance of blocks and treatment effects. Number of galls was squareroot transformed to improve normality and homogeneity of variances.

Suppression of the Tiny Floret Galler with injected, trunk and soil-applied systemic insecticides

At Frankston, Victoria (38^0.12'S,145^0.16'E), six systemic insecticide treatments were randomly allocated to sexually mature *A. mearnsii* trees with single trunks and basal diameters of 16-47 cm. Injection treatments were imidacloprid at 200 g l^{-1} a.i. as a soluble concentrate (Confidor® SL 200) at a rate of 5 ml per 10 cm of basal trunk circumference, dimethoate at 150 g l^{-1} a.i. at 10 ml per 10 cm of basal trunk circumference and oxydemeton-S-methyl at 50 g l^{-1} a.i. at 10 ml per 10 cm of basal trunk circumference. Injection was undertaken with a Sidewinder® tree injector at 600 psi into pre-drilled 6 mm diameter holes, 4 cm deep, arranged in spiral pattern around the trunk. Injection immediately followed drilling and holes were sealed with plastic plugs after application of insecticides. Additional treatments were imidacloprid at 200 g l^{-1} a.i. as a soluble concentrate painted on the basal 1-1.5m of main trunks, and imidacloprid at 4 g l^{-1} a.i. as a suspension concentrate was applied in 1 litre of tap water to the soil in a shallow depression surrounding the trunk. There were six replicates for each treatment and all were applied on 12 September 2002 when *A. mearnsii* was in bud.

During late October 2002, freshly emerged Tiny Floret Galler adults were collected from galls harvested from *A. mearnsii* sites in Western Australia. Adults (85-90) were aspirated into ventilated plastic containers and transferred to sleeve cages on branches of *A. mearnsii* with at least 30 panicles of fresh flowers. Two sleeve cages were placed on separate branches of each tree. Sleeve cages were 70-80 cm x 38 cm cylinders made of nylon gauze with a mesh diameter of 417 μ. The sleeves were left in place on the branches for 5-8 days after the release of insects. Branches were harvested during March 2003 and the number of infructescences and galls per infructescence were recorded. Gall numbers per branch were transformed using a fourth root function, which improved normality, and provided homogenous variance and normally distributed residuals. Transformed data was subject to ANOVA to detect differences between treatments.

Suppression of the Tiny Floret Galler with a foliar-applied synthetic pyrethroid

At Frankston, Victoria, 1m long flowering branches on the outer canopy of *A. mearnsii* were sprayed with a synthetic pyrethroid, β-cyfluthrin at 0.02 g l^{-1} a.i. mixed with the surfactant Agral® at 1 ml l^{-1}. A 5-l hand-pressurized garden sprayer with a coarse droplet setting was used to apply the insecticide to the point of run-off at 400-500 ml per branch. Trees were at least 5 m apart and a single branch holding at least 30 panicles of flowers was used for each of 10 replicates. Control trees were sprayed with tap water. Treatments were applied in a completely randomized design from 23-26 October 2002. Several hours after insecticide application, when the sprayed foliage was dry, freshly emerged Tiny Floret Galler adults were collected and confined to sleeve cages on branches as described above. A single, flowering branch was used from each tree.

In March 2003, branches were harvested and the number of infructescences and galls per infructescence were recorded. A student's *t*-test with separate variance was used to test the H_0 that there was no significant difference in gall numbers between treated and untreated trees. The number of galls per branch was $\log_{10}$ transformed to improve normality and homogeneity of variances.

Seed rain

At Pietermaritzburg, South Africa (29^0.32'S,30^0.27'E), seed traps were installed beneath the canopy of a commercial plantation of *A. mearnsii*. Seed traps were constructed of 80% grade shade-cloth and were attached to a circular metal frame with a diameter of 80 cm ($0.5m^2$). The traps were 30 cm deep and were positioned approximately 60 cm above ground level. Twenty trap positions were randomly selected in stands of *A. mearnsii* of 4, 6, 8 and 9

years since planting. Traps were installed in November 2001 and 2002 and debris within traps was collected in March of the following year. Seeds were separated by hand and counted. Seed viability was determined by combining subsamples of seed collected from each season and randomly selecting five replicates of 50 seeds for germination in soil after soaking seeds in boiled water for 12 hrs (Sherry 1971). Seedlings were counted after 25 days.

An ANOVA was used to test for the effect of tree age on seed fall. Seed number from 2003 was $\log_{10}$ transformed to improve normality and homogeneity of variance. Untransformed seed numbers were used for the 2002 season. Tukey's HSD test was used to separate means where effect of stand age was found to be significant.

Effect of fire on seed banks

At Pietermaritzburg, South Africa (29^0.32'S,30^0.27'E) *A. mearnsii* planted in 1990 was harvested for timber and bark in November 2001 and the 'slash' placed on plantation lines to dry before being burned on 17 January 2002. Three-months before burning (November 2001), 20 cylindrical soil cores (7 cm diameter, 38.4 cm^2 surface area) were randomly sampled to a depth of 7.5 cm from each of five 50 m transect lines running parallel and within slash lines. Twelve-days after burning transect lines were resampled, then again 17 months after burning using the procedures outlined above. These samples were taken from within the zone. Seedlings within the sample cores taken 12 days after burning were also counted. All seedlings were sprayed with herbicides 12 months after burning in preparation for planting, following normal silvicultural practice.

Modelling the impact of the Tiny Floret Galler

The projected impact of the Tiny Floret Galler on seed production in commercial *A. mearnsii* plantations in South Africa was modelled by utilsing seed-rain and soil-seed reserve data collected at Pietermaritzburg (see above) to describe seed input into the soil profile.

Seed-rain was calculated by averaging and rounding seed fall data collected for different ages stands at Pietermaritzburg. Viable seed-rain was determined by taking into account seed viability determined by germination testing of seed samples. A linear increase in seed production was assumed from sexual maturity (year three) at 100 viable seed/0.5m^2 to harvesting (year 10) at 800 viable seeds/0.5m^2. The annual rate of seed-rain increase was 100 viable seeds/year/0.5m^2. Post-dispersal seed mortality was assumed to be 10% of the accumulated reserves after 10 years. However, no data were collected or were available for *A. mearnsii* within South Africa.

Hypothetical reductions in seed-rain caused by the Tiny Floret Galler were calculated assuming a 70% reduction at year three followed by a 5% increase in seed reduction to years nine and 10, where 100% seed reduction is achieved. In a second scenario, the model assumed a 90% seed reduction at years three and four, 95% seed reduction at years five and six and 100% seed reductions for years seven to 10. The accumulated soil-seed reserves at post-harvest were calculated by totalling the annual soil-seed contributions.

Residual soil-seed levels remaining in the soil profile after slash-burning operations were calculated from data collected at Pietermartizburg, 17 months after burning. Viable soil-seed levels were calculated by assuming 80% seed viability. Furthermore, only 30% of seed were assumed available for germination in the following crop regeneration cycle due to deep burial. An additional 90% loss of viability was assumed to occur after storage in the soil for 10 years until the following crop regeneration practises are implemented. As the Tiny Floret Galler was assumed to reduce seed-rain, soil-seed carry-overs after the first crop rotation following the insects hypothetical release were assumed to drop by 90%.

Results

Suppression of the Tiny Floret Galler with a soil-applied synthetic pyrethroid

Experiment 1

There was no significant difference in the number of emergent adults of the endoparasitoid ?*Synopeas* sp. (Platygastridae) and the Tiny Floret Galler when deltamethrin was applied shortly before larvae emerged from galls ($F_{1,34} = 2.73$, $P = 0.10$). The interaction term between main effects (insecticide treatment, insect type) was also insignificant ($F_{3,34} = 1.09$, $P = 0.364$) and therefore the number of emergent Tiny Floret Galler adults and ?*Synopeas* adults were combined when assessing the impact of insecticide. There was a significant insecticide effect ($F_{1,34} = 37.89$, $P<0.001$), where deltamethrin reduced the mean total number of emergent insects at all application rates compared to the control ($P<0.05$) with a maximum reduction in emergence of 89.9% for 0.02 g l^{-1} and 0.04 g l^{-1} (table 7.1). There was no significant difference in emergence levels between 0.01 g l^{-1} and 0.02 g l^{-1} deltamethrin, and between 0.02 g l^{-1} and 0.04 g l^{-1} (table 7.1).

Experiment 2

There were significant main effects and interaction ($P<0.001$) when deltamethrin was applied after emergence of Tiny Floret larvae from galls, therefore only the number of emergent cecidomyiids were considered when assessing the impact of insecticide rate. Deltamethrin had a significant effect on emergence of the Tiny Floret Galler ($F_{3,17} = 21.5$, $P<0.001$) with adult emergence lower at all application rates when compared to control (table

7.1). There was no significant difference in adult emergence for deltamethrin applied at 0.01 g l^{-1} and 0.02 g l^{-1}. The largest reduction in adult Tiny Floret Galler emergence was 54% for deltamethrin at 0.04 g l^{-1}.

Suppression of the Tiny Floret Galler with injected, trunk and soil-applied systemic insecticides

At Frankston, Victoria, application of systemic insecticides into the basal trunk of *A. mearnsii*, and stem-wiping and soil application of imidacloprid had no detectable effect on gall formation by the Tiny Floret Galler ($F_{6,27}$ = 1.81, P = 0.13) (table 7.2). Similarly at Denmark, Western Australia, application of systemic insecticides by trunk injection had no detectable effect on gall formation by the Tiny Floret Galler ($F_{4,22}$ = 0.164, P = 0.95) (table 7.2). Block effects were also not significant ($F_{6,22}$ = 0.342, P = 0.90).

Determination of insecticide residues in flowering tissue of *A. mearnsii* at the time of addition of Tiny Floret Galler adults to branches showed large variation in concentrations (mg/kg) of all insecticides, with the greatest variation occurring in stem injection treatments (table 7.3). Scatter plots of gall numbers against insecticide residue concentrations showed no clear correlations. However, an inverse trend between gall numbers and residue of imidacloprid SL was apparent, five months after stem injection, but the correlation was not significant (P>0.1) with few sample points (n = 4) (figure 7.1).

Suppression of the Tiny Floret Galler with a foliar-applied synthetic pyrethroid

Flowering branches of *A. mearnsii* sprayed with β-cyfluthrin at 0.02 g l^{-1} a.i before introduction of Tiny Floret Galler adults produced a mean (± SE) of 9.9 ± 7.2 galls (branch^{-1}) after four months. Unsprayed trees produced a mean of 87.7 ± 12.6 galls. The difference between gall numbers on sprayed and unsprayed trees using log_{10}-transformed data was highly significant (t = 6.85, df = 12.6, P<0.001).

Seed rain

In 2002, seed fall from *A. mearnsii* significantly increased with the age of the plantation ($F_{2,39}$ = 80.36, P<0.001), but this trend was not apparent in the 2003 season where no detectable differences could be found in seed fall from trees of 4-8 y ($F_{2,54}$ = 0.33, P = 0.71) (table 7.4). Mean seed fall ranged from 127-995 seeds per 0.5m^2, but variability within tree age cohorts, particularly in 2003, was high. Mean seed viability's were 71.6% and 74.8% for 2002 and 2003, respectively, and these were not statistically different (P>0.05).

Table 7.1. Mean (± SE) number of emergent Tiny Floret Galler (TFG) adults at four rates of deltamethrin (g l^{-1}) when applied to the soil before emergence of larvae (L) or adults (A). Data are untransformed values. Means in rows followed by the same letter are not statistically different (P>0.05).

	Rate of deltamethrin (g l^{-1})			
TFG stage	0	0.01	0.02	0.04
L	131.0 ± 13.8 a	35.5 ± 7.4 b	14.4 ± 3.0 bc	7.4 ± 2.2 c
A	146.2 ± 7.4 a	96.8 ± 9.6 b	103.0 ± 2.2 b	68.0 ± 6.9 c

Table 7.2. Mean number of Tiny Floret Galler flower-head galls per tree (± SE) following application of systemic insecticide treatments at Frankston, Victoria and Denmark, Western Australia. Data are untransformed values. Method of application: SI=stem injection, SW=stem wiping, SA=soil application, SC=stem cartridges

Insecticide	Method of application	Galls (no. $tree^{-1}$)	
		Frankston	Denmark
Control		24.0 ± 6.8	50.3 ± 11.5
Imidacloprid SL	SI	34.6 ± 16.3	-
Imidacloprid SL	SW	39.0 ± 5.7	49.5 ± 7.3
Imidacloprid SC	SW	-	60.6 ± 14.3
Imidacloprid SC	SA	42.5 ± 26.2	-
Dimethoate	SI	30.9 ± 13.8	48.9 ± 13.3
Oxydemeton-s-methyl	SI	24.7 ± 7.9	-
Acephate	SC	-	62.0 ± 13.1

Table 7.3. Concentrations (mg kg^{-1}) of systemic insecticides residues in flowering branchlets of *A. mearnsii*, 5-6 months after application. Method of application as for table 7.2.

Insecticide	Method of application	Residue range (mg kg^{-1})
Imidacloprid SL	SI	0.53 - 8
Imidacloprid SL	SW	<0.02 – 0.58
Imidacloprid SC	SW	0.15 - 0.66
Imidacloprid SC	SA	0.02 1.2
Dimethoate	SI	1.5 - 16
Oxydemeton-s-methyl	SI	0.08 – 8.7
Acephate	SC	0.05 - 0.26

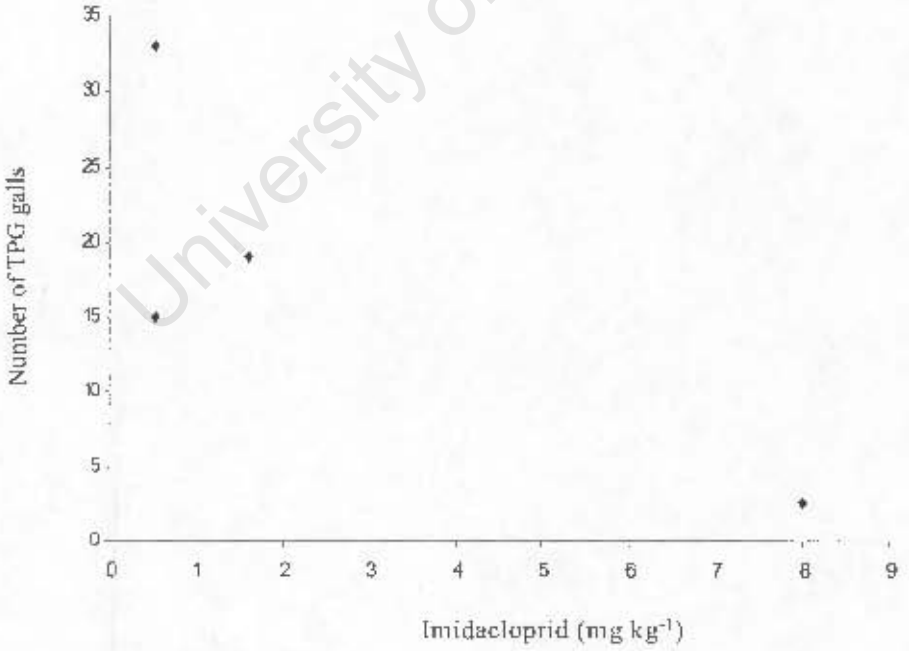


Figure 7.1. Gall formation by the Tiny Floret Galler on *A. mearnsii* at different residue concentrations of imidacloprid SL, five months after stem injection.

Table 7.4. Mean number of *A. mearnsii* seeds (± SD) caught in seeds traps ($0.5m^2$) in 2003 and 2003 under trees of different age at Pietermartizburg, South Africa. Means are untransformed data. Means in each row followed by the same letter are not statistically different (P>0.05) using Tukey's HSD test. Mean seed viability (%) (± SD) is for seeds from each season.

	Age (yr)				
Year	4	6	8	9	Viability (%)
2002	127.2 ± 82.4 a	518.1 ± 213.3 b	-	995.3 ± 160.8 c	71.6 ± 18.7
2003	309.3 ± 495.6 a	305.3 ± 535.8 a	585.5 ± 800.0 a	-	74.8 ± 22.9

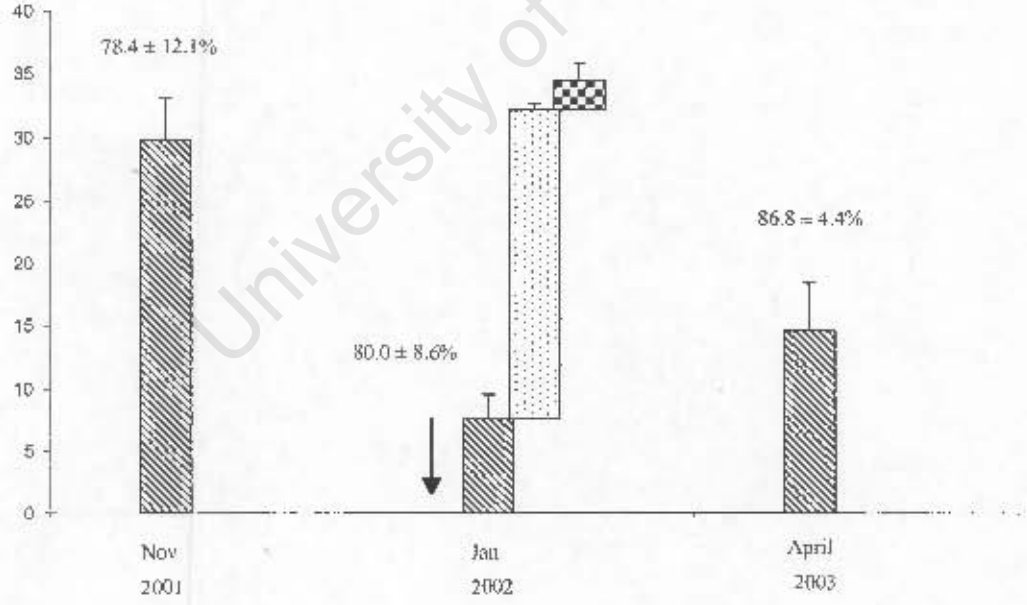


Figure 7.2. Mean number of seeds (diagonal-hatch), seedlings (stiple) and germinating seeds (check) in soil samples before and after slash burning in an *A. mearnsii* plantation at Pietermartizburg, South Africa. Column values have been offset to show 95% confidence values. Numbers above columns are the mean % viability (± SE) of ungerminated seed. The arrow indicates the date of burning.

Effect of fire on seed banks

After harvesting, the mean soil-seed reserve from a 10 yr commercial plantation of *A. mearnsii* was 29.8 seeds per 288 cm^3, most of which were viable (figure 7.2). Approximately 78% of seeds germinated 12 days after slash-burning in summer with 22% of seeds remaining in the soil seed bank. Seventeen months after burning, mean soil-seed reserves in burnt slash lines were 14.6 seed per 288 cm^3 with 86.8% remaining viable (figure 7.2).

Modelling the impact of the Tiny Floret Galler

Mean viable seed-rain of *A. mearnsii* in a commercial plantation at Pietermaritzburg was 160 seeds/0.5m^2 for four year old trees, 302 seeds/0.5m^2 for 6 year old trees, 430 seeds/0.5m^2 for eight year old trees and 730 seeds/0.5m^2 for nine year old trees. Measured seed-rain values closely matched the annual seed-rain values used in the impact model for the Tiny Floret Galler.

In the first model which assumes 70% reduction in seed-rain at sexual maturity *of A. mearnsii*, the predicted net soil-seed reserve from seed rain after an eight-year period (contributions from years three to 10) is 500 seed/m^2. In the second model, which allows for higher levels of seed reduction by the Tiny Floret Galler, the predicted net soil-seed reserve from seed rain after eight years is 126 seed/m^2.

After slash-burning following harvesting in a commercial *A. mearnsii* plantation, approximately 2080 viable seeds/m^2 remain in the soil profile (based on 10 seeds/38.4cm^2 core samples). Assuming only 30% of seeds are able to germinate successfully due to soil burial effects, and a 90% loss in seed viability occurs over the following crop rotation period (10 years), a predicted soil-seed carry over from the previous crop is 62 seeds/m^2. However, the Tiny Floret Galler is assumed to reduce seed-rain from between 70-100%, therefore residual soil-seed carry overs subsequent to the first crop rotation following release were assumed to be reduced by 90%. Therefore, the predicted longer-term soil-seed carry over between crop rotations is six viable seeds/m^2.

Discussion

The Cecidomyiidae is a large cosmopolitan family of flies with many species causing damage to plants of economic importance (Barnes 1946a, 1946b). *Dasineura* is the largest genus and occurs in all zoogeographic regions of the world where the species are typically mono- to oligophagus and utilize a broad range of host plants from annual herbs to trees. Insecticides are a common component in the management of injurious *Dasineura* with mixed levels of success dependent on predicting timing of effective spray application (Cross & Crook 1999),

accessibility of insecticides to eggs and larvae and large variation in susceptibility of species to pesticides. Preventative cultural methods (Barnes 1946a, 1946b), synchrony disruption (Huddleston *et al.* 1972) and use of non-susceptible host biotypes (Keep 1985, Kumar *et al.* 1992) can negate or complement the use of pesticides for control of pest cecidomyiids.

Despite a rich Cecidomyiidae fauna occurring on African acacias none has been recorded on any Australian *Acacia* in South Africa, including *A. mearnsii* where detailed phytophagous surveys are available (Hepburn 1966, 1967, 1979). Australian Cecidomyiidae do not occur as pests on *Acacia* in southern Africa, but the possible release of the seed-reducing Tiny Floret Galler may affect seed production in commercial wattle plantations of *A. mearnsii*, possibly necessitating control efforts where high levels of seed production are required. The rapid dispersal and increase in population densities of the multivoltine midge *D. dielsi* in South Africa for suppression of seed production of *A. cyclops* indicates the potential efficacy of wind-dispersed cecidomyiids for biological control purposes (Chapter 8).

Foliage-feeding Lepidoptera (Eucosmidae, Lasiocampidae, Psychidae) are sporadic, but often injurious pests of *A. mearnsii* in South Africa and require control by the use of synthetic pyrethroid insecticides. Gall-forming cecidomyiids on a range of crops can be effectively controlled with synthetic pyrethroids (Kolbe 1982, Northwood & Verrier 1986, Kolesik *et al.* 1992, Metlitskii *et al.* 1993, Malik *et al.* 1996). Cyfluthrin, registered for control of bagworm on wattle in South Africa, is highly effective at reducing gall formation by the Tiny Floret Galler on *A. mearnsii* under experimental conditions in Australia, indicating a potential for broader field-based application. Similarly, the pyrethroid deltamethrin applied as a soil-surface spray before emergence of Tiny Floret Galler larvae has potential for suppression of midge populations, but requires evaluation, together with more persistent multi-isomer pyrethroids, to determine dose-response mortality and efficacy under field conditions. High-density populations of the Tiny Floret Galler in south-west Western Australia could be utilised for insecticide evaluation without quarantine restrictions and the risk of harm to plantations in South Africa.

Adult Tiny Floret Galler emergence is well synchronized with flowering of *A. mearnsii*, irrespective of climate, providing a predictable period for application of insecticides to protect potential seed crops. Application of pyrethroids in September-October would simultaneously be effective for suppression of wattle bagworm (Atkinson 1999) and the seed-feeding beetle *M. maculatus* (Donnelly *et al.* 1992), which commences reproduction and has peak adult activity during these months (Impson & Moran 2003). Fruit formation by *A. mearnsii* appears unaffected by applications of pyrethroids, despite the tree being out-

crossing with partial self compatibility (Moffet & Nixon 1974, Moncur *et al.* 1991) and pollinated by non-specific insects (New 1984), making the use of pyrethroids compatible with seed-production in plantations.

Injection and implantation of systemic insecticides demonstrate no consistent pattern in control of injurious gall-forming cecidomyiids, particularly in woody plants. Application of systemic insecticides by injection techniques, bark-painting or soil-drenching eliminates the risk of non-target spray drift damage that can occur with foliar-applied insecticides. Systemic insecticides are translocated to flowering branchlets of *A. mearnsii* when applied basally to trees in late bud, but of the five insecticide products and four applications methods used in this study, none had a detectable effect on reducing gall formation of the Tiny Floret Galler on *A. mearnsii*. The reasons for the absence of a treatment response are not clear, but may include poor translocation of insecticides to the inner tissue layers surrounding floret gall chambers where feeding by larvae occurs. Alternatively, the Tiny Floret Galler may have high tolerance to systemic insecticides, but as other *Dasineura* are generally susceptible to these pesticides, this is unlikely. Lack of efficacy, increased risk of invasion of *Ceratocystis* by bark wounding (Morris *et al.* 1993), and higher product/application costs are disincentives for further research effort on injection-application of systemic insecticides to *A. mearnsii*.

In South African wattle plantations, insecticides are aerially-applied for suppression of canopy pests and although cost effective, pilot and aircraft costs far exceed the cost of insecticides. The average total cost for aerial application of the synthetic pyrethroid cypermethrin at 200 ml ha^{-1} is R130 ha^{-1} (H. Antel, pers. comm. 2002). In South Africa, around 1000 ha y^{-1} of *A. mearnsii* plantation is harvested for seed for crop regeneration by line sowing or planted seedlings and yields approximately 3 t of seed $annum^{-1}$ (SAWGU, pers. comm. 2002). The projected cost of aerial-application of cypermethrin to all seed-producing plantation of *A. mearnsii* for protection against biological control agents is estimated at R130,000-R200,000 $annum^{-1}$. In plantations regularly harvested for seed, spraying every 2-3 years is likely to be sufficient as both *Melanterius* and the Tiny Floret Galler are likely to take at least several years to reach threshold levels of damage after initial suppression with insecticides. Isolation of seed-producing plantations by geographic separation from *A. mearnsii* crops or by embedding seed-producing *A. mearnsii* within other timber crops such as pine or *Eucalyptus* may also help reduce the need for application of insecticides. These stands could be more difficult to locate by dispersing insects and rates of population growth may be slower than sites located in close proximity to plantations with established populations of biological control agents.

A development program to create sterile *A. mearnsii* for commercial forestry (Beck & Dunlop 2002, Dunlop & Beck 2002) would completely alleviate the need for insecticide control of seed-reducing biological control agents, if flowering and viable seed production can be eliminated. As a future prospect, pheromone trapping of cecidomyiids (Miller & Borden 1981, Venugopal 1985) and use of the entomophagous fungus *Beauveria bassiana* (Balsamo) Vuillemen (Seo *et al.* 1995) also have potential to reduce reliance on insecticides, but each requires investment in research and development.

In plantations of *A. mearnsii* where soil-seed reserves are used for crop regeneration, application of insecticides for the control of the Tiny Floret Galler and *M. maculatus* should not be required as seed production from regenerating clear-felled blocks is likely to occur over several seasons or more before these univoltine, flower-dependent insects are able to re-establish in large numbers. A field-data based model that assumes biological control agents will cause 70% seed reduction at sexual maturity of *A. mearnsii* (three years) and increases by 5% annum^{-1} to 100% seed reduction at nine years (harvestable age), predicts that soil seed reserves available for natural regeneration at the end of the rotation will be will be around 500 seeds/m^2. As around 80% of seeds in soil-stored reserves germinate after slash burning, a seedling bank of about 400 plants/m^2 should develop in the first season after burning to ensure that sufficient plants survive for subsequent manual thinning to 1 tree/ 2m^2. A more conservative model assuming 90% seed reduction at the commencement of seed production and 100% seed reduction after seven years will yield around 126 seeds/m^2, again sufficient for crop regeneration in most regions. Residual soil-stored seed remaining after the initial germination pulse may also contribute seedling densities in the following crop.

These models also highlight the high level of efficacy needed by seed-reducing biological control agents to bring about effective control of the target weed on their own, a problem recognized in biological control programs for other weeds, including invasive *Acacia* (Kriticos *et al.* 1999). Even with very low levels of seed production, integrated control techniques (Campbell & Kluge 1999, Campbell *et al.* 1999) will be required to effectively suppress *A. mearnsii* infestations in South Africa, but the efficacy of these operations will be greatly improved by the presence of biological control agents. Wattle farmers practising natural regeneration techniques and resource-poor communities that utilise *A. mearnsii* as a fuel source or for building materials are very unlikely to be affected by a biological control program restricted to seed-reducing agents.

CHAPTER 8

The Small Fluted Galler (*Dasineura dielsi*) in South Africa, a recently introduced biological control agent for the invasive Australian shrub *Acacia cyclops*

Abstract

Acacia cyclops is a woody invasive weed in South Africa and a target for biological control with seed-reducing agents. In southern Australia, two gall-forming Cecidomyiidae, *Dasineura dielsi* and *Asphondylia* sp. (Seed Galler), occur on the flowers and seeds of *A. cyclops*, respectively. *Dasineura dielsi* larvae form woody galls on the ovaries of open florets and prevent the development of fruit. *Asphondylia* sp. is associated with a *Camarosporium* sp. and causes deformation of pods and destruction of ovules. *Dasineura dielsi* was selected as a biological control agent for *A. cyclops* in South Africa and was approved for release after host specificity evaluation and consideration of the potential conflicts with domestic and commercial utilisation of *A. cyclops* as a source of fire wood. *Dasineura dielsi* became naturalised in South Africa in 2001 and after three years dispersed up to 450 km from a single liberation point at Stellenbosch, Western Cape. Dispersal was predominantly in the direction of the prevailing winds of the south-western Cape. At sites close to Stellenbosch where *D. dielsi* has been present longest, high gall densities occur on *A. cyclops* during the peak flower season in summer. Utilisation of *D. dielsi* by four indigenous South African hymenopteran parasitoids occurred after three years. Levels of parasitism in the Western Cape varied between sites. Monitoring is required to evaluate trends in the population status of *D. dielsi,* its parasitoids and seed production of *A. cyclops*. Importantly, field monitoring should determine the extent and nature of possible competitive interactions between *D. dielsi* and an introduced seed-feeding weevil, *Melanterius servulus*.

Introduction

Acacia cyclops was introduced into South Africa around 1835 (Roux 1961), primarily for sand stabilisation in the southwestern Cape region. *Acacia cyclops* is used as a valuable source of fuel wood, but the species has also become invasive in native vegetation where it disrupts ecological processes and contributes to losses of biodiversity (Musil 1993). Classical biological control has been adopted as the best method to suppress *A. cyclops* in South Africa. However, because the plants are beneficial, the choice of agents has been restricted to phytophagous species that lower seed-production without affecting vegetative growth of the host.

The first agent to be considered was the Australian curculionid beetle *Melanterius servulus,* which was released in South Africa in 1991 and after initial difficulties in establishing field populations, the insect is now widely naturalized (Impson *et al.* 2000). Larvae and adults of *M. servulus* feed on immature seeds and pods of *A. cyclops* and destroy developing ovules (Donnelly 1992). Seed-destruction by *M. servulus* ranges up to 95% at sites in southern South Africa, but the insects'ability to sustain high levels of seed destruction appears to be curtailed through interference by other animals and phenological asynchrony with reproductive patterns of *A. cyclops* (Impson *et al.* 2000).

Seed-reducing biological control agents need to be highly effective to have an impact on the population dynamics of their host (Noble & Weiss 1989, Kriticos *et al.* 1999), particularly for species, such as *A. cyclops*, that annually produce hard-coated seeds and form seed-banks in the soil (Milton 1980). Seasonally-low seed destruction by biological control agents, even at irregular intervals, may allow *A. cyclops* to produce sufficient seed to maintain population densities that are problematic for nature conservation. Integrated control techniques such as manual clearing and chemical control are compatible with biological control, if undertaken in a planned manner, and may reduce the threshold required for effective seed-reduction by biological control agents. However, as infestations of *A. cyclops* occur over vast areas of the southern Cape, financial constraints limit the area that can be treated with manual-clearing methods.

A combination of compatible biological control agents is likely to be more successful than a single agent in providing effective weed suppression (Denoth *et al.* 2002). Interspecific competition between agents can curtail the efficacy of multi-agent biological control systems, or limit some of the component species (Woodburn 1996, Causton & Pena-Rangel 2002). In South Africa, *A. longifolia* appears to be under effective biological control by the gall-forming hymenopteran *Trichilogaster acaciaelongifoliae*, together with *M. ventralis,* which destroys seeds that escape damage from the former agent (Dennill *et al.* 1999). The recently introduced *M. compactus* may also augment the damage caused by the rust fungus *Uromycladium tepperianum* on *A. saligna* (Morris 1999) by destroying seeds produced on fungus-infected trees.

In Western Australia, *A. cyclops* is a primary host to two gall-forming cecidomyiids that develop exclusively on reproductive organs of this host species. *Dasineura dielsi* (Small Fluted Galler) is widespread on *A. cyclops* in Western and South Australia, although population densities are mostly sparse and transitional. The galls of *D. dielsi* develop on the ovaries of open florets and form woody, multi-chambered galls. *Asphondylia* sp. (Seed Galler) feeds on ovules in immature pods causing pod deformation and seed destruction.

Asphondylia larvae develop in association with a mutualistic fungus *Camarosporium* sp. (Chapter 4). The *Asphondylia* sp. associated with *A. cyclops* is restricted to Western Australia and is mostly rare. Although both cecidomyiids have potential as biological control agents of *A. cyclops* in South Africa, only *D. dielsi* was selected for detailed evaluation as it was readily available in large quantities in Australia, was amenable to rearing under laboratory conditions and formed outbreak densities in Australia, indicating potential efficacy as a biological control agent. In addition, *D. dielsi* is not associated with obligate fungi that could complicate the process of host specificity evaluation, and possibly naturalisation, if approved for release.

Host specificity evaluation of *D. dielsi* indicated that this cecidomyiid is restricted to a narrow range of Australian acacias and therefore a low risk to African acacias, commercially important Australian acacias in South Africa or other plants of economic importance (Adair 2002). The biomass of *D. dielsi* galls produced per flower is lower than the biomass of fruit (Adair 2002b). Unless gall numbers become exceptionally abundant with the total gall biomass and developmental period exceeding that produced by fruit, *D. dielsi* is unlikely to create a resource sink and impair the vegetative growth of *A. cyclops*. Domestic and commercial industries utilising *A. cyclops* as a source of fuel wood are unlikely to be affected by the release of *D. dielsi* in the short-term as complete destruction of seed crops is unlikely and soil-seed banks will contribute to seedling recruitment. In the longer-term, if the biological control programme for *A. cyclops* reduces seed production permanently to levels at which natural infestation begin to decline, cultivation of crops of *A. cyclops* may meet market demands for fuel wood of this species (Adair 2002b).

Dasineura dielsi was approved by regulatory authorities as a biological control agent for *A. cyclops* in South Africa and populations of this insect established in the western Cape in 2001. This chapter reports on the spread of *D. dielsi* in South Africa and presents data on parasitoids reared from galls collected at several localities. The implications of this preliminary data for biological control of *A. cyclops* in South Africa are discussed.

Methods

After *D. dielsi* naturalised at Stellenbosch (33^0.58'S, 18^0.46'E) in June 2001, surveys of *A. cyclops* were undertaken in the Western and Northern Cape to determine the geographic range and rate of spread of *D. dielsi.*

Acacia cyclops plants were surveyed at varying distances radiating from the first detected population of *D. dielsi* at Stellenbosch. Populations of *A. cyclops* were searched for the presence or absence of *D. dilesi* galls and the position of the survey sites was recorded

using a Global Positioning System. Surveys were undertaken from 2001-2003 between March-June when galls were reaching maximum density on host trees and therefore were easiest to detect. The dispersal front of *D. dielsi* was determined by surveying sites at approximately 20 km intervals along highways and roads running through *A. cyclops* infested regions. For each year, surveys were discontinued when *D. dielsi* was absent for 3 or more locations (i.e. >50 km) in the same general direction after the last recorded occurrence.

In April 2003 at Strand Beach (34^0.06'S, 18^0.48'E), Somerset West (34^0.02'S, 18^0.49'E) and Stellenbosch (33^0.58'S, 18^0.46'E), sites where *D. dielsi* has been present longest in South Africa, 200-500 mature galls were collected at each site and placed in sealed cardboard boxes. Extraneous arthropods, mainly small spiders, were present in low numbers with collections of galls. Emerging insects were collected by placing a funnel connected to a clear specimen bottle over a hole in the side of each box. Insects attracted to the light were trapped in the specimen bottle and counted after emergence had finished. Potential parasitoids of *D. dielsi* were separated for identification and voucher specimens lodged with the South African National Insect Collection (Pretoria). Microhymenoptera, particularly those with multiple accessions, were assumed to be parasitoids of *D. dielsi*, although their precise relationship with *D. dielsi* was not determined.

Results

Dasineura dielsi spread rapidly in the Western Cape with dispersal occurring predominantly in a north-westerly and easterly direction from Stellenbosch (figure 8.1). After three years, *D. dielsi* occurred 450 km in a NNW direction (Hondeklipbaai 30^0.320'S,17^0.277'E) from the release point and 390 km in an easterly direction (Glentana 34^0.041'S, 22^0.325'E), but at these sites, like most sites on the invasion front, gall densities were low with often only 1-10 galls found on 10-20 sample trees. In contrast, at Strand Beach and Somerset West, where *D. dielsi* has been present for 1-2 years, several thousand galled flower-heads may occur on a single mature tree after the peak flowering period in summer.

At three sites, microhymenoptera were reared from *D. dielsi* galls, but these varied in abundance from 14.8% of the total number of *D. dielsi* adults at Strand Beach, and 1.6% and 0.7% at Somerset West and Stellenbosch, respectively (table 8.1). Four parasitoid species were identified: ?*Synopeas* sp.3 (Platygastridae), Genus indet.3 (Platygastridae), *Torymus* sp.3 (Torymidae) and *Mesopolobus* sp. 2. (Pteromalidae) (table 8.1). Two Platygastridae species, and particularly Genus indet.3, were the most frequent parasitoids at Strand Beach, where they emerged with adults of *D. dielsi* over six weeks, commencing within several days after collecting galls from the field. Platygastrids were absent at Stellenbosch and Somerset

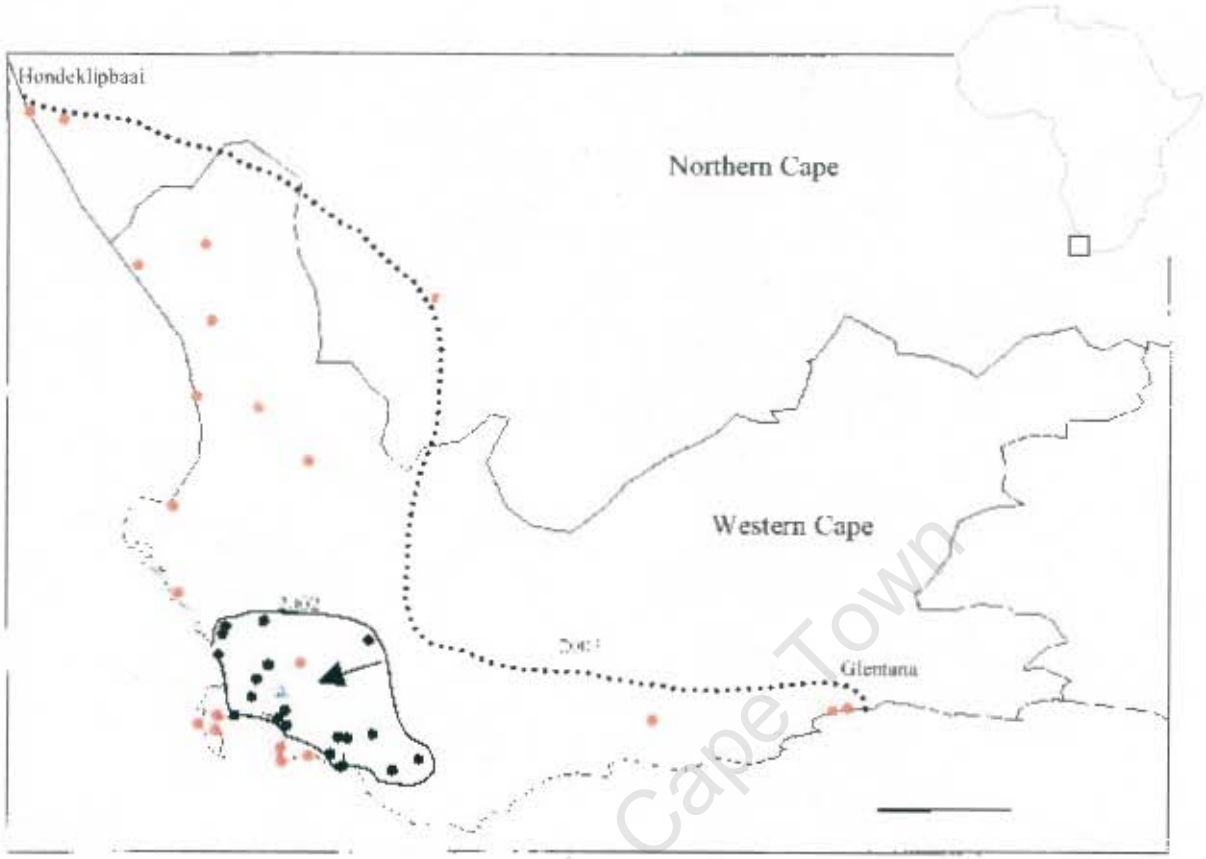


Figure 8.1. Distribution of *Dasineura dielsi* in South Africa. ▲= original release point (Stellenbosch) (indicated by arrow), ● = distribution in 2002 outlined with a solid line, • distribution in 2003 outlined with a dotted line. The scale bar is 100km.

Table 8.1. Hymenopteran parasitoids reared from *Dasineura dielsi* galls from three locations in Western Cape. * = two Platygastridae taxa (*?Synopeas* sp. 3 and Genus indet. 3. Numbers were combined because of difficulties in distinguishing these species)

Site	Number of *D. dielsi*	Total number of parasitoids	Platygastridae*	*Torymus* sp. 3	*Mesopolobus* sp. 3
Stellenbosch	865	6	0	1	5
Somerset West	751	12	0	0	12
Strand Beach	921	136	124	1	11

West where *Mesopolobus* sp. was most abundant. All microhymenoptera genera collected from *D. dielsi* galls from the three study sites include species recognised as parasitoids of other cecidomyiids in South Africa and elsewhere (Prinsloo 1980, CSIRO 1991, O. Neser, pers. comm. 2003).

Discussion

The south-west Cape region of South Africa experiences strong winds during most of the year with winds from the SE direction dominating in summer and winds from the NW in winter. Easterly winds occur intermittently throughout the year, particularly in the southern Cape. Adult *D. dielsi* are small fragile insects and the dispersal of this insect has been strongly influenced by the prevailing winds within the distribution of *A. cyclops* in South Africa. The rapid spread of *D. dielsi* on *A. cyclops* combined with dramatic rates of population build-up, once sites are colonized, negates the need for a mass-rearing and release program, applied to other biological control agents in South Africa (Gillespie *et al.* 2003). At current dispersal rates, *D. dielsi* is expected to occur throughout the entire *A. cyclops* distribution of South Africa and Namibia by 2005.

Most Australian gall-forming cecidomyiids from *Acacia* are univoltine, but *D. dielsi* is multivoltine with up to five generations per annum (Chapter 3). Short generation times of *D. dielsi* and high fecundity are likely to have contributed to rapid spread of this cecidomyiid in South Africa. In southern Australia, *D. dielsi* is present over most of the distribution of *A. cyclops* (Chapter 2) indicating broad climatic tolerance and no apparent host-agent limitations based on population differences of *A. cyclops*. These factors are also likely to have contributed to the establishment of *D. dielsi* over a broad range in South Africa.

Univoltine cecidomyiids under consideration as biological control agents for *A. mearnsii*, such as the Tiny Floret Galler and the Glabrous Bud Galler may exhibit much slower rates of spread than those recorded for *D. dielsi*.

The curculionid seed-feeding insect *M. servulus* is well established in South Africa as a biological control agent for *A. cyclops* (Impson *et al.* 2000). Interactions between biological control agents and the subsequent impact on host plant ecology remains a speculative, but important consideration in biological control science. Few studies have been directed at documenting these interactions. Although *D. dielsi* and *M. servulus* feed on inflorescence structures that are phenologically well separated, and therefore avoid direct competition for food resources, indirect competitive effects may become apparent in areas where *D. dielsi* and *M. servulus* are sympatric. *Dasineura dielsi* gall flowers of *A. cyclops* preventing the development of fruit, which are used by *M. servulus* adults and larvae as a source of food

(Dennill *et al.* 1999, Impson *et al.* 2000). Reduced fruit loads of *A. cyclops* caused by high densities of *D. dielsi* may curb *M. servulus* numbers. *Melanterius servulus* contributes to the biological control of *A. cyclops* in South Africa (Impson *et al.* 2000), but declines in populations of the weevil due to *D. dielsi* will only be consequential if seed destruction achieved by a combination of *D. dielsi* and *M. servulus* is less than destruction levels currently obtained by *M. servulus* alone.

A possible sequence of events will be that *D. dielsi* will invade areas occupied by *M. servulus* and although weevil numbers may decline, total seed reduction of *A. cyclops* could increase and show reduced seasonal variation. If this occurs, then biological control of *A. cyclops* in South Africa will have been enhanced considerably. Only detailed field monitoring of *M. servulus* at sites before and after the arrival of *D. dielsi,* and the respective contributions of the two agents to seed destruction, will reveal the nature of the interactions between these species. This information will provide important indicators to help in the assessment of the Tiny Floret Galler and the Glabrous Bud Galler as biological control agents of *A. mearnsii*. The rapid spread of *D. dielsi* in South Africa indicates that the need to establish field-monitoring sites is urgent.

Indigenous hymenopteran parasitoids may affect the ecology of *D. dielsi* on *A. cyclops* in South Africa. Four species of Hymenoptera reared from *D. dielsi* galls in South Africa are distinct from those reared from galls in Australia (O. Neser, pers. comm. 2003), including two species of the Platygastridae, a family that contains many cryptic species, which are still very poorly studied locally and generally difficult to distinguish using morphological characteristics. The platygastrid endoparasitoid (genus indet. 3) was most abundant from *D. dielsi* galls at Strand Beach, but relatively uncommon at other sites in the Western Cape. This parasitoid was absent along with other parasitoids at three survey sites elsewhere in the Western Cape where *D. dielsi* has recently arrived (J. Moore pers. comm. 2003). The four parasitoids recorded for *D. dielsi* in South Africa were not recorded in an extensive survey of cecidomyiid parasitoids from South African plants between 1999-2003, although conspecifics were present in all cases (Chapter 5). This indicates that parasitoids of *D. dielsi* in South Africa may originate from undetected cecidomyiids or other insect families or orders and have included *D. dielsi* in its host range. Further evaluation of parasitoid impact on *D. dielsi* in South Africa could establish whether the spectacular establishment of this cecidomyiid is a short-term event similar to the rise and fall of *Rhopalomyia californica* in Queensland (McFadyen 1985, Julien & Griffiths 1998), or whether sufficiently high population densities can be sustained to render this insect an effective biological control agent for the suppression of *A. cyclops.*

Conclusions and recommendations

Acacias form a diverse component of the Australian flora and are ecologically important, as are analogous indigenous taxa over much of South Africa. On both continents, gall-forming cecidomyiids utilize reproductive structures of their host acacias as a food source, but divergent speciation processes within the Cecidomyiidae in Australia and South Africa have produced faunas with few taxonomic similarities.

Australian *Acacia* species cause serious ecological damage in South Africa (Boucher & Marais 1995, Henderson 2001), while African acacias threaten to do so in Australia (Scott 1991). Gall-forming biological control agents have made effective contributions to the suppression of several invasive *Acacia* species in South Africa and Australian Cecidomyiidae expand the opportunities for further progress. In the case of *A. mearnsii*, eight cecidomyiid species reduce seed production by forming galls on buds, flowers or ovules. Three species, the Tiny Floret Galler, Glabrous Bud Galler and the newly discovered Egg Galler, have potential as classical biological control agents in South Africa based on host specificity, impact efficacy and minimal resource-loading criteria.

Detailed evaluation of the Tiny Floret Galler indicates this cecidomyiid is suitable for release on *A. mearnsii* in South Africa and will cause limited impact on commercial wattle plantations. In plantations or orchards used for seed collection, foliar-applied, pyrethroid insecticides could be used to suppress the Tiny Floret Galler, if it proves to be necessary, minimising the conflict of interest between weed control efforts and commercial forestry pursuits in South Africa.

Dasineura dielsi (Small Fluted Galler) also meets host specificity and safety evaluation criteria and has been approved for release as a biological control agent for *A. cyclops* in South Africa. The rapid establishment, spread and population growth of *D. dielsi* in the Western Cape suggest that this insect has considerable potential as a seed-reducing agent for *A. cyclops*, particularly with its capacity to locate and colonize small and isolated stands of *A. cyclops*. Although indigenous South African parasitoids utilize *D. dielsi* and have adopted this new host within three years, their impact and levels of parasitism throughout the distribution ranges and seasons remain to be ascertained.

Seed-reducing biological control agents need to be highly effective to reduce the impact and spread of alien woody weeds (Noble & Weiss 1989, Kriticos *et al.* 1999), particularly for *Acacia*, where large numbers of long-lived seed are produced in most seasons. Single species of seed-reducing biological control agents are unlikely to achieve acceptable levels of control of invasive weeds where dense infestations are dispersed over a

broad geographic range and seed production is a protracted seasonal event. Under these circumstances, there is a high probability that sufficient seeds will escape destruction to ensure the perpetuation of infestations. The integration of compatible suites of seed-reducing biological control agents that feed on reproductive structures at various stages of development are more likely to achieve effective levels of control than programs that utilise single-species. The biological control programs against *A. longifolia* in South Africa appears to have been successful because of the impact of the bud-galling hymenopteran *Trichilogaster acaciaelongifoliae* and the seed-feeding curculionid *M. ventralis*, where the efficiency of *M. ventralis* lies in its ability to locate the sparse pods that form on trees attacked by *T. acaciaelongifoliae* (Dennill & Donnelly 1991, Donnelly & Hoffmann, in press).

Similarly, in South Africa *A. saligna* is severely weakened by the gall-forming fungus *Uromycladium tepperianum,* which causes reduced seed production and decline in host trees, particularly during periods of moisture-stress (Morris 1999). However, alone, this biological control agent cannot adequately suppress seed production of *A. saligna* throughout its range. The seed-feeding weevil *M. compactus* was recently introduced to *A. saligna* infestations in South Africa to compliment control measures achieved by *U. tepperianum.*

The use of biological control agents to attack a range of phenological stages are also likely to be required for the successful suppression of *A. cyclops* and *A. mearnsii* in South Africa. Both acacias have a seed-feeding *Melanterius* species established, and although high seed-destruction levels have been recorded, this has been confined to a small proportion of release sites and appears to be subject to seasonal fluctuation. Further monitoring is required to evaluate the efficacy of *Melanterius* on these acacias.

The recent release, establishment and expansion of *D. dielsi* populations on *A. cyclops* in South Africa offer an opportunity to evaluate interspecific competitive effects between *D. dielsi* and *M. servulus* and how these may affect total seed production of the host species. Future monitoring efforts should concentrate on these possible interactions as this information may be of importance in considering the suitability for release of the Tiny Floret Galler as a biological control agent for *A. mearnsii* in South Africa, where *M. maculatus* is presently utilized as a seed-feeding biological control agent. Interspecific competitive effects are relevant to all multi-species biological control programs, but these rarely receive the attention from biocontrol practitioners they deserve.

There are large differences in the morphology and reproductive phenology of *A. mearnsii* and *A. cyclops*. This also applies to the ecology of the *Dasineura* species that occur on these acacias. Therefore, there are limited opportunities to accurately investigate the

effects on insecticides for control of gall-forming Cecidomyiidae or resource-loading impact on the growth of *A. cyclops* with planned extrapolation of results to analogous systems on *A. mearnsii*. If further evaluation of the Tiny Floret Galler is considered necessary, then wild outbreak populations of this insect on *A. mearnsii* in Western Australia provide the ideal locality for this research. Alternatively, experiments to refine insecticide-suppression of the Tiny Floret Galler could be considered in remote populations of *A. mearnsii* in South Africa. These may serve as quarantine sites, but as small cecidomyiids are dispersed readily by wind, there is a high risk that this approach would result in the Tiny Floret Galler invading *A. mearnsii* sites elsewhere in South Africa sooner than expected.

Sufficient data has been collected to demonstrate that the Tiny Floret Galler is suitable for release in South Africa for the biological control *A. mearnsii* seeds. The process of application for release from quarantine in South Africa entails public consultation and involvement with interested and affected parties that can extend over several years. The commercial wattle growing industry in South Africa is an essential organisation in the consultation process, and their involvement in the early stages of the research development program has avoided disruptive disputes over how to proceed (Adair 2002a). Although evaluation of indirect competitive effects between *Dasineura* and *Melanterius* is advisable before the release of the Tiny Floret Galler on *A. mearnsii* occurs, this need should not delay the release application process, which should commence immediately so that possibly unforeseen issues can be addressed.

While the primary focus of this project has been to determine the potential the Tiny Floret Galler and *D. dielsi* as biological control agents in South Africa, the ecology and biology of the Egg Galler and the Glabrous Bud Galler requires further attention, so that these insects may also be considered in a fully integrated biological control program for *A. mearnsii*. Both cecidomyiids have low gall biomass, narrow host ranges and can occur in outbreak populations in their native range. The Glabrous Bud Galler develops in young buds, an acceptable, yet previously untargeted organ for biological control of *A. mearnsii*. The use of these agents in combination, together with *M. maculatus,* may eventually achieve the levels of control that is required to render *A. mearnsii* a useful, but benign tree in South Africa.

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