The influence of mite predation on the efficacy of the gall midge, *Dasineura* sp., as a biocontrol agent of Australian myrtle, *Leptospermum laevigatum* (Myrtaceae) in South Africa

THESIS

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ABSTRACT

Dasineura sp. is a gall forming midge that was introduced into South Africa for the biocontrol of the Australian myrtle, *Leptospermum laevigatum*. It causes galls on both the vegetative and reproductive buds of the plant. Although *Dasineura* sp. was initially regarded as a potentially successful agent, galling up to 99% of the buds of the host plant, it has been preyed on by native opportunistic mites, which caused a decline in the performance of the midge as a biocontrol agent of *L. laevigatum*. This raised a concern about whether this fly will be able to perform effectively in the presence of its new natural enemies. Therefore, the objectives of this study were to:

1) ascertain whether mite abundance has seasonal variations;
2) determine if plant density and plant size have an effect on midge predation by the mites; and
3) determine if midge predation varies in different locations.

The study was conducted at three sites in the Hermanus area, Western Cape Province. Every three weeks for thirteen months, galls were collected and dissected so as to count and record the numbers of midge larvae, pupae, adults and mites that were found. Data collected showed that predation varied with season, and the mites were scarce during the flowering season. Predation also varied among the study sites and plant density had an effect on midge predation. Midges in smaller plants (saplings) were more vulnerable to predation than those in the bigger plants (plants from isolates and thickets). It was concluded that although mites have an effect on midge populations, they do not prevent their establishment on the plant. Therefore, a survey should be done in two to three years time to check if the midges are still persisting on the plant,

and recommendations are that a new agent should be released to supplement the midges.

Keywords: Biocontrol agent, *Dasineura* sp., Density, *Leptospermum laevigatum*, Mites, Predation.

Declaration

I hereby certify that the research work reported in this dissertation is the result of my own investigation, except where acknowledged.

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Thabisa Honey Mdlangu Date

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PREFACE

Eight percent of South Africa is severely infested with alien invasive plants, which use about 7% of the available surface water. This has lead to the extinction of many indigenous plant species (van Wilgen [n.d.]: 9-12). Other problems associated with the spread of invasive plants include: increases in the intensity of fires, destabilization of catchment areas through soil erosion and a decrease in the aesthetic appeal of natural vegetation (Vitousek *et al.* 1996). The current study focuses on *Leptospermum laevigatum* (J. Gaertn.) F. Muell. (Myrtaceae), commonly known as Australian myrtle. This invasive weed was introduced from Australia in the early 1800s, to stabilize drift sand on the Cape flats in the Western Cape Province. Subsequently it was used as a hedge plant in gardens and has invaded other areas including the Eastern Cape. It is particularly invasive in the unique and endangered Cape Floristic Kingdom and is now rated as the 5th most invasive weed plant in the Fynbos biome (Gordon 1999).

To overcome the danger posed by *L. Laevigatum* on the native plants, two natural enemies (biological control agents) of this plant were introduced to South Africa from Australia. The first biological control agent to be introduced was the leaf-mining moth, *Parectopa thalassias* Meyrick (Gracillariidae), which was released in 1996. According to Gordon (1999), the larvae form thin serpentine mines which develop into large blotch mines that cause premature leaf abscission. The leaf miner is not as effective as was expected on *L. laevigatum* since it only attacks young leaves. Nevertheless, repeated defoliation reduces the vigor and growth of the seedlings (Gordon, unpublished data). The second biological control agent introduced in 1984 was an undescribed gall midge, *Dasineura* sp. (Diptera: Cecidomyiidae). This gall midge forms galls in the reproductive

and vegetative buds of the plant. Damage by this agent results in reduced fruit production and restricted growth of the plant. Attempts to rear this insect in quarantine failed and it was reintroduced in 1994. However, before host specificity studies commenced, fly populations which seemed to be *Dasineura* sp. were discovered on *L. laevigatum* at a few localities in the Western Cape (Gordon,1999). When the midge was eventually released in certain areas, observations showed that up to 98.9% of the myrtle buds were galled and destroyed at some sites and the prognosis for biological control seemed to be extremely good (Gordon, unpublished data). However, further investigation showed a decline in the midge population and this was attributed to indigenous predatory mites. The objective of this work is to investigate the seasonal history and abundance of the predatory mites and their impact on midge populations.

Chapter 1 gives a brief background about the research topic, states the problem and the objectives of the study as well as the key questions asked. It also establishes the hypothesis and the limitations to the study.

Chapter 2 is an introduction to the *L. laevigatum* problem and factors thought to have contributed to the success of *L. laevigatum* as an invader in South Africa and other parts of the world. The biological control agent, *Dasineura* sp. and the predatory mites that are affecting its efficacy as a biological control agent are also discussed.

Chapter 3 deals with the laboratory materials and methods used in this study and the collection of insects from study sites, as well as the statistical methods used to analyze the data.

Chapter 4 deals with the results under two subheadings:
Section 4.2 covers phenology of the insect, number of midge generations per year and the stages that occur at different seasons. Section 4.3 covers the seasonal occurrence of mites and the mortality of midges caused by mites as well as other factors affecting predation.

Finally, Chapter 5 discusses the practical and theoretical implications of the study as well as the need for additional biological control agents.

CHAPTER 1

1.1. General Introduction

A successful weed biological control agent should be able to undermine the reproductive potential of the weed and this depends on the agent's fecundity, ecological range, susceptibility to parasitism and the harm it does to the host (Raghu and van Klinken 2002). Gall-inducing midges are dipterans which belong to the family Cecidomyiidae. Adults in this fly family are usually small with long antennae. Larvae are yellow to reddish, with a reduced head and they are legless, while pupae are enclosed in a cocoon or puparium. Gall midges feed on plants as immatures (Armstrong 1995). Similar to other gall forming insects, as the midge larvae feed, they cause abnormal growth or swellings (galls) within the infected plant tissues. A given midge species forms galls only on certain plant parts and the galls formed are usually very distinctive (Dreger-Jaufrett and Shorthouse 1992). However, parasitism, or predation may cause a decline in population density of a gall inducer and affect its potential as a biocontrol agent (Carlson and Mundall 1990). Native parasitoids may attack introduced gall inducers used for weed biocontrol and the nature of parasitism/predation of biological control agents can influence the success of the biological control agent (Dodd 1961). The presence of such natural enemies can have a negative impact on the establishment of a biocontrol agent and can limit the impact of the biocontrol agent (Sebolt and Landis 2004). Goeden and Louda (1976) reviewed cases in which activities of predators, parasitoids and pathogens were implicated as factors influencing the outcome of weed biocontrol projects. They concluded that

predation rather than parasitism or diseases has the greatest impact on the efficacy of biocontrol agents.

1.2. Problem statement

Biocontrol of the weed, *Leptospermum laevigatum* involves the introduction of two agents, *Parectopa thalassias* (a leaf mining moth) and *Dasineura* sp. (a bud galling midge). The moth has been doing well, attacking about 50% of the plant leaves, but the damage is done only on younger plants and mature plants are not affected. The midge on the other hand initially did remarkably well, galling up to 99% of the buds on both mature and small plants (Gordon unpublished data). However, the high levels of damage have not been sustained for reasons that have not been fully explained. One possibility is that indigenous mites, which have been observed to attack the midge larvae feeding inside the galls, curtail the abundance of *Dasineura* sp. and thereby the biological control of *Leptospermum laevigatum*.

1.3. Objectives

The current study was conducted to ascertain the effect of mite predation on the gall midge and to determine if the midge will be able to reach its full potential as a biocontrol agent in spite of the threat posed by the predatory mites.

1.4. Key questions

The questions asked are as follows:

- Do levels of mite predation vary seasonally? This will determine to what extent the mites prey on the gall midges and if mite predation levels decrease or increase in different seasons.
- Do the levels of mite predation vary with plant density? This will indicate whether gall midges on isolated plants are more susceptible to mite predation than those in thickets or vice versa.
- Does plant size negatively or positively affect midge predation? In this instance it will be possible to determine if the larvae and pupae in galls on saplings escape mite predation or not.

1.4. Hypothesis

Mites curb the efficiency of the gall midge as a biological control agent of *L. laevigatum*. It is hypothesized that there will be seasonal variations, and that mites would be mostly abundant around September during the flowering time when the midges are most effective. Thickets will have more midge predation because the trees are closer together, which makes it possible for the mites to move from one tree to another. Site and plant size will also have an effect on the predation of midges by mites.

CHAPTER 2

Literature review

2.1. Introduction

The Agricultural Research Council-Plant Protection Research Institute is involved in many projects on weed biological control in the Fynbos. This involves surveys for the biocontrol agents from the weed's country of origin, rearing of the biocontrol agents in quarantine, host specificity tests in the country of introduction, release of biocontrol agents at the sites where the weeds are invasive, and the evaluation of their establishment and effectiveness on the plant. The biocontrol agents include insects, pathogens and other arthropods. There are many different types of invasive species that are involved in the projects, including the *Hakea* spp., different species of *Acacias*, and Eucalyptus trees, to name a few. The current study focuses on *Leptospermum laevigatum* (Australian myrtle) which is affecting the Fynbos.

The Fynbos vegetation is unique to South Africa and it is the smallest of the six floristic kingdoms in the world. It consists of shrubs of different types and sizes. At least 68% of the plant species occurring in the Fynbos are endemic (BirdLife International 2003). The distinctive growth forms include proteoids, ericoids, restoids and geophytes (Pierce 1984). Of these, the tallest are the proteoids which can grow to a height of 1-3 m (Giddy 2008). Alien invasive plants pose a serious threat to this unique vegetation because the invaders tend to surpass the native plants in height and ability to consume water (Olckers *et al* 1998). These two characteristics have a strong negative impact on the natural flora (Kunwar 2003). Among the measures taken to curb the spread of

invasive plants in the Fynbos, is the use of natural enemies (biological control) that can reduce invasive weed populations to tolerable levels (Hawkins *et al*. 1997).

Many types of biocontrol agents have been used against their target weeds and have had their advantages and disadvantages. Some control agents have managed to reduce the aggressiveness of the weeds, but some have failed to establish. Although others have established, their impact has been negatively affected by climate, parasites, pathogens and mostly native predators (Goeden and Louda 1976), but nonetheless, the agents may still diminish the overall impact of the weeds (Hill and Olckers 2001).

Before a biocontrol agent is released in new areas, strict quarantine measures are followed to reduce the chances of importing it with natural enemies from its native region. However there are several cases where introduced natural enemies have been attacked by predators, parasitoids and pathogens native to the area of introduction (Sebolt and Landis 2004). For example, a galling midge, *Dasineura* sp., introduced into South Africa for the biological control of *Leptospermum laevigatum* is negatively affected by the presence of native mites which attack the gall midge when they are feeding and developing inside the galls (Gordon, unpublished data). The predatory mites are reported to have reduced the effectiveness of the midge as a biocontrol agent of *L. laevigatum* (Gordon pers.comm). Sebolt and Landis (2004) carried out studies to evaluate the potential for a biotic interference (the predators in this case) to restrict the effectiveness of the weed biocontrol agents. Their study consisted of laboratory and field experiments. In their study, the tests showed that the attack rates on eggs and larvae ranged from 10 to 100% in the laboratory, and the field study

showed that the attack rates ranged between 10 and 27%. From the results they obtained, the high rates of predation exhibited in the laboratory studies were not reflected in the field studies. As a result of their observations, they concluded that biotic interference, mainly predation, limits the population growth of natural enemies in the field, but it is unlikely to prevent establishment and spread under the conditions studied. In a study conducted by Reimer (1988), on the effect of predation on the biocontrol agent of *Clidemia hirta* (Linnaeus) D.Don (Melastomataceae), *Liothrips urichi* Karny (Thysanoptera, Phlaeothripidae), the results showed >40% mortality in open cages (cages exposed to predation) as compared to the <10% mortality in closed cages. Reimer (1988) concluded that predators have the abilty to reduce the efficacy of biocontrol agents. Chacon *et al.* (2008) studied the effect of biotic interference on the establishment of *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), a potential biocontrol agent of *Aphis glycines* Matsumura (Hemiptera: Aphididae) using a surrogate insect *Aphidius colemani* Viereck (Hymenoptera: Braconidae). The study was to assess how biotic interference, mostly by generalist predators, may affect the establishment of classical biocontrol agents of the soybean aphid, *A. glycines* in North America. This was to determine if the potential biocontrol agent was safe for release in the country where *A. glycines* was a problem. In this study, they released the surrogate insects in open field plots (plots exposed to predators) and also in closed field plots (plots protected from predators). The results showed that the surrogate insect increased the control of *A. glycines*, despite the indications that predation occurred. They concluded that biotic interference was not sufficient to eliminate the contribution of the surrogate insect to the suppression of the pest, meaning that the agent would

not suffer excessive predation. In this case, the attack by native predators did not have any impact on the efficacy of the biocontrol agent.

Hunt-Joshi *et al.* (2005) studied the effects of a predator, *Plagiognathus politis* (Miridae) on *Galerucella calmariensis*, a biocontrol agent of the purple loosestrife, *Lythrum salicaria* (an invasive weed). They set up field cages where predators were included and they also set up cages where predators were excluded. In the cages where predators were involved, the adult and immature mirid stages consumed large numbers of eggs and young larvae of *G. calmariensis*, whereas in other cages (without the predator), the agent was allowed to establish and persist in the field cages. The presence of predators allowed the weed to maintain greater leaf, stem and reproductive biomass than without predators. From the results, they concluded that, the predators consume large numbers of eggs and larvae of the biocontrol agent. Consequently, the efficacy of the agent could be greatly suppressed when release sites are inhabited by the mirid predators. These workers stated that it is very important to document interference by opportunistic predators, because it is usually subtle and may remain unrecognized even if release efforts are regarded to be unsuccessful. The different studies that have been reviewed above show the different effects of biotic interference on the efficacy of biological control agents. The success of a biocontrol agent is measured by its ability to disperse and establish viable populations in new host patches beyond those in which it was initially released (Sebolt and Landis 2004). In the current study the influence of biotic interference on *Dasineura* sp. was investigated.

2.2. ***Leptospermum laevigatum***

Leptospermum laevigatum (J. Gaertn) F. Muell., belongs to the family Myrtaceae, which originates from Australia. It is widely distributed in coastal areas from south-east Queensland through to the south-east of South Australia and Northern Tasmania (ANPSA 2010). It grows well in places with moist soil (Lam 2002). It can grow well in a range of climates (Cavanagh *et al.* 2007) with well drained, slightly acidic soil and withstands periods of drought (Holliday and Hill 1974). It is commonly known as Australian myrtle or Victorian tea tree. In many countries, *L. laevigatum* is grown as an ornamental plant and the dried leaves can be used for making tea, hence the name, "coastal tea tree" (Facciola 1990).

This plant is an evergreen shrub with oval or tear drop shaped dull green to grey leaves (Canopy 2006). It produces five-petalled white flowers (Plate 2.1), 15–20 mm across, which develop into semi-woody capsule fruits (Plate 2.2), which subsequently open to shed large numbers of seeds (Keighery 1994). Australian myrtle flowers in spring and the semi-fleshy, capsular fruits mature during the following year (Gordon 1999). The plant is serotinous (*i.e.* the seeds which are stored in the non woody capsules are only released after three years or after fires) (Gordon 1999). Lam (2002) reported that stress to plant parts (*e.g.* by fire, mechanical damage, herbicide application) triggers mass seed release from affected parts; and in the absence of such factors half of the seed would be released from capsules on reaching maturity. Therefore, prolific germination occurs during the following winter.

Plate 2.1: Flowers and leaves of *Leptospermum laevigatum* (picture taken at one of the field sites).

Plate 2.2: Fruits and leaves of *Leptospermum laevigatum* (picture taken at one of the field sites).

2.2.1. The invasiness of *Leptospermum laevigatum*

In South Africa, *L. laevigatum* is a declared weed and is targeted for control under the Conservation of Agricultural Resource Act 43 of 1983 (Klein 1999). It was introduced in this country during the 1800s to stabilize drift sand around the coastal regions and was also grown as a hedge plant (Shaughessy 1980). *Leptospermum laevigatum* became invasive in many climatic zones of South Africa especially due to the fact that there were no natural enemies to curb its growth. This invasive weed is widely distributed in the Western Cape and Eastern Cape (Fig. 1), with the worst infestations occurring at Hermanus, Kleinmond, Bredarsdorp (Western Cape) and Port Elizabeth (Eastern Cape) (Gordon 1999), where it rapidly replaces *Acacia saligna* (another Australian weed that is presently under successful biocontrol) in areas where the two invasive plants occur together (Plates 2.3 and 2.4). It is particularly invasive in the fynbos, where it outcompetes and even replaces the unique and endangered fynbos vegetation (Gordon 1999). In the Fynbos, fires are essential for plant production and regeneration. In the case of *L. laevigatum,* adult plants are readily killed by fire, but seeds are extremely well protected from fire in capsules which split to release a large mass of seeds which are dispersed by wind or water (Johnson 1978). The passage of fire creates an ash bed which provides both supplementary nutrients and a seed bed where seeds germinate (Gordon 1999; Lam 2002).

Leptospermum laevigatum is better at colonizing disturbed spaces more than any other species, yet it is also able to grow in seldom-disturbed places (Lam 2002). This plant has several features that contribute to its hardiness and allow it to survive. Some of those features include an ability to survive mechanical damage, water stress, and fast

growth. Furthermore, the influence of humans on indigenous vegetation means that site disturbance levels have increased in many ecosystems, tipping the balance in favor of opportunistic species like *L. laevigatum* (Lam 2002).

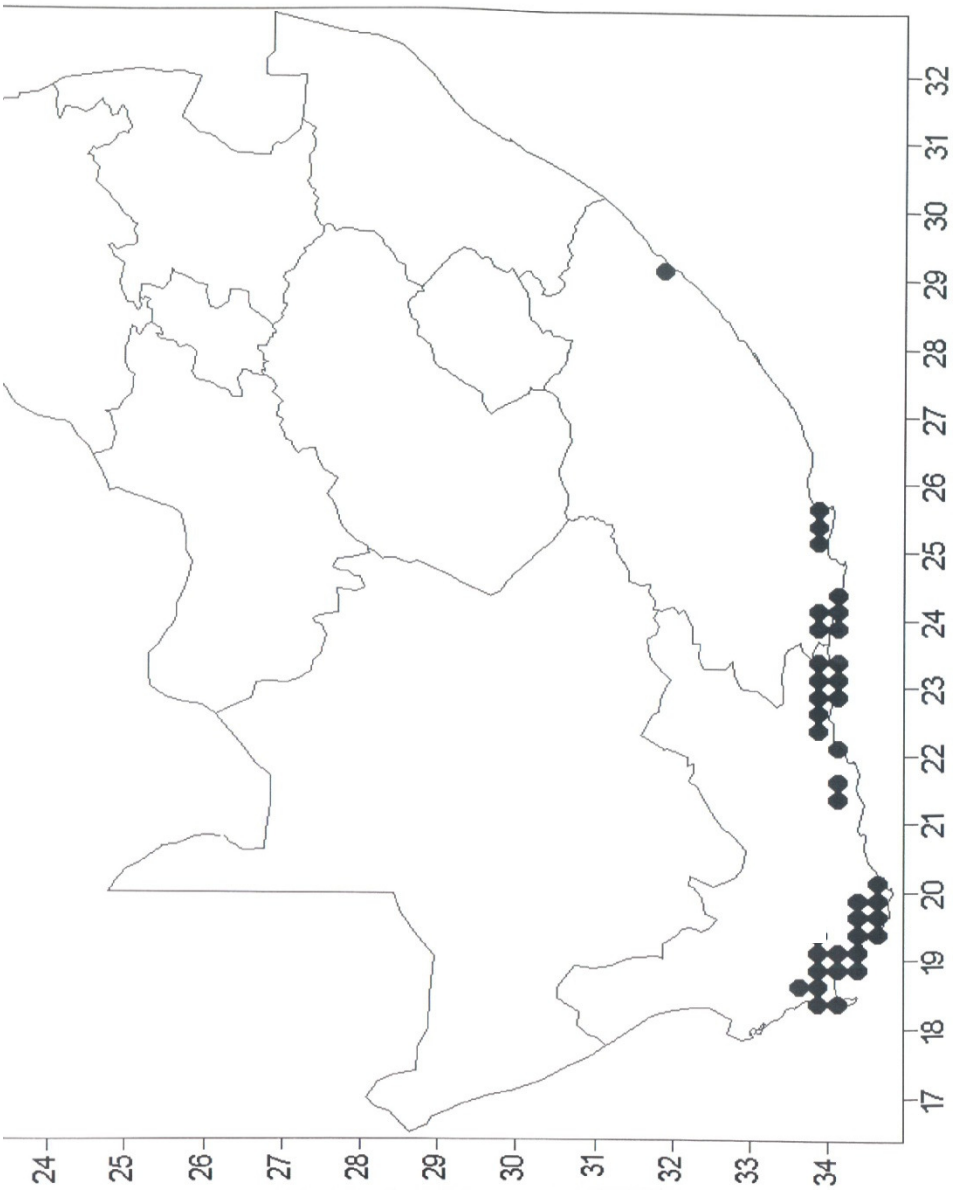

Fig. 1: Distribution of *Leptospermum laevigatum* in South Africa. The black dots represent the infestation of the plant in South Africa. (Map drawn by Lesley Henderson, ARC-PPRI, Pretoria).

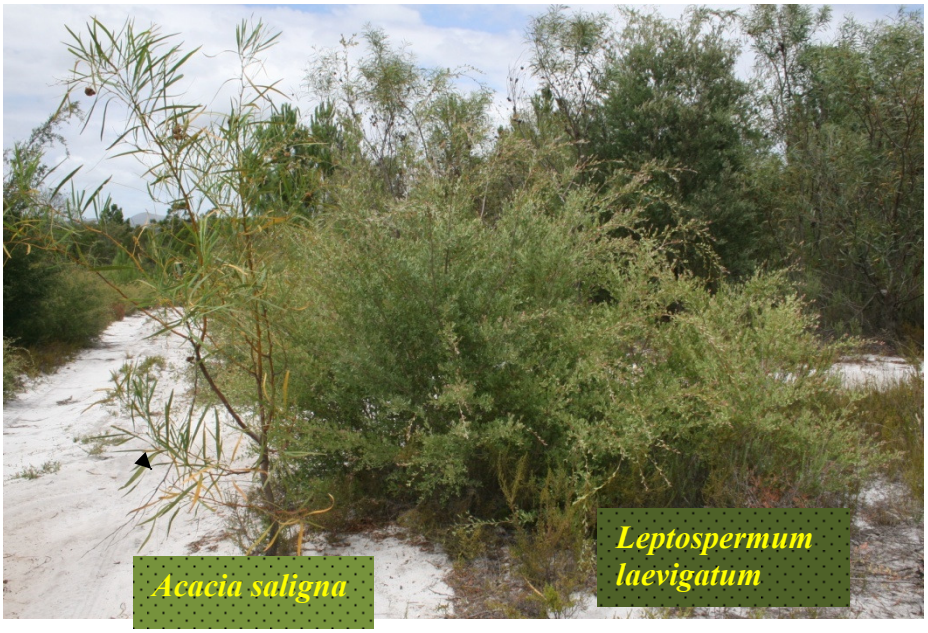

Plate 2.3: *Leptospermum laevigatum* and *Acacia saligna* where they occur together (Stanford, Western Cape).

Plate 2.4: The invasiveness of *Leptospermum laevigatum* in the Cape Floral Kingdom (Stanford, Western Cape).

2.2.2. Control of *L. laevigatum*

a) Mechanical control

Small seedlings can be controlled mechanically by hand pulling. However, mechanical control of saplings and mature trees does not kill the plants and results in multi-stemmed regrowth, which becomes increasingly difficult to control in follow-up operations (Gordon 1999).

b) Chemical control

Chemical control is extremely expensive and may be damaging to native plants, but nevertheless, the non-selective Tebuthiuron (Reg No: 1967/007147/07) is currently the only chemical registered for use on *L. laevigatum* (Gordon 1999). Herbicides are also regarded as one of the triggers for seed release (Lam 2002).

c) Biological control

The biological control of this plant has been opportunistic, and according to Gordon and Neser (1994), surveys for natural enemies of other invasive Australian plants have provided the opportunity to identify and collect potential agents for *L. laevigatum*. One of the agents introduced for the biological control of *L. laevigatum* in South Africa is a leaf mining moth, *Parectopa thalassias* (Lepidoptera: Gracillariidae) (Plate 2.5) which causes premature leaf abscission. This leaf miner was first released in April 1996 and has dispersed to many areas of South Africa where Australian myrtle occurs (ARC-PPRI 2002).

Plate 2.5: A leaf miner, *Parectopa thalassias* on a *Leptospermum laevigatum* leaf. (Photo: Tony Gordon).

Plate 2.6: A gall midge, *Dasineura* sp. on a *Leptospermum laevigatum* leaf. (Photo: Tony Gordon).

Larvae of the moth damage the younger leaves, and the older leaves are not attacked as they are not suitable for the moth larvae (ARC-PPRI, 2002). The female adult deposits eggs on the lower and upper surfaces of the leaves where they hatch in about 7 days. The emerging larvae penetrate the leaf and feed within the internal tissues (ARC-PPRI, 2002). The feeding results in the formation of a thin, linear tunnel, called a serpentine leaf-mine, which becomes visible through the leaf cuticle and later expands to form a large, irregularly shaped excavation or a blotch mine, which eventually appears inflated (ARC-PPRI 2002). Each larva completes its development in a single leaf, and if more than one egg is laid on a leaf, one of the larvae will kill the others. When the larva is fully developed, it exits the leaf mine, folds over the side of another leaf, where it spins a cocoon and pupates and the moth eventually emerges. In the country of origin, Australia, *P. thalassias* caused about 50% damage on *L. laevigatum* leaves. In South Africa, the levels of leaf damage are the same (ARC-PPRI 2002).

Dasineura sp. (Plate 2.6) was introduced in 1984 without any success and it causes galls on flower buds and auxillary buds of *L. laevigatum* (Gordon and Neser 1994). According to Gordon (1999), the midge was reintroduced in 1994 and although it was successfully cultured, no host-specificity tests were ever conducted, because populations of what appeared to be the same cecidomyiid were discovered at a few localities in the Western Cape between 1994 and 1996, suggesting that the insect had already become established (Gordon 1999). In 1996 the midge was found to be widespread around Stellenbosch, in the Western Cape. Evaluation studies to determine the efficacy of the midge as a biological control agent showed that the midge reduced the vegetative as well as reproductive potential of the plant by 98.9% at some

sites (Gordon, unpublished data). However, the numbers of galls declined sharply at most of these sites and were on average up to half of their previous levels. Further investigations revealed that predation by indigenous predatory mites was probably the cause for the decline in the population of *Dasineura* sp. (Gordon, unpublished data).

2.3. ***Dasineura* sp.**

Dasineura sp. is a gall midge belonging to Order: Diptera, Suborder: Nematocera, Family: Cecidomyiidae. Similar to other flies, it has a holometabolous life cycle. The female eggs are deposited on the surface of the flower ovary and in the place of normal fruits, galls develop (Adair *et al.* 2000). At the flowering time, eggs hatch into tiny larvae (Adair *et al.* 2000) which do not look anything like a fly. These larvae start feeding on the plant. The feeding by the larvae irritates the plant, and in response to this, the plant develops extra, thick layers of plant tissue, which evaginate around the larvae leading to gall formation that causes one to several chambers per gall (Gage 2005). Short and Castner (2005), believe that galls result when the cambium and other meristematic tissues react to stimuli produced by larvae and cause abnormal growths. Larval feeding occurs for a few weeks to several months (Adair *et al.* 2000). The larva eats and grows and sheds its skin until it pupates, and changes into an adult fly (Gordon 1999). All the features of the adult become recognizable in the pupae which consequently have a greater resemblance to the adult than to the larva (Baker 1991). The adults are minute flies (rarely more than 3 mm long), slender with long legs and antennae (Scholtz and Holm 1985). The gall midges are usually host specific which makes them ideal biological control agents (Abrahamson and Weis 1987). However,

sedentary insects are the most vulnerable to attack by opportunist predators (Hawkins *et al.* 1997) including mites.

2.4. Mites

The word “mite” comes from Old English and means “a very small creature”. These are minute arachnids, requiring magnification for identification (West Boulevard Vet Clinic Ltd 2009). Mites are the most diverse and abundant of all arachnids, but because of their small size (usually less than 1 mm), they are rarely seen and they have a high capacity for dispersal by wind (Broce *et al.* 2005). Mites belong to the order Acarina of the class Arachnida. Apart from the other arachnids, mites have the head, thorax and abdomen all fused together into one oval-shaped body. Antennae, wings or mandibles are absent in mites. Mites have the ability to colonize nearly every known habitat (*e.g.* in the ground, surface litter, fresh water and salt water) (Walter and Proctor 1999). Modes of reproduction are diverse and include: ovipari (laying eggs), ovivipari (eggs hatch inside the females), vivipari (females produce live young), parthenogenesis (reproduction without fertilization) and arrhenotoky (production of males from unfertilized eggs (Ueckermann pers.comm). Most mites begin their lives as eggs, laid singly or in masses. They are usually very particular about where they lay their eggs. They usually limit their maternal care in hiding their eggs in crevices, within silken webs, covering them with detritus particles or a jelly-like sheath or conveniently dying with them protected inside the mother’s body (Walter and Proctor 1999). However most of them oviposit and show much care in the choice of the site where they lay their

eggs. Some of them use an extrusible ovipositor, sometimes as long as the female's body, to place their eggs within crevices. Under favorable conditions, a single female can lay up to 500-800 eggs in her lifetime at a rate of 20-30 per day (Hallas 1991) and their developmental stages may take a few days to a few weeks, depending on the temperature, humidity, type of species and food supply (Ueckermann pers.comm). They grow very quickly if there is enough food, and if their density increases, a shortage of food will be reached quickly. In that case, the female predatory mite will move in order to lay its eggs and start a new colony elsewhere (Koppert Biological Systems 2005). Mites have three stages in life: egg, nymph (protonymph, deutonymph, and tritonymph) and adult. After the second nymphal stage, some of them pass into what is known as a 'hypopus stage', which is a diapause form, in which they are almost immobile and very resistant to desiccation. During this stage the body wall hardens and the suckers, which allow the mite to attach to other insects and animals for dispersal, appear on the underside (Hallas 1991). The nymphal stage of mites resemble the adult stage except that the nymph has three pairs of legs, while the adult has four pairs. Mites have a high prey-searching capacity. During this study, a number of mites were found attacking and feeding on the midge, *Dasineura* sp.

CHAPTER 3

3. MATERIALS AND METHODS

This chapter describes the procedures that were used to obtain the data on levels of mite predation on *Dasineura* sp.. The study consists of two phases, collection of samples in the field and later processing of these samples in the laboratory. This was designed so as to address the research questions stated in the first chapter.

3.1. Study sites

To study the phenology of *Dasineura* sp. and the effect mites have as predators on the midge, three study sites were selected: Stanford (34˚ 25.250' S; 19˚ 26.768' E), Onrus-manor (34˚ 24.412' S; 019˚ 10.064' E) and Fisher Haven (34˚ 22.181' S; 019˚ 08.509' E), these sites were all in the Hermanus area in the Western Cape, South Africa. The climate in the Hermanus area is Mediterranean with an average rainfall of 518 mm per annum, of which about 14% falls in July. Sites were selected because they have large stands of *L. laevigatum* and because *Dasineura* sp. had been established there. In this study, *L. laevigatum* were investigated in the following categories of trees: isolates (where trees were further apart at an average distance of 4.5m) (Plate 3.2), thickets (where trees were closer together) (Plate 3.1) and saplings (where trees were less than 1 m in height) (Plate 3.3). Saplings that were sampled ranged between 20 and 99 cm in height and their average height was 42.11±14.61 (Fig. A1). The study was conducted for 13 months from February 2008 to February 2009. The study sites, tree densities and the study period were designed in relation to the research questions.

Plate 3.1: Thicket trees at Stanford.

Plate 3.2: An isolated tree at Stanford.

Plate 3.3: Saplings (small trees) at Stanford.

3.2. Data collection

Stanford samples were collected once in every three weeks, and once in every six weeks for the other two sites. The difference in the time interval is mainly because of the workload, there were too many galls to dissect and therefore, Stanford was treated as the main study site and the other two sites were to compare and confirm the results. Trees on which sampling was done were selected as follows: five from isolates and five from thickets. The selected trees were marked and labeled as they were used throughout the study. From each tree, three 30 cm branches with galls were removed and taken to the ARC laboratories in Vredenburg, Stellenbosch for gall dissections. Branches with fresh galls (not more than a year old) were selected. These galls were weighed using a microbalance, and then dissected under a stereomicroscope. The numbers of midge larvae, pupae and exuvia (The remains of the exoskeleton after the insect had moulted into an adult and had emerged) were counted. The exuvia counts were used to determine the number of adult midges that had emerged. For each gall dissected, the following variables were considered:

i. Number of healthy larvae
ii. Number of attacked larvae
iii. Number of healthy pupae
iv. Number of attacked pupae (body fluids were sucked from these by the mites)
v. Number of adults that had emerged (these were obtained by counting the number of exuvia)
vi. Number of mites

The late larval stages (prepupae) were also recorded as pupae. A total of 2,304 galls were dissected. Mites were sent to Dr Eddie Ueckermann at ARC-PPRI, in Pretoria, for identification. Microscopic observations, measurements and pictures of the midge were carried out using a Leica EZ4D microscope.

3.2.1. Calculating the inter-tree distances

To obtain the inter-tree distances, random points were located in a stand for each tree density (isolated, thickets and saplings). Ten trees were selected for each and a total of 30 trees from each study site were located. The height of each of these trees was measured and the distance to the nearest tree was measured using a measuring tape and a ruler for smaller saplings.

The formula that was used to obtain the average inter-tree distances was:

$$d = n / 2x^2$$

where **n** is the number of trees and **x** is the average distance between the trees.

3.3. Data analysis

Statistical Analysis System (SAS) v. 8.2 (SAS, 2001) was used to analyze the data. Analysis of variance (ANOVA) at P = 0.05 level of significance was used to assess the effect that tree density and each site has on predation. One way ANOVA was carried out, using the completely randomized design. Means were separated using Duncan's Multiple Range Test (DMRT) procedure. Simple regression and correlation analyses were also performed to check the relationship between mites and attacked immature stages. For the correlation analysis between mites and attacked immature stages and their seasonal occurence at Onrus-manor and Fisher Haven, data were $\log_{10}$ transformed to meet the assumption of normality before the analyses were carried out.

CHAPTER 4

4. RESULTS

The average inter-tree distances

The average inter-tree distances between the *L. laevigatum* trees at each of the three sites were: Stanford (isolated trees: 0.29 m^2, thickets: 2.22 m^2, saplings: 0.482 m^2); Fisher Haven (isolated trees: 0.25 m^2, thickets: 16.83 m^2, saplings: 0.49 m^2) and Onrus-manor (isolated trees: 0.67 m^2, thickets: 35.56 m^2, saplings: 21.52 m^2). The heights of the isolated and thicket trees varied between 1 m and 7.28 m at Stanford, 1 m and 4.22 m at Onrus-manor and 1 m and 3.98 m at Fisher Haven.

4.1. Observations on the midges in the laboratory

When the *L. laevigatum* galls were dissected , different stages (larvae (Plate 4.1) and pupae of the midge) were observed. Larvae had an average length of 1.59 mm and an average width of 0.72 mm. These tiny insects are orange in colour and their larvae are segmented. The smallest larvae were usually found in the innermost layers of the gall (Plate 4.3), where they feed on the plant. When these larvae are ready to pupate they move to the outermost layers where they form cocoons (Plate 4.2) in which they lie motionless in preparation to pupate. They develop appendages during the pupal stage, and very tiny adult flies emerge. The female adult (Plate 4.4) has a long ovipositor of about 1 mm. The male adults (Plate 4.5) have elaborate (plumose) antennae which have a greater length than the simple (filliform) antennae found in females. The

smallest adult that was recorded was about 1.5 mm and the largest was about 3 mm in length.

Plate 4.1: The larva of a *Dasineura* sp.

Plate 4.2: The prepupa of a *Dasineura* sp.

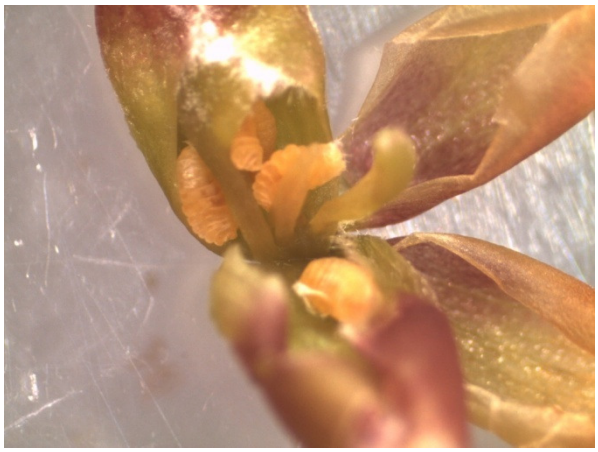

Plate 4.3: *Dasineura* larvae inside the *Leptospermum laevigatum* gall.

Plate 4.4 : The female adult of *Dasineura* sp.

Plate 4.5: The male adult of *Dasineura* sp.

4.2. Phenology of *Dasineura* sp.

Phenology is the study of the annual cycles of plants and animals and their response to seasonal changes in their environment. It can be used for correlation with insect emergence and pest control (Diver 2002), and for predicting exotic insects' establishment (Bretts *et al.* 2007). Estimating the likely number and timing of generations that an insect might undergo if introduced into a new area is useful for incorporation into an assessment of its establishment potential (Baker 1991). Cecidomyiids differ in the number of generations in a year. Some of them are univoltine (only one generation occur in a year) and some are multivoltine (two or more generations occur in a year) (Adair *et al.* 2000). *Dasineura mali* has about four to five generations (Shaw *et al.* 2005) and *Dasineura tetensi* has only two (Hellqvist 2001). Mostly, their populations increase rapidly during summer and this coincides with the maximum shoot growth (Shaw *et al.* 2005).

At Stanford, where samples were collected at three week intervals, larvae, pupae and adults were seen on the plant throughout the year, but their numbers varied in different seasons. The results are presented in Fig. 2 - Fig. 4, where the larval stages peaked in June, July and August. The highest peak was observed in July. Larval numbers were low in May, October,November and again in January and February and the lowest was observed in May (Fig. 2).

The pupal stages peaked in September, October, and December (Fig. 3). The highest peak was observed in September. The lowest numbers of pupae were observed in July. Adults emerged mostly in October, November, December and January the

following year and the highest peak was observed in October (Fig. 4). Data collected throughout the year at Stanford, showed that the peak levels (recorded as mean values per gall per month) of larvae, pupae and adults were 3.262, 1.353, and 1.197 respectively.

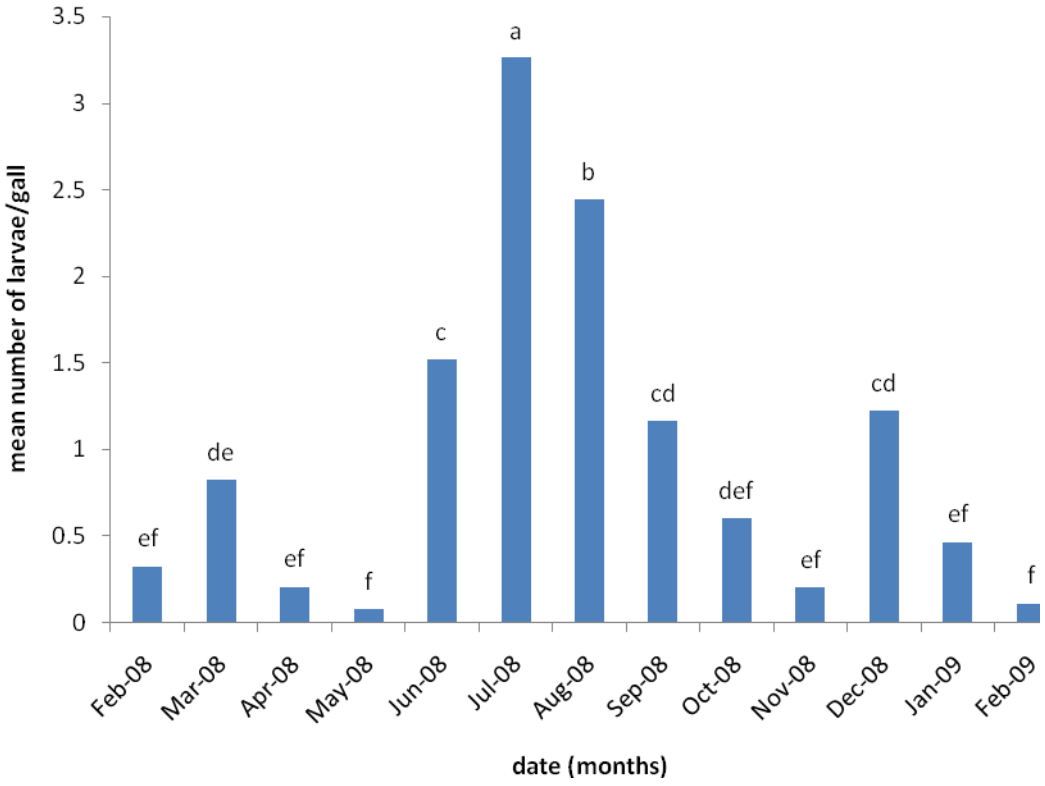

Fig.2: Mean numbers of *Dasineura* sp. larvae per gall of *Leptospermum laevigatum* at monthly intervals between February 2008 and February 2009 at Stanford. Months followed by the same letter are not significantly different from each other.

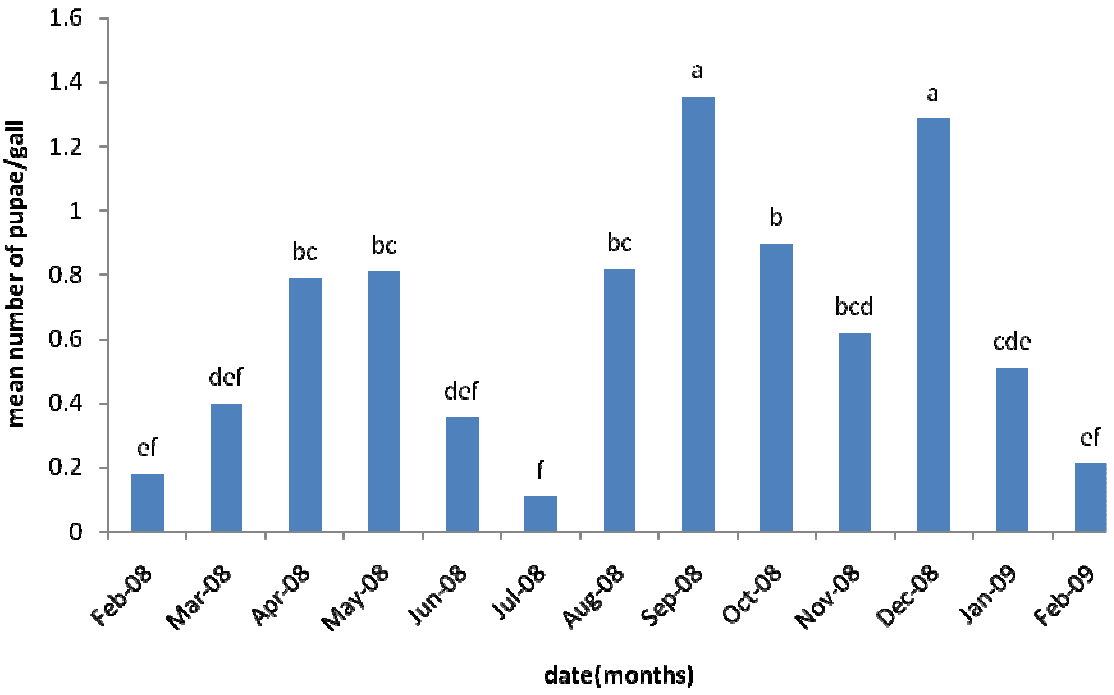

Fig. 3: Mean numbers of *Dasineura* sp. pupae per gall of *Leptospermum laevigatum* at monthly intervals between February 2008 and February 2009 at Stanford. Months followed by the same letter are not significantly different from each other.

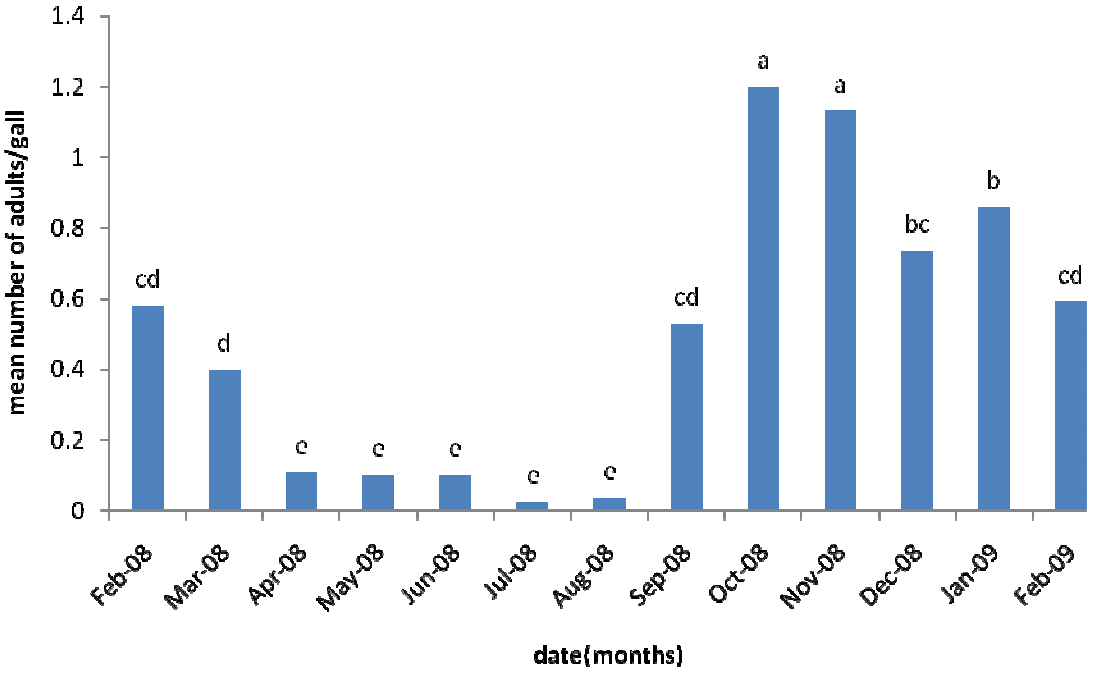

Fig. 4: Mean numbers of *Dasineura* sp. adults per gall of *Leptospermum laevigatum* at monthly intervals between February 2008 and February 2009 at Stanford. Months followed by the same letter are not significantly different from each other.

Galls that were dissected were weighed. Gall weight ranged between 1.31 and 57.4 mg (Fig. A2). Regression analyses using simple linear regression that was performed to check if gall weight can be used to predict the number of midges found inside, gave an R^2 value of 0.260, meaning that there is a 26% chance of reliability (Fig. 5). There is a very weak relationship between gall weight and the numbers of midge individuals found inside. Therefore the number of midges inside the galls cannot be predicted by the weight of the gall.

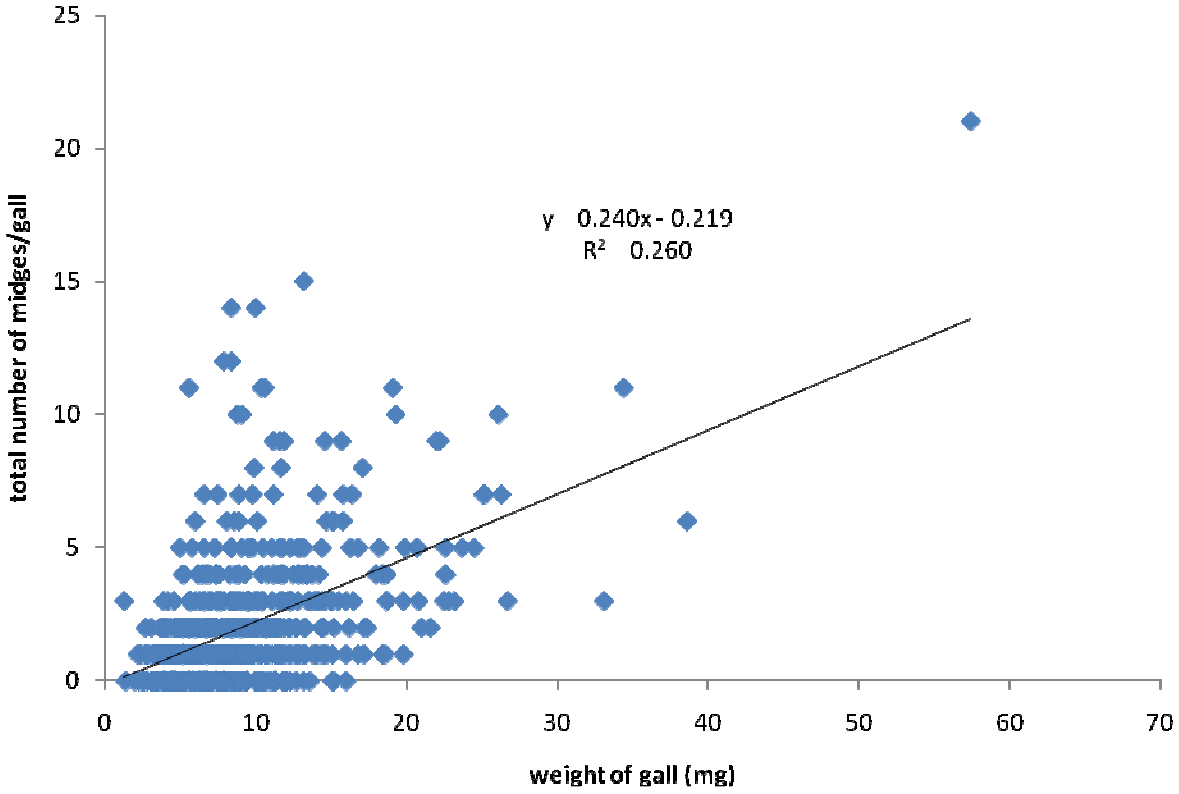

Fig. 5: Regression analysis to determine if the weight of the gall (in mg) can be used to predict the numbers of midge individuals found inside.

4.3. Predation on the midges

Observations during the study showed that mites attack the late larval stages of the midge. This is the prepupal stage (Plate 4.2) when the midge larva has stopped feeding, when it has spun a cocoon and is lying inside the gall without any movements. Before the larva forms a prepupa, it moves to the outermost layers of the galls where mites lie there in wait for their prey. When the prepupa is formed the mites tear the cocoon and make their way inside, and then they start sucking body fluid of the motionless midge. Mite identifications have shown a variety of mites attacking the midges; these mites come from different families. Some of the mites are predators (they feed on small insects), but some of them are just opportunists (they feed on the plant or they are just there on the plant without doing any harm to the midges). Background research on these mites shows them as being generalist feeders (Ueckermann 2007). The types of mites that were found are listed in Table 1.

Table 1: The mites that attack the midges, sorted in alphabetical order according to family.

Mites encountered in the gall	Family	Feeding guild
Tyrophagus putrescentiae	Acaridae	Stored product mite
Tetratriophtydeus myacanthus Ueckermann	Edbakerellidae	Predatory mite
Triphtydeus immanis Kuznetzov	Edbakerellidae	Predatory mite
Glyciphagous sp.	Glyciphagidae	Stored product mite
Oribatei sp.	Oribatidae	Saprophytic mite
Meyerius latus van der Merwe	Phytoseiidae	Predatory mite
Typhlodromips swellendamensis	Phytoseiidae	Predatory mite
Pyemotes ventricosus	Pyemotidae	Parasitic mite
Tarsonemus sp	Tarsonemiidae	Fungivorous mite
Tydeus grabouwi	Tydeidae	Predatory mite
Brachytydeus sp.	Tydeidae	Predatory mite
Tydeus spathulatus Oudemans	Tydeidae	Predatory mite

Of the 12 mite species found in the galls, seven were predatory. The most common family found was Tydeidae, followed by Phytoseiidae together with Edbakerellidae and these are all predatory groups.

4.4. The seasonal effect of mites on *Dasineura* sp.

Stanford

There are seasonal variations in the abundance of mites on the plants at Stanford, throughout the year (Fig. 6). Predation was measured by the presence of attacked pupae in the galls.

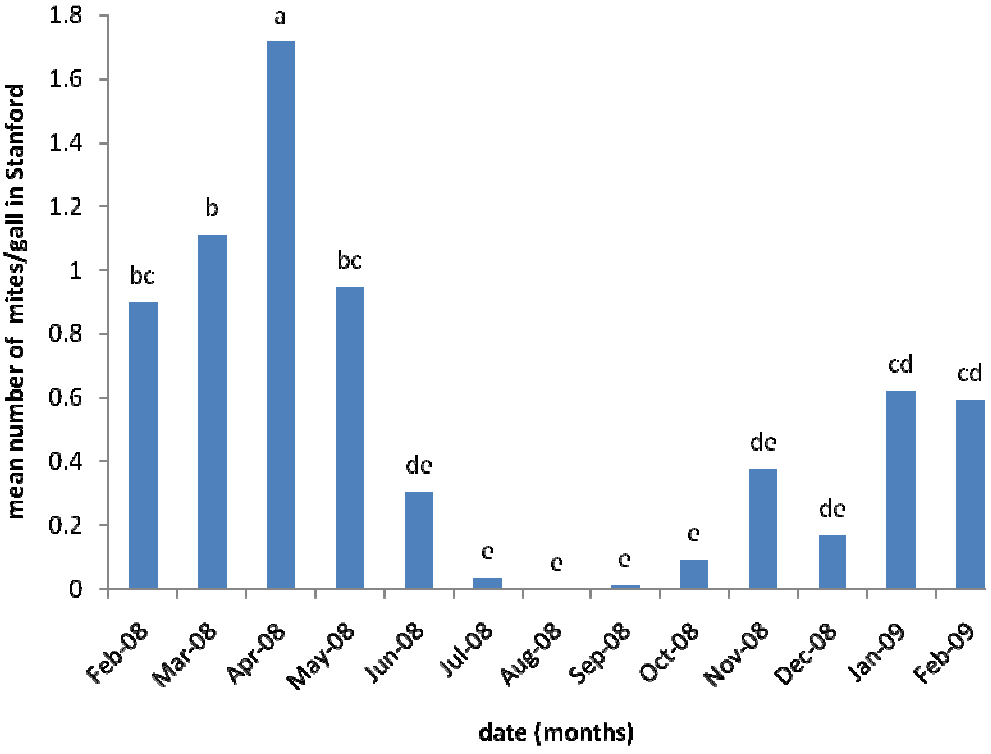

Fig. 6: The seasonal occurrence of mites in *Dasineura* sp. galls on *Leptospermum laevigatum* at Stanford.

At Stanford, the numbers of mites were more abundant between February and May, 2008 (Fig. 6). The peak population was observed in April, while there was no gall infestation in August, 2008. The proportion of pupae that were attacked by the mites in each month (Table 2), and the level of predation (Fig. 7) per month were calculated by simple ratios. The levels of attacked pupae (%predation) were high in February, April, May, June and November, with the highest peak in May when 100% predation was recorded. Generally, level of predation increased with increasing mite infestation and *vice versa* (Fig. 6 and 7). The highest levels of predation were observed in May and the numbers started to drop in July. Between July and October, the levels of predation were low (Fig. 7). The levels of predation then started to pick up in November as the populations of mites started to build up again. There was no predation recorded in August.

The relationship between average number of mites and pupae attacked per gall is presented in Table 3. The analysis showed a positive relationship between the two variables, except in February 2009. Significant relationships were recorded between February 2008 and June, July and August, and November to December of the same year.

Table 2: The average number of attacked pupae of the midge throughout the season at Stanford, South Africa.

Season	n	ATT	TOT
February '08	119	0.126^{ef}	0.177^{ef}
March '08	126	0.206^{de}	0.397^{def}
April '08	210	0.576^{b}	0.786^{bc}
May '08	110	0.809^{a}	0.809^{bc}
June '08	214	0.350^{cd}	0.355^{def}
July '08	84	0.024^{ef}	0.107^{f}
August '08	81	0.000^{f}	0.815^{bc}
September '08	170	0.106^{ef}	1.353^{a}
October '08	66	0.121^{ef}	0.894^{b}
November '08	130	0.400^{c}	0.615^{bcd}
December '08	53	0.113^{ef}	1.283^{a}
January '09	77	0.351^{cd}	0.507^{cde}
February '09	71	0.127^{ef}	0.211^{ef}

n: number of galls examined/season.

ATT: average number of attacked pupae/ gall.

TOT: average number of pupae/ gall (healthy + attacked pupae)

Values in the same column followed by similar letter are not significantly different at P = 0.05.

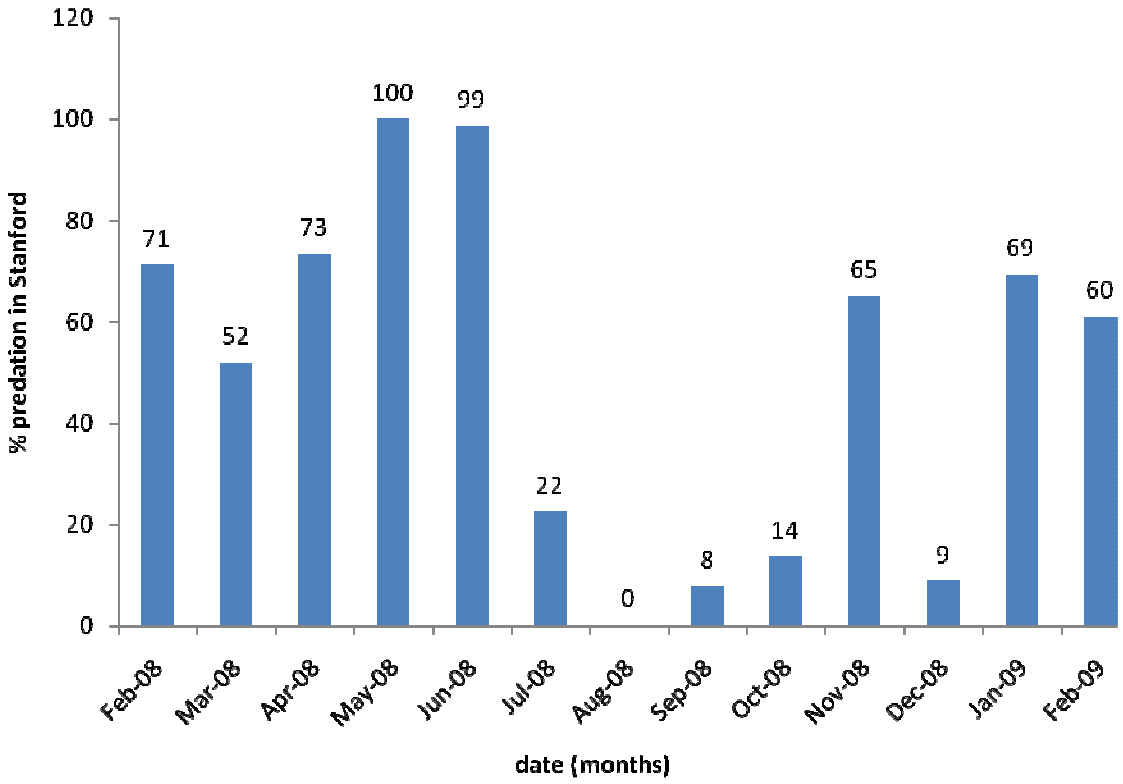

Fig. 7: Proportion (in percentages) of midge pupae attacked by mites on *Dasineura* sp. galls at Stanford

Table 3: Correlations between relative abundance of mites and numbers of attacked pupae in *Leptospermum laevigatum* galls collected at Stanford.

Date	Correlation coefficient
19-Febr-08	0.38313***
11-March-08	0.49731***
03-April-08	0.63635***
22-April-08	0.40936***
12-May-08	0.27888**
03-June-08	0.39218***
30-June-08	0.17197
21-July-08	0.48270***
11-Aug-08	0.48270***
03-Sept-08	0.12283
22-Sept-08	0.12283
13-Oct-08	0.04309
04-Nov-08	0.73324***
25-Nov-08	0.55164***
15-Dec-08	0.68897***
13-Jan-09	0.10708
03-Febr-09	-0.08322

,* Significant F-test at $P \leq 0.01$ and $P \leq 0.001$, respectively.

Onrus-manor and Fisher Haven: At Onrus-manor (Fig. 8), the attacked pupae were most abundant between March and June, declined between July and September, and started to rise again in October to January the following year. The mites also followed the same pattern; the greater the number of mites, the higher the number of pupae attacked.

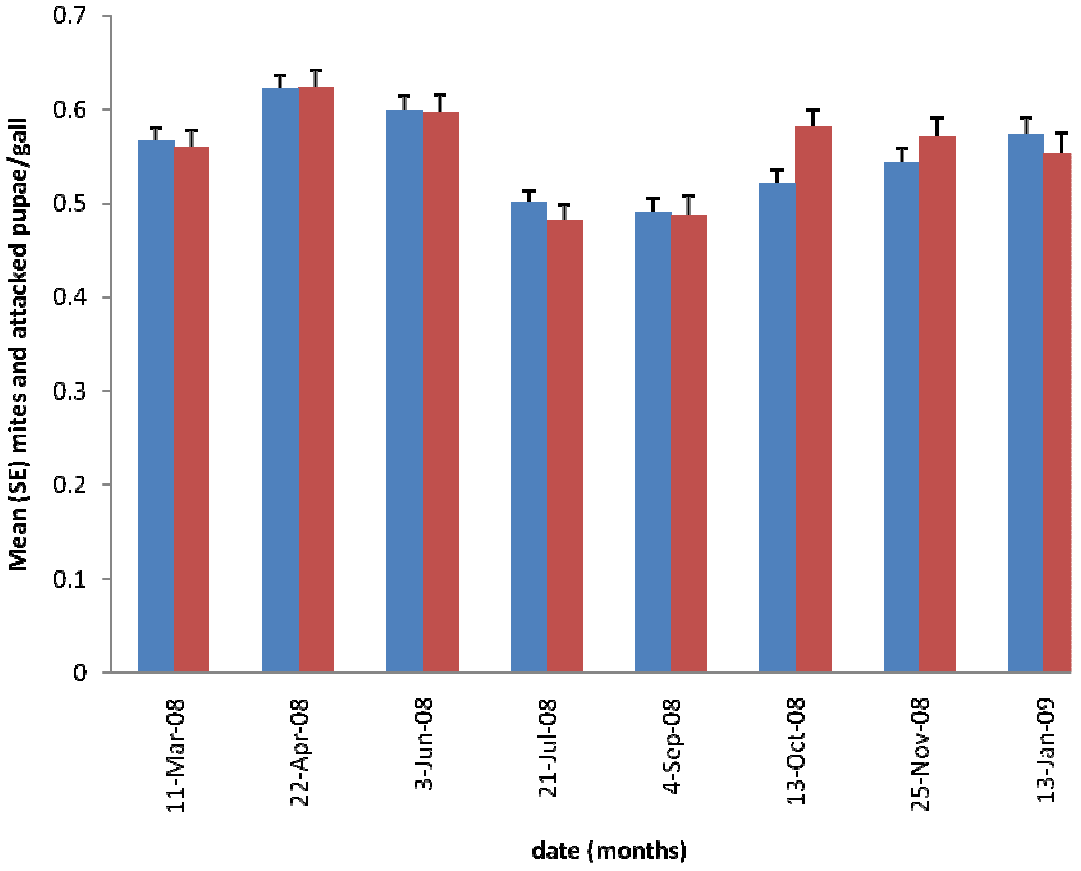

Fig. 8: The seasonal occurrence of mites (mean ± SE per gall) and the measure of predation in *Dasineura* galls on *Leptospermum laevigatum* at Onrus-manor. The blue bars represent the attacked pupae and the red represent mites.

Similarly, at Fisher Haven (Fig. 9) mite populations were most abundant in April, and so were the numbers of pupae attacked. There was a decrease between July and September, and mites started to build up again in October until January the following year.

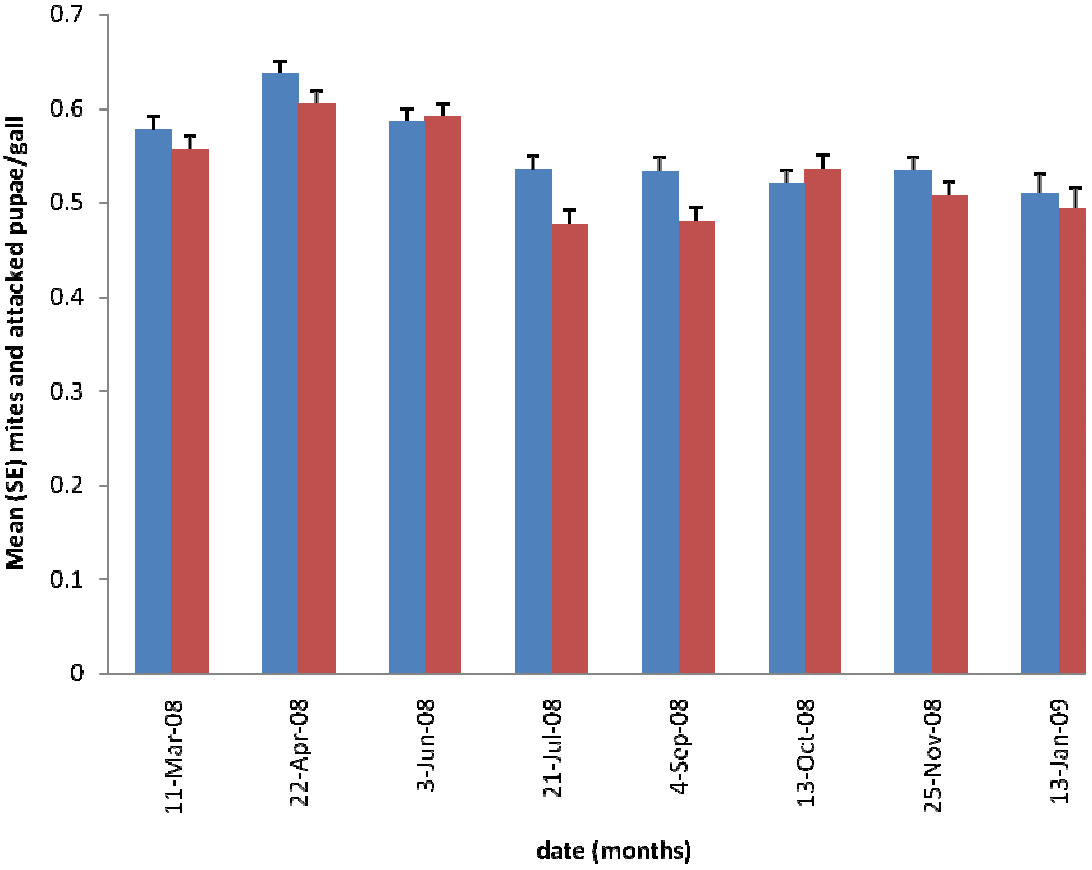

Fig. 9: The seasonal occurrence of mites (mean ± SE per gall) and the measure of predation in *Dasineura* galls on *Leptospermum laevigatum* at Fisher Haven. The blue bars represent the attacked pupae and the red represent mites.

Onrus-manor and Fisher Haven had similar percentages of predation (Fig 10 & 11). The highest percentages were observed between March and June and again between October and January. Onrus-manor (Fig. 10) had 100% predation in March and April and in September there was only 4% predation. Fisher Haven (Fig. 11) had 100% predation in March and June and again in January. In September, the lowest percentage predation (39%) was observed.

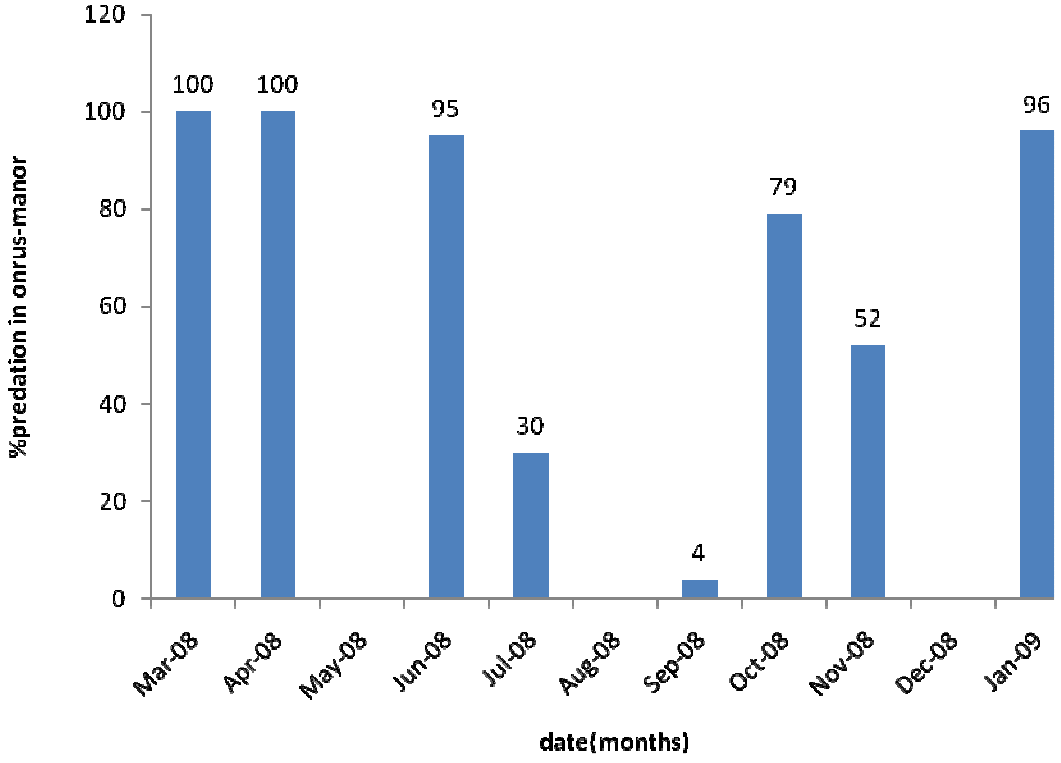

Fig. 10: The measure of predation (in percentages) by mites on *Leptospermum laevigatum* galls at Onrus-manor.

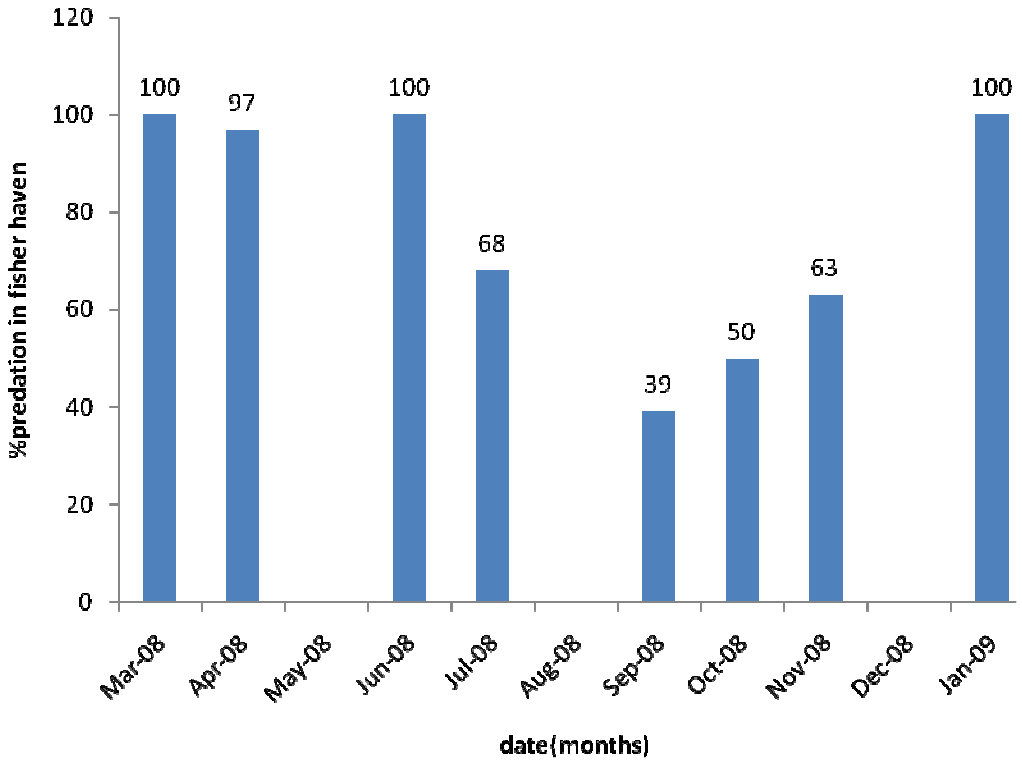

Fig. 11: The measure of predation (in percentages) by mites on *Leptospermum laevigatum* galls at Onrus-manor.

The relationship between mite densities and the pupae attacked at Onrus-manor are presented in Table 4. There is a significant positive relationship between mites and attacked pupae in March, April, October and November.

Table 4: Correlation analysis at Onrus-manor, between the numbers of attacked pupae and mites.

Date	**Correlation coefficient**
11-March-08	0.40719**
22-April-08	0.49375**
03-June-08	0.26880
21-July-08	0.12713
04-Sept-08	0.18949
13-Oct-08	0.38233**
25-Nov-08	0.32712*
13-Jan-08	0.32733

*,** Significant F-test at $P \leq 0.05$ and $P \leq 0.01$, respectively.

The relationship between mites and the pupae attacked at Fisher Haven is shown in Table 5. There is a significant positive relationship between mites and attacked pupae except in April 2008 and January 2009.

Table 5: Correlation analysis at Fisher Haven, between attacked pupae and mites.

Date	Correlation coefficient
11-March-08	0.54148**
22-Apr-08	0.24128
03-June-08	0.30558*
21-July-08	0.30558*
04-Sept-08	0.21637**
13-Oct-08	0.56680***
25-Nov-08	0.66065***
13-Jan-08	0.05558

*,**,*** Significant F-test at P≤ 0.05, P≤ 0.01 and P≤ 0.001, respectively.

4.5. Mite population dependence on midge numbers

Correlation analysis between mites and total number of pupae (healthy and attacked pupae added together), showed a significant positive relationship between the two (Spearman's rank correlation, $r_s = 0.218$, $p < 0.001$). However, there was a very weak relationship, ($R^2 = 0.0089$) which indicated that population densities are independent of the numbers of midges in the study areas (Fig. 12).

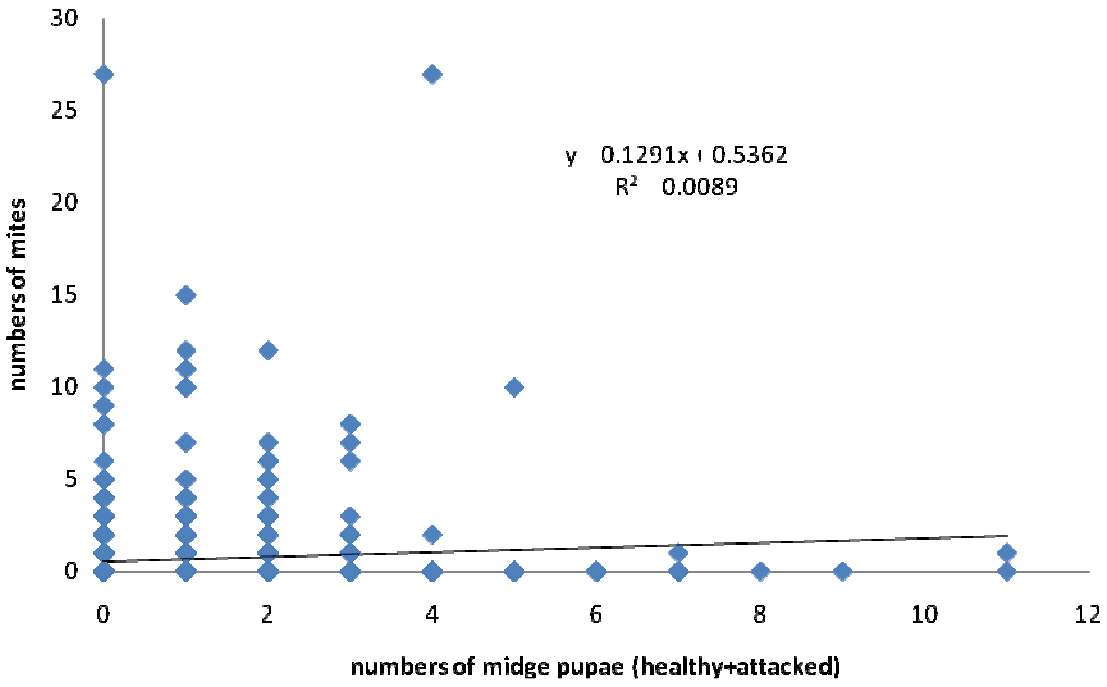

Fig. 12: Regression analysis to determine if mite populations are density dependent on the pupal numbers. Pupae are used because they are the stage most vulnerable to attack by mites.

4.6. The effect of tree density on mite predation of *Dasineura* sp.

The role played by tree density on predation by mites is shown in Table 6 and 7. At Stanford, there was no significant difference in the abundance of mites among isolated trees, saplings and the thickets. At Onrus-manor, there was also no significant difference between the three tree densities, meaning that density had no effect on the abundance of mites at this site. Thickets had the highest number of mites, followed by isolated trees and saplings had the lowest number of mites. At Fisher Haven, the highest numbers of mites were observed in thickets, followed by saplings and then the isolated trees. The mean values of mites at this site differed significantly, meaning that density had an effect on the abundance of mites at this site (Table 6).

Table 6: The average numbers of mites at the three sites (Stanford, Onrus-manor and Fisher Haven) arranged according to densities.

	Stanford			**Onrus-manor**			**Fisher Haven**		
	Iso	Sap	Thick	Iso	Sap	Thick	Iso	Sap	Thick
Average number of mites	0.624^{a}	0.635^{a}	0.605^{a}	0.810^{a}	0.549^{a}	0.966^{a}	0.168^{c}	0.546^{b}	1.06^{a}

Values in the same row, per site, followed by similar letter are not significantly different at P = 0.05. Iso, Sap and Thick represent the three tree densities: Isolates, Saplings and Thickets, respectively.

Table 7: The average number and percentage of attacked pupae of the midge at each tree density per site.

	Stanford			**Onrus-manor**			**Fisher Haven**		
	Iso	**Sap**	**Thick**	**Iso**	**Sap**	**Thick**	**Iso**	**Sap**	**Thick**
ATT	0.178^{c}	0.535^{a}	0.178^{c}	0.603^{a}	0.617^{a}	0.798^{a}	0.540^{b}	0.678^{b}	1.019^{a}
TOT	0.570^{b}	0.973^{a}	0.547^{b}	0.922^{a}	0.060^{a}	1.303^{a}	0.938^{b}	0.744^{b}	1.269^{a}
%PRE	31.23	54.98	32.54	65.40	10.28	61.24	57.57	91.12	80.30

Values in the same row, per site, followed by similar alphabet are not significantly different at P = 0.05. ATT is the average number of attacked pupae/gall, TOT is the average number of pupae/gall (healthy + attacked pupae) at each site and %PRE is the percentage of predation at each site. Iso, Sap, and Thick stand for the three tree densities, Isolates, Saplings and Thickets respectively.

The mean numbers and percentages of attacked pupae are shown in Table 7. At Stanford, saplings had the highest percentage of pupae attacked, followed by the thickets and then isolated trees. The mean numbers of attacked pupae in saplings were significantly different to the mean numbers in both isolated trees and thickets. There was no significant difference between isolated trees and thickets. At Onrus-manor, isolated trees had the highest percentage of pupae attacked, followed by thickets and then saplings. However, the mean numbers of attacked pupae between the three densities did not differ significantly. Fisher Haven had the highest percentage of pupae attacked in saplings, followed by thickets and then isolated trees. Thickets differed significantly from the other two densities in the numbers of attacked pupae.

Correlation analysis (Table 8) between mites and attacked pupae showed a strongly significant positive relationship between mites and numbers of attacked pupae, for each plant density at each site.

Table 8: Correlation analysis between mites and numbers of attacked pupae at each study site. Values represent correlation coefficients (r).

Density	Stanford	Onrus-manor	Fisher Haven
Isolates	0.49***	0.31**	0.25**
Saplings	0.50***	0.65***	0.63***
Thickets	0.35***	0.47***	0.42***

, * Significant F-test at $P \leq 0.01$ and $P \leq 0.001$, respectively.

4.7. The effect of site on mite predation of *Dasineura* sp.

The levels of mite abundance were not significantly different at the three sites (Table 9). However, the levels of pupal attack were significantly lower at Stanford than at the other two sites. Fisher Haven had the highest percentage of attacked pupae (Table 10), followed by Onrus-manor and Stanford showed the lowest level of attacked pupae.

Table 9 The average numbers of mites/gall at each site.

Site	Number of galls	Mites
Stanford	1511	0.619[a]
Onrusmanor	338	0.749[a]
Fisher Haven	342	0.585[a]

Values in the same column, followed by similar letter are not significantly different at P = 0.05.

Table 10: The average number and percentage of attacked pupae of the midge at each site.

Site	n	ATT	TOT	%PRE
Stanford	1511	0.296[b]	0.640[b]	46.25
Onrus-manor	338	0.660[a]	1.077[a]	61.28
Fisher Haven	342	0.740[a]	0.973[a]	76.05

Values in the same column, followed by similar alphabet are not significantly different at P = 0.05.
n: number of galls examined/season.

ATT: average number of attacked pupae/ gall.

TOT: average number of pupae/ gall (healthy + attacked pupae)

%PRE: percentage of predation/ site.

CHAPTER 5

5. DISCUSSION AND CONCLUSION

Biological invasions worldwide threaten the biodiversity, ecosystem dynamics, resource availability, human health (Ricciardi *et al.* 2000) and national economy (Larson *et al.* 2000). Their impact on the environment may be distressing by reducing the carrying capacity of the environment (Banerji 1958). About 30-70% loss of water in the areas with dense stands of alien invasive plants has been reported (Geldenhuys 1986). Gall inducers have been used in many biocontrol programs to combat weeds (Diatloff and Palmer 1987) because of their host specificity (Abrahamson and Weis 1987; Stilling and Rossi 1995).

Dasineura sp. on *Leptospermum laevigatum* completes four to five generations in a year (Gordon pers.comm). Their adults are mostly abundant in October during the flowering season of *L. laevigatum*, which could be an advantage for them as biocontrol agents of this plant. This pattern resembles that of *Dasineura mali* Keifer (Todd 1959) and even their numbers of generations in a year are similar. The generation which occurs in October is the most damaging, as it coincides with the period of maximum shoot growth on their host plant.

This study showed a close ecological relationship between the insect and its host plant, with the most effective developmental stage (larvae) occurring prior to flowering of plant. The phenological studies of the insect showed its developmental stages (larvae, pupae and adults) occurring throughout the year, with peaks at different seasons. During the study period, larvae had their peak between June and August, pupae

peaked between September and December, while the adults peaked between October and January the following year.

However, biotic invasions such as predation reduce the effectiveness of the agent on the plant (Goolsby *et al.* 2000). Hawkins (1990) reports that “parasitism or predation that causes a major reduction in population density of a gall inducer obviously affects its efficacy”. This is true for *Dasineura* sp. on *Leptospermum laevigatum*, because since it has been attacked by the native mites, it has never reached its initial levels of control again. As stated by Cornell and Hawkins (1993), the high proportion of predators attacking these midges tend to have a wide host range, and they belong to different families. The study has shown factors that may have had an influence on the predation by mites.

In Stanford, the presence of mites on the plant varies in abundance seasonally. Mites are mostly common on the plants between February and May with the highest peak occurring in April. This coincides with the presence of pupal stages which are more vulnerable to attack by mites. During the winter season, when the midges are in their larval stages, the mites become scarce on the plant with their populations recovering in October. Climate may be the reason for the scarcity of mites during winter on the plant. Predation on the midges was also abundant mostly between February and June, with 100% predation levels occurring in May. In August there was 0% predation, this is because there were no mites either during that time.

Ehler (1978) reported that parasitism of the gall making cecid *Rhopalomyia californica* varied according to the parasitoid assemblage in the gall; the same was found in my

study with season, when the mites were more abundant, predation was even more. Comparing with the other two sites, predation levels were also high between March and June, with 100% predation occurring in March and April for Onrus-manor, and at Fisher Haven 100% predation levels were found in March, June and January. These two sites showed a similar trend to that at Stanford. Harris and Shorthouse (1996) report that predation is especially a problem for gall-inducing biocontrol agents.

Further analyses have shown that mite abundance does not depend on the abundance of the midges on the plant. Mites inhabit the plant regardless of the numbers of midges present.

Tree density had no influence on mite abundance at Stanford and Onrus-manor, but in Fisher Haven, they differed significantly. In Stanford, saplings had the highest percentages of pupae attacked, in Onrus-manor, isolated trees had the highest percentage of pupae attacked and in Fisher Haven, saplings had the highest percentage predation.

Site had no difference for the abundance of mites, but in predation, Stanford differed significantly from the other two sites. Fisher Haven had the highest predation percentage (76%), followed by Onrus-manor (61%) and then Stanford (46%). Ecological differences at the study sites may be influencing the activity of mites. The majority of the mite species that were identified were opportunists.

During the study, a number of mites were found in the outermost layers of the gall. From June to September, although mites were there in the outermost layers, there were healthy, tiny larvae that were found in the innermost layers of the gall, feeding inside the gall. Mites had no access to these larvae, as they were concealed inside the gall. The presence of mites did not affect the most effective stages of the midges (the feeding larvae). Nevertheless, these mites had an impact on the populations of midges, as they have the potential to reduce the numbers of midges emerging from pupae. These mites would attack the midges as they were ready to pupate and emerge. The tendency of the midges to move to the outer layers of the gall for pupation (which makes it easier for them to emerge), made them more vulnerable to mite attack. Predation increased with the increase in mites.

Mites can feed on a midge number that is greater than their own number. Some of these mites, namely *Pyemotes ventricosus* would become imbibed (swollen) after feeding on the midges. Sometimes, as galls were dissected, mites had escaped already from the gall and only the attacked pupae would be found in the galls. At times, there would be a large number of mites attacking only one pupa in a gall. These two instances account for the months where there was no significant relationship between mites and attacked pupae. The highest number of mites that were found inside a single gall was 27.

The study showed that season, site and tree density has an effect on the predation of midges by mites. However, these mites do not stop the midges from establishing and damaging the plant as biological control agents. The advantage these midges have is that, when they are at their peak (from June to September), the mites are at their

lowest numbers. These midges continue forming galls, despite the presence of mites, but the only concern is that the mites might decrease the populations of midges until they are not effective as biocontrol agents of the plant. The recommendation is that more data should be collected, maybe in three years from now to check if the midges are still persisting on the plant. Another control agent could be considered to supplement *Dasineura sp*. Because of the gradual decrease of the populations of the midges, it might be impossible for them to reach their initial damaging levels, as recorded by Gordon (unpublished data). The initial hypotheses that site, density and season have an effect on the predation of the midges by mites were accepted.

Discussion of problems

Mite species could not be quantified due to the inability to identify the mites without mounting them. This rendered it impossible to tell the percentages in which each species occur. Understanding that this could have added more value to the study, it does not render the thesis inadequate, because a lot of information has been generated from the study. This includes 1) the seasonal abundance of mites, which I believe is more important to evaluate their effect on the midges in different seasons; 2) the percentages of damage by the mites on the midges; 3) the midge stages that are most vulnerable to attack by mites; 4) the different factors (site, density and plant size) that could have had an influence on the predation of the midges. The proportion of the midges that escaped predation was also observed. An honours project which would focus on identifying the mites could be created to gather such information, so as to be able to tell which ones are more dangerous to the midge.

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APPENDICES

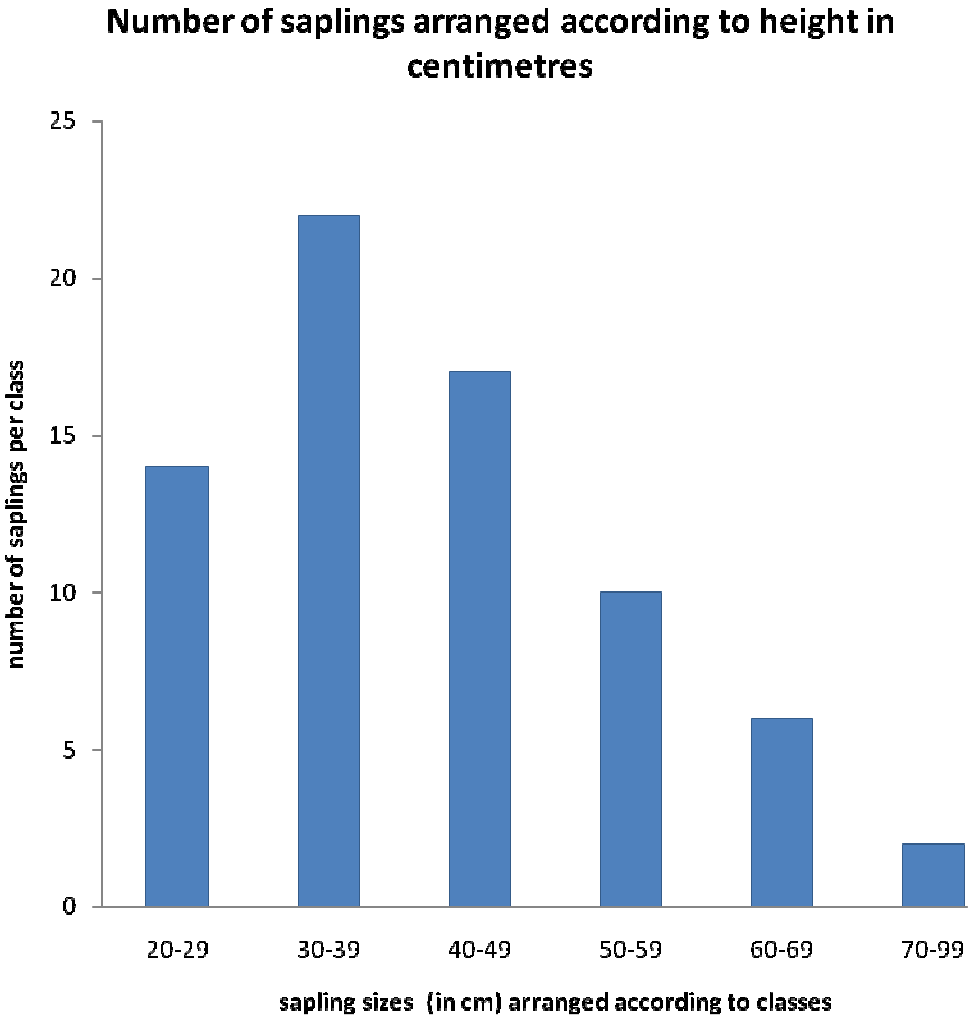

Fig. A1: Saplings have an average height of 42.11 ± 14.61cm.

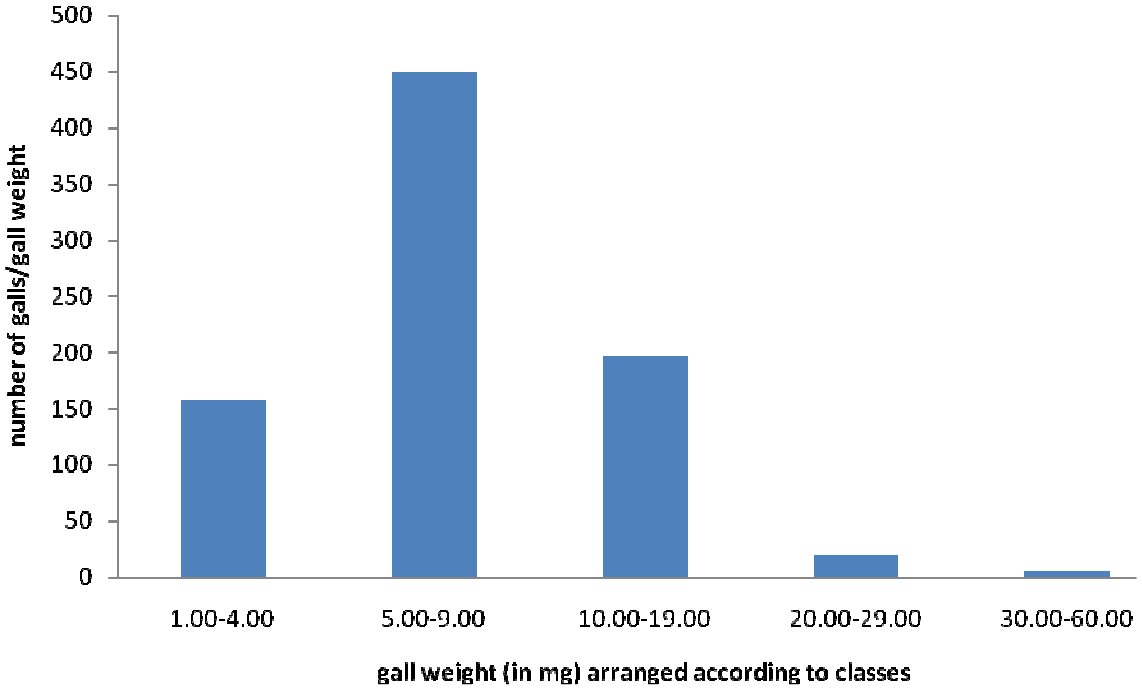

Fig. A2: Number of galls grouped according to their weight from the smallest to the largest. Galls weigh on average 9 ± 0.005 mg. The largest gall weighed 57.4 mg and the smallest weighed 1.31m g. The greater the weight, the fewer the number of galls that were found.