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

by

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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.