Ecology of the *Fergusonina* fly and *Fergusobia* nematode gall association in South Australia

by
Elise Head

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Statement of originality

I hereby declare that the research work presented in this thesis is original and has not been previously submitted in full or in part to any other university or institution for any kind of degree. This thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis being available for photocopying and loan by any interested person.

Elise Head

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Abstract

Attempts were made to grow *Fergusobia* nematodes in a dual aseptic culture with *Eucalyptus camaldulensis*. Callus tissue was grown from *E. camaldulensis* stem pieces in aseptic conditions. Calli were prone to deterioration after 14 days unless transferred to fresh growth medium. Lower levels of solutes (25% Murashige and Skoog salts, 25% plant vitamins and 0.5% sucrose) were more successful than published concentrations.

Fergusobia J2 nematodes were surface sterilised with either Hibitane or washed with water to prepare them for inoculation of callus (Hay, 1994). *Fergusobia* subsequently recovered from plates of callus were all dead, which raised questions of how the nematodes are suited to Murashige and Skoog salt solutions.

The survival of *Fergusobia* in aqueous solutions was then observed. It has been assumed that *Fergusobia* live about 2 days after dissection. Amphimictic nematodes from *E. camaldulensis* axial bud galls were used for a survival study. Nematodes lived for as long as 12 days in fresh tap water and 11 days in 1% M.S. solution. They were more active in tap water than in 1% M.S. solution. Nematodes in a dish together with dissected gall material died within 2 days. Nematodes in a separate dish with fresh tap water and clean gall fibres were observed to gather around the fibres. Observations of *Fergusobia* could be made within fresh solutions providing deteriorating gall tissues were removed from the dish.

Gall production was attempted on *E. camaldulensis* grown in the glasshouse. These tree saplings were pruned to encourage new growth and periodically treated for infestations of scale insects leading to growth of sooty mould. Two forms of cage construction were used: (1) 1 m^3 screened cages and (2) acetate sleeves as used by Goolsby *et al*., 2000. Within the 1 m^3 cages containing flies, the growing points on saplings were blackened, possibly due to over-exploitation by ovipositing flies. One growing point caged in an acetate sleeve showed oviposition scars but did not produce a mature gall. Production of galls in the glasshouse was hindered by a lack in coincidence of flies emerging from mature galls and the flush of new growth following pruning. The production of galls within the glasshouse was not achieved.

The phenology of *E. camaldulensis*, a host for the *Fergusonina*/*Fergusobia* mutualism, and gall ecology were observed in a two-year, non-destructive, field study in the Urrbrae Wetlands, Adelaide, South Australia. Tree growth and gall development was observed in the lower regions (0

– 2 m) of young trees. Three bud forms, terminal leaf bud, axial leaf bud and flower bud galls were monitored on the trees.

The densities of galls were highly seasonal. Greatest density of growing points, axial leaf bud and flower bud galls occurred mostly during mid-winter to spring, whereas that of terminal leaf bud galls occurred during mid-spring to summer.

Galling of flower buds did not appear to influence flowering and more flower buds and flowers occurred in the second year of the study as the trees matured.

Trees mostly had medium (30-70%) levels of leaf damage, but there were seasonal trends in damage levels. Low scores for leaf damage were associated with increases in flower bud and flower production. Leaf damage, including sooty mould, appeared to increase during the cooler winter months. There were no significant seasonal relationships between levels of leaf damage and either growing point density or the occurrence of galls. When trees were compared with each other, those with lower leaf damage were more likely to have more growing points. The appearance of the canopy and the likelihood of a tree to have galls varied greatly between the trees. One tree was particularly susceptible to leaf attack, rarely had new leaves and produced no mature galls during the study. The colouring of leaves varied between trees, which indicates possible genetic variations causing some trees to be more likely hosts for *Fergusonina*/*Fergusobia*.

Both new vegetative growth and terminal leaf bud galls were concentrated on the northern and eastern quadrants. Axial leaf bud and flower bud galls occurred more on the western or southern quadrants where they were possibly more protected from sun exposure on the northern or eastern quadrants. Axial galls on the northern side of one tree became reddened while those in the southern and western quadrants remained green. Reddening of axial galls may increase their likelihood of parasitism and predation by birds.

Each of the three gall forms occurred within certain positions in the canopy. The tree host resource is partitioned effectively, with the three gall forms occurring on three different host structures. Additionally, the two vegetative forms terminal leaf bud and axial leaf bud galls occur on different shoot regions and in different seasons. The numbers of the galls is probably also affected by biotic and climatic influences. Parasitism, plant canopy shading, nutrient levels and host genetics are possible influences.

Assuming an interval of 4 weeks between oviposition and first observation within the current study, terminal leaf bud galls had an average longevity of 11 weeks and axial leaf bud galls an average longevity of 14 weeks. Flower bud galls had longevities of 14 to 27 weeks from oviposition to senescence, assuming an interval of 6 weeks between oviposition and first observation within the current study. Flowers and flower buds occur irregularly within the eucalypts so it would be advantageous for flies and nematodes developing within flower bud galls to have extended or variable longevities to allow fly emergences to coincide with new flower buds.

Not all of the galls recorded matured to produce adult flies. Nearly half of the terminal leaf bud galls initiated were aborted, recorded as absent, parasitised or eaten (45% of initiated galls). Of the three gall forms, they were the most prone to obvious parasitism and as many as 12 hymenopteran species have been reared from terminal leaf bud galls on *E. camaldulensis* (Taylor *et al*., 1996). These galls obviously provide a resource for many species within multiple trophic levels. Fourteen percent of axial leaf bud galls were absent or eaten and birds were seen breaking off and feeding on the galls. More than half (55%) of the initiated flower bud galls disappeared during the period of observation, possibly due to the foraging of birds. Destructive sampling and rearing out of parasitoids from both axial leaf bud and flower bud gall forms is needed to establish what species exist within them.

Terminal leaf bud galls ranged from 7.5 to 30.1 mm in diameter and 10.0 to 43.6 mm in length. Flower bud gall size varied, with the largest being 15.0 mm by 22.3 mm. Axial leaf bud galls, ranged from 2.6 to 13.0 mm in diameter and length ranged from 2.3 to 10.5 mm. The larger axial leaf bud galls were nodular and appeared to have multiple locules. Destructive sampling and rearing out of flies is needed to establish the relationship between size and numbers of flies emerging.

Terminal leaf bud galls increased in size, including many locules and exit holes per gall. Axial leaf bud galls were much smaller than the terminal leaf bud galls and 99% had only one to three exit holes. The rounded shape and presence of few locules within the axial leaf bud galls indicate that this form is limited to a shape and size producing few flies. The observation of greater size of terminal leaf bud galls suggests that these galls may have multiple foundresses. Twelve of the 13 flower bud galls with exit holes had either one or two holes. In flower bud galls on *E. camaldulensis*. the operculum remains sealed and the characteristic *Fergusonina* “window” appears at the side of the flower bud before fly emergence through a single hole. Destructive sampling is also necessary to determine parasitism of each of the gall forms.

1. Introduction

1.1. Plant galls and gall forming processes

1.1.1. The definition and function of cecidogenesis

Gall formation, or cecidogenesis, is one form of a plant response to invertebrate herbivory. This results in a swelling or neoplastic out-growth of plant tissue, usually as a defensive response to foreign organisms. The formation of a gall involves complex interactions between plants and organisms and is evidence of reciprocal adaptations that are highly specific between the plant host species and gall-inducer.

Gall-inducing organisms are highly specialised, primarily sessile herbivores, which redirect the development of a region of plant tissue and benefit from the resources that the resultant gall provides. Gall formation represents complex physiological and biochemical responses of plants to gall-inducer herbivory. As an adaptive strategy, gall formation may include both advantages and compromises to host and gall-inducer (Ananthakrishnan, 1984; Price *et al.*, 1987; Mani, 1992). Host plants may respond to the foreign invader by isolating the organism to a specific plant region where it may be vulnerable because it is sessile (Cornell, 1983; Mani, 1992). Given gall-inducers usually become immobilised within a gall, it may not be able to escape competition with other gall organisms and may be vulnerable to specialised predators or parasites. However, gall-inducers benefit by having a microhabitat buffered from exposure to the external environment, where nutritional resources are available locally, and they are protected from most general predators. The adaptive advantages to gall-inducers have been hypothesised as microenvironmental, nutritional, and protection from enemies (Ananthakrishnan, 1984; Price *et al.*, 1987). These variables alter according to plant host and gall species, gall type or size, and season (McCalla *et al.*, 1962; Shekhawat *et al.*, 1978; Hartnett and Abrahamson, 1979; McCrea *et al.*, 1985).

Galls give limited buffering against temperature changes, but provide humid conditions which reduce desiccation, particularly for larvae that have a seasonal diapause (Abrahamson and Weis, 1987). Gall tissue may also act as a buffer against changes in salinity and environmental variations which affect the host plant (Martel, 1995; Fay *et al.*, 1996; Martel, 1998).

Gall inducers are able to intercept and redirect nutrients from the host plant, as well as prolong the production of new growth. During periods of rapid gall growth, the gall animal has access to proliferating layers of nutritive cells and additional nutrients that the host plant imports from other tissues. In many galls, the redirection of nutrients allows them to become metabolic sinks for photosynthates, soluble nitrogen, amino acids, minerals and phosphorus (Wallace, 1974; Bird and

Loveys, 1975; Stinner and Abrahamson, 1979; McCrea *et al.*, 1985; Abrahamson and McCrea, 1986; Paquette *et al.*, 1993). Many galls also have increased concentrations of secondary plant compounds such as phenolics and tannins, which vary both seasonally and within gall tissues (Shekhawat *et al.*, 1978; Hartley, 1998). The presence of secondary compounds may represent plant defence since they decrease the nutritional value of gall tissue. However, they may also be sequestered by the gall-inducer to protect it from attack from such agents as fungi, parasitoids (which lay their eggs on or into primary gall-inducers) or generalist folivores (Shekhawat *et al.*, 1978; Cornell, 1983; Taper and Case, 1987; Gfeller *et al.*, 1995; Hartley, 1998).

Debate exists, however, over the ability of galls to protect gall organisms from enemies (Price *et al.*, 1980; Weis and Abrahamson, 1985; Price *et al.*, 1987; Hawkins *et al.*, 1997). By living within plant tissues, a gall insect for example, may avoid direct predation from generalist insectivores, but then be prone to parasitoid attack. Small galls or galls induced late in the season are more susceptible to parasitoids with short ovipositors, and an increase in gall diameter generally provides more protection against parasitoids (Price *et al.*, 1980; Weis and Abrahamson, 1985; Price and Clancy, 1986; Zwolfer and Arnold-Rinehart, 1994). Although larvae within larger galls may escape parasitoid attack, they are more prone to bird or rodent predation and excessive competition with other gall organisms (inquilines) such as lepidopteran larvae and fungi (Price *et al.*, 1980; Weis and Abrahamson, 1985; Brooks and Shorthouse, 1997; Dezousa *et al.*, 1998; G. Taylor, pers. comm.). Aside from the arguably protective nature of gall size, other aspects of gall morphology provide protection for the gall inhabitants, such as the presence of surface resins and trichomes (spines) which impede the action of small predators and parasitoids (Price *et al.*, 1980; Stone and Cook, 1998).

1.1.2. Gall-inducing taxa and descriptions of gall forms

Galls are induced by a vast array of heterotrophic taxa, which include representatives from the Fungi, Eubacteria, Protista and Animalia. The Cyttariaceae are cecidogenic fungi that produce galls on tree twigs; some Eubacteria cause crown galls, and protists such as club-root induce tumours on crucifers (Talbot, 1971; Agrios, 1997; Hartley, 1999).

Within the kingdom Animalia, gall-inducing nematodes and arthropods are summarised in Table 1. This table includes the majority of confirmed gall-inducing taxa, but is not exhaustive. Many more species of invertebrates are gall-associated or have been reared from galls.

Table 1: Summary of gall-inducing invertebrates.

Taxon	Order	Family with gall-inducers	Gall type
Phylum Nematoda			
Class Adenophorea	Dorylaimida	Longidoridae (1)	terminal root galls of agricultural hosts
Class Secernentea	Tylenchida	Criconematidae (1)	root galls of agricultural hosts
		Pratylenchidae (1)	necrotic lesions that become gall-like on agricultural hosts
		Heteroderidae (1)	root-knot galls of agricultural hosts
		Tylenchidae (1)	stem/bulb galls and galls of wheat seeds
		Aphelenchoididae (2, 3)	galls of fig plant leaves, fruit and seeds
		Sphaerulariidae (4)	nematode/fly gall association within leaves and flower buds of myrtacaeous hosts
Phylum Arthropoda			
Class Arachnida	Acarina	Tarsonemida (5, 6)	mites cause simple curled leaves with hypertrophy
		Eriophyidae (5, 6)	mites cause diverse galls of leaves, stems and buds
Class Insecta	Thysanoptera	Phlaeothripidae Terebrantia (7)	thrips cause simple and globose leaf galls and flower bud galls
	Lepidoptera	Tortricidae (8, 9)	micro-moths cause diverse leaf and stem galls
	Coleoptera	Curculionidae (10, 11)	weevils cause simple to organoid galls on fruits, flowers stems and shoots
	Hemiptera	Tingidae (8)	lace bugs cause mostly floral galls on specific hosts
		Cercopidae (8)	spittle bugs inhibit growth and cause wrinkled leaves on many hosts
		Psyllidae (12)	psyllids cause pit to pouch and organoid galls on dicot leaves
		Eriosomatidae (13)	aphids cause simple leaf and shoot galls to complex galls on various hosts
		Adelgidae (13)	aphids cause covering galls on pine and spruce hosts
		Phylloxeridae (13)	aphids cause simple pit to dehiscent pouch galls
		Coccidae (14)	scale insects cause simple galls on leaves
		Asterolecaniidae (14)	pit scale insects cause simple to organoid galls
		Diaspididae (14)	armoured scale insects cause simple galls
		Eriococcidae (14, 15)	scale insects cause sexually dimorphic galls from simple to adorned globular form
	Diptera	Cecidomyiidae (16)	gall midges cause simple to pouch or piston galls which often open by dehiscence on leaves, stems and roots
		Tephritidae (17)	fruit flies cause a variety of gall forms on leaves, stems and shoots of phanerogams, Compositae and ferns
		Chloropidae (18)	induce shoot galls on phanerogams
		Agromyzidae (16, 19)	leaf miners which induce complex galls
		Anthomyiidae (20)	induce simple galls on subterranean stems
		Fergusoninidae (4)	fly/nematode gall association within leaves and flower buds of myrtacaeous hosts
	Hymenoptera	Tenthredinidae (21)	sawflies cause complex galls on leaves and stems of various hosts
		Agaonidae (22)	wasps cause galls within fig fruit
		Pteromalidae (23, 24)	wasps cause complex woody galls on shoots of various hosts
		Eurytomidae (23, 25)	wasps cause woody galls on twigs of citrus and gall fruits of other hosts
		Torymidae (12)	induce bud galls on *Hakea* and other Australian trees
		Tanaostigmatidae (23); (26); (27)	induce galls in leguminous and Euphorbiaceae hosts
		Eulophidae (23, 28)	induce galls on eucalypt leaves, shoots and stems
		Cynipidae (29)	wasps cause a range of complex galls on various host organs
		Braconidae (30)	induce stem galls on *Banksia* trees

Source: (1)Maggenti, 1981; (2)Hunt, 1993; (3)Kjellberg *et al.,* 2005; (4)Taylor *et al*., 1996; (5)Westphal, 1992; (6)Channabasavanna and Nangia, 1984; (7)Ananthakrishnan, 1992; (8)Dreger-Jauffret and Shorthouse, 1992; (9)Martel, 1995; (10)Le Pape and Bronner, 1987; (11)O'Brien and Pakaluk, 1998; (12)Hodkinson, 1984; (13)Wool, 1984; (14)Beardsley, 1984; (15)Gullan, 1984 (16)Rohfritsch, 1992; (17)Freidberg, 1984; (18)Vandevyvere and de Bruyn, 1998; (19)Eckberg and Cranshaw, 1995; (20)Gassman and Shorthouse, 1990; (21)Price, 1992; (22)Bronstein, 1992; (23)Boucek, 1988; (24)Bagatto and Shorthouse, 1994; (25)Askew and Blascozumeta, 1998; (26) Prinsloo and Lasalle, 1995; (27) Weekley, 2000; (28) Redak and Bethke, 1995; (29) Askew, 1984; (30) Austin and Dangerfield, 1998.

All plant tissues are susceptible to galling, including roots, stems, leaves, flowers and fruits, with leaves being the dominant host tissue of gall insects. A wide variation in gall forms results from different types of tissue being disrupted and plant organs reorganised. In most cases undifferentiated meristematic plant cells are the target of gall organisms, although some species of cynipid wasps induce galls on fully developed tissues by inducing even senescing autumn leaves to produce secondary meristematic cells (Cornell, 1983). In addition to the influence of host species and tissue type, the developmental stage or sex of the gall-inducer can also affect gall form. Some galls comprise a single chamber that houses one or more individuals, but most galls are an aggregate of multiple chambers, each with at least one primary gall-inducing organism. Although galls have been classified according to a number of criteria (e.g. Felt, 1940; Abrahamson and Weis, 1987; Rohfritsch, 1992; Gullan and Cranston, 1994), here they are loosely categorised as simple or complex, based on their degree of structural complexity and the way they develop.

Simple galls are also described as kataplasmic galls (Abrahamson and Weis, 1987); they are usually irregular in size and shape and possess poorly differentiated tissues. Examples of simple galls are tumour or callus galls, which are formed from undifferentiated parenchymal tissue that becomes meristematic. Tissue conversion occurs in response to increased plant growth regulator activity and wounding stimuli associated with the gall-inducer. Typically, the simplest galls (such as pit, roll and fold galls) are caused by non-insect agents. In general, nematodes produce simple galls of roots and leaves and can inflict severe damage upon agricultural hosts depending on the intensity of infestation (Nickle, 1991). Because of the aquatic nature of nematodes, most nematode galls occur on underground plant tissues. When nematodes are found within aerial plant galls, they are often in a close association with arthropod vectors. These carry and deposit the nematodes inside suitably humid plant tissues, to induce galls in association with the arthropod gall larvae. Further discussion of these associations will follow in Sections 1.2 and 1.3.

Complex galls are also described as prosoplasmic galls (Abrahamson and Weis, 1987). They form a consistent external shape and have tissues that are differentiated into well-defined zones. They result from significant modification of the original host organ, virtual or entire encapsulation of the gall organism and plant tissues which are differentiated into distinct layers usually including lining cells referred to as nutritive tissue (see Fig. 1). Most of the arthropod taxa listed in Table 1 produce galls of the complex category.

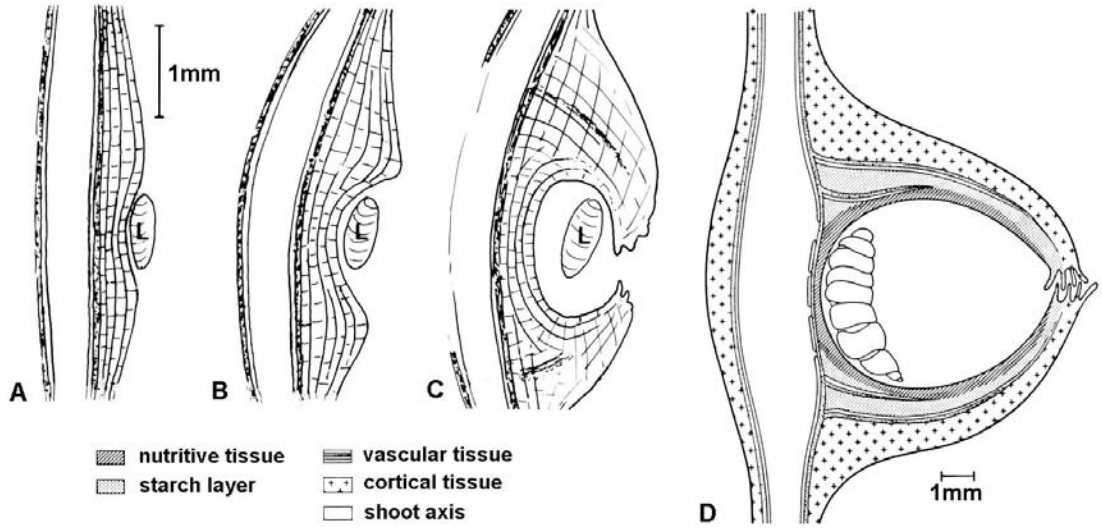


Fig. 1: An example of a complex gall, formed by the cecidomyiid *Geocrypta galii*, showing developmental stages and tissue layers (from Rohfritsch, 1992).

1.1.3. Gall formation processes

The ability of an animal to induce a gall and maintain the production of gall tissues is not fully understood, but the physiological response of the plant follows a generally predictable pattern. Following initiation, galls must grow and be structured to allow the release of the mature gall inducer when the gall matures. Many galls have regions that the inducer can easily chew through to escape and some have dehiscence mechanisms related to physiological and chemical changes to gall growth (Rohfritsch, 1992).

Various stimuli provided by the gall-inducer, including both mechanical and chemical factors, act upon the genotype and phenotype of plant tissues to produce a phenotypic gall response (see Fig. 2). This combination of cecidogenic actions is difficult to imitate experimentally because multiple factors act over time to produce a gall and each stimulus has a slightly different function that is intimately coordinated with plant phenology.

Many gall-inducing insects, particularly within the Diptera and Hymenoptera, oviposit into or onto plant organs and the developing larva then either continues or induces gall production from within the plant. Sawflies (Tenthredinidae) induce galls on willow leaves soon after the oviposition event, before the larva emerges from the egg (Hovanitz, 1959). In sawflies a combination of wounding by the sawing action of the ovipositor and the placement of the egg bathed in oviposition fluids causes the early gall response (Rohfritsch, 1992). Compounds within the oviposition fluid stimulate the

growth of preformed galls but further development and maturation of sawfly galls depends on the emergence and continued presence of the sawfly larva (McCalla *et al.*, 1962; Hovanitz, 1959).

Fig. 2: Relationships among gall organism and plant genotypes and the environment in the development of gall phenotype (adapted from Weis *et al*., 1988).

Feeding within galls elicits mechanical irritation and may introduce oral secretions to stimulate gall responses. Physical injury causes both physical and metabolic changes to plant tissues (Edwards and Wratten, 1986). These changes include increased water loss, local changes in nutrients and raised levels of growth regulators, increased respiration, and increased protein synthesis as part of the repair process (Edwards and Wratten, 1986). Damage also increases the production of secondary metabolites such as phenolic polymers which may represent an active defence against herbivory (see Fig. 3; Edwards and Wratten, 1986). Mechanical injury to plant tissues from feeding clearly impacts physical and chemical processes associated with gall formation. The degree of mechanical irritation resulting from feeding within galls varies between taxa because of differences in mouthpart structure. Gall organisms such as the tylenchid nematodes and larvae of both the Thysanoptera and Hemiptera feed via piercing and sucking using a probing spear or stylet (Maggenti, 1981; Ananthakrishnan, 1992; Miles, 1999). *Meloidogyne* species, the root-knot nematode (RKN) is known to use genes acquired from *Rhizobacteria* for gall induction (Opperman and Conkling, 1994). Aphids and other Hemiptera also inject salivary solutions into plant tissues as they penetrate them, and pemphigid gall aphids probe leaf petioles repeatedly in a species-specific pattern thought to aid in gall formation (Miles, 1999). Cecidomyiid larvae have reduced, sharp mouthparts which puncture cells and feed using a sucking action, without provoking cell necrosis (Rohfritsch, 1992). Cynipid wasp larvae break open the cell walls of the gall lining with strong, chitinous mandibles and suck the juices of the nutritive tissue (Bronner, 1992). The tissue lining of the gall is progressively renewed within cecidomyiid and cynipid galls (Bronner, 1992; Rohfritsch, 1992). Other dipteran larvae such as Tephritidae, Chloropidae and Anthomyiidae feed with a rasping action upon solid plant material and produce faeces within galls (Freidberg, 1984;

Gassman and Shorthouse, 1990; Dreger-Jauffret and Shorthouse, 1992; Vandevyvere and de Bruyn, 1998). The larvae of cecidogenic coleopterans, lepidopterans, and wasps (other than cynipids) chew within their galls and destroy the lining, yet are able to maintain the plant tissue hypertrophy and hyperplasy associated with galls.

Increases in concentrations of particular chemicals and molecular factors have been implicated, but no single compound has been isolated as the causative agent of gall formation. The source of the chemicals effecting gall formation is also ambiguous, since they may be manufactured by the gall maker or redirected from plant host origins.

Plant growth regulators such as auxins and cytokinins are associated with the hypertrophic and hyperplasic response of many gall tissues (McCalla *et al.*, 1962; Dimalla and van Staden, 1977; Shekhawat *et al.*, 1978; van Staden and Davey, 1978; Mapes and Davies, 1984; Miles, 1999). Increases in the auxin indole acetic acid (IAA) are associated with rapidly growing organs such as those that are often chosen as gall sites, wounding or damage from herbivory and oxygen deprivation of plant tissues (see Fig. 3; Edwards and Wratten, 1986; Miles, 1999). Increased IAA levels are also found in the saliva and whole larvae of gall insects and within gall tissues (Shekhawat *et al.*, 1978; Mapes and Davies, 1984; Wool, 1984; Miles, 1999). Cytokinins are plant growth regulators that promote plant cell division and have been isolated from the accessory glands, labial glands, faeces and whole larvae of various gall insects, and from whole samples of *Meloidogyne* root-knot nematodes (McCalla *et al.*, 1962; Dimalla and van Staden, 1977; van Staden and Davey, 1978; Elzen, 1983).

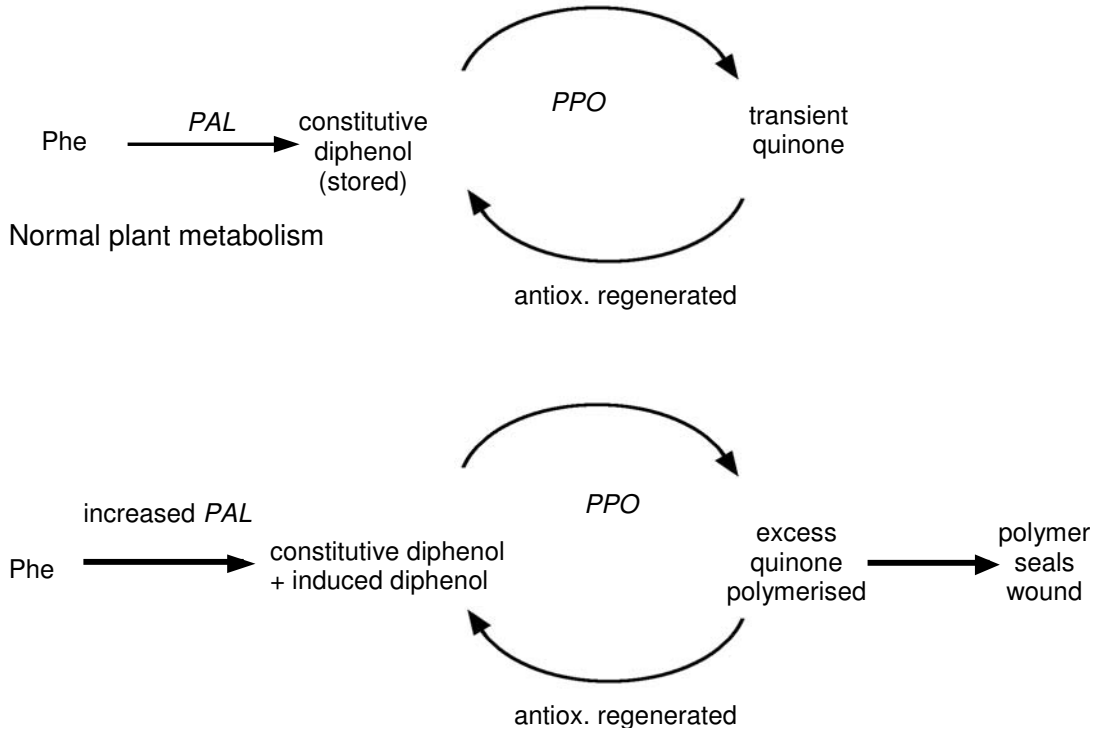


Fig. 3: Models of the wounding and hypersensitivity response pathways in plants; antiox = antioxidant system; const. = constitutive; *PAL* = phenylalanine ammonia lyase; Phe = phenylalanine; *PPO* = polyphenol oxidase (from Miles, 1999).

Secondary plant compounds such as phenolics are associated with chemical defences to wounding and herbivory, as stimulants to insect feeding and oviposition, and are often found in higher amounts within gall tissue (Shekhawat *et al.*, 1978; Hartley, 1998; Hartley, 1999; Roininen *et al.*, 1999). The adaptive function of phenolics within galls has been debated (eg. Taper and Case, 1987; Weis *et al.*, 1988; Hartley, 1998). It has been hypothesised that the adaptation of galling provides enhanced nutrition relative to other modes of herbivory and implies that galled tissues should have lower levels of nutritionally poor compounds such as phenolic chemical defences relative to unaffected plant material (Price *et al.*, 1987). In many cases there are higher concentrations of phenolics within gall tissues which may actually aid gall formation because phenols inhibit IAA oxidase causing a build up of IAA auxin (Miles, 1999). In addition, increased concentrations of phenols may have a protective function for gall organisms (see Fig. 3; Shekhawat *et al.*, 1978; Cornell, 1983; Taper and Case, 1987; Hartley, 1990; Hartley, 1998; Hartley, 1999; Miles, 1999).

Although the presence of growth regulators alone can experimentally stimulate hypertrophy and hyperplasy, mixtures of these chemicals do not produce the morphological differentiation associated with complex galls (Cornell, 1983). For this reason it has been suggested that gall-inducing organisms genetically manipulate plant tissue as gall tissue is formed (Opperman and Conkling, 1994). Crown-gall formation, for example, results from genetic manipulation of plant tissue as the bacterium *Agrobacterium tumefaciens* controls the metabolism of gall tissue by the transfer of plasmid DNA into plant host cells (Ream and Gordon, 1982). The formation of root-knot galls by plant parasitic nematodes such as *Meloidogyne* species also includes alteration of plant host gene expression (Opperman and Conkling, 1994). In the case of insect galls, it has been suggested that genetic transformation of plant tissues may occur via viroid-like particles acting in mutualistic association with gall organisms to form highly differentiated, complex gall structures (Cornell, 1983; Price, 1992). Viroid particles may act as regulator genes, which could override normal plant growth and stimulate the structural sequences necessary for the abnormal development of plant tissue to form galls (Price,1992).

1.2. Ecology of galls and gall associations

The gall habitat provides a resource that supports multiple trophic levels of organisms (Askew, 1980; Price *et al.*, 1980; Askew, 1984; Price, 1992). In addition to the host plant and its primary gall-inducer, many other organisms including fungi, bacteria and various arthropods are associated with galls (Wiebes-Rijks and Shorthouse, 1992; Graham, 1995; Taylor *et al.*, 1996). There is a spectrum of interactions among the gall organism communities that include competitive, predatory,

parasitic, commensal, and mutualistic processes. Plant gall communities are formed from an assemblage of various interacting species centred around the habitat of a gall or gall host. Some examples of gall assemblages are given in Figs 4, 5 and 6. Gall communities may represent as many as 75 associated species in the case of the cynipid (*Biorhiza pallida*) oak gall (Mani, 1964). Many ecological processes occur within gall species, particularly within galls that contain multiple individuals. Galls of homopterans and thrips, for example, are initiated by a colonising foundress which produces many parthenogenetic offspring (Wool, 1984; Crespi *et al*., 1997). Aphid foundresses are known to compete for gall sites and some galls are communally induced (ie. have multiple foundresses) (Wool, 1984; Ngakan and Yukawa, 1996; Miller, 1998). This creates opportunities for competition and kin selection within the gall, where the fitness of some gall aphid individuals is reduced by an increase in the mean number of foundresses (Miller, 1998). Intraspecific fighting is common within thrips and aphid galls, and galls are often usurped by conspecifics or members of other species (Akimoto and Yamaguchi, 1997; Crespi *et al*., 1997). These taxa exhibit complex life cycles and reproductive strategies that often create complex social systems (Wool, 1984; Crespi *et al*., 1997). More than 50 species of aphid and many species of thrips possess morphologically specialised soldiers, which defend the galls against intruders (Foster and Rhoden, 1998; Morris *et al*., 1999).

Gall induction and formation may represent the combined efforts of more than one taxon via apparently symbiotic associations (Currie, 1937; Graham, 1995). For example, fergusoninid flies and nematodes act together to produce galls on Myrtaceae, while cecidomyiid flies and fungi together form galls on Lythraceae (Currie, 1937; Graham, 1995). The description and ecology of *Fergusonina* fly and *Fergusobia* nematode initiated galls will be covered in Section 1.3.

Inquilines share the gall resource and generally act as competitive herbivores, often leading to the death of the primary gall-inducer (Swanton, 1912; Price, 1992; Brooks and Shorthouse, 1997). In studies of the primary gall-inducing cynipid species *Diplolepis nodulosa*, up to 65% of an annual population sample were killed by inquilines, and parasitoids caused a further 17% mortality of inducers within the same year (Brooks and Shorthouse, 1997).

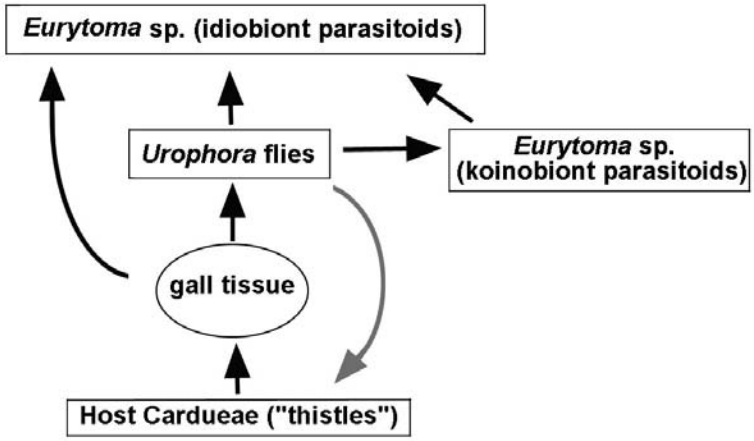


Fig. 4: Energy flow within the *Urophora* fly gall. The grey line indicates stimulation for gall production. The idiobiont *Eurytoma* wasp parasitoids either act as ectoparasitoids which kill the *Urophora* host immediately or are phytophagous inquilines. The koinbiont *Eurytoma* parasitoids are endoparasitoids which feed upon the developing *Urophora* fly larva (adapted from Zwolfer and Arnold-Rinehart, 1994).

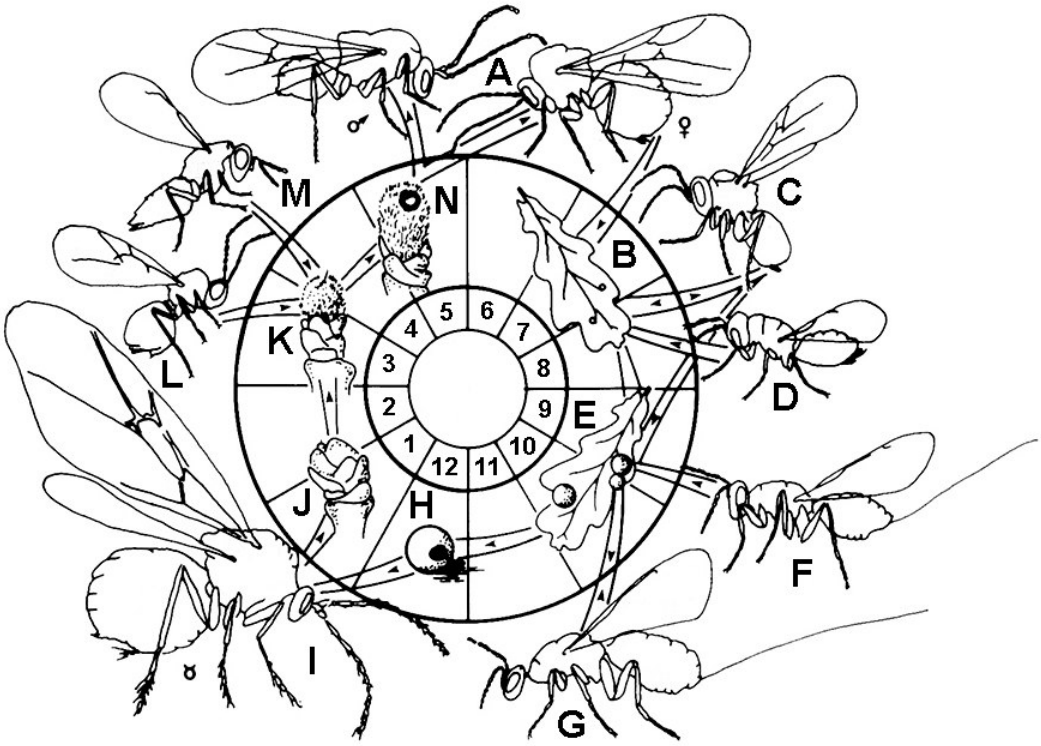


Fig. 5: Life cycle, gall forms and associated inquilines and key parasitoids of the oak cynipid *Cynips quercusfolii*. Months of the year are shown on the inner circle. A, male and female of the sexual generation; B, immature agamic leaf galls; C, *Synergus* inquiline; D, *Eurytoma* parasitoid; E, mature agamic leaf galls; F, first generation *Torymus* parasitoid; G, second generation *Torymus* parasitoid; H, agamic gall post emergence; I, agamic adult; J, oak bud; K, immature sexual gall; L, *Synergus* inquiline; M, *Mesopolobus* parasitoid; N, mature sexual gall post emergence (from Wiebes-Rijks and Shorthouse, 1992).

Gall parasitoids include ectoparasitoids, that lay their eggs upon the host larva, and endoparasitoids, that lay their eggs within the gall inducer. In both cases the emerging parasitoid larvae develop at a cost to the gall herbivore, causing the death of the herbivore in most cases (Zwolfer and Arnold-Rinehart, 1994). In a study of *Diplolepis triforma* (Cynipidae) stem gall communities, more than 85% of the sampled summer population of gall-inducers were killed by parasitoids (Wiebes-Rijks and Shorthouse, 1992). Some species of parasitoid are highly host specific, but many are opportunistic and parasitise various gall hosts (Zwolfer and Arnold-Rinehart, 1994; Stinner and Abrahamson, 1979; Wiebes-Rijks and Shorthouse, 1992; Askew, 1980). The parasitoid guild associated with galls can include many interacting species (as can be seen in the assemblages depicted in Fig. 4, 5 and 6). The structure of this guild may alter through the seasons, particularly where there is alternation of generations of gall species and each generation produces galls of different characteristics (Askew, 1980).

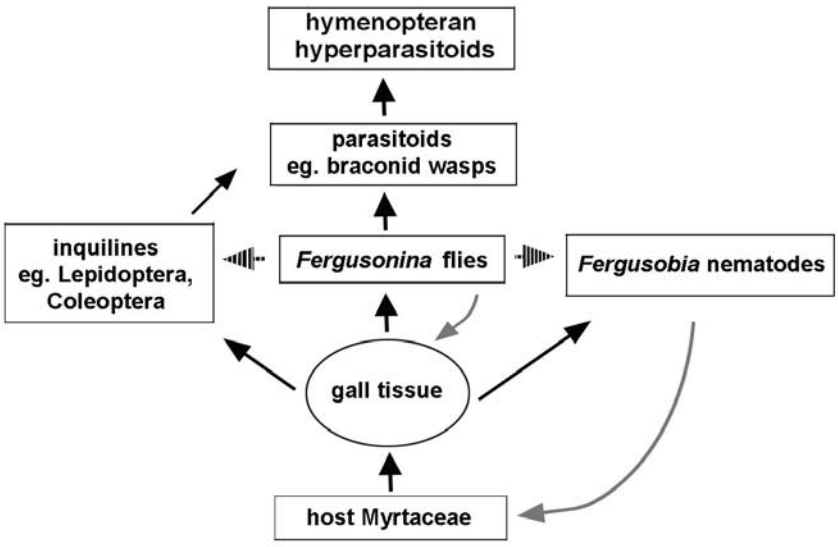


Fig. 6: Energy flow within the *Fergusonina* fly/*Fergusobia* nematode gall. Grey lines indicate stimulation for gall production, stippled lines indicate variable energy flow.

Plants provide essential cues to enable predators and insect parasitoids to select gall hosts (Vinson, 1976; Price *et al.*, 1980; Weis and Abrahamson, 1985). For instance, parasitoids restrict their search patterns to galls within specific host plants and plant regions (Price *et al.*, 1980). The tendency to parasitoid attack of cecidomyiid and cynipid gall-inducers is relative to the position of galls within leaves, and cynipid oak gallers within catkins may be less parasitised than in leaf galls (Askew, 1961; Plantard and Hochberg, 1998). The survival and community structure of many gall insects relates to gall size, which usually varies with plant genotype and may be correlated with the length of parasitoid ovipositors (Weis and Abrahamson, 1985; Price and Clancy, 1986). Gall size

determines the structure of the parasitoid complex within some cynipid galls, as they are attacked by different species of parasitoid during the growth of galls (Plantard *et al.*, 1996). As a consequence of these relationships, it has been suggested that plant hosts and parasitoids are mutualists, since plants assist parasitoids to recognise gall hosts, and parasitoids aid in plant defence (Hartnett and Abrahamson, 1979; Price *et al.*, 1980; Weis and Abrahamson, 1985). Against this argument, many galls possess plant defences such as chemicals, secretions and trichomes that hinder predation and parasitism of gall herbivores (Price *et al.*, 1980; Taper and Case, 1987; Stone and Cook, 1998).

The ecosystem of a gall relates closely to the host plant as a primary food source and habitat. Most gall-inducers choose particular plant organs or regions of specific host plants. Host recognition depends on factors such as visual and chemical cues, governed by the host plant genotype (Price *et al.*, 1980; Price *et al.*, 1999; Roininen *et al.*, 1999). Figure 2 depicts the relationship between the genotypes of both plant and gall-inducer to produce gall phenotype. The plant host, therefore, impacts gall characters through its phenology, morphology and chemistry, which subsequently affects the community structure within the gall (Price *et al.*, 1980; Taper and Case, 1987).

The biogeography of gall organisms is controlled by factors such as host distributions, climate and the palaeogeological relationships of land masses. Cecidomyiid gall midges are distributed on all continents; cynipine gall wasps are confined mostly to holarctic regions; thrips (Thysanoptera) occur mostly (85% of gall species) in the oriental and Australian zoogeographic regions; and scale insects (Hemiptera) are primarily tropical or Australian (Felt, 1940; Ananthakrishnan, 1984; Beardsley, 1984 Gagne, 1984). The northern continents share many gall species such as sawfly and cecidomyiid genera on willow hosts; adelgid aphid genera on spruce and conifer hosts; and cecidomyiid genera on pines (Gagne, 1984). There are also biotic affinities throughout southern regions which include thrips genera and the *Fergusonina* fly and *Fergusobia* nematode gall association which are Australasian (Ananthakrishnan, 1984; Evenhuis, 1989; Harris, 1982). However, because of its relatively long period of geographical isolation, Australia possesses many endemic species of gall insects such as eriococcid scales (Beardsley, 1984; Gullan, 1984).

The structure of vegetation is correlated with trends in assemblages of herbivorous and parasitoid species (Askew, 1980; Price *et al.*, 1980). In general, trees provide more sites for gall formation than shrubs or herbs which then attract diverse species of polyphagous parasitoids (Askew, 1980). Agromyzid fly galls are an exception, being most numerous on herbaceous plants (Askew, 1980). Plants growing in association with the host plant can affect gall communities by providing nectar and pollen sources for adult parasitoids and predators (Price *et al.*, 1980).

It is generally considered that plant fitness is neither affected or reduced by gall formation, but in rare cases plants are dependent upon gall organisms for their reproduction (Ananthakrishnan, 1984; Price *et al.*, 1987; Bronstein, 1992). Such an obligate mutualism has developed between *Ficus* species and the agaonid fig wasp pollinators. Female fig wasps enter the fig inflorescence, disperse pollen and then lay their eggs within the accessible flower ovaries (Kathuria *et al.*, 1997; Center *et al.*, 1999). These flowers form galls containing the developing wasp larvae and the remaining pollinated, uninfested flowers develop into seeds (Kathuria *et al.*, 1997). Although pollination occurs at a cost to the plant, the wasps are specifically dependent on the fig inflorescences for their development.

1.3. The *Fergusonina* fly and *Fergusobia* nematode gall association

Flies of the genus *Fergusonina* (Diptera: Fergusoninidae) and nematodes of the genus *Fergusobia* (Tylenchida: Sphaerulariidae) induce galls on various Myrtaceae hosts, listed in Table 2.

1.3.1. Life cycles

Fig. 7 summarises the complex life cycle of each genus. A fly egg accompanied by juvenile nematodes is oviposited within undifferentiated, meristematic plant organ tissue (Fig. 7a; Currie, 1937). A phytophagous fly larva emerges from the egg, at about the same time as adult nematodes are first found in the gall. Flies progress through two additional larval instars before pupating within the gall (Fig. 7 b,c ; Currie, 1937). Meanwhile, the nematodes progress through a dicyclic life cycle, which includes both parthenogenetic and sexual (gametogenetic) stages (Fisher and Nickle, 1968). The phytophagous juvenile nematodes deposited by the fly feed and develop into parthenogenetic females (Fig. 7 a,d). These produce a second generation, which develops into mature gametogenetic (amphimictic) females and males that mate (Fig. 7d,e,f). The resulting fertilised, pre-infective females enter only female third instar fly larvae where they lose their outer cuticle and become parasitic within the fly (Fig. 7 g,h; Currie, 1937; Fisher and Nickle, 1968; Giblin-Davis *et al.*, 2001a). Either during pupation or after emergence of the adult fly, the parasitic female nematodes deposit many fertilised eggs into the fly's haemolymph, which develop into juvenile female nematodes (Fig. 7 i; Currie, 1937; Fisher and Nickle, 1968). These nematodes travel to the fly oviducts from where they can be oviposited with a fertilised fly egg to begin a new cycle (Fig. 7 a; Fisher and Nickle, 1968).

Depending on gall type and plant host, more than one generation of flies may be produced annually. Those fly species which form within flower-buds would be expected to complete a generation per flowering season. Some species may have a diapause of unknown duration, particularly if growth is retarded relative to environmental conditions. Further studies of *Fergusonina*/*Fergusobia* gall species are required to clarify factors producing differences in length of gall cycles.

Table 2: Plant host records for the *Fergusonina* fly/*Fergusobia* nematode association.

Host Myrtaceae	Location	Source
Myrtoidea		
Syzigium jambolanum	India	(1, 2)
S. luehmannii	Queensland	(5)
Leptospermoidea		
Angophora apocynifolia	Queensland	(3, 4)
A. floribunda	New South Wales	(5)
A. subvelutina	Queensland	(3)
Corymbia abbreviata	Western Australia	(5)
C. citriodora	Queensland	(6)
C. intermedia	Queensland	(3)
C. maculata	New South Wales	(5, 7, 8)
C. ptychocarpa	Queensland	(5, 6)
C. tesselaris	Queensland	(3)
C. torreliana	Queensland	(5)
C. trachyphloia	New South Wales	(5)
Eucalyptus (Monocalyptus)		
E. acmenoides	Queensland	(3)
E. amygdalina	Victoria; Tasmania	(5, 7)
E. diversifolia	South Australia	(5, 6)
E. haemostoma	New South Wales	(5)
E. macroryncha	New South Wales	(4, 7)
E. obliqua	South Australia	(4, 5, 6)
E. pauciflora	New South Wales	(7)
Eucalyptus (Symphyomyrtus)		
E. albens	New South Wales; Victoria	(5, 7, 8)
E. aromaphloia	South Australia	(5, 6)
E. baueriana	Tasmania	(5)
E. baxteri	Victoria	(5)
E. blakelyi	Australian Capital Territory	(2, 5, 7, 8)
E. brevifolia	Western Australia	(5, 6)
E. camaldulensis	New South Wales; South Australia; Victoria	(7, 5, 6)
E. capularis	Western Australia	(5)
E. confluens	Western Australia	(5)
E. coolabah	South Australia	(5, 6)
E. cosmophylla	South Australia	(6)
E. crebra	Queensland	(7, 8)
E. dalrympleiana	South Australia	(5)
E. dealbata	New South Wales	(5)
E. deglupta	Papua New Guinea; Philippines	(9)
E. drepanophylla	Queensland	(3)
E. fasciculosa	South Australia	(6)
E. gomphocephala	Western Australia	(7)
E. interstans	Queensland; South Australia	(5, 6)
E. intertexta	South Australia	(5, 6)
E. johnstonii	Tasmania	(5)
E. leucoxylon	South Australia	(5, 6, 10)
E. lesouefi	Western Australia	(5)
E. macrorrhyncha	Australian Capital Territory; South Australia	(5, 6)
E. marginata	Western Australia	(5, 6)
E. melanophloia	Australian Capital Territory	(7, 8)
E. melliodora	Australian Capital Territory; New South Wales	(5, 7, 8)
E. microcarpa	South Australia	(5, 6)
E. odorata	South Australia	(6)
E. ovata	Tasmania	(5)
E. polyanthemos	Australian Capital Territory	(7, 8)
E. populnea	Queensland; New South Wales	(3, 5)
E. pruinosa	Western Australia	(5, 6)
E. robusta	New South Wales	(5)

(**Table 2, continued**)

Host Myrtaceae	Location	Source
E. rudis	Western Australia	(5, 7, 8)
E. siderophloia	Queensland	(5, 6)
E. sideroxylon	Australian Capital Territory; New South Wales	(7)
E. tereticornis	Queensland; Victoria	(5, 7, 8)
E. viminalis	South Australia	(5, 6)
Eucalyptus sp.	Queensland	(2)
Eucalyptus sp.	South Australia	(5)
Leptospermum laevigatum	Victoria	(12)
Melaleuca armillaris	New South Wales	(11)
M. cajuputi	Queensland	(11)
M. dealbata	Queensland	(11)
M. decora	Queensland	(5)
M. fluviatilis	Queensland	(11)
M. leucadendra	Queensland; Western Australia	(5, 11)
M. linariifolia	New South Wales	(5)
M. nervosa	Queensland; Western Australia	(5, 6, 11)
M. nodosa	New South Wales	(5)
M. quinquenervia	New South Wales; Queensland	(11)
M. stenostachya	Queensland	(11)
M. viridiflora	Queensland	(11)
Metrosideros excelsa	New Zealand	(5, 6)

(1) Harris, 1982; (2) Siddiqi, 1986; (3) Colbran, 1964; (4) Australian Museum Collection; (5) K. Davies, pers.obs.; (6) G. Taylor pers.obs.; (7) Currie, 1937; (8) Tonnoir, 1937; (9) Siddiqi, 1994; (10) Davies and Lloyd, 1996; (11) Taylor, 2004; (12) R. Adair, pers. obs.

Fig. 7: Life cycles of the *Fergusonina* fly/*Fergusobia* nematode gall association (after Siddiqi, 1986). Not to scale.

1.3.2. Gall forms and morphology

Fergusonina flies follow the pattern of many other gall-inducing insects by selecting leaf meristematic tissue as the dominant plant tissue attacked (Harris, 1982; Taylor *et al.*, 1996; Giblin-Davis, 2000; Giblin-Davis *et al*., 2004a). The Myrtaceae are evergreen hosts with seasonal production of new leaves, providing a regular yet seasonal supply of new plant tissues for fly oviposition. Galls of flower-buds are also common, occurring during seasonal floral production (Currie, 1937; Tonnoir, 1937; Fisher and Nickle, 1968).

Descriptions of fly and nematode species, together with molecular analyses, suggest that each gall form within each host represents a unique species association (Giblin-Davis *et al.*, 2004b and c; Ye *et al.*, 2007). As suggested for cynipid, eriococcid and psyllid galls, morphology of *Fergusonina*/*Fergusobia* galls may be a taxonomically valuable character to assist in species identification (Gullan, 1984; Taylor, 1990; Shorthouse, 1993; K. Davies, pers. comm.).

Many of the *Fergusonina*/*Fergusobia* gall forms have been described subjectively by the collector. In some collections, information about the plant organ forming the gall, and even the plant host species, are unknown (G. Taylor and E. Head, unpub. obs.). Fly larvae live and feed within individual gall chambers or locules, where they are accompanied by nematodes. Galls may be unilocular (containing only one fly larval chamber) or multi-locular aggregates of many fly larval chambers. Some *Fergusonina*/*Fergusobia* galls comprise amorphous developments of juvenile vegetative host structures, with the volume dependent upon the number and arrangement of the fly chambers. The morphologies of galls represent the interactions of a unique *Fergusonina*/*Fergusobia* complex within specific organs of a unique plant host species, and are generally consistent for each complex. Flies that specifically choose lateral or axial leaf buds can only deposit a few eggs and nematodes into these very small structures, producing galls with a single or limited number of chambers. Galls arising from terminal leaf buds are generally much larger, being formed from many more fly eggs laid into a growing point consisting of a cluster of small leaves. Descriptions of the external morphology and histology of gall forms from various hosts are given in Taylor *et al.* (2005) and Giblin-Davis *et al.* (2004a) respectively. Examples of external morphology of gall forms are shown in Fig. 8 (p. 21).

Unilocular vegetative bud galls

Pea leaf galls

These are also known as spherical axial leaf bud galls and are unilocular and pea-sized (2-3 mm; Taylor *et al*., 2005). Typically, they are a stalked axial bud gall or occur as a “pea” at a leaf tip. They have been collected from *E. camaldulensis*, *E. leucoxylon* and *E. microcarpa* (Fig. 8a; K. Davies, G. Taylor and E. Head, unpub. obs.).

Unilocular leaf galls

These are also unilocular, but are hemispherical (not pea-like), and protrude from only one side of a newly expanded leaf. Examples have been collected from *E. marginata* and *E. pauciflora* (K. Davies and G. Taylor, pers. comm.).

Multi-locular vegetative bud galls

Elongate shoot (stem/petiole) galls

Formed at the axes of shoots, these galls form as a swollen stem with one to four chambers and contain elongated fly larvae. The tip of the galled stem or leaf petiole may have small protruding leaves. In one host species the galls are cryptic and chambers can only be seen when held up to bright light. They have been recorded from *E. brevifolia*, *E. camaldulensis*, *E. leucoxylon*, and *E. porosa* (Fig. 8 b; K. Davies and G. Taylor, pers. comm.).

Axial leaf bud galls

Also known as axillary bud galls, these have differing morphologies and occur on a number of plant hosts (Taylor *et al.*, 2005). They always form at a leaf or stem axis and usually have one to six chambers. The galls are usually small (3-5 mm) but can be as large as 10 mm on *Angophora floribunda* (K. Davies, pers. comm.). Larger axial bud galls appear to be a composite of multiple locules and may support as many as 20 fly larvae. Axial bud galls on some hosts have fleshy or hirsute protuberances as in those found on *A. floribunda*, *E. fasciculosa*, and *E. largiflorens* (K. Davies and G. Taylor, pers. comm.). Galls of this form that bear numerous hair-like projections have also been referred to as “Moss” galls and have been collected from eucalypt, *Melaleuca* and *Angophora* hosts (Fig. 8c). “Cabbage” galls comprise proliferations of invaginated leaf structures and are another example of axial bud galls only collected from a *Melaleuca* host (Fig. 8 d; Giblin-Davis *et al.*, 2004a).

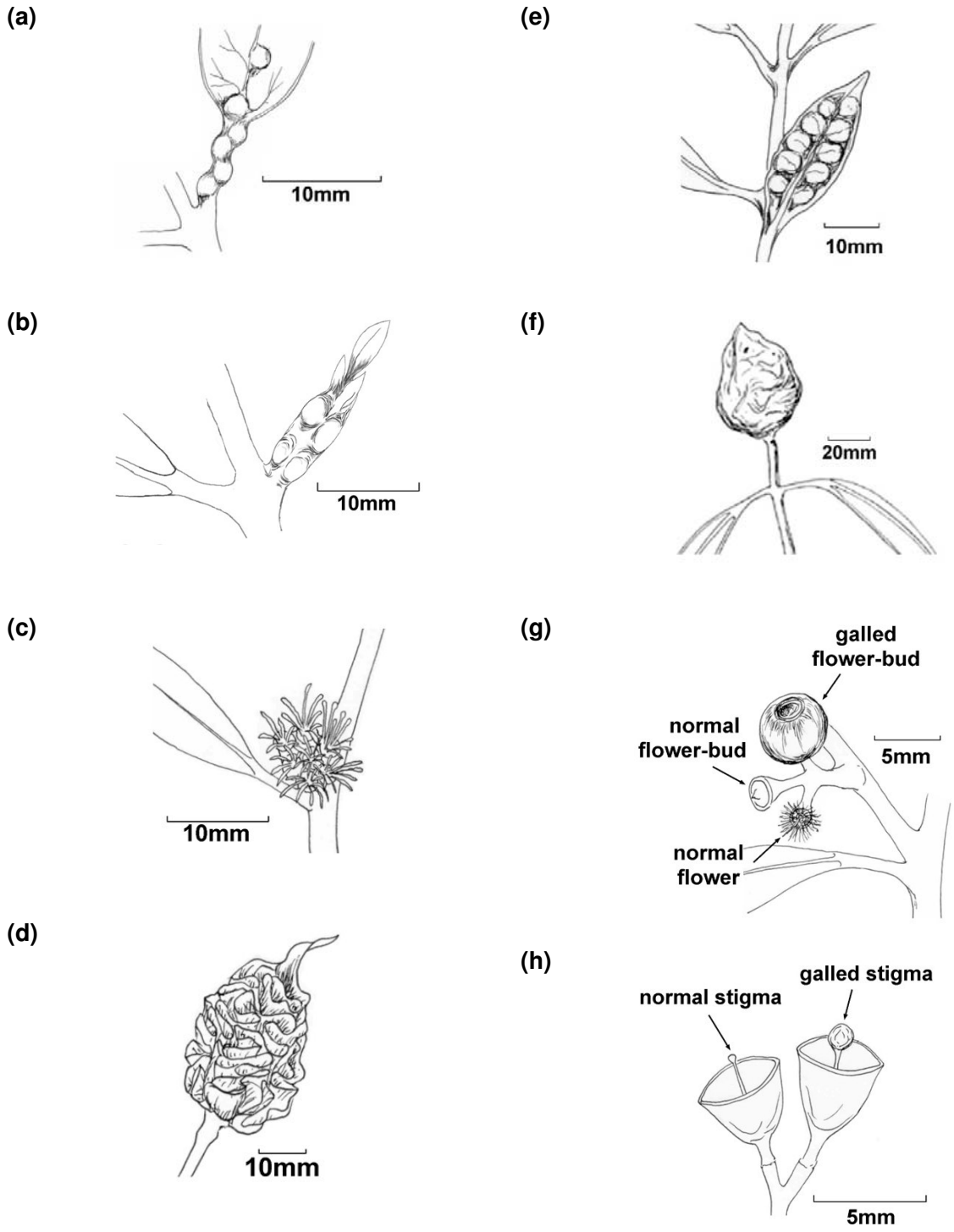


Fig. 8: External morphology of common *Fergusonina*/*Fergusobia* galls of Myrtaceae: (a) pea leaf gall on *E. camaldulensis*, (b) elongate shoot gall on *E. leucoxylon*, (c) "moss" gall on *E. fasciculosa*, (d) "cabbage" gall on *M. dealbata*, (e) flat leaf gall on *E. albens*, (f) terminal leaf bud gall on *E. camaldulensis*, (g) flower bud gall on *E. microcarpa*, (h) stigma gall on *E. fasciculosa*.

Flat-leaf galls

Flat-leaf galls are recorded from *Corymbia* and *Eucalyptus* hosts and are formed along the leaf lamina (see Fig. 8e). Usually the entire leaf is thickened (to 5-10 mm) from the aggregation of chambers that have coalesced walls (e.g. on *E. leucoxylon*). These galls support 10 to 20 fly larvae. However, a variant of this from host plants in Queensland, has rows of locules developing on the leaf lamina, without the thickening of the whole leaf (K. Davies and G. Taylor, pers. comm.).

Terminal leaf-bud galls

These occur on most of the recorded Myrtaceae hosts including *Angophora*, *Eucalyptus* and *Melaleuca* (see Fig. 8f). They are formed at the terminal growing point from a cluster of small leaves or shoots. Morphology varies among hosts but generally terminal leaf-bud galls are quite large (approx. 50 mm in diameter) and are multi-locular, spherical or ovate, bulbous structures containing 10 to 400 fly larvae accompanied by their associated nematodes. Some forms are elongate and woody, but those from *Melaleuca stenostachya* are covered in convoluted leaf tissue (Taylor *et al*., 2005; Giblin-Davis *et al*., 2004a)

Flower bud galls

Flower bud galls develop on many eucalypt and *Melaleuca* hosts. The flower bud swells and contains as many as 20 locules after initiation and within many hosts adult flies emerge as the operculum opens at gall maturity (Fig. 8g; Taylor *et al*., 2005; Giblin-Davis *et al*., 2004a). Within *E. camaldulensis* flower bud galls, however, adult flies emerge from the side of the gall through single exit holes.

Stigma galls

These are swellings of the flower stigma and contain 1 to 2 fly larvae. Stigma galls have been recorded only from *E. fasciculosa* (Fig. 8h; Taylor *et al*., 2005).

1.3.3. Plant host species and biogeography

Fergusonina/*Fergusobia* galls have been recorded from the myrtaceous genera *Angophora*, *Eucalyptus*, *Corymbia*, *Leptospermum*, *Melaleuca*, *Metrosideros* and *Syzygium* within the Australasian and Oriental zoogeographic regions (Currie, 1937; Harris, 1982; Siddiqi, 1986; Siddiqi, 1994; Taylor *et al*., 2003; Scheffer *et al*., 2004; Taylor *et al*., in press; K. Davies and G. Taylor, pers. comm.; R. Adair pers. comm.). Minimal collection of galls has occurred outside Australia and most gall material from an array of host species has come from coastal south-eastern Australia

(including Tasmania) and coastal Queensland (Taylor *et al*., 2005; K. Davies, pers. comm.). Galls are commonly found on broad-leaved *Melaleuca* hosts in the tropical North of Australia (K. Davies and G. Taylor, pers. comm.) Some specimens have also been collected from inland sites within South Australia, New South Wales and Western Australia, although galls are rarely found within areas of dry vegetation such as Mallee scrub (K. Davies, pers. comm.).

Observations of host phenology were made in a non-destructive two year study of population dynamics of *Fergusonina/Fergusobia* galls on *Melaleuca quinquenervia* at different sites in Queensland and northern New South Wales (Goolsby *et al*., 2000). These studies were encouraged by the potential use of the fly/nematode complex for biological control of *M. quinquenervia*, which is a woody weed in the Florida Everglades (e.g. Giblin-Davis, 2000). This study noted similarities between one site at Morayfield (Queensland) and sites in southern Florida which had stands of *Melaleuca* seedlings that had regrown after clearing with little competition from other woody plants (Goolsby *et al*., 2000). The CLIMEX program was used to assess the suitability of Miami, Florida, and to compare it with host locations in northern NSW and Queensland, by matching the long-term meteorological data of target locations (Goolsby *et al*., 2000). The Morayfield site had a high density of galls and it was predicted that if introduced as a biological control agent, *Fergusonina/Fergusobia* galls would develop on hosts in similar conditions within Florida (Goolsby *et al*., 2000).

1.3.4. Gall initiation and growth

As with all galls, *Fergusonina*/*Fergusobia* galls represent the combined effects of organisms and plant responses to produce the gall phenotype. In the case of *Fergusonina*/*Fergusobia*, it is likely that both fly and nematode species act upon plant tissues to cause the formation of these complex galls.

Gall initiation is thought to primarily involve the action of nematodes before the eclosion of the fly larva from the egg (Currie, 1937; Giblin-Davis *et al*., 2001b). However, materials within the fly oviposition fluid or the fly egg itself may contribute to gall initiation, in addition to the stimulus of nematode activity. It is presumed that the nematodes secrete metabolically active chemicals from their large, granular eosophageal glands during plant feeding (Giblin-Davis *et al*., 2004a; K. Davies, pers. comm.).

The initiation and growth of galls has been described in detail for flower-bud galls on *E. macrorhyncha* (Currie, 1937), *E. camaldulensis* (Fisher and Nickle, 1968) and shoot-bud galls on

M. quinquenervia (Giblin-Davis, 2000). Within these hosts and gall types, gall development occurs within about 4 weeks of fly oviposition and nematode deposition, before emergence of the fly larvae (Currie, 1937; Fisher and Nickle, 1968; Giblin-Davis, 2000). Before eclosion of the fly egg, juvenile and parthenogenetic female nematodes surround the unhatched fly eggs within crypts induced within young growing host tissues (Currie, 1937; Giblin-Davis, 2000). Early tissue response suggests a hypoplasic reaction that involves the inhibition of normal meristematic cell differentiation (dedifferentiation), followed by hypertrophy and hyperplasy associated with overgrowth and abnormal proliferation of cells (Currie, 1937; Giblin-Davis, 2001b). The crypts produced are lined with layers of hypertrophied cells (Currie, 1937; Giblin-Davis, 2001b). Once the fly emerges from the egg there is an increase in the granulation of lining cells, more layers of hypertrophied callus are formed, and the crypts become more defined in shape (Currie, 1937; Giblin-Davis, 2000). The layers lining the gall form nutritive tissue (Currie, 1937).

By the late third instar stage of the fly larva (Fig. 7 g), the outer layer of the chamber becomes lignified, the nutritive tissue is consumed by the fly larva, and the gall tissue dries (Currie, 1937). The third instar larval cuticle hardens to form a puparium and only the outer, dried, lignified shell of the gall crypt remains (Currie, 1937). Nematodes that do not enter female flies and those that exist within the locules of male flies, eventually die. The adult fly emerges and uses the ptilinum to force its way out of the puparium by breaking through a thin area or “window” of the outer gall structure (Currie, 1937; G. Taylor and K. Davies, unpub. obs.).

1.3.5. Ecology of the association

Initially, the gall complex was described and 17 species of fly larvae dissected from gall forms collected from coastal south-eastern Australia and Perth (Western Australia), on various *Eucalyptus* hosts (Currie, 1937). Observations were also made on gall ecology and parasitoids from some of the gall forms were documented (Currie, 1937). Since then, parasitoids from terminal leaf bud galls on *E. camaldulensis* from South Australia were described (Taylor *et al.*, 1996) and parasitoids from *M. quinquenervia* shoot-bud galls from the Queensland and northern New South Wales coast were reported (Taylor *et al.*, 1999; Davies *et al.*, 2001; Goolsby *et al.*, 2001). This work has been supplemented by biological observations and collections from a variety of Myrtaceae hosts (Taylor, *et al.,* 2005; K. Davies and G. Taylor, pers. comm.). Comprehensive studies of the gall complexes on the *Melaleuca* genus have been conducted, including molecular analysis of host relationships and ecological observations (eg Giblin Davis, 2000; Goolsby *et al.*, 2000; Davies, *et al.*, 2001; Scheffer *et al.*, 2004; Ye *et al.*, 2007). *Fergusonina* flies from nine *Melaleuca* species were phylogenetically analysed using mtCOI (mitochondrial cytochrome oxidase subunit I) and were

found to be specialists of their hosts (Scheffer *et al*., 2004). In this study of *Fergusonina* phylogenies, and on the basis of morphological identification, *F. turneri* was believed to form galls on both *M. quinquenervia* and M. *fluviatilis*. However, the species comprises two cryptic species which respectively specialise on each of the two hosts (Scheffer *et al*., 2004). Phylogenetic analyses using SSU (nuclear ribosomal DNA near-full length small subunit), D2/D3 (nuclear ribosomal DNA partial large subunit D2/D3 domain), and mtCOI of *Fergusobia* nematodes from a variey of myrtaceaous hosts and gall forms, showed that the genus is broadly divergent and contains many monophyletic clades (Ye *et al*., 2007). The clades are generally consistent with host plant species and gall type, but phylogenetic analysis indicates host switching has occurred in many *Fergusobia* lineages (Ye *et al*., 2007).

From seasonal ecological studies of *M. quinquenervia*, the galls follow an annual cycle and are most abundant in August/September (Goolsby *et al*., 2000). Gall density and leaf bud density on *M. quinquenervia* are strongly correlated, and both are negatively correlated with temperature (Goolsby *et al*., 2000). Additionally, the related parasitoids and inquilines of *Fergusonina/Fergusobia* galls on *Melaleuca* were studied to gain information on the multi-trophic community structure of the galls and to make predictions of how gall growth may be affected if flies were released as biological control agents in Florida, where no native parasitoids exist (Davies *et al*., 2001).

Unfortunately, for many of the gall forms and hosts of the *Fergusonina/Fergusobia* association, little ecological information has been recorded. Many *Fergusonina* flies stored at The National Museum, Sydney, for example, were collected by sweep net or light trapping methods and are not accompanied by corresponding nematode and host plant data (K. Davies and G. Taylor, pers. comm.). Often gall material has been collected from unknown tree sources, and important ecological information regarding the gall species relationships and tree host phenology is missing.

1.4 Research aims and project significance

Fergusonina fly and *Fergusobia* nematode galls are restricted primarily to Australia and represent gall organisms which exist as obligate mutualists (Taylor *et al*., 2003; Giblin-Davis *et al*., 2004b and c). Instances of obligate mutualistic gall species are extremely rare and in the case of fly and nematode gall induction this relationship is unique. The association therefore presents an ideal case study for processes of gall formation and the evolution and ecology of cospeciation. In order to gain greater understanding of the mutualistic nature of the association and the ecology of the gall complex within a host species, the following research was proposed.

1.4.1. Rearing of flies and nematodes

Many aspects of gall formation are poorly understood and involve highly specific, complex interactions between the plant host species and gall-inducer. It is not certain whether flies or nematodes are both necessary for gall formation. The study aimed to rear flies and nematodes separately for experimental manipulations of plant and gall material to determine if each species alone could produce galls. Rearing flies and nematodes separately was expected to highlight the adaptive benefits of this presumably coevolved association. In addition, the successful culturing of nematodes could provide a useful tool for further studies of the *Fergusonina*/*Fergusobia* complex.

1.4.2. Life histories and gall ecology

Gall ecology involves complex interactions, often at many trophic levels. Gall inducing species occur prolifically in many geographic regions, but few studies have focussed on gall formation and the ecology of interactions within plant hosts in the Southern Hemisphere. This study aimed to document the life histories of the *Fergusonina/Fergusobia* gall species within one host, *E. camaldulensis*, to examine life cycle strategies such as generation length and host organ selection in relation to seasonal factors and host phenology.

2. Culture of Fergusonina/Fergusobia *in controlled conditions*

2.1. Introduction

Culture of both *Fergusonina* and *Fergusobia* under controlled conditions was attempted because regular supplies of flies and nematodes are essential for experimental manipulation of plant and gall material, but are sparsely and only occasionally available from field collections. The study aimed to rear flies and nematodes separately to show how each species independently influenced gall formation. Growing plant parasitic nematodes successfully within a dual culture would also provide a useful tool for further studies. The study also aimed to develop methods to produce galls on glasshouse trees, for examination of the stages of gall growth and to provide a supply of flies and nematodes.

2.2. Dual culture of *Eucalyptus* callus and *Fergusobia*

2.2.1 Methods

In order to grow a dual culture of plant host and nematodes it was first necessary to formulate a protocol for growing *Eucalyptus* callus tissue in aseptic conditions. To micropropagate plant callus on gel media, the methods of Aryan and Scott (2000) were used as a starting point. Batches of MS (Murashige and Skoog, 1962) plant growth medium were prepared in small (50 mm) and large (90 mm) petri dishes, and 30 mL sterile plastic tubes. The gel formulation was then modified to suit dual culture of plants and nematodes according to Hutangura *et al.* (1998). Reduction of sucrose was suggested to reduce the blackening of woody tissues, and media with reduced solutes were more successful for the dual culture of plants and nematodes (Aryan and Scott, 2000; Hutangura *et al.*, 1998). The solute media according to Hutangura *et al.* (1998) comprised:

25% strength MS salt base (Murashige and Skoog, 1962; supplied by SAFC Biosciences Pty. Ltd., Brooklyn, Victoria)

25% strength plant vitamins (125 µl/500 mL; Aryan and Scott, 2000)

0.5% sucrose (2.5 g/500 mL)

0.6% agar (3 g/500 mL)

To stimulate the growth of callus, 2,4-D (dichlorophenoxyacetic acid) was added to the culture medium at 0.3 mg/L as recommended (E. Scott, pers. comm.; Aryan and Scott, 2000).

Surface sterilisation and aseptic growth of callus of *E. camaldulensis*

Surface sterilisation of *E. camaldulensis* was undertaken following the protocols set out in Aryan and Scott (2000), in addition to the modifications below.

- Actively growing stems from young glasshouse grown *E. camaldulensis* trees were harvested using small shears and the shoots were cut into 15 – 30 mm pieces using a scalpel
- stem pieces were rinsed in dilute detergent (5%) and scrubbed with a small brush
- washed in running water for at least 30 min.
- sterilised 15 min. with 1% active hypochlorite bleach solution
- rinsed three times with autoclaved distilled water

After surface sterilisation, stem pieces were placed on fresh sterile growth medium, the plates sealed with Parafilm and grown in a 12 h light:12 h dark 25°C growth room.

Surface sterilisation and inoculation of callus with *Fergusobia*

After the successful aseptic growth of *E. camaldulensis* callus, the tissue was inoculated with *Fergusobia*. *Fergusobia* juveniles (J2's) were dissected from adult female *Fergusonina* flies, which emerged from axial leaf bud galls on *E. camaldulensis*, 8 days earlier. Under sterile conditions within the laminar flow cabinet, flies were dipped in alcohol then rinsed with sterile water. Within aseptic conditions using an alcohol sprayed dissecting microscope, flies were dissected and juvenile nematodes were collected by pipette and stored temporarily in a glass cavity block in 2 mL of sterile tap water.

The nematodes were surface sterilised following the methods of Hay (1994). These involve the use of an autoclaved sintered glass funnel with clamp, stopper and flask with side attachment assembled in the laminar flow cabinet to surface sterilise and filter the nematodes by suction. The funnel is attached to a pump outside of the laminar flow cabinet to allow filtering.

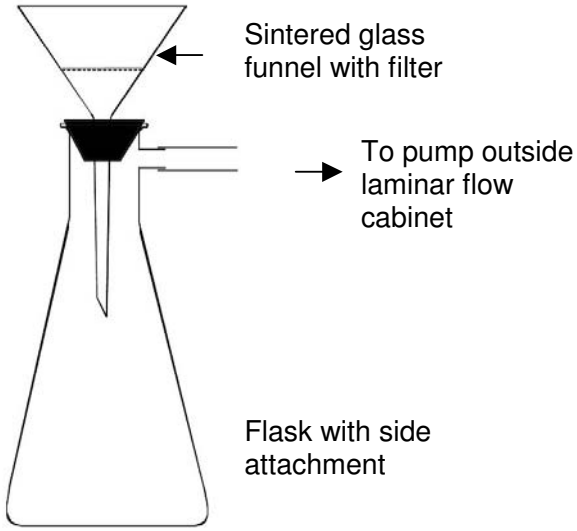


Fig. 9: Flask set-up for surface sterilising nematodes

It was not known if *Fergusobia* would be damaged or killed by the surface sterilisation process including the Hibitane solution (Schering-Plough Animal Health Limited, North Ryde, N.S.W.) recommended (Hay, 1994; K. Davies, pers. comm.). For this reason, the total available *Fergusobia* juveniles were divided into two cavity blocks to be treated with either:

- sterile tap water only, and
- 0.5% Hibitane in addition to rinsing with sterile tap water.

The sterile tap water only treatment also provided a control for the rinsing and aspirating process. The nematodes to be treated with sterile tap water only were washed and aspirated 3 times, after being transferred to an autoclaved 0.2 µm Milli-Q filter placed in the funnel. Nematodes were transferred using a pipettor with 0.5 mL sterile cut pipette tips. The tips of these were cut to broaden the aperture and to minimise damage to the nematodes during transfer. The nematodes were then washed off the Milli-Q filter into sterile tap water within a sterile cavity block.
For the Hibitane treatment, nematodes were transferred to an autoclaved 0.2 µm Milli-Q filter placed in the funnel. Then 1 mL of autoclaved 0.5% Hibitane solution was pipetted onto the nematodes and left for 15 min. The Hibitane solution was then aspirated off and the nematodes washed and aspirated 3 times with sterile tap water. After surface sterilisation the nematodes were washed off the Milli-Q filter into sterile tap water within a sterile cavity block.

Following both sterile tap water only and Hibitane treatments, all the nematodes appeared active and intact when checked under the microscope before inoculating the callus tissue.

Inoculation of callus

Approximately 20 nematodes in sterile tap water were collected from the tap water only and Hibitane treatments and pipetted onto callus as follows:

- 30 mL tubes containing gel with callus (3 replicates). The gel was set on an angle to maximise the contact of nematodes with the callus.
- large (90 mm diameter) petri plates containing gel with callus (4 replicates). The gel was scored with a sterile knife and the callus set in the grooves created, to maximise contact of nematodes and callus.

2.2.2 Results

Within approximately 14 days, callus grew on the tips of the stems. The calli began to deteriorate after two weeks, and it was necessary to transfer cut pieces of the callus tissue to fresh growth medium before the inoculation with nematodes. This was possibly due to the chemical properties of the *Eucalyptus* tissue which is known to contain high levels of phenolics causing browning and deterioration (Aryan and Scott, 2000). Some plates of callus were discarded throughout this procedure due to their deterioration.

Four days post inoculation, none of the tubes and plates was contaminated, indicating that surface sterilisation was successful. Within 3 of the 4 plates with nematodes from both the water only and Hibitane step treatments, nematodes were seen but were not moving and appeared to be dead, although they did not show obvious signs of deterioration. Within the 2 remaining plates nematodes could not be found. Only dead J2 nematodes were recovered from all 6 inoculated tubes from both the water only and Hibitane step treatments. This raised questions about the suitability of plant growth media for *Fergusobia*, which may have inappropriate levels of salts or be unsuitable for the movement of semi-obese nematodes (Siddiqi, 1986). This led to testing the survival of *Fergusobia* nematodes in tap water and other solutions.

2.3. Survival of *Fergusobia* in vitro

2.3.1 Methods

Following dissection from gall material many species of *Fergusobia* nematodes appear to be sensitive and rarely live beyond one day (K. Davies, pers. comm.). This study compared the survival of a species of plant parasitic *Fergusobia* in two solutions relevant for dual culture with host plant material. Tap water was chosen as the first solution, because it is known to be compatible for short-term observations of *Fergusobia* (Currie, 1937; K. Davies, pers. comm.). Murashige and Skoog (MS) medium at 1% concentration in pure Milli Q water was chosen as the second solution, because it is the growth medium suitable for the culture of plant host callus (Murashige and Skoog, 1962).

Axial bud galls from the host *E. camaldulensis* were chosen for this study. During the plant parasitic stage many nematodes exist in each gall. Approximately 300 amphimictic female and male nematodes were extracted from 6 galls in fresh tap water from *E. camaldulensis* in the South Parklands, Adelaide, South Australia, on 25 January 2003, at 10:30. For survival observations, 1 mL sub-samples of 50 nematodes were collected in tap water. Before beginning observations in either tap water or MS solution at 11:00, nematodes in each treatment were rinsed in the appropriate medium and then placed in a white, 2 mL plastic weighing dish. The 1 mL level of each dish was marked. The movements of 10 nematodes were then observed with a dissecting microscope for 60 s. A “movement” was determined by the twisting or bending of the body, which occurs particularly in response to light, and is an indicator of survival. After observation a glass square was placed lightly over the dishes to minimise evaporation, but also to allow some gaseous exchange. When the level of each solution fell to below 1 mL, additional pure Milli Q water was added to each of the tap water and MS dishes to maintain the concentration of salts within each solution. In addition to these two solutions, the nematodes remaining in the gall dissection dish were also monitored. This gave an indication of the activity usually witnessed by others after gall dissection (K. Davies, pers. comm.). It contained 50 to 100 nematodes in 2 mL tap water but also contained some dissected plant gall material. In order to test the behaviour of nematodes in response to clean gall fibres, an additional white weighing tray of nematodes in fresh tapwater was monitored for the first week of the observation period. After each day the majority of tapwater was pipetted off and refreshed with clean unsterilised, tapwater.

2.3.2 Results

The results of the activity study are presented in Fig. 10. Generally, nematodes were more active and lived slightly longer (13.5 h) in the fresh tap water than in 1% MS solution (Fig. 10). Over a period of 103 hours (25/1/03 11:00 to 29/1/03 19:30) the nematodes were recorded as “very active”. This can be seen within Fig. 10 as a large peak in activity recorded for tap water nematodes at the end of Day 5. Even at the end of Day 7 (31/1/03 22:00) there was considerable activity of nematodes in tap water in particular (Fig. 10). By Day 8 (1/2/03 18:30), some dead nematodes were noticed in the 1% MS solution and by Day 9 (2/2/03 9:30) dead nematodes were present in the tap water sample. At the completion of Day 9 (2/2/03 22:45) many of the nematodes in both solutions were dead, but many of the smaller nematodes were still quite active. Within the 1% MS solution, at the end of Day 11 (4/2/03 22:30) the nematodes had ceased moving and many were degrading. At Day 12 (5/2/03 9:00) the nematodes of the tap water solution had ceased moving and were dead and degrading.

Observations of the activity of the dissected material nematodes were also recorded. Within the first few hours, the nematodes were active (Fig. 10). After 31 h all activity ceased and the dissected gall tissue browned and the nematodes began to deteriorate. By 35.5 h (26/1/03 22:30) all of these nematodes were dead and degrading (Fig. 10).

Within the dish of regularly refreshed tap water and gall fibres the nematodes were seen gathering around and orienting themselves anteriorly towards the gall fibres. This gathering occurred during times of dark within the natural daylight hours. The activity was not recorded for this dish. By the end of seven days these nematodes had ceased moving and begun to degrade.

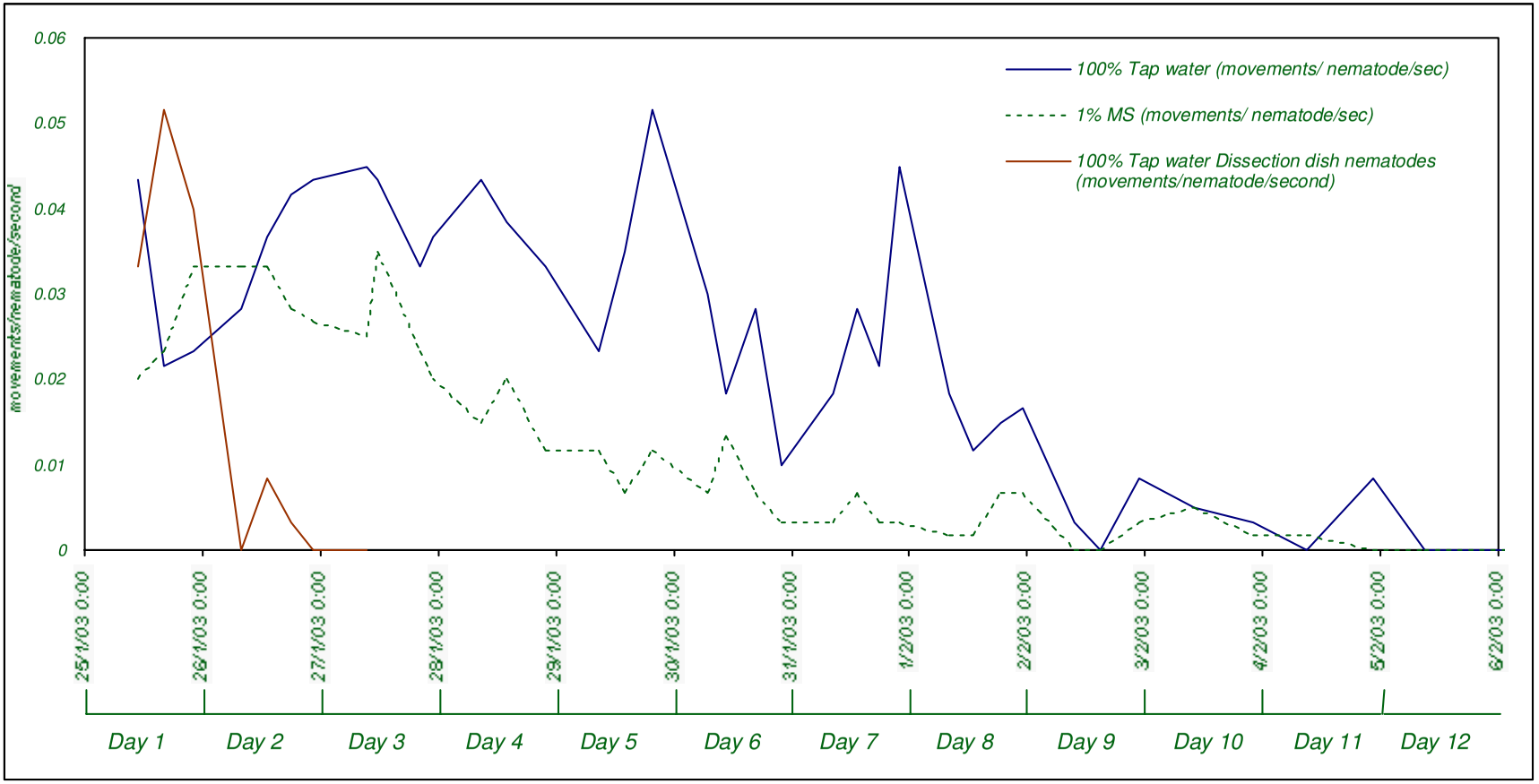


Fig. 10: Activity of *Fergusobia* in 100% tap water (solid line) and 1% MS solution (broken line).

2.4. *Fergusonina* releases on caged trees

2.4.1 Methods

Twenty-four glasshouse grown *E. camaldulensis* trees were chosen for fly releases from the 120 glasshouse grown trees available for the study. Four to six weeks prior to the predicted availability of flies from maturing galls, the apical stems of the entire available glasshouse grown trees were pruned to stimulate the growth of new leaves. Once at least 20 growing points were available on 24 of the trees, mature terminal leaf bud galls were collected from local field sources. The trees were set up in two ways as follows:

1. 16/3/02: 4 trees approximately 600 mm tall within 1 m x 1 m x 1 m fine mesh cages. These cages were set up in a 25°C constant temperature room, with a 12L:12D light cycle. A mature terminal leaf bud gall was added to each of the 1 m^3 cages and the flies were allowed to emerge. A tube of sugar water was added to the cages for the flies to feed on.
2. 25/4/03: 20 trees approximately 600 mm tall. These were positioned in a glasshouse, and acetate confinement sleeves with fine mesh windows were positioned over the available growing points. Details and photos for construction of these confinement sleeves can be found in Giblin Davis *et al*., 2001. A single male and female fly from terminal leaf bud galls were added into each sleeve tube with a small tube of sugar water.

Trees were monitored for two months after release of the flies.

2.4.2 Results

1. No oviposition scars were observed on the trees in the 1 m^3 cages and no sign of gall formation was recorded after 69 days. After 23 days post release, some of the growing points turned black on one of the trees.

2. No signs of gall formation occurred within the 20 trees with confinement sleeves after 69 days since the fly releases. Some oviposition scars were noticed on two trees 7 days after introduction of the flies. After 41 days the growing points were still green and raised “bumps” accompanied the oviposition scars. By 69 days the raised bumps and associated oviposition scars were not visible.

2.5. Discussion

After overcoming problems with early deterioration, the protocols for the growth of eucalypt callus were successfully refined and meristematic tissue was grown. Unfortunately, this did not culminate in the successful dual culture of *Fergusobia* on callus within aseptic conditions. *Fergusobia* are apparently sensitive to many stresses and, given their semi-permeable cuticle and epidermis, the levels of salts present probably contributed to this. Additionally, *Fergusobia* are semi-obese and move slowly. This would make finding and penetrating into the callus difficult, even if they could then actually feed on the tissue. When the J2 nematodes are initially oviposited into the plant tissue by the fly they may be surrounded by fluids of the fly and those of the plant. The fluids may provide the nutrition for early development, until they can begin effective probing and gall stimulation. Simply being oriented near suitable meristematic host tissue may not be enough to enable recognition, feeding and growth. Additionally, the juvenile nematodes (J2s) were extracted from flies that were already 8 days old. In natural circumstances, female flies probably mate and oviposit quickly, allowing the plant parasitic J2 nematodes rapid access to feed on meristematic plant tissue. After 8 days the J2s may have had depleted energy reserves and be close to death. This may have made them less than optimal for inoculation of the eucalypt callus. *Fergusobia* are aquatic organisms that obviously survive for some time during their lifecycle within aqueous conditions, such as when immersed in oviposition fluids at the time of fly egg oviposition. This led to considering how aqueous media could be applied to the growth of *Fergusobia* in controlled conditions.

It appears that *Fergusobia* are indeed affected by their surrounding media, particularly when stored with the dissected gall material (Fig. 10). The outcome is surprisingly different, however, when the nematodes are stored in clean solutions, particularly fresh tap water (Fig. 10). Survival up to 12 days was unexpected. Clearly, the deteriorating dissected gall material must release substances that become toxic to the nematodes within hours.

The results of this simple survival study have led to consideration of storing *Fergusobia* in aqueous solutions with plant material. In addition to the treatments formally assessed, another dish of nematodes in clear tap water with gall fibres was observed for several days. Particularly during late evenings, the nematodes were seen orienting themselves around the fibres. Feeding could not be observed with the low power of the dissecting microscope. It is unlikely that there would be sufficient traction to allow feeding upon the plant fibres. Such an aqueous medium could be refreshed regularly, to avoid deterioration. Providing the nematodes received sufficient nutrition, the amphimictic females and males may be able to mate to produce the pre-parasitic female

generation. This could be difficult, however, because the aqueous medium may not provide sufficient traction to allow this. In addition, the cues for progression from the pre-parasitic to the parasitic form would be absent. Experimental addition of ecdysteroids could be made to test if the insect moulting hormone stimulates the adult moult in the nematodes.

The release of flies onto caged trees did not lead to gall production. Initially, this could have been due to crowding. It is likely that within the 1 m^3 cages, flies were over-exploiting the growing points which led to a hypersensitive reaction and the blackening of growing points on one of the trees. Such hypersensitive reactions have been observed with flies on caged *M. quinquenervia* (Giblin-Davis, pers. comm.). Unfortunately, following construction of the acetate confinement sleeves, no mature galls from *E. camaldulensis* were available from the field to supply the necessary numbers of flies for releases. For the two trees with confinement sleeves that had evidence of oviposition, abortion of galls probably accounts for the lack of gall growth (Currie, 1937; K. Davies and G. Taylor, pers. comm.). Production of galls in the glasshouse was achieved by a team of researchers with *Melaleuca* (e.g. Giblin–Davis 2000; Giblin–Davis *et al*., 2001b). Clearly, for the successful production of galls on *E. camaldulensis*, maturation of galls and fly emergence must coincide with availability of trees with the correct meristematic tissues. Although 120 *E. camaldulensis* trees were grown in glasshouse conditions, few of these had the necessary growing points available when flies emerged from mature galls. In addition to the lack of available growing points on the trees, many suffered from scale infestations which attracted ants and sooty mould outbreaks. Such infestations also occurred commonly on *E. camaldulensis* trees in the field. Any trees in the glasshouse with these infestations were isolated and treated then and allowed to recover and grow fresh stems and leaves, but this depleted the number of healthy trees available for attempts to produce galls. Obviously, additional time, labour, galls and trees are required to enable the successful production of galls on caged or glasshouse trees.

Much of the early work within the project focussed on the culture of nematodes and galls, a time-consuming exercise. Flies and nematodes could not be cultured reliably within the available time frame, so greater focus was placed on the non-destructive field study.

3. Ecology of Fergusonina/Fergusobia *galls in Urrbrae Wetlands*

3.1. Introduction

In order to study the field ecology of some *Fergusonina* fly species and how they relate to the phenology of a eucalypt host within South Australia, a two year study was conducted within the Urrbrae Wetlands. The aim of the study was to clarify early informal observations of life histories and to examine the seasonality of galls on *E. camaldulensis*. It was planned to obtain ecological information from a different plant host and climate that could be compared with the population ecology of *Fergusonina*/*Fergusobia* galls on *M. quinquenervia*.

This study of *E. camaldulensis* galls was non-destructive. Other non-destructive field surveys have been conducted for gall species of Australian trees (Goolsby, *et al*., 2000; Gullan *et al*., 1997). Senesced galls often remain on trees for extended periods into following generations and they may be a necessary visual cue for the flies to recognise suitable hosts, although the senesced galls are not included within gall counts (Goolsby, *et al*., 2000; Gullan *et al*., 1997). Also, removing active galls reduces the number of flies available for future gall initiations. It is therefore essential for gall surveys of this nature to be non-destructive.

This study aimed to make observations of host phenology, local climate, gall occurrences and gall characteristics. Within this framework the following questions were posed:

- What gall types occur and when are they abundant at Urrbrae Wetlands?
- How does tree phenology relate to gall growth?
- How do tree growth and gall occurrence compare with environmental factors such as monthly average, mean daily maximum and minimum temperature and total monthly rainfall?
- Is there a relationship between the density of new leaves and new galls?
- Does leaf damage affect density of new leaves and gall occurrence ?
- Are galls distributed uniformly between the trees?
- Are galls distributed uniformly within each tree relative to distances to trunk and ground and orientation within the tree?

Gall surveys are often biased against taller, more mature trees because of difficulty in reaching their upper regions (Goolsby *et al*., 2000; Gullan *et al*., 1997). Australian field studies have shown

that galls are more abundant on saplings or on regrowth of Myrtaceae than on the foliage of older trees (LeBreton and Vaarwerk, 1993; Gullan *et al*., 1997; G. Taylor and K. Davies, pers. comm.). Additionally, gall insect species reared in glasshouse conditions initiate galls only on the new foliage of actively growing shoots (Goolsby *et al*., 2000; Gullan *et al*., 1997). Sap sucking insects have also been shown to prefer coppice rather than mature trees, perhaps because young leaves and shoots have a preferable nutrition than older vegetation (Yen, 1989). Therefore, this field survey was non-destructive and focussed on the new growth regions of young trees.

3.2. Methods

The Urrbrae Wetlands were reclaimed from grazing land and revegetated with trees, shrubs and grasses endemic to the area in 1997, and are depicted in Fig. 11. The site is within the City of Mitcham and is 6 km South of the business centre of Adelaide. Many of the trees are *Eucalyptus camaldulensis*, grown from local seed sources, have been widely planted in the wetlands, and fly/nematode galls had been previously sighted at this location. These trees provided an ideal location for the observations of gall and tree phenology.

Twenty trees were chosen, situated within two areas within the wetlands site (Fig. 11). Area A contained ten trees, five of which edged the main pond and grew with tall grasses and sedges (Fig. 12 and 13). Access to these five trees was limited because they were on the edge (within 0.3 m) of the unlined main pond (Fig. 13). The remaining five trees of Area A were within 12 m of the main pond. During the study, two trees of Area A were pruned by the wetlands staff, resulting in the observations of one (Tree 6) being discontinued and the other (Tree 9) modified to omit observations of the southern side. Area B initially contained ten trees with little undergrowth, situated within nine to thirty-five metres of a dam lined with plastic (Fig. 14 and 15). Observations of the full perimeter of these trees were possible. One tree (Tree 11) was lost from the study when it was removed for alterations to the wetland near it.

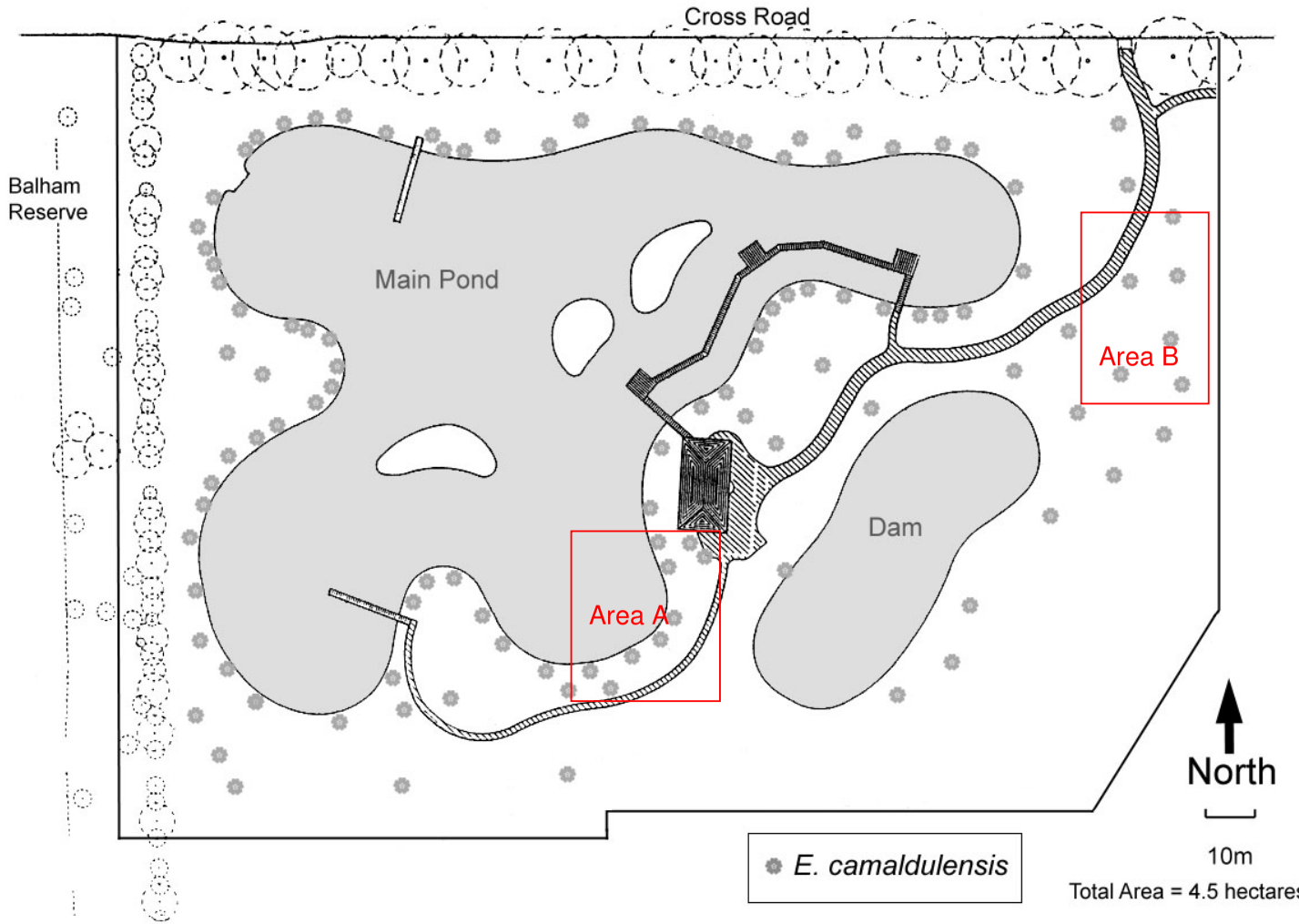


Fig. 11: The Urrbrae Wetlands site, Cross Road, Netherby, South Australia, showing approximate areas (A and B) of trees studied.

Figure 12: North-facing views of Area A, Urrbrae Wetlands.

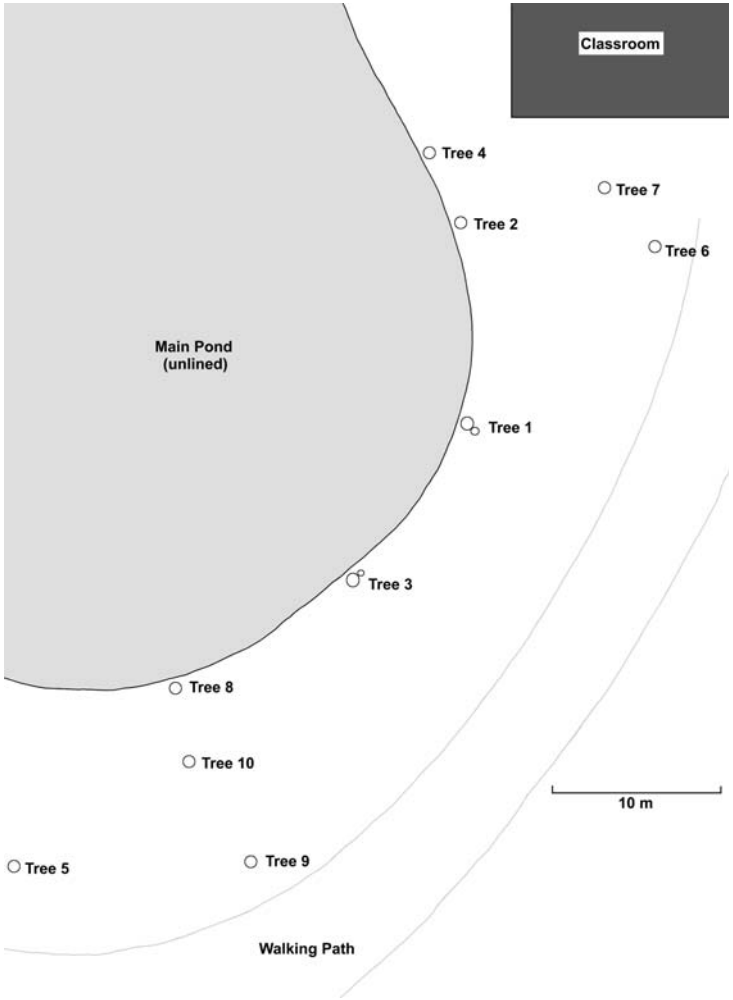


Figure 13: Area A of Urrbrae Wetlands, showing positions of trees and Main Pond.

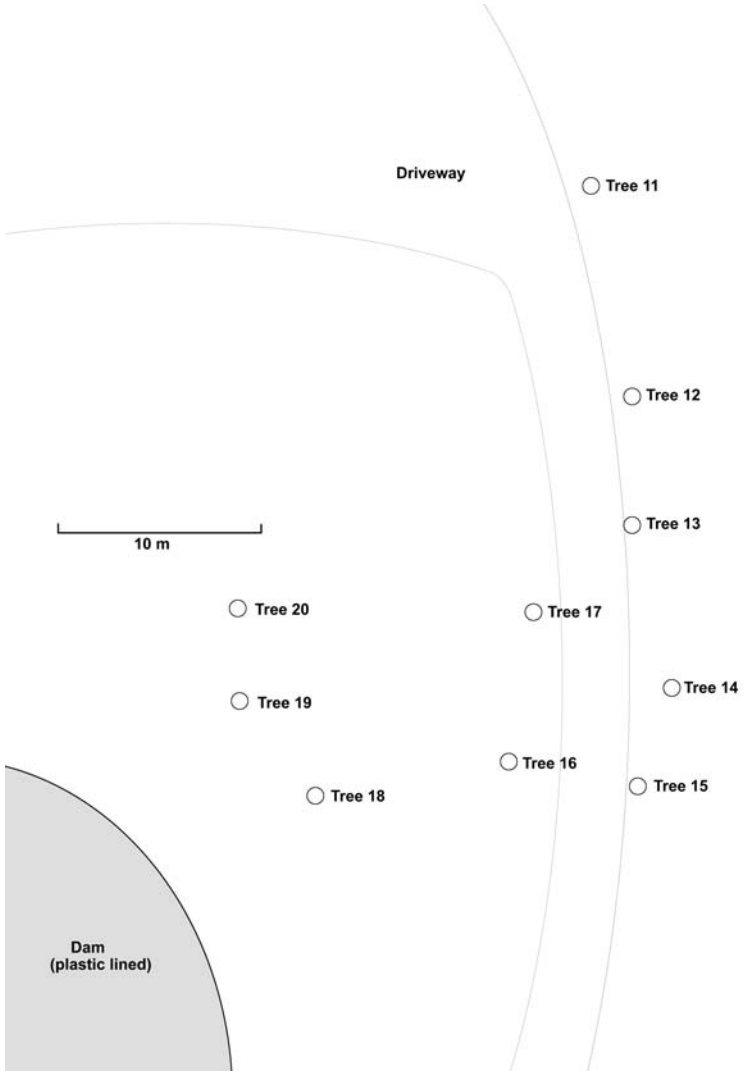
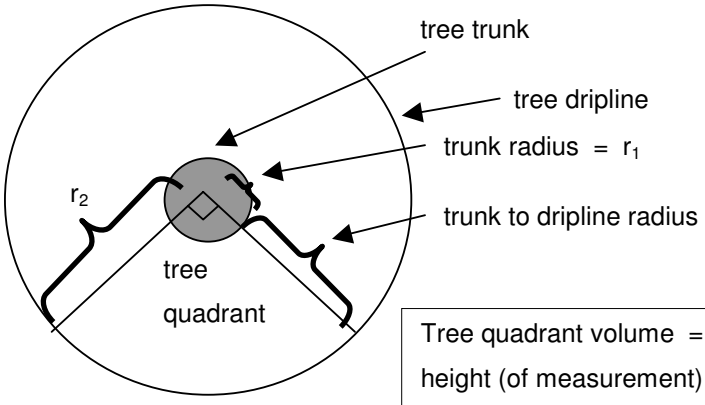


Figure 14: Area B of Urrbrae Wetlands, showing positions of trees and Main Pond.

Figure 15: South-facing views of Area B, Urrbrae Wetlands.

For each tree, tree and gall measurements were collected, mid-month, for each accessible quadrant from ground level up to a height of 2 m. Some of the trees had widely differing canopy size and some inaccessible quadrants, and these were therefore not directly comparable. A measure of growing point or gall density (ie per m^3) was therefore preferable to simple counts per tree. The following calculation was used to enable the comparison of quantitative density measures (Fig. 16). The measurements of trunk circumference and dripline radius were taken as these were necessary for these calculations.



Tree quadrant volume = tree quadrant base area (m^2) x height (m)

height (of measurement) = 2 m

Tree quadrant volume = tree quadrant base area x 2 m^3

tree quadrant base area = (area large circle - area small circle) x 90/360

$= (\pi r_2^2 - \pi r_1^2) \times 1/4$

$= \pi$ { (trunk to dripline radius m + trunk radius m)^2 - (trunk radius m)^2}

Fig. 16: Calculation of tree quadrant volume

Tree observations were recorded as follows:

1. Growing point count: The numbers of regions at the tip of a stem, containing meristematic leaf or stem buds and leaves less than or equal to 10 mm in length, and suitable for oviposition, were counted per quadrant. Any one stem could have many growing points along it.
2. Tree growth: Measurements of tree height were taken at about 3 monthly intervals using an Abney level 10 m from each tree. The drip line radius and trunk circumference were measured (± 10 and ± 1 mm, respectively), to determine outward growth and density calculations.
3. Flowering: The presence of flower buds or flowers was recorded each month.
4. Leaf damage: Scored according to the following scale: low (< 30% leaves heavily damaged), medium (30-70% leaves heavily damaged) or high (>70% leaves heavily damaged). Additionally, photographs were taken with a digital camera to enable visual comparisons of canopy condition.

Each gall was numbered and recognised using a small plant tag and then checked monthly. Observations of galls were made as follows:

1. Gall type: Three gall types occurred on *E. camaldulensis* at this site. These were terminal leaf bud, axial leaf bud or flower bud galls. The fly and nematode species responsible for each gall form are given in the results section 3.3.2.
2. Gall stage: The stage of development of each gall was recorded as either:
    - N(ew), a freshly initiated gall, formed from primordial leaf tissues,
    - C(urrent), a gall which was maturing, or
    - S(enesced), a gall which had usually dried out and had its maximum number of exit holes.
3. Size measurements: Where practicable, Vernier calipers were used to measure the diameter and length of each gall (to ± 0.010 mm).
4. Exit holes and parasitism: Presence or absence of exit holes was noted and counted where possible, particularly at gall maturation. *Fergusonina* exit holes are typically 0.03 to 0.05 mm in size. Obvious signs of parasitism or predation were recorded where exit holes uncharacteristic of *Fergusonina* flies were present, such as large exit holes with frass, or where browsing damage was evident. Very small exit holes (<< 0.03 mm) indicated wasp parasitism.
5. Gall position: In order to record the position of the gall within the tree, the compass direction to trunk in compass degrees from North (± 2º) and the distances from the trunk and ground (± 10 mm) were recorded for each gall tag.
6. Additional comments: Brief comments regarding gall colour, condition and other factors were noted.

Manual measurements and automatic recordings of total rainfall, daily maximum and daily minimum temperatures were taken within 1 km of the wetlands site by staff of the Urrbrae Education Centre. Analysis of data was performed by JMP Version 5 (SAS Corporation). In order to nest the data in trees for analyses, averages per tree were used.

For comparisons of data, primarily non-parametric statistical methods were used because the data were not normally distributed. The measurements collected were often ranked or ordinal, and because the galls were highly seasonal, there were many months when means per tree could not be calculated and compared. Where applicable, data were grouped within trees because the trees were repeatedly measured. Where a relationship might exist between 2 variables the Spearman's rank correlation was used and each variable ranked. The non-parametric Mann-Whitney U test was used to compare two samples when t-tests were not applicable. Kolmogorov-Smirnov goodness of

fit tests were used for data grouped within trees to test the goodness of fit of observed to expected cumulative frequency distributions.

3.3. Results

3.3.1. Tree phenology and canopy condition

At the commencement of the study in May 2002, all of the trees examined were approximately 5 years old, and ranged in height from 2.6 to 9.8 m. At the completion of the study in April 2004, tree height ranged from 4.5 to 12.1 m. The fraction of trees with flower buds and flowers were positively correlated and generally increased with time (Fig. 17; Spearman's Rank Correlation, $r_s = 0.528$, $p = 0.0132$, $N = 23$ months). The presence of flowers on the trees tended to be seasonal, with some months when no trees had flowers, and periods from late spring to early summer of each year when 0.2 or more of trees were flowering (Fig. 17). During the study, flower buds were present on at least 0.3 of the trees at all times. In January 2003, more than 0.6 of trees had flower buds and the proportion with buds was above this for the remainder of the study (Fig. 17). The increase in flower buds and flowers later in the study was expected because the trees matured as the study progressed.

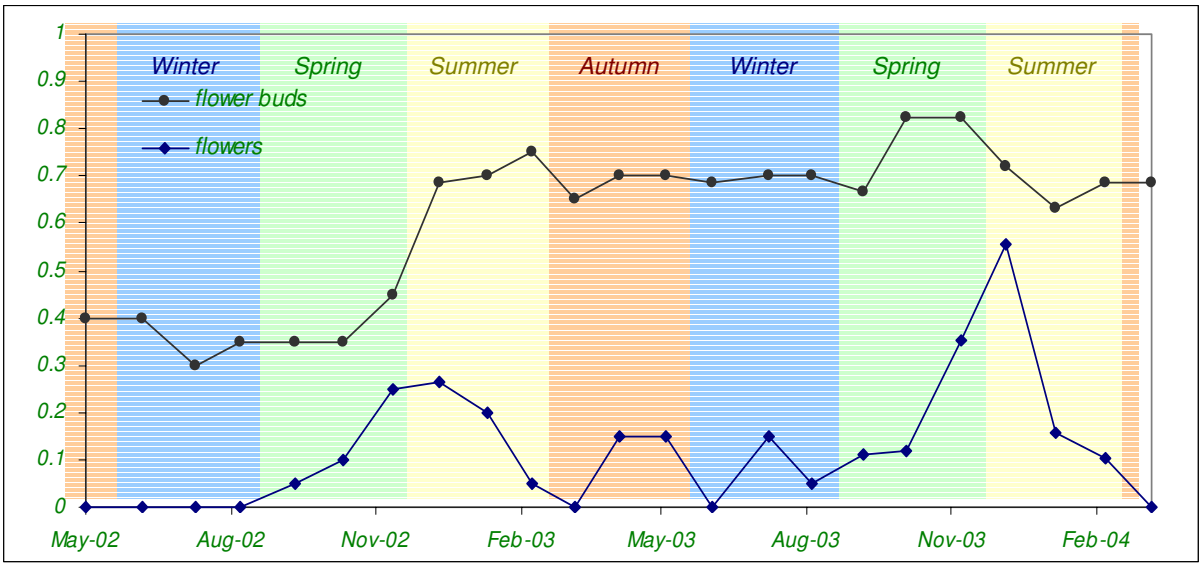


Fig. 17: Fraction of trees with flower buds and flowers throughout seasons.

During the period of the study, 0.77 of the trees were scored with medium (30 to 70%) leaf damage. Low (<10%) leaf damage occurred in 0.13 of trees and only 0.10 of trees were scored as having high (>70%) leaf damage. The seasonal distribution of tree leaf damage is shown in Fig. 18.

During the study, when the fraction of trees with low leaf damage increased, the fraction of trees with flower buds increased (Spearman's Rank Correlation, Rho = 0.587, p = 0.0059, N = 23 months). This indicates that flower buds are more likely to occur when the trees have low levels of leaf damage. Additionally, the fraction of trees with flowers had a weak positive correlation with the fraction of trees having low leaf damage (Spearman's Rank Correlation, p = 0.086, N = 23 months).

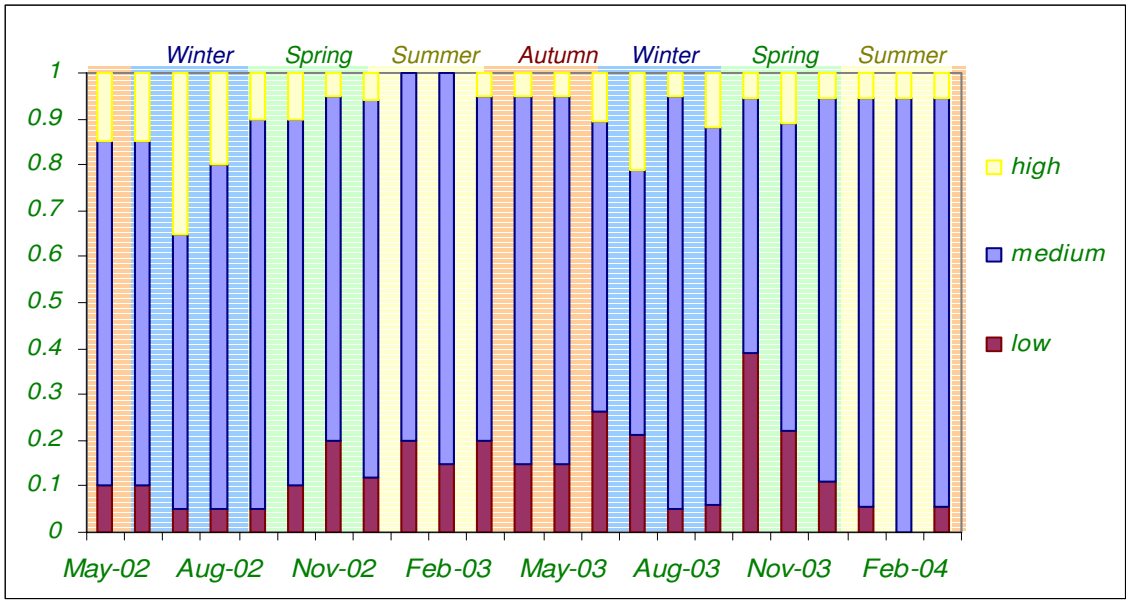


Fig. 18: Fraction of trees with levels of leaf damage throughout seasons.

The abundance of new growth on the trees was strongly seasonal, with greater density of growing points during late winter, spring and summer (August to February) in both years of the study (Fig. 19). There was little new growth in autumn and early winter, but more new growth during late winter/early spring (August, September and October) of 2002 than in the corresponding period of the following year (Fig. 19). Average density of growing points in the 2002 to 2003 late spring/summer months (November, December and January) was similar to the averages for these months in the following year (Fig. 19).

The fractions of trees with either low, medium or high leaf damage were compared with the tree average densities of growing points to see if there was a pattern between the monthly levels of leaf damage and the density of new growth. No significant correlations were found (Spearman's rank correlations, $p > 0.05$, N = 23 months).

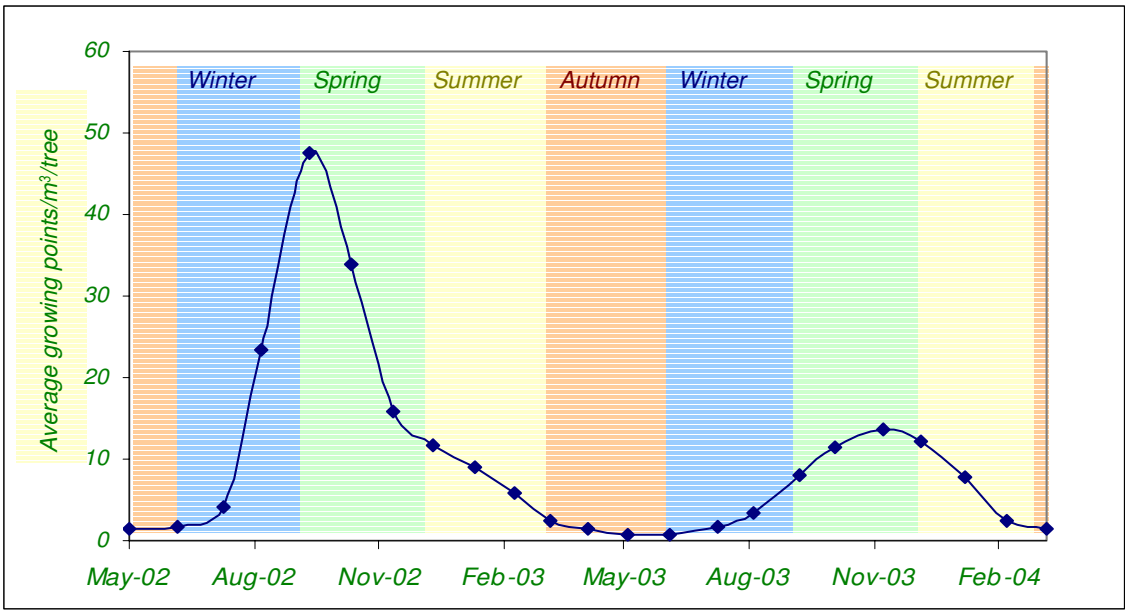


Fig. 19: Seasonality of growing point density.

3.3.2. Seasonality of gall growth

Three gall types were recorded from *E. camaldulensis* at this site; terminal leaf bud, axial leaf bud and flower bud galls (Fig. 20). The structure of these three gall forms was reviewed and described in Chapter 1, Section 1.3.2. Terminal leaf bud galls are produced by the fly *Fergusonina flavicornis* and the undescribed nematode *Fergusobia* sp. A. Axial leaf bud galls are produced by the undescribed species *Fergusonina* sp. and *Fergusobia* sp. B. The flower bud galls are produced by the fly *Fergusonina tillyardi* and the nematode *Fergusobia curriei*. No other gall forms were found on this host at this location.

The seasonal occurrences of the three gall types are shown in Fig. 21 .

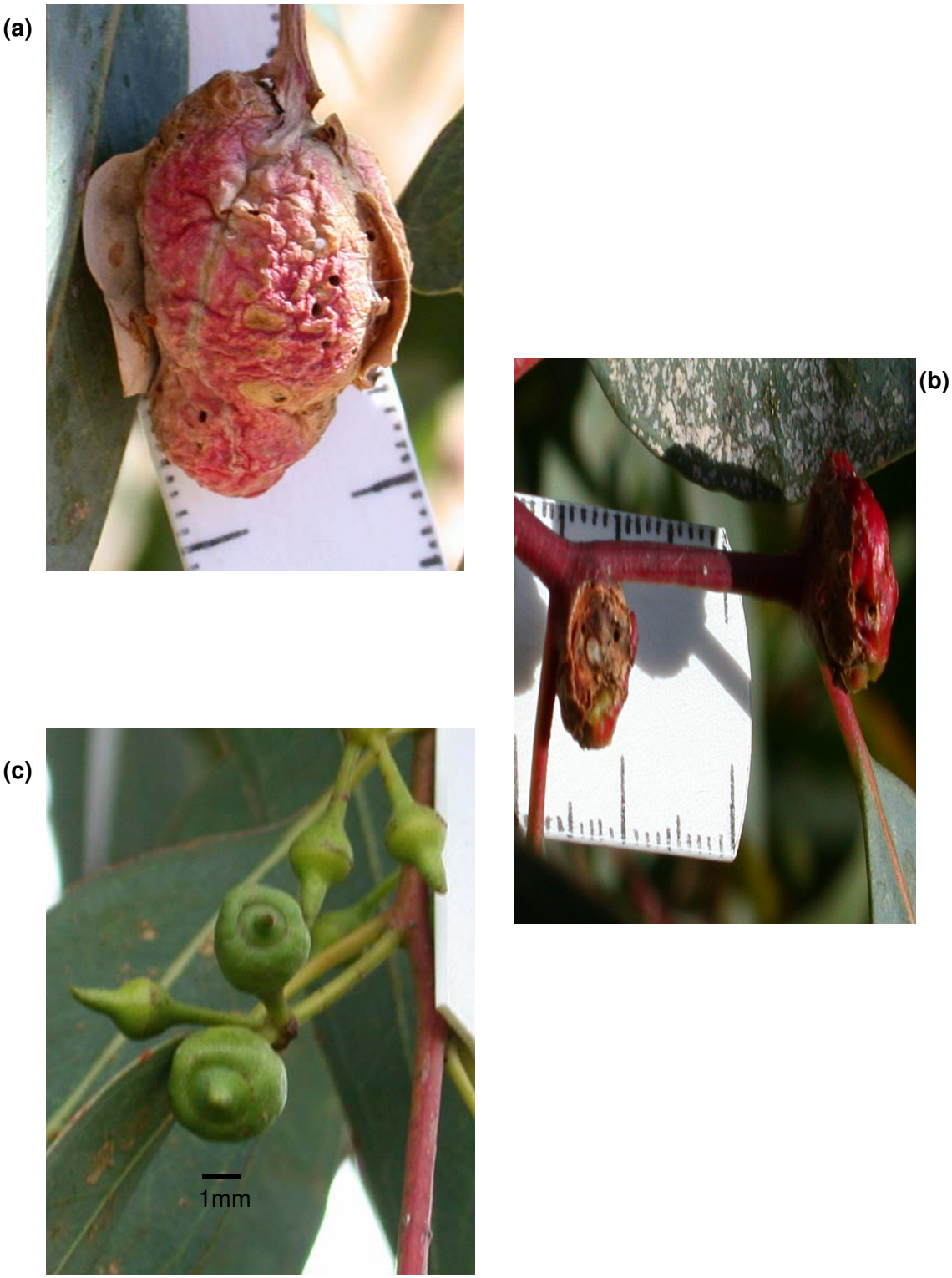


Fig. 20: Examples of (a) terminal leaf bud, (b) axial leaf bud, and (c) flower bud galls (1 gradation = 1mm).

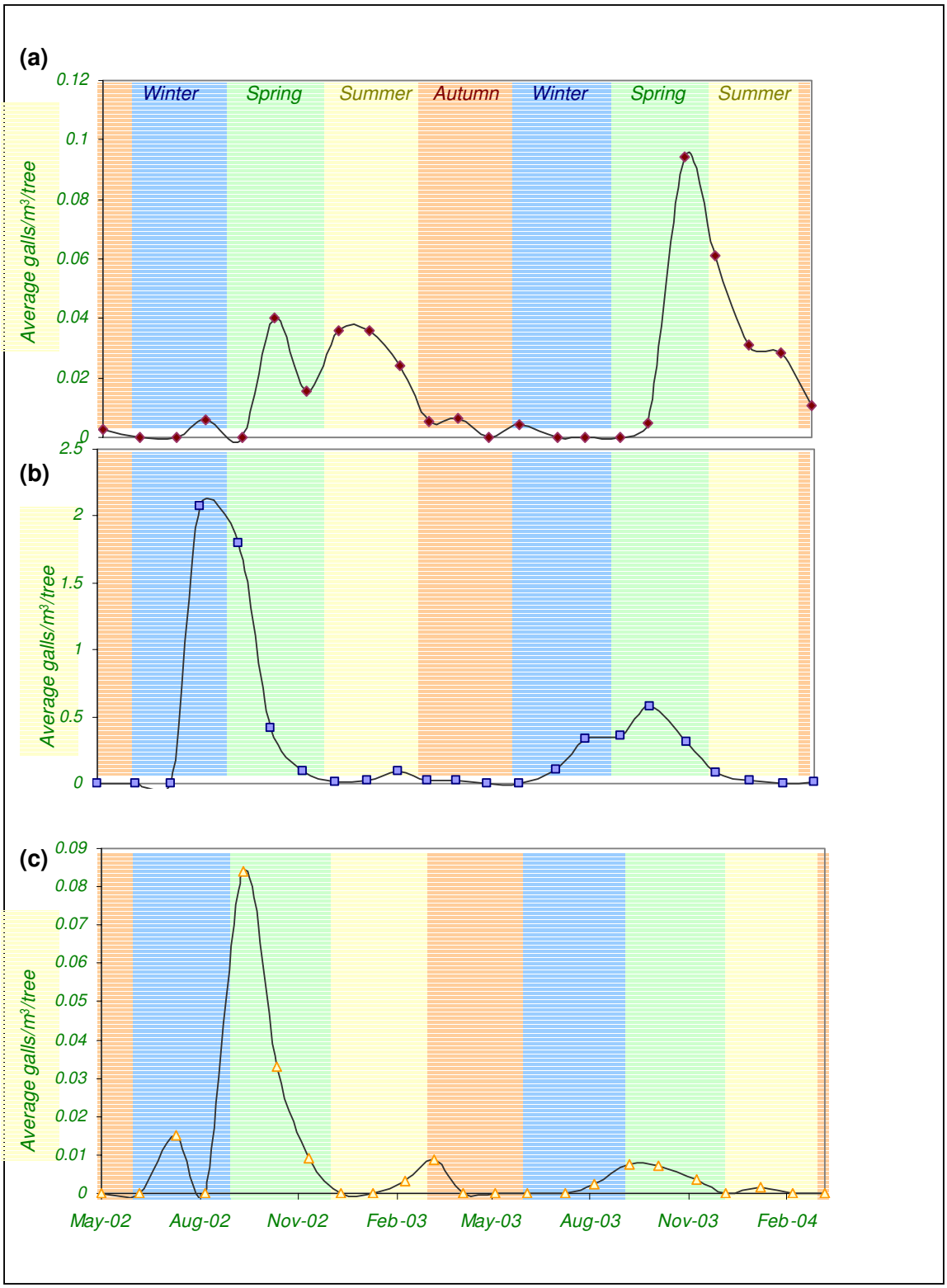


Fig. 21: Seasonal occurrences of (a) terminal leaf bud gall density, (b) axial leaf bud density and (c) flower bud density.

Each gall type had an annual seasonal pattern, with few galls developing during autumn (March to June, Fig. 21 a, b and c). Terminal leaf bud galls appeared primarily during mid-spring to summer (October to March, Fig. 21 a). There was a greater abundance of terminal leaf bud galls in October, November and December of 2003 than October, November and December of the previous year. Axial bud galls were abundant during the mid-winter and spring months (August to December, Fig. 21 b). There was a greater abundance of axial leaf bud galls in August and September of 2002 than in August and September of the following year (ANOVA, $p < 0.05$). Flower bud galls were present during the winter and spring (August to December) in 2002 and 2003 (Fig. 21 c). Additional flower bud galls were found in March 2003, September and October 2003, and January 2004 (Fig. 21 c). There was a positive correlation between the monthly averages of densities of axial leaf bud galls and flower bud galls (Spearman's rank correlation, Rho = 0.518, $p = 0.0246$).

Increases in the proportion of trees with flower bud galls were weakly correlated with decreases in the proportion of trees with flower buds (Spearman's Rank Correlation, $r_s = -0.378$, $p = 0.0763$, N = 23 months). This might indicate that the abundance of flower buds was reduced when flower bud galls were present on the trees. However, the overall impact of flower bud galls on flowering would probably be low because the densities of flower bud galls recorded was very low compared to the generally large number of flower buds observed. Only 42 flower bud galls were recorded throughout the study, but in the months when trees had flower buds there were often hundreds per tree. Since flower buds were not counted, it is not possible to assess any quantitative impact of flower bud galls on flower buds. The fraction of trees with flowers was not correlated to the proportion of trees with flower bud galls (Spearman's Rank Correlation, $r_s = -0.102$, $p = 0.6338$, N = 23 months).

Average monthly densities of growing points were regressed against average monthly densities of each of the gall type to determine if there was any relationship between new growth and gall abundance. There was a strong positive relationship between axial bud galls and growing point density (simple linear regression, $p = 0.0001$). Flower bud gall density was also positively correlated with growing point density (Spearman's Rank Correlation, $r_s = 0.533$, $p = 0.0124$). This was not expected as new leaf growth is not necessary for the initiation of flower bud galls. Terminal leaf bud galls require new plant growth sites for gall initiation, but density of terminal leaf bud galls and of growing points was not related (simple linear regression, $p = 0.381$).

Spearman's Rank Correlations between the monthly fraction of trees with either low, medium or high leaf damage and average growing point densities were not significant. Neither were there any correlations between the monthly proportion of trees with either low, medium or high leaf damage

and the monthly averages of terminal leaf, axial or flower bud gall densities. This indicates that the seasonal occurrence of galls was not dependent upon the level of leaf damage during the two year study.

3.3.3. Climate

The monthly values of local total rainfall and mean daily maximum and minimum temperature are shown in Fig. 22. Generally, rainfall increased during the winter months of the two years of the study, although 2002 was a relatively dry year. The year 2002 had the least rainfall recorded since 1994 and the driest Spring (September to December) since 1995 (Australian Bureau of Meteorology, Adelaide climate data; Fig. 22 a). The greatest densities of growing points were recorded during the late Winter and Spring of 2002 (Fig. 19). A negative relationship was found between rainfall and growing point density, indicating that rainfall was not a necessary factor in the encouragement of new growth at this site and in this study (Spearman's Rank Correlation, r_s = -0.529, p = 0.0132, N = 23 months). Since increase in rainfall was not correlated with increases in density of growing points, Area A trees located closer to the unlined main pond were compared with Area B trees next to the lined dam to determine if the new growth was concentrated in the Area A trees. Analysis of variance in new growth between the two areas of trees showed no differences, indicating that access to the nearby main pond water did not affect the density of this new growth within the trees. No relationship was found between growing point densities and mean daily maximum or minimum temperatures.

During the summer of 2002/2003, December temperatures were above average and more typical of January averages according to long term weather data. This early increase in mean daily maximum temperature roughly coincided with a slight drop in terminal leaf bud density at the end of Spring 2002 (Fig. 21 a and Fig. 22 b). Generally, however, average monthly density of terminal leaf bud galls was strongly positively correlated with monthly averages of daily maximum and minimum temperatures (Spearman's Rank Correlations, r_s = 0.803, p = 0.0002; r_s = 0.799, p = 0.0002, N = 23 months). This positive correlation between mean daily temperatures and terminal leaf bud galls supports the observation that the galls were more abundant during late spring and summer. There was also a strongly negative relationship between rainfall and density of terminal leaf bud galls (Spearman's Rank Correlation, r_s = -0.607, p = 0.0044, N = 23 months).

Seasonal comparisons between densities of both axial bud and flower bud galls revealed no relationships with total monthly rainfall, mean daily maximum and minimum temperatures. For the axial bud and flower bud gall forms, climate does not obviously influence gall seasonality, but seasonal changes in climate variables appear to be strongly associated with the density of terminal leaf bud galls.

Leaf damage, including the presence of sooty mould on leaves, increased during the cooler winter months and may have been influenced by changes in climatic variables. Correlations between the proportion of trees scored as having high damage and total monthly rainfall were not significant. However, there was a strongly negative relationship between the proportion of trees with high leaf damage and mean maximum daily temperature (Spearman's Rank Correlation, Rho = -0.588, p = 0.0058). This indicates that when the temperature was lower, there was a greater fraction of trees with high leaf damage. However, there was no correlation between the proportion of trees scored as having low leaf damage and mean monthly maximum temperature, indicating that low leaf damage is not significantly influenced by temperature.

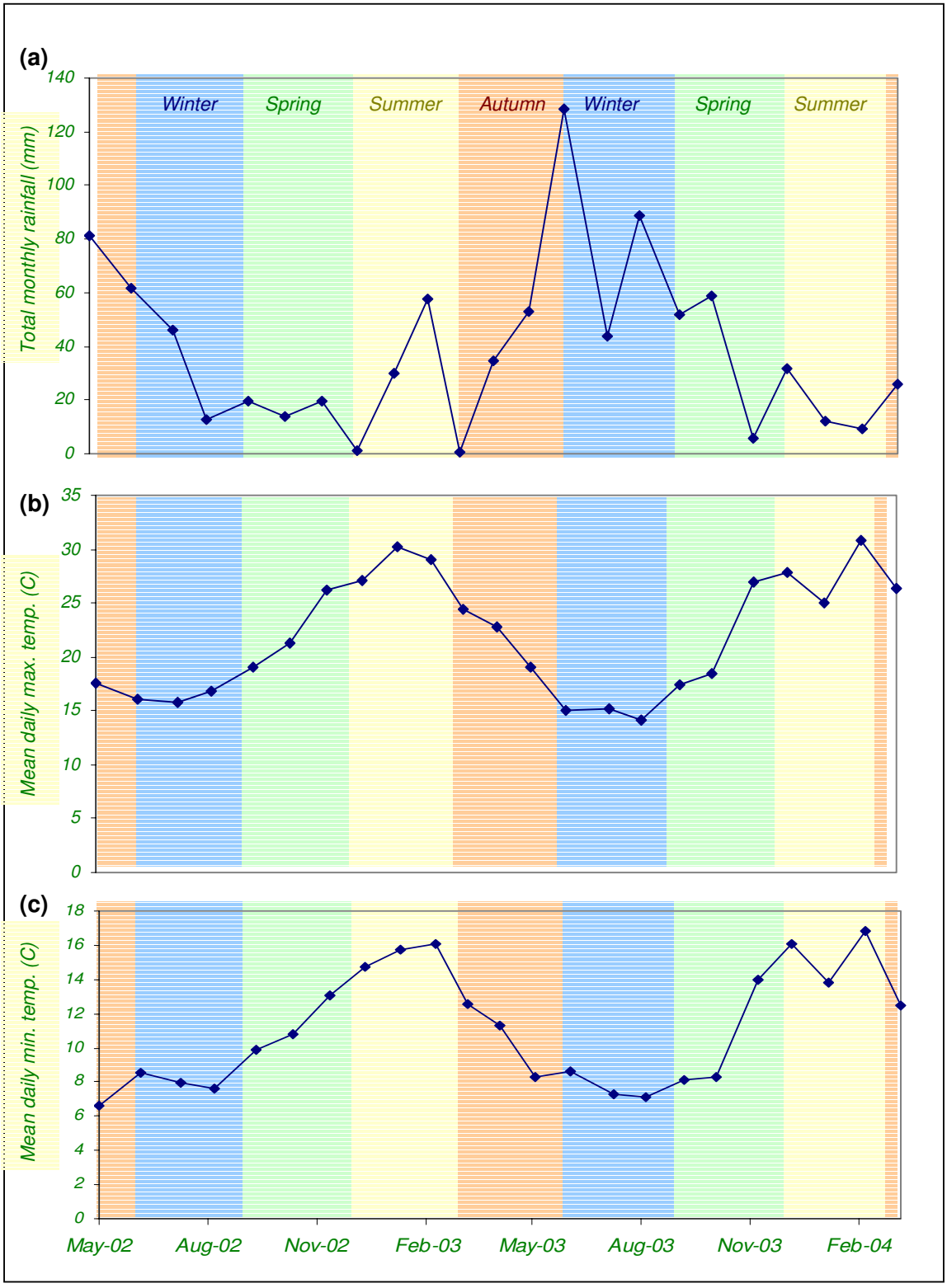


Fig. 22: Monthly averages of (a) monthly rainfall, (b) daily maximum temperature, and (c) mean daily minimum temperature at Urrbrae Education Centre.

3.3.4. Between tree growth, condition and gall occurrences

Individual tree characteristics were compared to determine if there were any obvious trends between each tree's growth, flowering, level of leaf damage and potential as a gall host. Area A trees (Trees 1 to 10) and Area B trees (Trees 11 to 20) were also compared, particularly to assess the possible effects of an additional water source provided to Area A by access to the unlined main pond.

For each of the twenty trees in the study, the initial and final tree heights are shown in Fig. 23 and the occurrence of flower buds and flowers are shown in Fig. 24. Fig. 24 shows the length of time as the fraction of months that each tree had flower buds and flowers. The initial and final heights of trees within the two areas were significantly different, with Area A trees taller than those of Area B (Table 3).

Table 3: Mean tree heights within Areas A and B.

	N	Area A (mean ± S.E.)	Area B (mean ± S.E.)	t-test Area A v's B prob.
Initial height	10	7.24 m ± 0.55	5.07 m ± 0.366	p = 0.0041
Final height	10	10.02 m ± 0.521	7.78 m ±0.561	p = 0.009

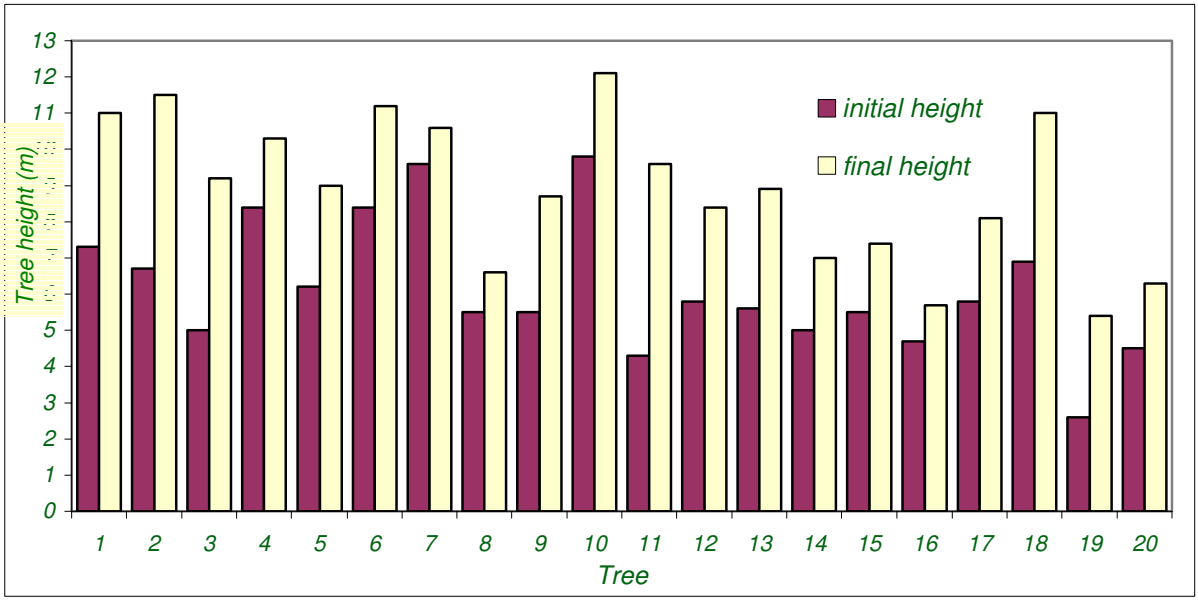


Fig. 23: Tree initial and final heights.

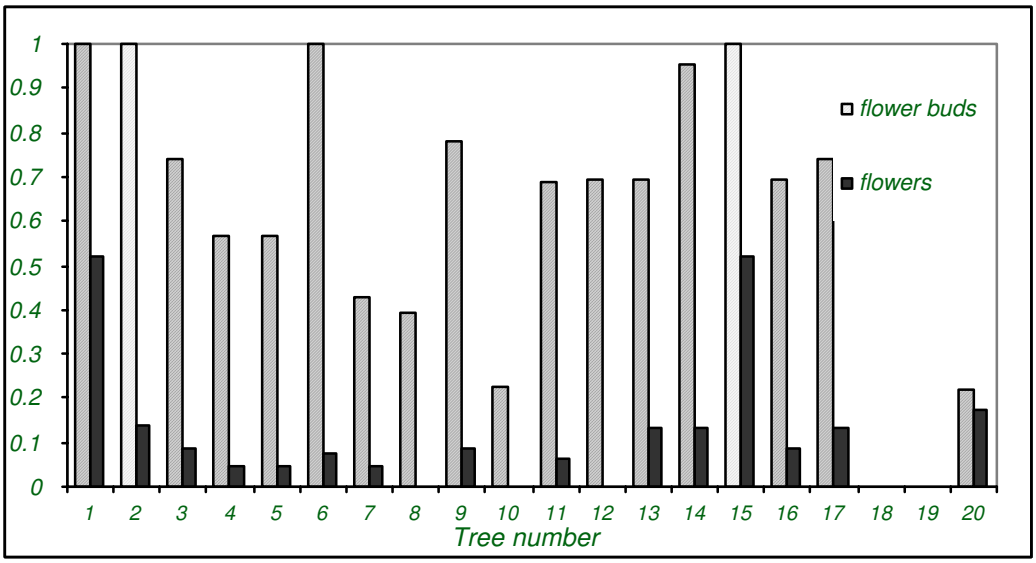


Fig. 24: The time (fraction of months) flower buds and flowers occurred on trees.

Although the heights of Area A and Area B trees varied, the proportion of months that Area A and Area B trees produced both flower buds and flowers did not differ (Mann-Whitney U tests, $p > 0.05$, 2-tailed).

To see if flowering was a function of tree height, the initial and final tree heights and tree growth were compared with the time (percentage of months) that trees possessed flower buds or flowers. There was no correlation between tree height, growth and flowering, indicating the likelihood that flowering was not related to height.

Trees 1, 2, 6 and 15 produced flower buds continually through the months of the study and each of these trees also had flowers (Fig. 24). Trees 8, 10 and 12 had flower buds for some months of the study but did not produce flowers, and Trees 18 and 19 did not produce flower buds or flowers at all (Fig. 24). As expected, the occurrence of flowers on trees with flower buds was strongly positively correlated (Spearman's Rank Correlation, Rho = 0.684, $p = 0.00429$, N = 20 trees).

The monthly densities of growing points for each tree were averaged and are shown in Fig. 25. In order to compare whole numbers of growing points per tree by Kolmogorov-Smirnov goodness of fit tests for grouped data, only those trees that were measured for the whole study period and for all 4 quadrants were used for comparisons (ie Trees 1, 2, 3, 4, 6, 7, 8, 9 and 11 were omitted). The tests showed that growing points were not distributed evenly between trees (Kolmogorov-Smirnov

goodness of fit tests, p<<0.001, n = 11 trees). Tree 13 had the greatest density of new growth (22.6 growing points per m^3) within the part of the tree measured and Tree 20 had the least new growth (with 2.0 growing points per m^3). The individual monthly gall densities for each tree were averaged and are shown in Fig. 27. These were compared to the growing point densities of each of the trees. Spearman's Rank Correlations showed no positive relationships between trees with greater density of growing points and those with greater gall density for each of the gall types. However, Tree 13, which had the most new growth, had the greatest density of leaf bud galls of the trees in the study (Fig. 25 and 27 b).

To assess a possible effect of the main pond on new tree growth, growing point densities were compared between Area A and Area B, and the growing point densities of Area A trees were compared to the distance of the trees from the main pond to see if there was a negative correlation between new growth and distance. Area A and Area B had similar densities of growing points and the growing points of Area A trees did not vary with the distance from the main pond, so it is unlikely that new growth within the lower regions of the trees was affected by proximity to the main pond (ANOVA, F = 1.029, p = 0.3237, N = 20 trees; Spearman's Rank Correlation, Rho = -0.298, p = 0.3706, n = 10 trees).

The averages of growing point densities of trees were compared to the length of time trees were scored with low, medium or high level leaf damage. The length of time for these comparisons was calculated as the fraction of months that each tree was scored with each level of damage. The level of leaf damage recorded in the trees is shown in Fig. 26. There were no relationships between medium or high levels of leaf damage and growing point densities, but there was a positive relationship between the time trees had low leaf damage and growing point density (Spearman's Rank Correlation, Rho = 0.469, p = 0.0411, N = 20 trees). This indicates that trees with lower leaf damage were more likely to develop growing points.

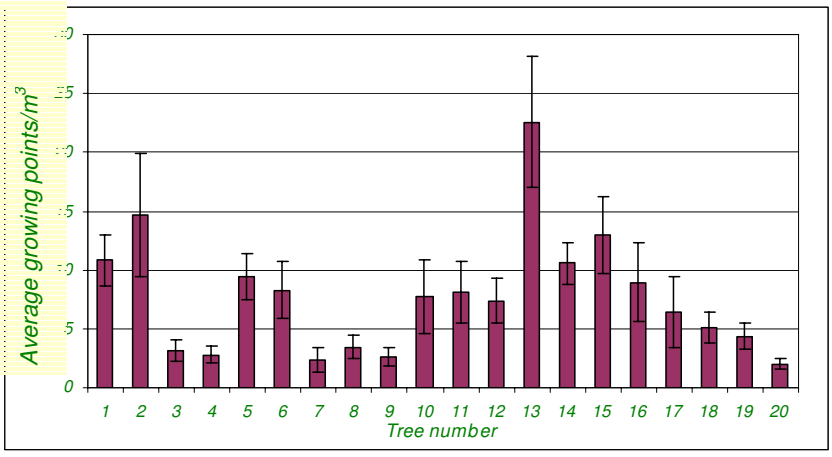


Fig. 25: Density of growing points for trees (average growing points/m^3 ± S.E.).

The proportion of trees in Area A with highly damaged leaves was greater than those of Area B for 12 of the 23 months studied (Mann-Whitney U tests, $p < 0.05$, 1-tailed). Additionally, the time Area B trees had low leaf damage was significantly greater than for Area A trees (Mann-Whitney U test, $p = 0.0034$, 1-tailed). The leaves of Area A trees were particularly prone to the attack of lerps or psyllids. These psyllids form sugary casings and produce exudates, associated with the presence of sooty mould, causing blackening of leaves and increasing the level of damage of the leaves in general. Five of the Area A trees which edged the main pond and grew among tall grasses and sedges and often had severely damaged, blackened leaves, particularly during the winter months. The construction of a lerp by a psyllid is determined by humidity (White, 1970). The branches of the pond edge trees touched the grasses possibly providing greater humidity for increased lerp production. To determine if proximity to the main pond affected the level of leaf damage within the Area A trees the proportions of months trees were ranked with either low or high damage were compared to the distance of the trees from the main pond. No correlation was found (Spearman's Rank Correlation, $p > 0.05$, $N = 10$ trees).

The density of the different gall forms varied greatly between the trees. Of the twenty trees measured, 15 had terminal leaf bud galls, 17 had axial bud galls, 5 had flower bud galls (Fig. 27) and few trees (Trees 1, 6, 11, and 15) had all gall forms. As for growing points, the total numbers of galls per tree were compared by Kolmogorov-Smirnov goodness of fit tests for grouped data, using only those trees that were measured for the whole study period and for all 4 quadrants (ie Trees 1, 2, 3, 4, 6, 7, 8, 9 and 11 were omitted). The tests showed that the respective gall forms were not distributed evenly between trees (Kolmogorov-Smirnov goodness of fit tests, $p<<0.001$, $n = 11$ trees). This supports a suggestion that some trees were more susceptible to gall formation than others within the same site and area (Fig. 27). The levels of damage varied between trees, with trees such as Tree 15, 16 and 19 repeatedly scored with low, or medium leaf damage (Fig. 26). Attempts to compare the damage ranks for gall densities in each month by ANOVA revealed no significant relationship between the levels of leaf damage of each tree and gall occurrence, but these comparisons are unreliable as averages could not be calculated for many months when galls did not occur. Tree 15 was scored with the least damage over the study (with 15 of the 23 months of measurements scored as low damage). This tree was one of the few with all gall forms, including 3 axial bud galls, and the highest totals per tree of 32 flower bud galls and 57 terminal leaf bud galls (Fig. 27). Tree 16, however, also had low leaf damage over many months, but only a few axial galls developed on it. Tree 8 was scored with the highest damage, with 19 of the 23 months of measurements having high leaf damage (Fig. 26), and had the least number of galls, with one terminal leaf bud gall which aborted a month after initial measurement (Fig. 27 a). However, there was no negative correlation between the times the trees had high leaf damage and the average

densities of terminal leaf bud, axial bud or flower bud galls developing on them. Similarly, there was no positive relationship between trees with low leaf damage in most months of the study and the average densities of terminal leaf bud, axial bud or flower bud galls on them

Comparisons between the time trees had medium leaf damage and density of axial bud galls revealed a weak correlation, indicating that axial bud galls were more likely to occur on trees that have medium rather than low and high levels of damaged leaves (Spearman's Rank Correlation, r_s = 0.384, p = 0.0938, N = 20 trees). Tree 13 had the highest abundance and density of axial bud galls but no other gall type, and Tree 5 also had a high number of axial bud galls and no other gall form. Both Trees 13 and 5 were repeatedly scored with medium leaf damage. However, other trees (such as Trees 6, 9 and 20) were continually scored with medium leaf damage, but had few axial galls (Fig. 26 and 27 b). No correlations were found between the time trees had medium leaf damage and densities of either terminal leaf bud or flower bud galls.

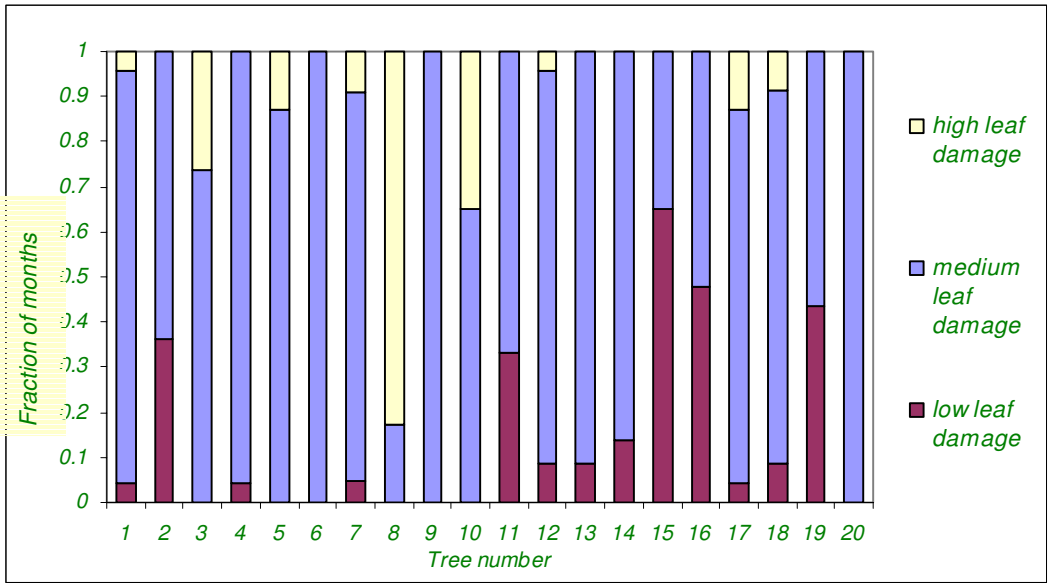


Fig. 26: Time (fraction of months) trees had high, medium or low leaf damage.

The length of time that flower buds occurred on the trees was not correlated to the level of leaf damage of the individual trees (ranked as either low, medium or high). Additionally, there was no correlation between the time that each tree was scored with either low or medium leaf damage and the occurrence of flowers. The length of time of trees scored with high leaf damage was weakly negatively correlated with the length of time that the trees had flowers, indicating that trees with highly damaged leaves are slightly less likely to flower (Spearman's Rank Correlation, r_s = -0.42, p = 0.0672, N = 20 trees).

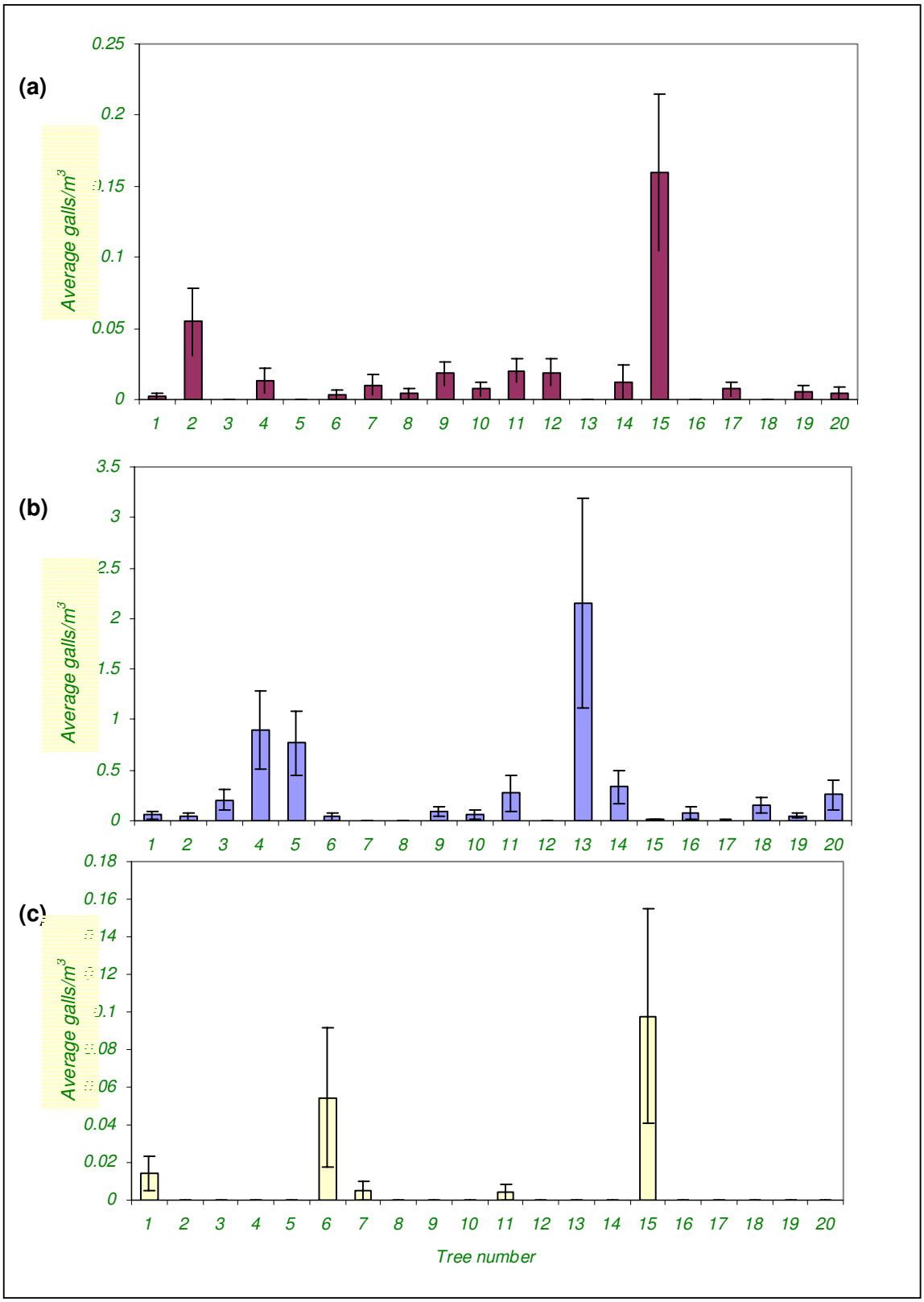


Fig. 27: Density of (a) terminal leaf bud galls, (b) axial leaf bud galls, and (c) flower bud galls for individual trees (ave. galls/m^3 ± S.E.).

The average density of flower bud galls on trees was positively correlated to the length of time trees had flower buds, supporting the assumption that flower bud galls occur on the trees with flower buds (Spearman's Rank Correlation, $r_s = 0.421$, $p = 0.0667$, $N = 20$ trees). There was no negative relationship between trees that produce flower bud galls and the length of time trees have flowers, indicating that flower bud galls did not reduce the likelihood of host trees producing flowers (Spearman's Rank Correlation, $r_s = 0.316$, $p = 0.1686$, $N = 20$ trees).

Area A and Area B trees had similar average densities of terminal leaf bud, axial bud or flower bud galls, so it is unlikely that gall occurrence within the trees is affected by proximity to the main pond (ANOVA, $p > 0.05$, $N = 20$ trees).

3.3.5. Orientation and distribution of galls and new tree growth

In order to compare total and average numbers of growing points with galls per quadrant, only those trees that were measured for all 4 quadrants were used for these calculations (ie Trees 1, 2, 3, 4, 7, 8, and 9 were omitted). The average growing points per quadrant are shown in Fig. 28. Both the northern and eastern quadrants of the trees had significantly more growing points per quadrant than the western or southern quadrants (Paired t-tests, $p < 0.05$, 1 - tailed). There was a similar abundance of growing points in the northern and eastern, and the southern and western quadrants of the trees (Paired t-tests, $p > 0.05$, 2 - tailed).

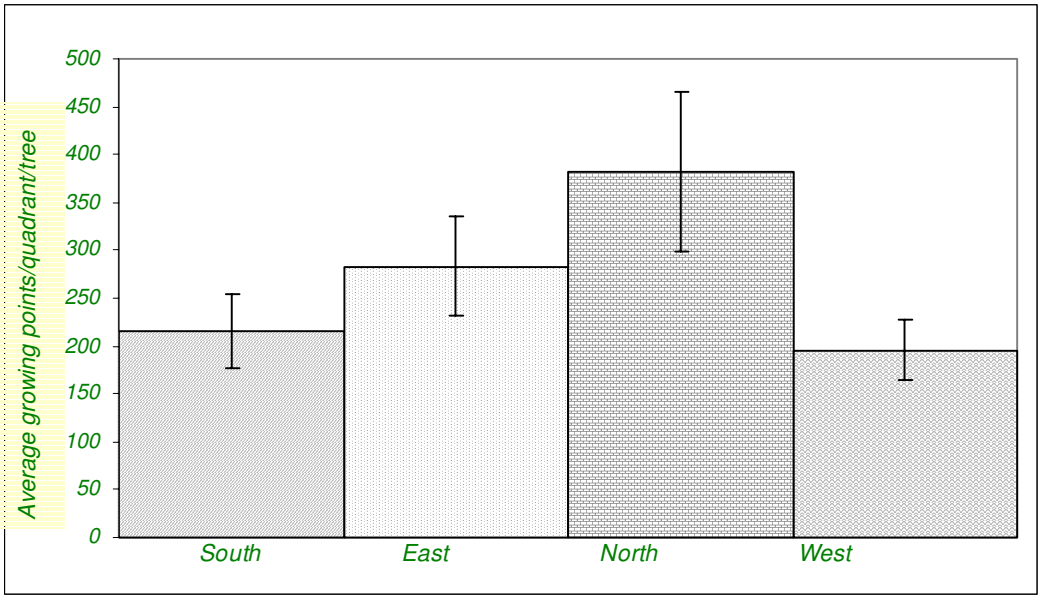


Fig. 28: Total growing points per quadrant per tree (ave. ± S.E.)

During spring of 2002 photos were taken of each quadrant of the trees. Fig. 29 depicts the canopy of Tree 13 which had the maximum number of galls during the study. For all trees, the southern quadrants appeared to have the least leaf damage and the western quadrants the greatest number of damaged leaves. This is supported by the average calculations during spring 2002 (September and October) of the fraction of leaves damaged within tree quadrants.

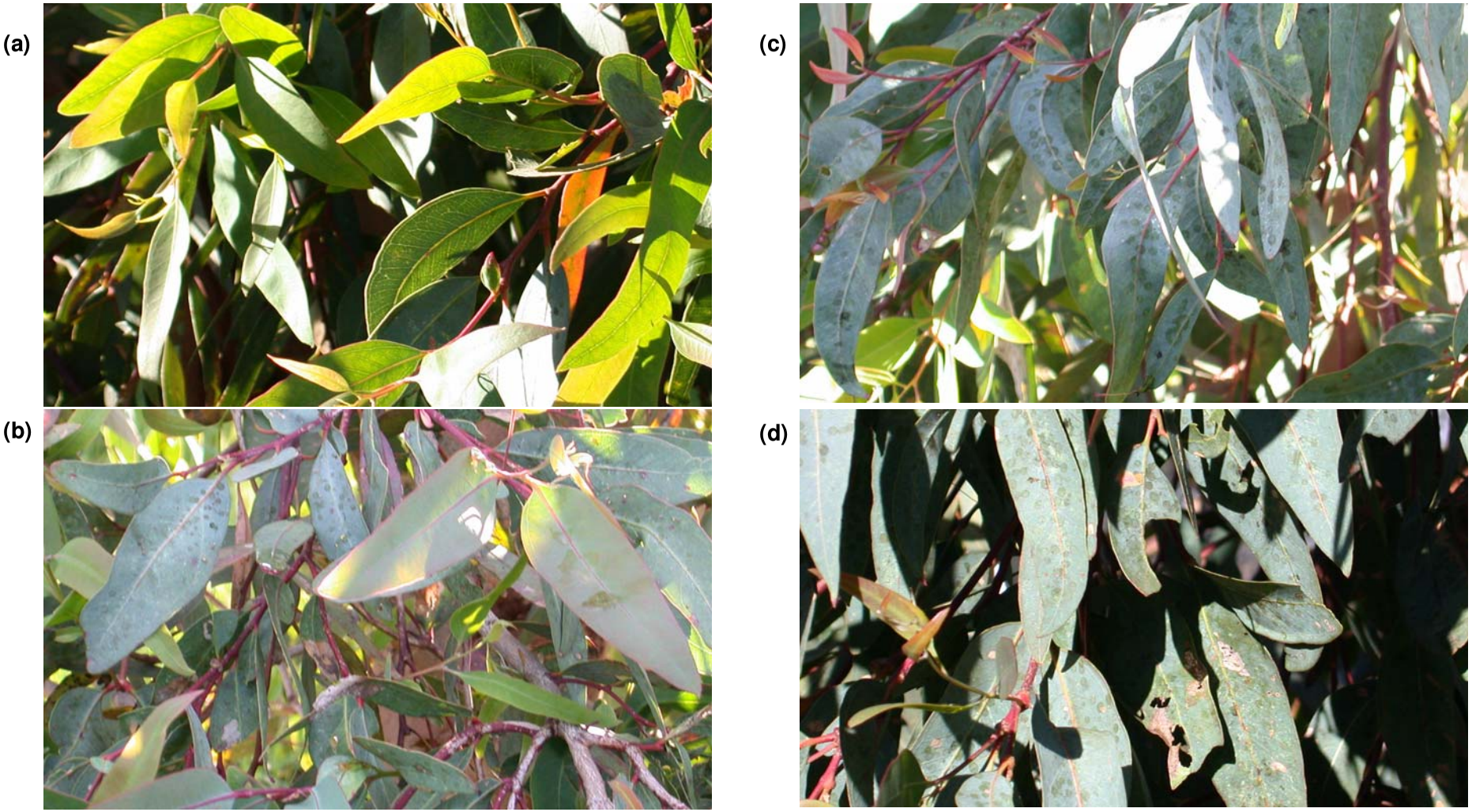


Fig. 29: Condition of leaves in canopy of Tree 13, 28.9.02, (a) South, (b) East, (c) North, and (d) West.

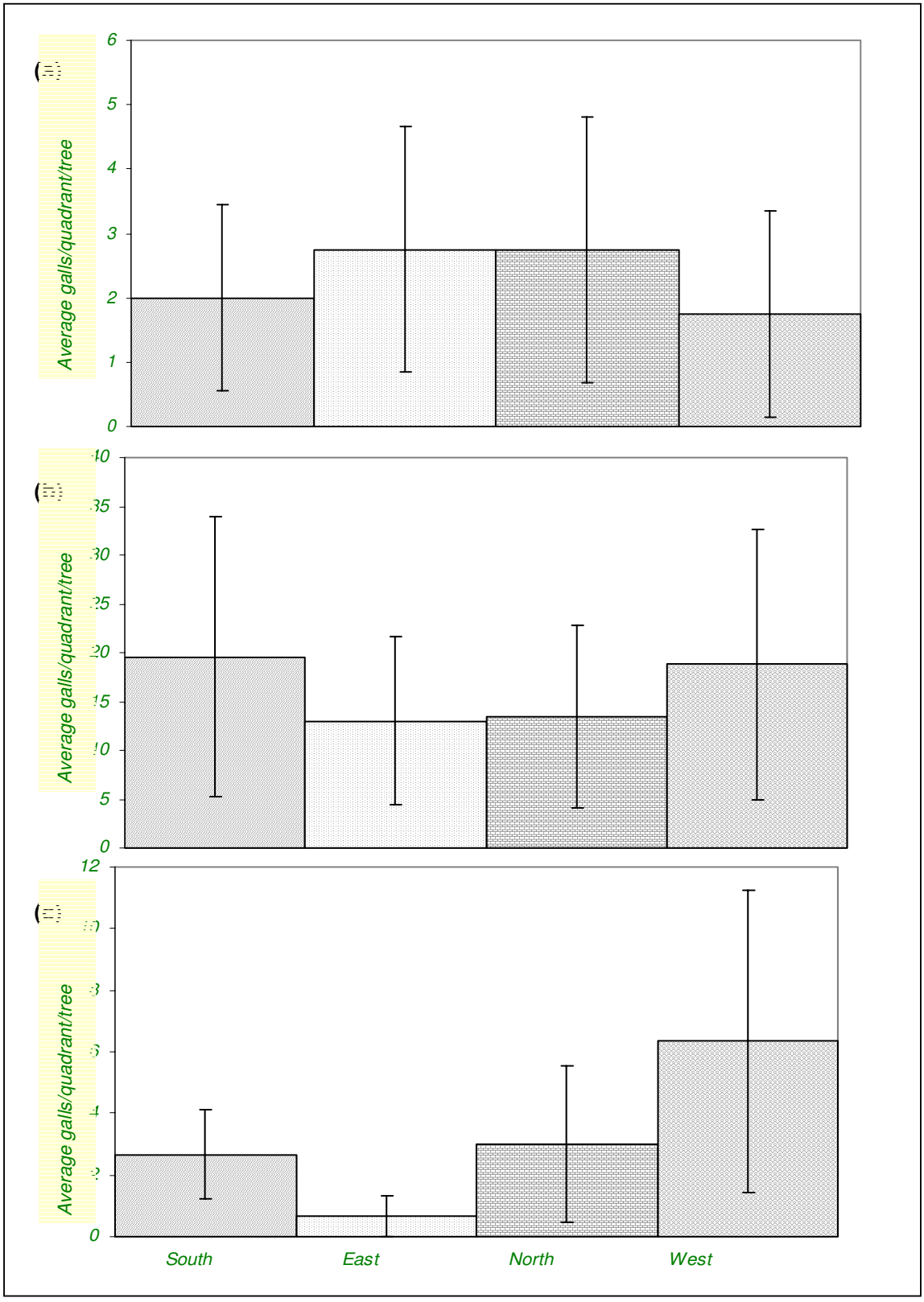


Fig. 30: Average total galls per quadrant per tree of (a) terminal leaf bud galls, (b) axial leaf bud galls, and (c) flower bud galls (± S.E.).

The relative numbers of galls per quadrant for each of the gall types were calculated per tree for trees measured in all 4 quadrants (ie Trees 1, 2, 3, 4, 7, 8, and 9 were omitted). The averages of galls per quadrant for terminal leaf bud galls (8 trees), for axial bud galls (11 trees) and for flower bud galls (3 trees) are shown in Fig. 30. Terminal leaf bud galls occurred more in the eastern and northern quadrants than in the western quadrants (Paired t-tests; $p<0.05$, 1-tailed). Although there was high variability in the data, axial bud galls tended to occur more in the southern and western quadrants, and the flower bud galls tended to occur more on the western and least in the eastern quadrants (Fig. 30).

The orientations of galls were additionally divided into 45° sectors but Paired t-tests were not reliable due to very little data for comparisons. Kolmogorov-Smirnov Goodness of Fit tests were able to be applied to the totals of galls per 45° sector to test for the evenness of distribution of galls around the trees. The results are shown in Table 4 and support clumping of each of the gall types within certain orientations within the trees.

Table 4: Results of Chi-squared Goodness of Fit tests for the distribution of galls within sectors around trees.

Gall form	Probability for 45° sectors	Number of galls
Terminal leaf bud	0.044	74 (within 8 trees)
Axial leaf bud	0.0001	899 (within 11 trees)
Flower bud	0.0053	38 (within 3 trees)

Within the trees, the distances of galls to the ground and to the tree trunks were also measured. Terminal leaf bud galls were recorded from 0.44 up to 2 m (the upper limit of measurement) and from between 0.31 to 2.2 m from the trunk. Axial leaf bud galls were recorded from 0.1 up to 2 m (the upper limit of measurement) and from between 0.02 to 2.02 m from the trunk. Flower bud galls were recorded from 0.64 up to 1.98 m and from between 0.44 to 1.85 m from the trunk. The averages per tree of the distances of galls to the ground and to the tree trunks of each of the gall forms within the tree canopies are shown in Fig. 31.

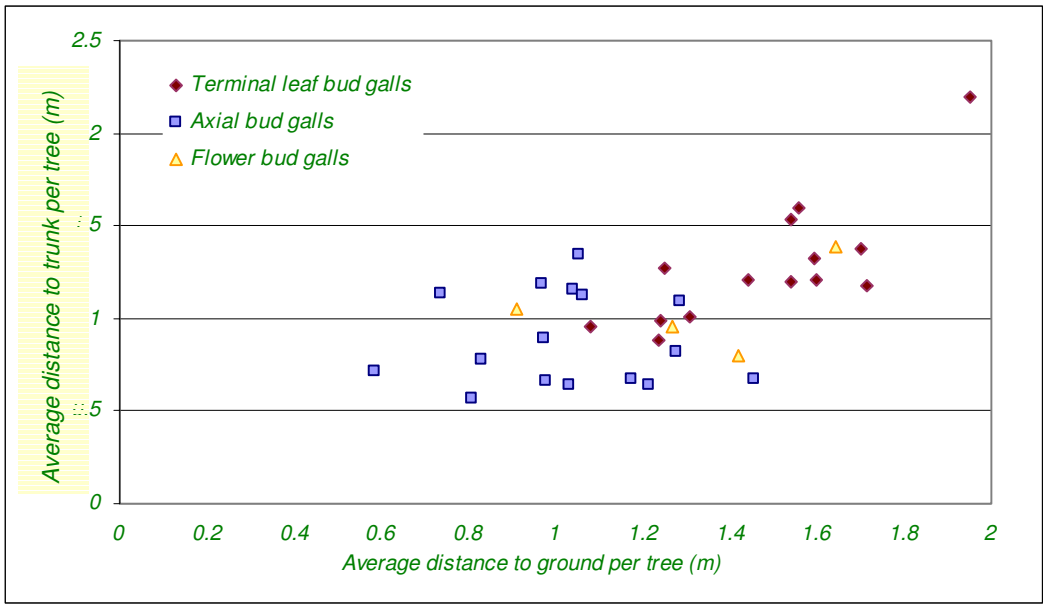


Fig. 31: Position of galls within trees (average distances to ground and trunk) of each gall form.

Terminal leaf bud galls occurred higher in the canopies and further from the trunks of the trees than axial bud galls (Paired t-tests, $p < 0.0001$, $p = 0.0014$, respectively, 1-tailed; Fig. 31). Axial bud galls and flower bud galls occurred at similar distances to the ground and distances to the trunks of trees (Paired t-tests, $p > 0.05$, 2-tailed; Fig. 31).

Terminal leaf bud galls were present at similar distances to the ground as flower bud galls (Paired t-test, $p > 0.05$, 2-tailed) but further from the trunks of trees than flower bud galls (Paired t-test, $p = 0.0031$ 1-tailed; Fig. 31).

During the study, the average distance (nested in trees) of galls to the ground and to the tree trunks did not change for each gall form (Spearman's Rank Correlation, $p > 0.05$). Since senescence of lower tree branches occurred as the trees aged, it was expected that an increase in the average distance of galls to the ground would occur with time. This was not the case and Kolmogorov-Smirnov Goodness of fit tests revealed that the galls of each form were not evenly distributed within

the ranges that they occurred ($p<0.001$). The total number of galls per month was compared to the average monthly distances of the galls from the ground and tree trunks (Figs 32 and 33). It appears that the majority of terminal leaf bud, axial bud and flower bud galls were concentrated within the tree canopies between 1 to 1.5m to the ground and trunk (Figs 32 and 33).

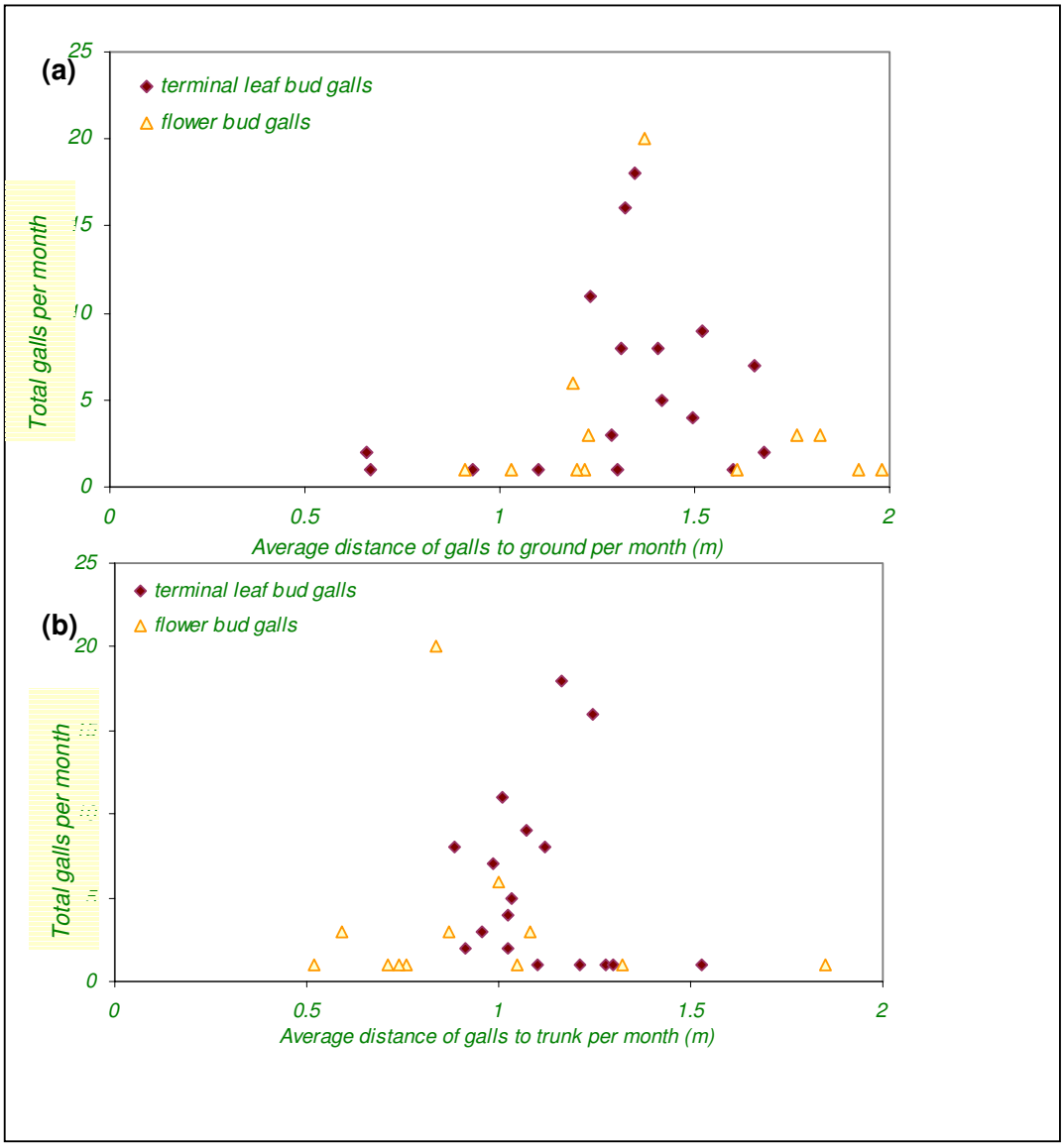


Fig. 32: Total galls per month for monthly average terminal leaf bud and flower bud gall (a) distances to ground and (b) distances to trunk.

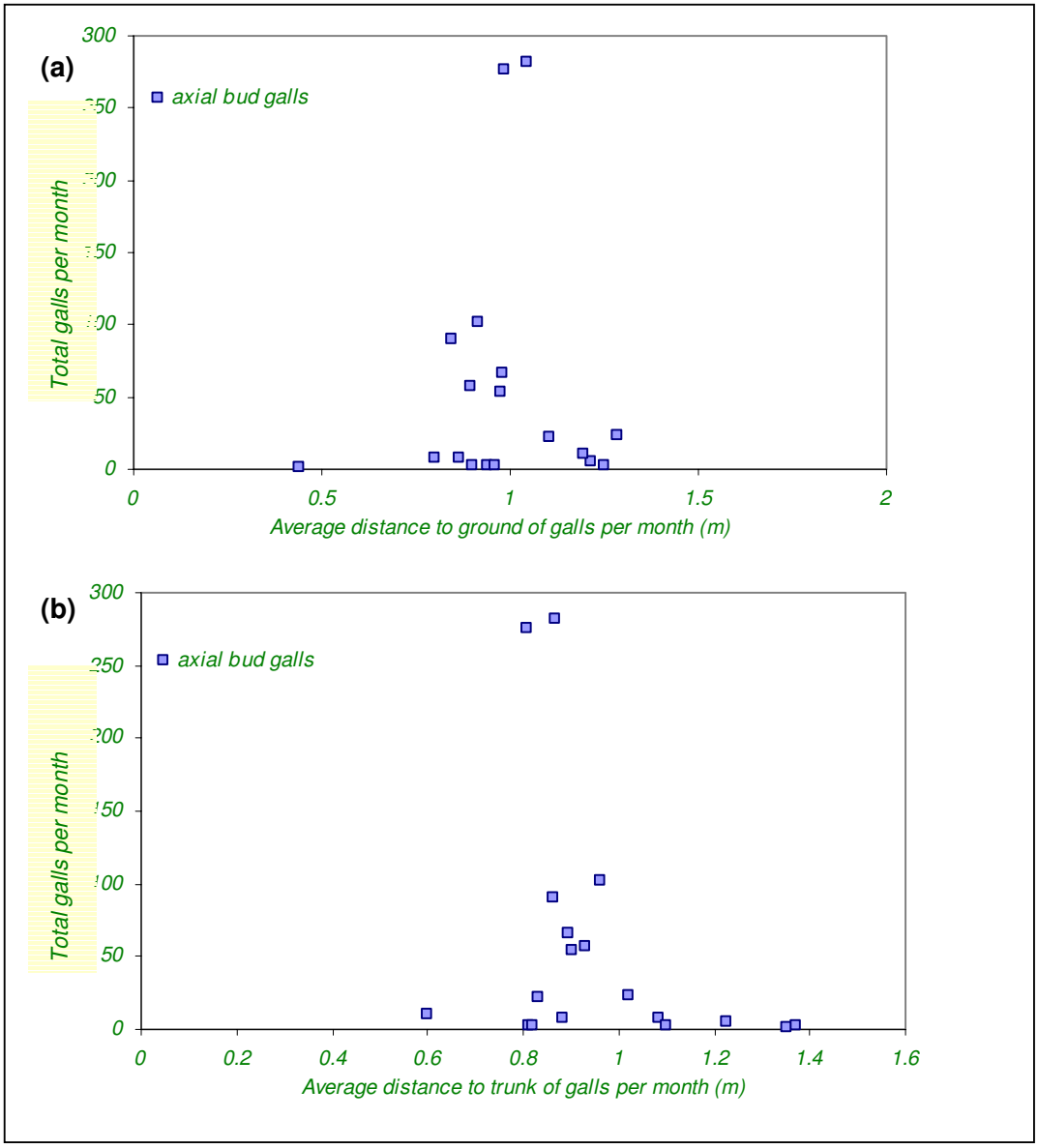


Fig. 33: Total galls per month for monthly average axial leaf bud galls (a) distances to ground and (b) distances to trunk.

3.3.6. Gall characteristics and outcomes

Observations were recorded for 78 terminal leaf bud, 810 axial bud and 38 flower bud initiated galls. Of these, only some were suitable for longevity studies and exit hole measurements because many initiated galls aborted or were missing at subsequent observation times. Since observations of galls were recorded once per month, the longevity or time since they first became visible to senescence could only be approximated. With this in mind, terminal leaf bud galls had an average longevity of 7 weeks (50 days), axial leaf bud galls an average longevity of 10 weeks (72 days) and flower bud galls an average longevity of 11 weeks (75 days, Table 5).

Of the galls observed, approximately 72% of terminal leaf bud galls, 64% of axial bud galls and 34% of the flower bud galls reached maturity and had exit holes (Table 5). Many galls either aborted, were absent, parasitised or eaten (Table 5). An accurate estimate of parasitic events could not be determined, since galls that were aborted or absent may have also been parasitised. Of the terminal leaf bud galls observed, 45% were aborted, absent, parasitised or eaten. Terminal leaf bud galls often possessed obvious signs of parasitism (30% of initiated galls). Such signs of parasitism included very large exit holes and accompanying frass associated with other gall inquilines. Terminal leaf bud galls were rarely eaten or had signs of obvious predatory grazing (1%). Of the axial leaf bud galls observed, 19% were either aborted, absent, parasitised or eaten. Axial bud galls showed less obvious parasitism (3%) than the terminal leaf bud galls, but were more likely to be absent (11%) or eaten (4%). Birds were seen breaking off and feeding on axial bud galls while observations were being recorded. Amongst the flower bud galls, 63% were absent, parasitised, or aborted. More than half (55%) of the initiated flower bud galls were absent at future visits, but none were observed to be eaten.

Of all the galls recorded, 41 terminal leaf bud, 213 axial bud and 19 flower bud galls were measured for maximum size at maturity. Terminal leaf bud galls were the largest gall form with an average diameter and length of 14.0 and 24.3 mm respectively. There was a large variation in diameter of these galls, because although most were generally ovoid in shape, some were very elongated in form. Flower bud galls had an average diameter of 9.0 mm and length of 11.8 mm and were spherical to slightly ovoid in shape (Table 5). Axial bud galls were generally smaller (average diameter 6.1 mm, average length 4.7 mm, Table 5), than both terminal leaf bud and flower bud forms and were typically spherical. The largest axial galls had multiple lobes which may have represented a cluster of smaller galls, or clumping of individual locules.

Table 5: Gall characteristics

Gall characteristic	Terminal leaf bud galls	Axial leaf bud galls	Flower bud galls
Galls measured	78	810	38
Galls that reached senescence	61	664	12
Longevity	7 weeks (50 days, N = 58)	10 weeks (72 days, N = 524)	11 weeks (75 days, N = 10)
Galls that produced exit holes	56	517	13
Galls parasitised	23	26	1
Galls aborted	3	6	2
Galls absent	8	89	21
Galls eaten or grazed	1	36	0
Gall diameter (ave, range, mm)	13.96, 7.50-30.10 (N = 41)	6.07, 2.55–13.00 (N = 213)	8.96, 5.00–15.00 (N = 19)
Gall length (ave. range, mm)	24.27, 10.00-43.55 (N = 41)	4.74, 2.10–10.50 (N = 213)	11.81, 7.90–22.30 (N =19)
Exit holes/gall (ave. range)	12.80, 1–60 (N = 56)	1.28, 1–6 (N = 517)	1.54, 1–6 (N = 13)

The maximum diameter and length of galls of each gall type were averaged and compared with numbers of exit holes per gall between trees to determine if there was a relationship between gall size and exit holes per gall.

For terminal leaf bud galls, the number of exit holes per gall was skewed, with 71% of galls having 1 to 12 exit holes per gall. As average terminal leaf bud gall size increased, there was an increase in the average number of exit holes per gall and this was evidenced by both gall diameter and gall length being linearly related to the tree averages of number of exit holes per terminal leaf bud gall (Spearman's Rank Correlations, Rho = 0.811, p = 0.015; and Rho = 0.681, p = 0.041, respectively, N = 10 tree averages).

For axial bud galls the number of exit holes per gall was skewed, with 77% of galls having just one exit hole per gall. Eighteen percent of the axial bud galls had two exit holes and 4% had 3 exit holes. Two axial galls (0.4%) had 4 exit holes and one axial bud gall had 5 exit holes (the size of this gall was not measured). The largest number of exit holes recorded per axial gall was 6, recorded only once. Generally, average axial bud gall size increased with greater exit hole number, and a linear relationship was found between the average number of exit holes per axial leaf bud

gall and gall diameter but not gall length (Spearman's Rank Correlation, Rho = 0.55, p = 0.048 for gall diameter, N = 14 tree averages).

Of the 13 flower bud galls that produced exit holes, ten (77 %) had 1 exit hole, two (15%) had 2 exit holes, and only one (8%) had 6 exit holes (the maximum number of holes per flower bud gall recorded). Exit holes occurred only in the flower bud galls on Tree 15. No relationship was found between the number of exit holes per flower bud gall and gall size.

3.4. Discussion

3.4.1. Seasonality of tree growth and new galls

During the study the trees matured and increased in height, and after January 2003, the proportion of trees with flower buds increased to above 60% (Fig. 17). Flowering was seasonal, occurring during late spring to early summer, with flowers on a greater percentage of trees in the second year (Fig. 17).

Most of the trees were scored with medium leaf damage during the study (77.2%) and there were seasonal trends in damage levels (Fig. 18). Low scores for leaf damage were associated with times of increase in flower bud and flower production. Leaf damage including sooty mould appeared to increase during the cooler winter months and was highly negatively related to mean maximum daily temperature. Cool, moist and humid conditions increased the effect of sooty mould and blackening of leaves, associated with higher levels of leaf damage. Although leaf damage appeared to increase during the cooler winter months, there were no significant seasonal relationships between levels of leaf damage and either growing point density or the occurrence of galls.

New growth was strongly seasonal, with growing points occurring mostly during late winter, spring and most of summer (Fig. 19). There was greater new growth during the first year, possibly due to the younger age of the trees. In 2002, new growth was still occurring in lower branches that would senesce as the trees matured (Fig. 19). The lack of relationship between seasonal levels of leaf damage and new growth indicates that the trees did not replace highly damaged leaves with new growth within the regions measured (0 to 2m above ground). Clearly the trees were maturing during the study, increasing in height, producing less new growth on lower branches, and producing more floral structures.

Three *Fergusonina*/*Fergusobia* gall forms were found within the trees observed at this location. The record of terminal leaf bud galls was consistent with other collections but the dominance of axial

leaf bud galls at this site was unexpected. The axial leaf bud galls occurred prolifically on a few trees at the Urrbrae Wetlands and this had not been observed at other collection sites in South Australia (K. Davies and G. Taylor, pers. comm.). Axial bud galls were observed on lower branches of mature *E. camaldulensis* trees in Victoria (K. Davies, pers. comm.).

New growth, axial leaf bud galls, and flower bud galls followed a similar seasonal pattern, occurring during mid-winter to spring (Figs 19 and 21 b and c). Terminal leaf bud galls appeared later, during mid-spring to summer (October, to March, Fig. 21 a). This is in contrast to collection dates and emergences recorded by Taylor *et al.* (1996) for terminal leaf bud galls on *E. camaldulensis* at Goolwa, approximately 50 km south-east of the Urrbrae wetlands site. In the Goolwa study, terminal leaf bud galls appeared in the autumn from late March and matured up to 4 weeks later (Taylor *et al.*, 1996). At the Urrbrae wetlands site, few galls of any type were found on *E. camaldulensis* during the autumn months. This may reflect differences in microclimates of the two sites, as Goolwa is coastal.

The mostly leaf bud *Fergusonina*/*Fergusobia* galls recorded in the *Melaleuca* study were abundant during late winter to early spring and closely followed increases in leaf bud densities (Goolsby, *et al.*, 2000). The axial leaf bud galls of the current study followed similar abundances in August and September, and in 2002, in particular, strongly followed growing point density (Fig. 21 b and Fig. 19). From these observations, it appears that some *Fergusonina* species are able to rapidly exploit localised increases in leaf bud densities.

Although flower buds were prolific on some trees at various times of the year, low numbers (42) of flower bud galls were recorded. There was no relationship between flower bud galls and the length of time trees had flowers. Within *E. camaldulensis* and *M. quinquenervia* flower bud galls do not appear to suppress flowering (G. Taylor pers. comm.; Goolsby *et al.*, 2000). Within the ecological study of *M. quinquenervia*, flower bud galls were included, but were believed to represent few of the total galls because the trees measured were immature (Goolsby, *et al.*, 2000). Currie (1937) observed the trends of flower buds and flower bud galls over a 5 year period in one *E. macrorhyncha* host tree. In the first year there were a reasonable number of flower buds but no flower bud galls, followed by a year with many flower buds and so many flower bud galls that few buds matured to flowering stage. In following years, there were many flower buds again but no flower bud galls. Currie concluded that there were large fluctuations in flower bud gall flies from season to season mainly due to the erratic formation of flower buds within the eucalypt host. He suggested that parasitism was reduced in the flower bud galls due to their more specialised structure (Currie, 1937). He also suggested that flower bud galls might be more heavily parasitised

than terminal leaf bud galls because the former are smaller in size (Currie, 1937). Within the current study, flower bud galls were rare in proportion to the number of available new flower buds present on the trees. This gall form is cryptic as it often appears as a slightly swollen bud and it is possible that some flower bud galls were missed. The flower bud galls did not appear to significantly affect flowering, the occurrence of flower buds was erratic, and the young trees may not have flowered consistently or prolifically enough to allow a large enough sample of flower bud galls for reasonable assessments of seasonal trends of the gall form and parasitism of them.

The strong positive relationship between growing point density and axial bud galls links the availability of new growth with potential leaf gall sites. Goolsby *et al*. (2000) also found that *Fergusonina* gall density on *Melaleuca* hosts followed increases in leaf bud density. In the current study, flower bud gall density was also positively correlated with growing point density, but new leaf growth is not necessary for the initiation of flower bud galls and this indicates that other seasonal factors may be influencing both new growth and flower bud galls. Goolsby *et al*. (2000) found that *Fergusonina* leaf bud galls followed a seasonal sine curve and theorised that the population dynamics were influenced by a range of biotic and climatic factors. In the current study, densities of terminal leaf bud galls and growing points were not positively related although terminal leaf bud galls require new plant growth sites for gall initiation. Because this gall type is only found at the end of stems or branches, not all of the leaf buds that occur along the branches would be available for gall formation. As for the axial bud galls, flower bud galls, and growing points, other factors such as biotic and climatic influences must affect the abundance of the terminal leaf bud galls.

The levels of parasitism, plant canopy shading, and nutrients present change throughout the seasons and would affect the seasonal occurrence of each of the gall forms and new growth. From previous taxonomic studies of the *Fergusonina* gall forms, each gall type is characterised by a fly and nematode species complex, which is associated with parasitoid species that are often host specific (Taylor, *et al*., 1996; Davies *et al*., 2001). The range of factors that would affect the seasonal abundances of the parasitoids associated with each gall form would also influence the abundance and seasonal patterns of galls. Currie (1937) theorised that differences in parasitism between gall forms contributed to the fluctuations of flower bud galls, in particular, compared to leaf bud galls. From an ecological perspective, although axial bud galls and flower bud galls occur concurrently and within the same tree in some cases, they fill different niches because the galls are formed from axial leaf buds and flower buds respectively. These fly species could coexist even in the one tree and avoid competition for growing point or flower bud initiation sites.

3.4.2. Climate and gall growth

Since new growth apparently was independent of rainfall, and the proximity of trees to the unlined main pond did not affect new growth, trees may have access to ample ground water at the Urrbrae site.

Terminal leaf bud galls were more abundant during late spring and summer and were positively correlated with mean daily temperatures. Their density was negatively related to rainfall. In contrast, Goolsby *et al*. (2000) found no relationship between rainfall and density of *Fergusonina* galls on *Melaleuca*. Higher summer temperatures appeared to reduce both *Melaleuca* bud density and gall densities in the following winter (Goolsby *et al*., 2000). In the current study, there was no relationship between growing point densities and mean daily maximum or minimum temperatures. The difference between the effect of climatic trends upon the current study and the *Melaleuca* study could be due to geographical variation (Goolsby *et al*., 2000). The *Melaleuca* study was conducted in northern New South Wales and Queensland, which have a sub-tropical to tropical climate with hot, wet spring-summers and cool, dry winters (Goolsby *et al*., 2000). The Adelaide region of the current study has a temperate climate with rain occurring mostly in the cooler, autumn to winter months and dry, hot summers (Fig. 22).

No relationship was found between rainfall and gall density within the study of leaf bud forms on *Melaleuca* (Goolsby *et al*., 2000), or with the axial leaf bud and flower bud galls of the current study. However, on a larger environmental scale, few *Fergusonina*/*Fergusobia* galls are found in regions of Australia with low rainfall (K. Davies, pers. comm.). It is suggested that much of the inland of Australia is usually too dry and harsh to support *Fergusonina*/*Fergusobia* galls (K. Davies, pers. comm.). For both the *Melaleuca* study and this current study, correlations of gall abundance and rainfall may not be significant, because the sites were coastal and had higher rainfall than inland Australia.

3.4.3. Host selection and gall position

Tree height varied between trees and between areas, although all of the trees were of the same age and seed provenance. Trees of Area A were significantly taller than trees of Area B. Area A trees were closer to the unlined main pond. This may have given them a source of water supporting faster growth than the trees of Area B. The density of new growth, however, within the trees of Area A and Area B did not differ, supporting that access to nearby main pond water did not affect the numbers of growing points. Additionally, Area A trees appeared more prone to damage than Area B trees, particularly by psyllids. Trees with lower levels of leaf damage were more likely to have

growing points. There were no obvious relationships between the levels of leaf damage and gall occurrence although axial bud galls slightly favoured trees with medium levels of leaf damage.

There were noticeable visual differences in leaf colour between trees and some appeared more susceptible to leaf damage. For example, Tree 8 had leaves in poor condition, affected by leaf skeletonisers, lerps and sooty mould. This tree did not develop flower buds, rarely had new growth and no successful galls were observed on it during the study. Some trees were more susceptible to attack by other invertebrates, and the likelihood of a tree being a host for *Fergusonina*/*Fergusobia* varied greatly amongst trees with medium leaf damage. There were also differences in the densities and types of gall forms between the trees, even when they were within a few metres of each other. For example, Tree 12 had 6 terminal leaf bud galls during the study, but no axial or flower bud galls (Fig. 27 a). Tree 13, only 5.5 metres away, had many (613) axial bud galls but no other gall type (Fig. 27). These trees were both within Area B, along an undisturbed path, with a similar aspect and degree of disturbance (Fig. 14). The colouring of the leaves of Tree 12 and 13 differed, and their density of new growth varied widely. These observations indicate possible genetic variation between the trees causing differences in phenology and the likelihood of being a host for the *Fergusonina*/*Fergusobia* complex.

Within trees, the northern and eastern quadrants had significantly more growing points than the western or southern quadrants (Fig. 28). This is consistent with the observation that more new growth occurs on the northern side of trees grown in the southern hemisphere (White, 1969; Currie, 1937). The leaves facing the North are more exposed to the sun and have a higher photosynthetic rate than leaves of other orientations (White, 1969). The higher photosynthetic rate is associated with higher nitrogen content and increased herbivory and parasitic attack on these leaves (White, 1969). The average monthly number of galls per quadrant per tree differed only for terminal leaf bud galls in the eastern and northern quadrants (Paired t-tests; $p<0.05$, 1-tailed; Fig. 30 a). Terminal leaf bud galls tended to occur where new growth was concentrated, suggesting that North-facing leaves may be preferred by flies for oviposition sites, or that galls grow more successfully on the northern sides of trees (Fig. 28 and Fig. 30 a). White (1969) found that psyllid densities were greater on the north-facing leaves of eucalypt hosts. Axial leaf bud and flower bud galls occurred more within the more shaded and possibly more protected western or southern quadrants, than the northern or eastern (Fig. 28, Fig 30 b and c). Regions of trees more exposed to the sun may also have higher temperature. Galls may abort because extreme heat (>40°C) appears detrimental to *Fergusobia* during the initial stages of gall induction (K. Davies, pers. comm.). Additionally, the levels of leaf damage varied between quadrants, with the southern having the least and the western quadrants the greatest number of damaged leaves (Fig. 29). There were

no obvious trends between the orientation of leaf damage and gall occurrence, but the southern quadrants had the fewest damaged leaves and the most axial leaf bud galls (Fig. 28 and 30 b). It is possible that some *Fergusonina* flies are deterred by leaf damage due to appearance or increased volatiles and choose the southern quadrants for oviposition to avoid competition from other herbivores (White, 1969). However, many flower bud and axial bud galls occurred in the western quadrants, where leaf damage was highest (Fig. 30 a and b). In addition, a greater fraction of leaves was damaged in the western than the northern or eastern quadrants, not consistent with the observation that greater herbivory occurs on north-facing leaves (White, 1969). Some axial leaf bud galls in the shaded regions of Tree 13 remained green, and did not redden as did those exposed to the sun. Reddening of axial galls may make them more visible and prone to attack by parasitoids and predators. Axial bud galls in the more shaded southern and western quadrants may therefore be more successful.

The uneven distribution of gall totals per 45° sector around the trees supported clumping of the galls within certain orientations. This supports that oviposition by *Fergusonina* flies was concentrated within particular stems or regions with suitable new growth for gall sites. While not quantitatively measured, growing points were spread from the lowest branches to the limit of measurement (2 m) and higher, yet the galls of each form were concentrated within certain distances from the ground (Fig. 31, 32 and 33). This indicates that *Fergusonina* flies choose oviposition sites non-randomly, perhaps at distances from the ground that favour flight. *Fergusonina* are small flies that may concentrate their flight within a “boundary layer”. Many small diurnal flies have greater densities from ground level up to an interface or a height of discontinuity, beneath which free flight and control of movements is possible (Taylor, 1974). Above the boundary layer, wind speeds are too great to allow control of flight (Taylor, 1974).

Despite the obvious bias of the study in assessing only the accessible region of the trees, from ground level up to 2 m, none of the gall forms increased their distance from the ground, expected as lower branches senesced with time. The majority of galls were concentrated within specific distances from the ground and trunk, suggesting that there was an optimal region for gall growth that not only reflects growing point availability, but also positions within the tree that may be either preferred by ovipositing flies or more likely to allow success of gall growth from protection by canopy shading. It is not known if there were other preferred regions higher in the canopy because these areas were not assessed. In particular, flower bud galls may have occurred higher on the trees since there were often many flower buds higher in the canopy where it was difficult to distinguish galls from buds. Terminal leaf bud galls occurred higher in the canopies and further from the trunks of the trees than axial bud galls. This is probably because new shoots on which axial leaf

bud galls form occur lower on the stem and closer to the trunk than terminal growing points. Axial leaf bud and flower bud galls occurred at similar distances to the ground and to the trunks of trees. Each of the three gall forming fly/nematode complexes utilise different meristematic resources that occupy specific levels or regions within the canopy.

3.4.4. Gall characteristics and outcomes

From other studies of *Fergusonina*/*Fergusobia*, initial swelling or early gall development is noticeable approximately one month after fly oviposition (Currie, 1937; Giblin-Davis *et al*., 2001b). Therefore, it is assumed for the current study that oviposition had occurred some weeks before the initial gall observation. In *Melaleuca* shoot bud galls, the life cycle has been estimated as either 2 months or 10 to 14 weeks from oviposition to senescence (Goolsby *et al*., 2000; Giblin-Davis *et al*., 2001b). Assuming that there was an interval of 4 weeks between oviposition and first observation within the current study, terminal leaf bud galls had an average longevity of 11 weeks and axial leaf bud galls an average longevity of 14 weeks. This corresponds closely to the estimates given in the *Melaleuca* studies (Goolsby *et al*., 2000; Giblin-Davis *et al*., 2001b). From previous studies, flower bud galls took approximately 6 weeks from oviposition to early development, and mature galls were observed and dissected at 6 months (Currie, 1937). The length of lifecycles in flower bud galls is variable and generally longer than the leaf or shoot bud galls. Assuming that there was an interval of 6 weeks between oviposition and first observation within the current study, flower bud galls had longevities of 14 to 27 weeks from oviposition to senescence (Currie, 1937). Flowers and flower buds occur irregularly within the eucalypts so it would be advantageous for flies and nematodes developing within flower bud galls to have extended or variable longevities to allow fly emergences to coincide with appearances of new flower buds. It is not known what physiological processes or cues enable such variable maturation and emergence times of the flower bud gall flies.

Many of the galls studied did not reach maturity and did not produce exit holes. Galls may abort or become parasitised. Nearly half of the terminal leaf bud galls initiated were aborted, absent, parasitised or eaten (45% of initiated galls). Of the three gall forms, they were the most prone to obvious parasitism (30% of initiated galls), probably because of their larger size. As many as 12 parasitic hymenoptera species have been reared from terminal leaf bud galls on *E. camaldulensis* in previous studies and these galls obviously provide a resource for many species within multiple trophic levels (Taylor *et al*., 1996). Parasitism was not as obvious within the axial bud galls (3% of initiated galls). Destructive sampling and rearing out of parasitoids from axial leaf bud galls is needed to establish what species exist within this gall form. It is likely that the genera of Hymenoptera in these galls will be similar to those of *Fergusonina*/*Fergusobia* shoot bud galls on

Melaleuca and terminal leaf bud galls on *E. camaldulensis.* Many of the wasp genera associated with these two gall forms are shared (Taylor, *et al*., 1996; Davies *et al*., 2001). Fourteen percent of axial leaf bud galls were absent or eaten. Birds (parrots and noisy miners, *Manorina melanocephala*) were seen breaking off and feeding on the galls, indicating that predatory grazing reduces the numbers of these galls that mature. Parrots have been observed grazing upon *Fergusonina*/*Fergusobia* flower bud galls on *E. camaldulensis* and are suspected of grazing upon terminal leaf bud galls on *E. cosmophylla* (Taylor *et al*., 2005; K. Davies and G. Taylor, pers. comm.). Within the current study, more than half (55%) of the initiated flower bud galls disappeared during the period of observation, possibly due to the foraging of birds. Parasitism was not observed within flower bud galls and Currie (1937) suggested that parasitism was reduced in them due to their more specialised gall structure. As for the axial leaf bud galls, flower bud galls need to be destructively sampled and their parasitoids reared out, because it is not known what other species occur in this gall form.

Terminal leaf bud galls ranged from 7.5 to 30.1 mm in diameter and 10.0 to 43.6 mm in length. Published records of terminal leaf bud galls give ranges of 12 to 20 mm in diameter and 18 to 50 mm in length (Taylor *et al*., 2005). Flower bud galls documented by Currie (1937) on *E. macrorhyncha* had a maximum size of 14 mm, grown from the normal flower bud size of 3 mm. Currie reported a difference in flower bud gall size without wasps (average diameter of 5.7 mm) and with wasps (average diameter of 9.3 mm). Within the current study on *E. camaldulensis*, flower bud gall size varied, with the largest being 15.0 mm by 22.3 mm. Normal flower buds on this species are similar in size to those on *E. macrorhyncha*, being approximately 3 mm in diameter. It is not known if the larger flower bud galls contained wasps. The size of axial leaf bud galls on *E. camaldulensis* has not been previously documented. For axial leaf bud galls, diameter ranged from 2.6 to 13.0 mm and length ranged from 2.3 to 10.5 mm. The larger axial leaf bud galls were nodular, and appeared to have multiple locules.

Larger terminal leaf bud galls had more exit holes per gall. Axial leaf bud galls were much smaller than the terminal leaf bud galls and 99% had only one to three exit holes. The rounded shape and presence of few locules within the axial leaf bud galls indicate that this form is limited to a shape and size producing few flies. However, the terminal leaf bud galls increased in size with a less defined structure, including many more locules, and producing many more flies. The observation of greater size of terminal leaf bud galls has led to the suggestion that these galls may have multiple foundresses (Taylor and Davies, 2000). The initial growing point within the shoot bud is a small plant organ a few millimetres in size and would not be large enough to support the reported hundreds of oviposition scars and emergent flies at gall maturity (Taylor *et al*., 1996; Taylor and

Davies, 2000). It is possible that as terminal leaf bud galls grow from the initiation by a single fly and nematodes, further flies oviposit into the gall to increase its size. The axial bud galls do not appear to increase in size in this manner. Only 13 flower bud galls were seen with exit holes and 12 of these had either one or two holes. The dehiscence mechanism has not been described for flower bud galls on *E. camaldulensis*. Adult flies on other eucalypt hosts studied by Currie (1937) emerged from flower bud galls through the operculum which opens at the top of the flower bud to allow flies to escape. This was not observed on *E. camaldulensis*, where the operculum remains sealed and the characteristic *Fergusonina* "window" appears at the side of the flower bud before fly emergence through a single hole. Further destructive study of these galls is necessary to determine the number of fly larvae that the gall can support, and to determine the extent of parasitism. In addition to destructive sampling to determine fly numbers and parasitism of each of the gall forms, it would be useful to also sweep or light trap the site to enable correlation of the fly numbers with each of the gall forms.

4. General Discussion and Conclusions

Culturing

Production of callus from *E. camaldulensis* within aseptic conditions was successful, but the callus was prone to deterioration with time. The levels of salts within growth media were crucial to the success of callus growth. Tap water is still considered the most appropriate medium for short term observations of *Fergusobia*.

Culturing *Fergusonina*/*Fergusobia* galls in glasshouse conditions was not successful within the current study due to limitations in time and lack of gall material at times when flushes of growth were stimulated in saplings in the glasshouse. *Eucalyptus* grown in glasshouse conditions were prone to attack by coccid scales, which depleted new growth and caused sooty mould outbreaks and damage to leaves. Growing *Eucalyptus* trees in glasshouses requires careful manipulation to produce saplings suitable and available for *Fergusonina* oviposition and gall growth. Further experimentation is needed to determine the optimal densities of flies per tree for successful production of galls. The successes of the culturing aspects of the project were hindered by limits in time and may be overcome with future work. Further work should test the survival of *Fergusobia* in aquatic media with plant material and exudates.

Seasonal gall growth and climate

The three *Fergusonina*/*Fergusobia* gall forms studied on *E. camaldulensis* in the Urrbrae Wetlands followed a strongly seasonal pattern. Terminal leaf bud galls usually occurred in the drier months of spring-summer, with a strongly negative relationship between rainfall and density of these. Axial leaf bud and flower bud galls occurred generally in the winter-spring months. The climatic variables of maximum and minimum daily temperature and rainfall did not obviously influence gall seasonality of these two gall forms. Within the seasonal occurrences of axial leaf bud galls there was a strongly positive relationship with growing point density. However, the density of terminal leaf bud galls and growing points was not related.

No relationship was found between growing point densities and mean daily maximum or minimum temperatures. When the temperature was lower, there was a greater fraction of trees with high leaf damage. Seasonally, there were no correlations between levels of leaf damage and gall occurrences.

The climatic variables total monthly rainfall and mean daily temperatures, do not obviously influence gall seasonality for the axial leaf bud and flower bud galls. Factors such as gall

parasitism, the amount of shade from the plant canopy, and changes in nutrients present in different seasons may affect the seasonal densities of the respective gall forms, and should be investigated. Geographic differences of sites and occurrences of *Fergusonina*/*Fergusobia* galls indicate that the gall-formers adjust to localised conditions to ensure survival. The gall complex is largely confined to coastal regions of Australia where hosts are plentiful and the climate is cool temperate to tropical with adequate rainfall and humidity. The gall itself is able to provide adequate protection for the aquatic nematodes within the plant parasitic life stage, unless temperatures exceed 40°C.

Variation in growth, canopy condition and gall occurrence between trees

The densities of growing points and gall forms varied greatly between the trees. Some trees were more susceptible to gall formation than others within the same site and area. Trees with more new growth had increases in gall abundance, particularly with the axial bud galls. Galls are rarely observed in the upper regions of tree canopies (K. Davies, pers. comm.). The apparent lack of galls in the upper canopy may be due to older trees having unsuitable leaf chemistry for gall formation. Within mature *E. regnans* trees, shoot growth is slower near the tops of tall trees and the leaves are small with thick cuticles, whereas saplings 5m tall have large, thin leaves (Ashton and Attiwill, 1994). Other studies have suggested that the leaves of young trees are preferred for gall formation (Goolsby *et al*., 2000; Gullan *et al*., 1997). This may explain the lack of galls above a certain height. Flower bud galls may be an exception, because flower bud abundance increases with tree maturity. Within one mature *E. microcarpa* host within the Waite Arboretum, flower bud galls are particularly prolific and not apparently reduced with height (K. Davies and G. Taylor, pers. comm.).

Within the current wetlands study, flower bud galls did not seem to reduce the likelihood of a tree producing flowers. A longer study is necessary, particularly to observe their possible occurrence higher in the tree canopies. Flower bud galls may increase to levels that suppress flowering (Currie, 1937).

The length of time that trees had medium leaf damage and the density of axial bud galls was weakly correlated, indicating that axial bud galls are more likely to occur on trees with medium rather than low or high levels of damaged leaves. It is likely that genotypical variation affects the likelihood of a tree being a suitable host because leaf colouring differed between trees with wide differences in gall density, although the trees were of similar age, degree of disturbance and aspect.

Orientation and distribution of galls and new tree growth

For all trees, the southern quadrants appeared to have the least leaf damage. Comparisons of 45° sectors around the circumference of the trees showed that the galls were clumped within particular regions around each tree (Table 4). This was expected as the flies would seek regions with growing points for oviposition. *Fergusonina* have been observed returning to buds in which oviposition had already taken place, indicating that inhibitory marking secretions may not be produced by the flies (Giblin-Davis, 2000). This observation also supports the possibility of multiple foundresses within the larger galls in particular (G. Taylor pers. comm.; Taylor *et al*., 2005). Greater genetic variability for nematodes is possible if more than one female fly oviposits into a bud. Flies may oviposit repeatedly into newly initiated galls to increase the gall mass. Alternatively, new leaves may be overexploited by flies, producing a hypersensitive response in the growing point. This hypersensitive response was observed in the caged tree gall cultures.

Gall position

Terminal leaf bud and flower bud galls occurred higher in the canopies than axial bud galls. Axial bud and flower bud galls occurred at similar distances to the ground and distances to the trunks of trees. During the study, it appeared that there was an optimum position for each gall form, supported by the observation that the average distance of galls to the ground and to the tree trunks did not change over two years. It is likely then, that younger trees would be favoured for these gall forms because older trees would not have the lower branches favoured by the respective fly species.

Gall characteristics

Many of the galls did not mature to produce exit holes and adult flies. Galls are prone to abortion, parasitism, being eaten or becoming absent. Each of the gall forms showed great variation in longevity. Flower bud galls had particularly long life spans, remaining green without exit holes for almost 7 months before senescence. Axial bud galls were collected at the South Parklands (South of Adelaide city centre) in January 2003 for the nematode survival study (Chapter 2, Section 2.3.1), but at Urrbrae Wetlands this gall form was not observed in January. The differences in seasonal occurrence and lifespan of galls indicates that some *Fergusonina* species might enter a diapause during their life cycle. Plant parasitic nematodes (females and males) were recovered from axial leaf bud galls collected in the South Parklands. It may be at this plant parasitic stage that diapause is most favoured. *Fergusonina* flies within these axial galls were still in the egg stage. If host plant physiology and climate are suitable, fly development may be delayed and the plant parasitic nematodes may cycle until conditions are suitable for further development. How this is controlled

would require further studies, particularly using plant ecdysteroids to examine nematode development and coordination with the fly life cycle.

This non-destructive ecological study has provided observations of tree phenology, gall growth and gall characteristics. It is likely that flies within the gall system undergo a resting stage or diapause, as suspected by Currie (1937) for flower bud galls. Following the growth of galls under Adelaide climatic conditions has allowed comparison with the study of galls on *Melaleuca* in Northern New South Wales, Queensland and Florida (e.g. Goolsby *et al.*, 2000).

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