Investigation into the effects of parasitoids on a gall midge *Dasineura sp.* [Cecidomyiidae], a biological control agent of Australian myrtle, *Leptospermum laevigatum*.

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Abstract:

Leptospermum laevigatum is one of the top five invading species in the fynbos biome and its biocontrol is of high conservation priority in the Western Cape. The first species used as a biocontrol agent was a leaf-mining moth *Parectopa thalassias* that became established and wide-spread. The second agent a *Dasineura sp* [Cecidomyiidae] gall midge whose origin in unknown but was probably introduced accidentally. As it was not screened before release an investigation into the effect of parasitism on its effectiveness as a control agent is important. Midges were found to select plants for oviposition that have high growth rates and result in large galls. This results in plants with lots of large galls. These plants are conspicuous to parasites and levels of parasitism are highest at these sites. Once the plant has been selected by the parasitoid, gall selection for oviposition was not related to density of the midges within. The spatial scale showed the dispersal of the midge was primarily related to the prevailing westerly wind. Parasitism levels followed the spread of the midge and increased as midge densities increased. The midge is still spreading and indications show parasitiods do not prevent establishment into new areas. As this is the midges most vulnerable phase, once they are established they should persist. However even at the sites with high number of galls the plants still produced fruits with seeds. Thus high parasitism levels could reduce the population densities of the midge and inhibit is usefulness as a biocontrol agent. A further biocontrol agent that attacks these reproductive parts could result in the successful control of this invasive species.

Introduction:

Australian myrtle, *Leptospermum laevigatum* was introduced into South Africa, primarily for sand-dune stabilisation, in the early 1800s [Gordon 1999] and subsequently has been used for hedges. From these deliberate plantings *L. laevigatum* has spread into the surrounding countryside. Australian myrtle is now one of the five most invasive plants in the fynbos biome [Macdonald and Jarman 1984]. One of the most important factors that makes *L. laevigatum* a major invader is post-fire seed recruitment in the fire-prone fynbos. The plants produce strong lateral roots with a fine mat of rootlets. Water uptake by these roots is very effective and few other plant species are able to survive in the immediate vicinity of myrtle [Bromilow 1995]. Another concern is that in areas where the two species co-occur, *L. laevigatum* is replacing *Acacia saligna*. Which is being successfully controlled by a gall-forming rust fungus *Uromycladium tepperianum*. Thus negating the success of this biocontrol project [Gordon 1999].

Chemical control of *L. laevigatum* is undesirable to natural ecosystems while mechanical control does not always kill the shrubs and can result in multi-stemmed regrowth, which is difficult to control. Both these methods are expensive and labour intensive. Thus biological control of *L. laevigatum* is desirable and important in the conservation of the fynbos biome. A leaf-mining moth was the first species released for control and rapidly became established. The second species to be considered is an unidentified gall-forming midge *Dasineura sp.* [Cecidomyiidae] that galls the flowers and axillary buds. Attempts to screen the midge were stopped as populations of a seemingly identical cecidomyiidae were discovered at a few localities in the Western Cape between 1994 and 1996 [Gordon 1999]. The first established populations were discovered at Botrivier and the midge is most likely to have spread from this site

[John Hoffman, personal communications]. The origin of this species is unclear although it appears to be the same Cecidomyiidae and was probably introduced accidentally. The gall midge has become widely established.

Edwards *et al* [1996] stated that introduced insects would experience lower levels of parasitism and predation than they do in their native environment and thereby have a greater impact on their host plant. A large effort is made to clear biocontrol agents from their own natural enemies, but once released nothing can be done to prevent exposure to indigenous parasitoids. Although indigenous parasitoids often have little impact on introduced insects they have on occasions reduced their effectiveness as biocontrol agents [Turner *et al* 1990]. As the midge was accidentally released and not screened it could have possibly brought in parasitoids from its area of origin. Thus an investigation into the levels of parasitism on the midge is important in assessing its use as a biocontrol agent.

Susceptibility of biocontrol agents to attack from parasitoids is influenced by the feeding niche with poorly-concealed endophytic agents the most susceptible [Askew and Shaw 1986]. Endophytic hosts as natives usually support parasitoid complexes containing large proportions of generalists whereas external feeders may support relatively more specialists [Cornell and Hawkins 1993]. As introduced insects are more likely to be attacked by generalists, galling species will be able to support a large parasitoid complex due to the generalist nature of parasitoids attacking them. While specialist parasitoids might evolve appropriate traits to exploit this novel resource [Askew and Shaw 1986]. Evidence of native parasitoids attacking introduced galling insects has been shown by Moore 1989. This study found the effectiveness of a gall midge *Cystiphora schmidti* [Cecidomyiidae] as a biological control agent was limited by a parasitic wasp *Tetrastichus* sp. [Hymenotera].

The *Dasineura* cecidomyiid forms multiloculer galls on the flower and auxiliary buds. Although the midges gain protection from the plant tissue surrounding them there is a trade-off between protection and visibility [Rossi et al 1992]. Also due to limitations of the ovipositor length of parasitoids large galls can prevent parasitoids from reaching their hosts [Rossi et al 1992].

The ability of parasitoids to use a novel resource could depend on the taxonomic isolation of the new invader and thus its chemical, phenological and structural distinctness from indigenous galling insects making host shifts more difficult [Cornell and Hawkins 1993]. However ecological factors such as habitat or host-fruit texture could be more important in determining host-parasitoid associations than phylogenetic factors [Edwards et al 1996]. Although there is some evidence that for communities associated with invaders tend to stabilise over a relatively short period of time [Cornell and Hawkins 1993], predicting these outcomes is difficult for any species [Schonrogge et al 1996].

Parasitoids were found to start utilizing *Dasineura sp* soon after its introduction. The main aim of this study was to assess the levels of parasitism on the midge to determine whether the parasitoids are abundant enough to affect the ability of the midges as biocontrol agents of *L. laevigatum.* The investigation into levels of parasitism on the midge can also be used to understand the development of communities associated with invading species and to study aspects of the dynamics of community structure [Schonrogge *et al* 1996].

This study was done by looking at the relationships between the gall midge, parasitoids and galls on the plant. The first part of the paper deals with the relationship between levels of galling by the midges and the plants. The levels of parasitism and midge densities were then analysed. Lastly the differences

between the sites were investigated to look for trends in the dispersal of the midge and subsequent parasitoid assemblages.

Methods:

The study area for this project spanned 80Km along the south coast of Western Cape, South Africa. The study area included six different sites from Gordon's Bay, Pringle Bay, Klienmond, Botrivier, Hawston to the last site at Hermanus. This gives a spatial scale, as populations of the midge were first recorded at Botrivier. At each site five shrubs were randomly selected from a stand of *L. laevigatum* and three branches of approximately equal size were removed from varying heights on the plant. The total number of galls from each branch was counted and four randomly selected galls from each branch were weighed. These weighed galls were dissected under a microscope and the number of cocoons recorded as empty [unparasitised], parasitised or aborted was recorded. Empty cocoons were transparent, while parasitised cocoons had the remains of the destroyed midge inside and aborted cocoons had the midges still intact inside the cocoons.

Percentage parasitism was calculated as the number of parasitised cocoons divided by the total number of cocoons. Weight of the galls was regressed against the total number of cocoons for all the dissected galls. This was done to look at the effects of the midge on the size of galls. A correlation analysis between average weight of galls per plant and the total number of galls per plant was used to determine whether there were interplant differences in gall size. Percentage parasitism was correlated with the number of galls per plant and the total number of cocoons found in each gall to determine any density dependent behaviour by the parasitoids.

To compare sites, percentage parasitism and average number of galls per plant were calculated for each site. The data was analysed using ANOVA and a post hoc test using least squared differences was used to show significant differences between sites. The sites were then grouped according to the results from the post hoc test. Direction of the prevailing westerly winds was included in the analysis for its effect on dispersal. The ratio of parasitised cocoons to unparasitised cocoons was analysed using a Chi-squared test. Critical values and formulae for the Chi-squares test were obtained from Zar 1984.

Results:

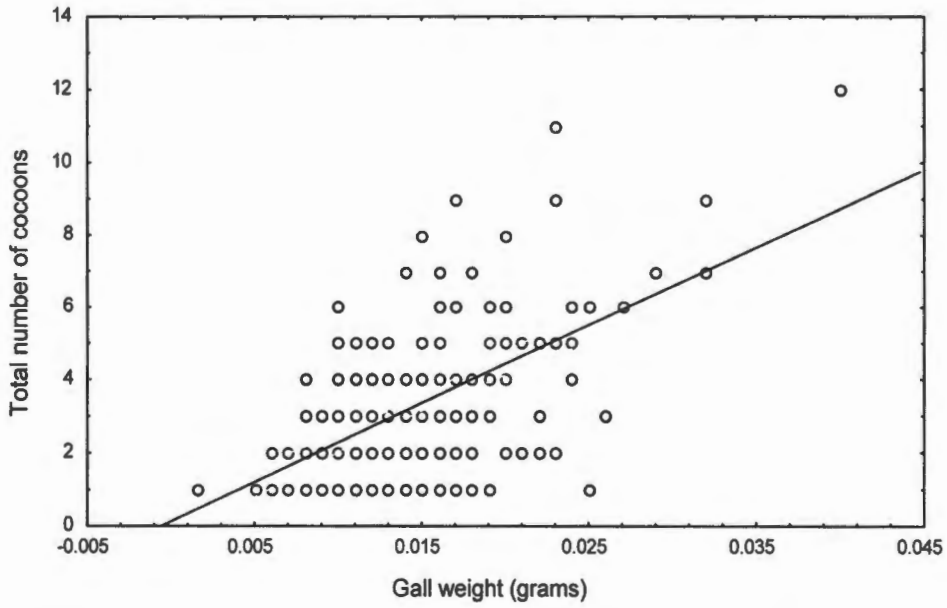

Fig 1: Total number of *Dasineura* cocoons found in each gall plotted against the weight of each gall for all *Leptospermum laevigatum* plants at all sites [R^2=0.341, p = 0.000].

The number of cocoons per gall increased with size [Fig. 1].

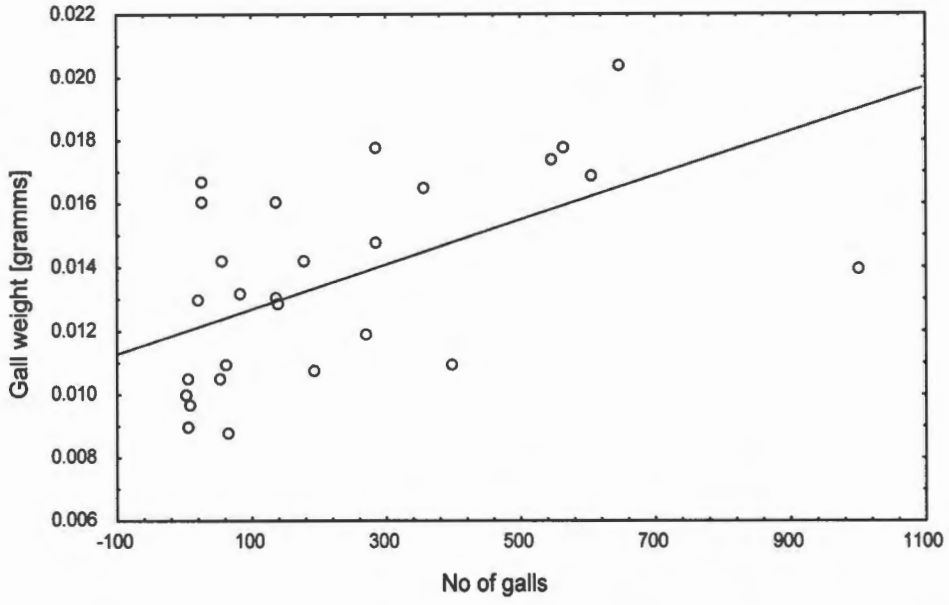

Fig 2: The average weight of *Dasineura* galls per *Leptospermum laevigatum* plant plotted against the number of galls per plant [R^2=0.0242, $p < 0.0078$].

The relationship is linear with the average weight of the galls increasing as the number of galls on a plant increase [Fig. 2].

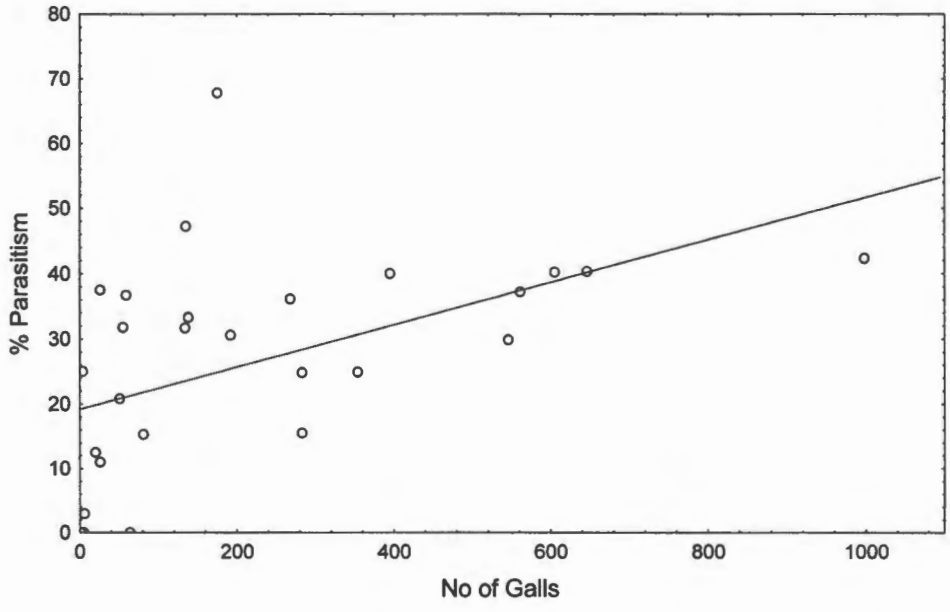

Fig. 3: Average percentage parasitism per *Leptospermum laevigatum* plant plotted with the number of *Dasineura* galls for each plant at all sites [R^2=0.2386, $p < 0.0084$].

There is considerable variation in the percentage parasitism for plants with less than 300 galls [Fig. 3]. Above this level there was a steady increase in percentage parasitism as the number of galls per plant increased.

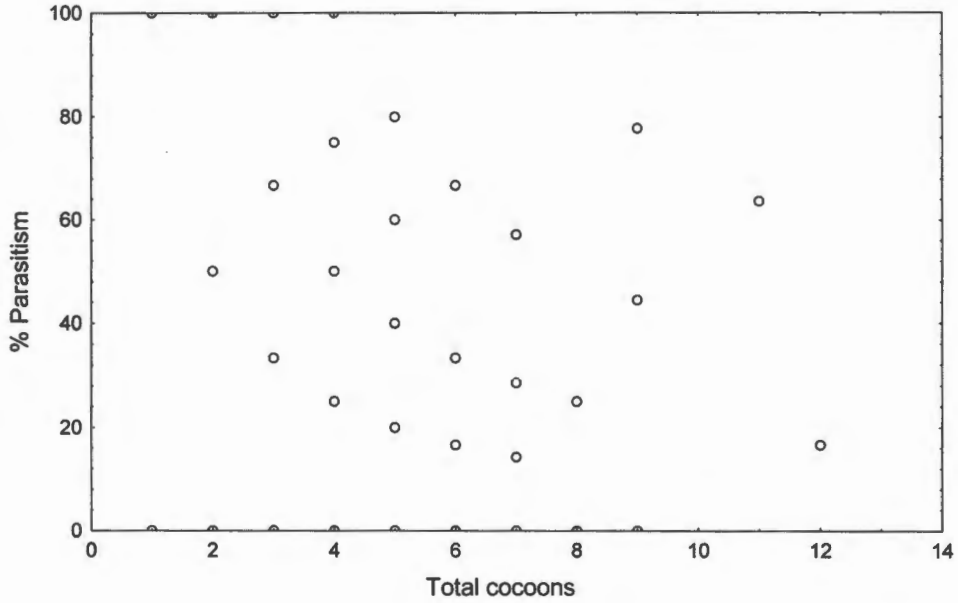

Fig. 4: Percentage parasitism for each gall in relation to the total number of *Dasineura* cocoons found in each gall on *Leptospermum laevigatum* [R^2=0.0018, $p < 0.8408$].

Fig. 4 shows a large amount of variation in the data but a slightly decreasing trend in percentage parasitism can be detected as the total number of cocoons increases. The insignificant correlation [$R^2 = 0.018$] lets us extrapolate that parasitism is largely unaffected by the number of cocoons in a gall.

Analysis across sites:

Table 1: Levels of parasitism of *Dasineura sp.* across all six sites including overall percentage parasitism, distance and direction from initial established site at Botrivier.

Site and distance from release site	Distance from Botivier [Km]	Direction from Botrivier	Percentage parasitism
Botrivier	0	-	42.5
Klienmond	7.2	West	27.73
Hawston	8.4	East	31.12
Hermanus	12.5	East	33.29
Pringle Bay	26.4	West	2.5
Gordon's Bay	31.1	West	12.06
Overall			30.22

Pringle Bay had the lowest levels of parasitism while Botrivier where the midges have been established the longest had the highest levels. Only Pringle bay and Gordon's bay had levels significantly lower than the total percentage parasitism. Hawston and Hermanus had the highest levels of parasitism after Botrivier and are both found on the western side of this site.

Table 2: The average number of gall midge galls per branch at each site. Included is the standard deviation and groups according to the ANOVA and post hoc tests.

Site	Number of galls [std dev.]	Groups
Botrivier (0km)	164.13 [137.03]	A
Klienmond (7.2km)	67.39 [47.5]	B
Hawston (8.4km)	25 [17.45]	C
Hermanus (12.5km)	149.3 [90.65]	A
Pringle Bay (26.4km)	1.6 [0.89]	C
Gordon's Bay (31.1km)	11.33 [21.42]	C

The ANOVA showed $F_{[5,83]} = 15.235$ and a post hoc test using least squared difference method allowed the sites to be grouped according to significant differences. The sites with large numbers of galls per branch were grouped together [A] and the sites with low numbers were grouped together [C]. Klienmond is placed in a separate group [B] due to its intermediate number of galls per branch.

Table 3: Contingency Table of number of parasitised and unparasitised cocoons at all sites.

Site	1	2	3	4	5	6	Total
Parasitised	9	1	30	59	44	70	213
Unparasitised	63	16	74	103	92	151	499
Total	72	17	104	162	136	221	712

Percentage parasitism was not solely density dependant but also varied between sites [X^2=19.143, 5 degrees of freedom, $p < 0.001$].

Discussion:

The positive relationship between gall size and number of midges in each gall [Fig. 1] could be caused by two factors. Either midges attack plants that are rapidly growing and produce large galls or the size of the galls depends on the number of its occupants. Gall size is often correlated with plant vigour or growth and an increase in gall number and size was found on fertilized plots [Rossi et al 1992]. Thus the relationship is more likely to be the midges select plants for oviposition that are growing rapidly. Support for this hypothesis come from the fact that plants that produced large galls also has higher numbers of galls [Fig. 2] indicating that they were being selected preferencially by the midges.

Parasitism was influenced by the number of galls per plant but not by the number of midges per gall. Plants with large numbers of galls are conspicuous and therefore selected by parasitoids. Once the parasitoids has selected a plant for oviposition the selection for galls was not related to midge densities. Also cocoon number is related to gall size and parasitism was unaffected by gall size. Gall size usually has an effect on percentage parasitism due to parasites ovipositor length and hence protection of hosts from the gall walls. However the change in gall weight were relatively small and changes to the thickness or diameter of the galls are neglible.

The analysis across sites gives a spatial scale from Botrivier the site where the midge was first recorded to Gordan's Bay the furthest site from Botrivier. As it

is still early in the establishment of midges this gives a good illustration of dispersal from initial site and the subsequent assemblage of parasitoids.

The two furthest sites had low levels of parasitism and numbers of galls per branch. The midges although present at these two sites are probably not well established and parasites have not yet targeted this novel resource.

The midges have spread from Botrivier predominately to the eastern side due to the prevailing westerly wind in these areas. The only discrepancy from this pattern is the low levels of galling at Hawston. This site was situated next to a rubbish dump and the plants were covered in black grime and pollution. This may have affected the midges selection of plants. However the percentage parasitism was unaffected and remained consistent with sites of a similar distance from Botrivier.

The Chi-squared test from Table 3 confirms the fact that parasitism levels are not solely density dependent but vary between sites. Factors affecting the parasitism levels include galls per plant and ecological factors. Although parasitism is evident at all sites and heavily at some it appears parasitism only becomes a factor once the midges have reached a critical level or been present for a sufficient period of time. Thus the parasitoids are not stopping the midges from establishing into new area. As insects are most vulnerable during establishment phase [Olckers 1995] the midge will surely persist once established.

Even though the percentage parasitism can reach high levels up to 43.5% due to the generalist nature of parasitoids attacking endophytic hosts [Cornell and Hawkins 1993], the parasitoids do not have an effect on the formation of the galls or the size of galls. The number of parasitoids attacking invaders tend to

increases over the first 150 years after introduction [Cornell and Hawkins 1993]. Thus levels of parasitism could possible increase to levels where the parasitoid can reduce the population densities of an agent and thus hinder its biocontrol effectiveness [Hill and Hulley 1995]. However similar constraints on parasitoid assemblage size operate in both native and foreign localities [Cornell and Hawkins 1993].

In this study parasitism has not yet reached levels high enough to stop the dispersal of the midge. However even the sites with high numbers of galls per plants still produced seeds in capsules. Thus the high parasitism levels reached at places where the midge has become establishes could reduce the gall midge [Cecidomyiidae] effectiveness as a biocontrol agent for Australian myrtle *Leptospermum laevigatum*. Another biocontrol agent that attacks these reproductive parts could result in the successful control of this invasive species.

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