PATTERNS OF PARASITISM AND EMERGENCE IN THE GALL MIDGE *DASINEURA DIELSII* (Diptera: Cecidomyiidae), A BIOLOGICAL CONTROL AGENT OF *ACACIA CYCLOPS* IN SOUTH AFRICA

N.M. WHEAT

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SUPERVISORS: D.M. RICHARDSON & J.H. HOFFMANN

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ABSTRACT

Acacia cyclops A. Cunn. ex G. Don is an invasive alien plant that invades fynbos and coastal dunes. Several acacia species are grown commercially in South Africa and this has limited biocontrol agents to those that reduce only reproductive capacity. *Dasineura dielsii* was released in 2002 as a biocontrol agent for *A. cyclops*. This gall-forming midge destroys inflorescences and prevents seedpods from forming, but allows continued harvesting of wood. Insects overwinter as larvae within their galls. This study examined the levels of parasitism experienced by dormant *D. dielsii* larvae, as well the trigger that causes them to break dormancy. Gall clusters were sampled over autumn and early winter, and were dissected to determine occupancy. The effects of temperature and light on dormant larvae were also examined. Observations showed that as the season progressed, more *D. dielsii* larvae entered dormancy. At the same time, a greater proportion of dormant larvae were parasitized. Parasitism was highest, at 18.5%, at the end of the study period, but this level of parasitism is not enough to reduce the biocontrol power of *D. dielsii*. A positive relationship between mass of gall clusters and the number of galls they contain was established. Larval dormancy could not be artificially broken by either light or temperature, and it appears that neither factor alone can trigger a break in dormancy.

INTRODUCTION

Ever since people first started to spread across the globe, they have been taking things with them from place to place. This included plants. As more people started travelling, more plants were moved and more plants became established in places other than their natural distributions. Plants are moved to new areas for many different

reasons. Some are valued for their use in agriculture or forestry, others are ornamental, or perform a specific function e.g. dune stabilisation. Some are transported accidentally - seeds may arrive in a new area with a container of imported goods. Some naturalised plants escape the confines of the area of their introduction. They thrive in the new area and reproduce in copious amounts. They are able to spread far and wide beyond their place as garden ornamentals or fodder plants. Hence, they are termed invaders or invasive alien plants (Richardson et. al. 2000).

Alien plants were first introduced to South Africa by European colonists. The colonists brought with them a variety of plants, including species intended for food, fodder, and forestry. This was only the beginning. Since those early times, nearly 750 species of trees and nearly 8000 species of shrubs and small plants have been introduced to the country, mostly from Australia, America and Europe (Baskin 2000). Of these, only a few hundred have become invasive alien plants. But these few have spread over a large area and are the cause of many problems.

Invasive alien plants can alter ecosystems and processes that sustain indigenous vegetation. Alien invasive plants cause a reduction in stream flow (Le Maitre et. al. 2002), changes to the fire regime (Richardson & van Wilgen 2004; Le Maitre et. al. 2002), soil erosion (Enright 2000) and ultimately cause a loss of biodiversity (Stohlgren et. al. 2003; Musil 1993). All of these effects have severe implications for the indigenous flora of the country. Invasive alien plants use up more water than indigenous flora does (Bosch & Hewlett 1982). In a dry country such as South Africa, this in itself is a good enough reason to treat invasions seriously and implement control operations. In addition, the invasive aliens often contain oily compounds in their leaves, making them more susceptible to burning. This alters the fire regimes of the fire sensitive ecosystems, which has a detrimental effect on indigenous plants. Ultimately, if alien invasive plants are allowed to continue their spread across the country, they may cause a loss in the diversity of indigenous species. In an area such as the Cape, this would have enormous implications. The indigenous vegetation of this region is so diverse that it constitutes an entire floral kingdom – the fynbos biome. So, what is being done to stop this invasion?

South Africa is one of the world's leading countries in the field of combating alien plant invasions. We do not have the strict border controls that other countries, such as New Zealand, have in place to stop the income of alien plants. Instead, there are many laws governing which plants are allowed to be grown in South Africa and where, as well as projects to remove infestations of declared weeds. The Conservation and Agricultural Resources Act of 1983 (CARA) paved the way to promote the control of alien invasive plants. This act divided invaders into one of three categories. Category 1 plants must be controlled and removed from wherever they are. They are not allowed to be planted or propagated e.g. *Lantana spp*. Category 2 plants pose a threat to the environment but are allowed to be used for commercial purposes, but must be contained in special areas e.g. *Pinus pinaster*. Category 3 plants are those with ornamental value. Only existing specimens may remain, with no further propagation allowed e.g. *Jacaranda mimosifolia* (Working for Water Annual Report 2002/2003). Initiatives like the Working for Water project meet the requirements of this law by removing infestations of alien plants.

However, there are some conflicts between those that benefit from the alien invasive plants and those that want to remove them for conservation purposes. Several alien invasive plants are allowed to be cultivated for commercial gain, but this requires a permit and cultivators must exercise strict control over the spread of these plants. Large corporations may be able to purchase the necessary permits and implement control measures, but smaller businesses and poor communities making an informal living off the plants would stand to lose. Many poor communities harvest firewood from woody invasive trees, both for personal domestic use and for informal trading. The plants from which they harvest the firewood are not cultivated by the communities and thus will be destroyed during removal projects. These communities cannot afford to run plantations and would be left without a source of income and firewood.

Several solutions to this type of conflicting interests have been proposed. Among them is the replacement of alien invasive plants with other economically beneficial indigenous plants e.g. replace alien trees harvested for firewood with indigenous plants that can be harvested for their flowers (Higgins et. al. 1997). But what should be done when an invasive alien plant serves a unique role in the ecosystem (Parker et.

al. 1999)? For example, when invasive alien trees provide nesting opportunities for indigenous birds (Richardson & van Wilgen 2004). An elegant solution is provided by biological control. Biological control agents can be used to reduce the reproductive capability of adult plants but cause no other harm, allowing for continued use of the adult plants for harvesting or nesting.

only initially, by far the cheapest in the long run.

Biological control, or biocontrol, is the control of an alien invasive plant or animal using natural enemies of the alien in question. This science is just over a century old, with well-documented cases from the late 19th century. Countries such as America, New Zealand and South Africa are the forerunners in this field. Biological control is more time consuming and expensive than other means of invasive plant control. It requires research into which agent, usually an insect, would be suitable for which plant. Once an agent had been selected, extensive tests must be performed to assess the impact that it will have on both the plant and the environment. Once this is done, the insects must be collected in their country of origin, transported to the country of infestation and reared in sufficient numbers, ready for release. After all of this has been done, and the agent had successfully established on the alien invasive plant, no further input is required, which makes this the best long-term solution (Baskin 2000).

and cheapest

In South Africa alone, as of the year 2000, 103 biocontrol agents had been released for the control of 46 invasive alien plant species (Baskin 2000). Most of these are insects (wasps, beetles, weevils and midges) or fungi. Success rates are good, with 22 of the alien plant species considered under control since biocontrol measures have been taken against them.

There are, however, some problems associated with biocontrol. In recent years, it has become more difficult to obtain biocontrol agents from some countries. Bureaucracy in these countries will not allow investigations on their native flora and fauna. This means that many potential biocontrol agents are out of our reach (Zimmermann & Neser 1999).

Out of the top ten worst invasive plants in the Cape Peninsula, five are Australian acacia species (Ukavuka handbook). In addition, 13 species of acacia are listed under the CARA legislation as either weeds or invaders (categories 1 and 2). Most of these

acacia species were introduced to South Africa during the 19th century, for various uses. They have become invasive in many types of vegetation, not only in the Cape, but also across the whole country. In the Cape, they invade mountain catchment areas, mountain and lowland fynbos as well as coastal dunes (Dennill et. al. 1999). Hence, members of this genus have been the subject of many biocontrol efforts. The first biological control agent of an acacia species was released in 1982, on *A. longifolia.* As of 1999, biocontrol projects had been initiated against six invasive acacia species. Additional agents have since been released on other species of acacia (Dennill et. al. 1999; Hoffmann et. al. 2002).

In Australia, acacia species have a range of natural predators, including species belonging to the orders Diptera, Hymenoptera and Coleoptera (Adair et. al. 2000; Hoffmann et. al. 2002; Impson et. al. 2003). In order for these to function as biological control agents, they must have very narrow host ranges. An agent must not be able to spread to indigenous South African acacia species, like *Acacia karoo,* and must only destroy seeds and/or flowers. In addition, the insects chosen as biocontrol agents must be able to sustain populations without human intervention. Despite this, some agents may fail to establish on the intended plant, for whatever reason, or they may only survive for one season after release.

In the early 1990s, *A. cyclops* A. Cunn. ex G. Don was targeted for biocontrol. *A. cyclops* was introduced into South Africa ca. 1845 in order to stabilise the shifting dune sands of the Cape flats and other sandy areas (Roux 1961; Avis 1989). It quickly became established (naturalised), possibly because of the similar climatic and soil conditions in South Africa to its country if origin, Australia. In the 1920s, dune stabilisation became a concern to the government, who began widespread dune reclamation projects. Unfortunately, they did not comprehend the potential of invasive alien plants at the time, and used *A. cyclops* extensively. The tree quickly reached invasive proportions and began altering dune ecosystems (Hellström 1996).

Table 1: Acacia species targeted for biocontrol and the agents used

Acacia species targeted	**Biocontrol agent**
A. cyclops	*Melanterius servulus* (type A), *Dasineura dielsii*
A. dealbata	*Melanterius* spp.
A. longifolia	*Trichilogaster acaciaelongifoliae, Melanterius ventralis*
A. mearnsii	*Melanterius maculatus*
A. melanoxylon	*Melanterius acaciae*
A. pycnantha	*Trichilogaster* spp.
A. saligna	*Uromycladium tepperianum*

By the 1990s, *A. cyclops* had invaded large areas of coastal dunes as well as inland fynbos. Many local communities were living off the proceeds of selling firewood harvested from these trees. Thus, large scale, mechanical removal was not an option in many infested areas. The only control option was biological control using seed and flower feeding agents. Two biological control agents have been used to curb the spread of *A. cyclops*. These are the seed-feeding weevil, *Melanterius servulus* Pascoe type A (Coleoptera: Curculionidae) and the gall-forming midge, *Dasineura dielsii* Rübsaamen (Diptera: Cecidomyiidae).

Melanterius servulus is a univoltine weevil, one of several *Melanterius* species used for the biological control of acacia species in South Africa. It was first released in 1991, but failed to become established. In 1994, the weevil was reintroduced at several sites throughout the Western Cape, after which it became well established (Denill et. al. 1999). *M. servulus* damages the seeds and seedpods of *A. cyclops* in several ways. Eggs are inserted through the seedpod wall into the developing seeds. This action damages the pod wall and may allow fungus to enter the seedpod, which further damages the seeds within. The larvae then consume the entire seed while they are developing. Larvae pupate in the soil below trees and overwinter as adults. In addition to larval feeding, adults feed on and destroy unripe seeds (Impson et. al. 2004). Seed damage reaches up to 99% in some situations (Dennill et. al. 1999).

In addition to the seed-feeding weevil, a second biological control agent was released in the early part of 2002. The Fluted Galler, *Dasineura dielsii* is a univoltine, gall-forming midge. It uses the flowers of *A. cyclops* by depositing its eggs directly onto the surface of the developing ovary. The hatching larvae cause the ovary tissue to form galls of several chambers. The galls on a single inflorescence form small clusters of up to 50 galls. Larvae pupate within the gall chambers. Once emerged, adult midges live for only a few days (Adair et. al. 2000). Mature larvae may overwinter, or enter diapause, within the galls. Generally, diapause is used as a means of surviving adverse environmental conditions, like extreme heat or cold (Wellso 1991). The following spring, insects emerge as adults to reproduce.

Neither of these biocontrol agents is without problems. *M. servulus* is a slow disperser – it doesn't increase its range very quickly. *A. cyclops* is capable of spreading faster than the weevil biocontrol agent used to control it spreads. In addition, the weevil doesn't disperse very far. Hence, if *A. cyclops* densities are reduced because of biocontrol, then the distance between trees may exceed the maximum dispersal distance of the weevil (Adair et. al. 2000). Biological control agents belonging to the family Cecidomyiidae, such as *D. dielsii*, are routinely parasitized by hymenopteran parasitoids, which sometimes reduce the efficacy of the insects as biological control agents (Adair et. al. 2000; Harris & Shorthouse 1996; Wehling & Piper 1988). *Dasineura dielsii* may not be an exception. Studies have shown that 40% of biocontrol agents that are established in South Africa are parasitized by native parasitoids (Hill & Hulley 1993).

The aim of this study was to examine *D. dielsii* to determine whether levels of parasitism by native parasitoids are high enough to curtail its effectiveness as a biological control agent in South Africa. As part of this study, patterns of emergence after overwintering were examined. The "triggers" that cause the onset and subsequent break of diapause in *D. dielsii* are, at present, unknown.

In this paper, the extent of parasitism on *D. dielsii* will be examined and the following key questions will be addressed 1) Are dormant *D. dielsii* midges subject to parasitism by native parasitoids? and 2) If so, what is the level/extent of the parasitism?

In addition to this, patterns of emergence will be studied in an attempt to address the key questions 1) What causes overwintering or dormant insects to emerge in spring? 2) Is this stimulus seasonal e.g. temperature? and 3) Does this allow *A. cyclops* to set seed while *D. dielsii* remains dormant?

METHODS

Study area

This study was performed on midges forming galls on a mature specimen of *Acacia cyclops* A. Cunn. ex G. Don. The tree was situated on the lower eastern slopes of Table Mountain, on the University of Cape Town's upper campus (33° 57.19S, 018° 27.88E). This area receives winter rainfall with an average of 99.82mm monthly. Temperatures range from an average of 16° C during the winter months, to an average of 24° C during the summer months, although extremes are noted. The soil is sandstone derived rocky soil, which is similar to the soil of its land of origin (Milton 1980).

Host plant

A. cyclops is a shrubby evergreen tree with flattened, linear leaves. Along the coast, it usually takes the form of a dense shrub, but further inland it can form a tree of up to 6m in height (Impson et. al. 2004). Bright yellow flowers are produced seasonally. Peak flower production occurs between October and February, although some flowering may occur as early as August. Non-seasonal flowering has also been noted during the winter months. Once the flowers are pollinated, characteristic seedpods begin to form. As they mature, the pods change from green to brown and curl up, eventually splitting and releasing black seeds (Van Wyk & Van Wyk 1997). Most of these seeds fall to the ground where they can lie dormant for several years. This allows a large seed bank to accumulate in the soil. Some of the seeds are dispersed over a wide area by birds (Glyphis et. al. 1981). Hence, when one adult tree is removed, hundreds of juveniles spring up to replace it. This is a problem for the control of this plant.

Patterns of parasitism

Dasineura dielsii insects cause the inflorescences of *A. cyclops* to form clusters of galls instead of seedpods. These gall clusters are initially green but turn brown with age. Only developed gall clusters (i.e. those having turned at least 90% brown) were sampled. Most midges had emerged as adults from the galls by the time the gall clusters had reached this stage. But, some larvae enter a state of arrested development and overwinter within the ripe galls, producing adults that emerge the following spring. Any insects occupying the ripe, brown gall clusters at the time of the study were assumed to have entered a state of dormancy.

Collection of gall clusters took place from late March to early May, spanning mostly autumn and early winter. On each sampling occasion, thirty randomly chosen gall clusters were collected, placed in a sealed bag and frozen. Freezing killed any insects, including midges and parasitoids inside the clusters, and created a "snapshot" of what was occurring inside the galls at the time of collection. Thirty green gall clusters that were expected to turn brown over the course of the following two weeks were marked and left to develop on the tree. All remaining brown gall clusters that were not collected were removed from the branch, so that only gall clusters that had turned brown during the preceding two weeks were collected on any sampling occasion. The gall clusters that were marked for collection were expected to turn brown over the two weeks preceding the collection date. However, not all marked gall clusters developed as expected. Hence, several additional brown, but unmarked, gall clusters were collected on each sampling occasion.

In addition to the 30 initial gall clusters sampled at the beginning of the study period, 30 of last seasons gall clusters were collected. Gall clusters remain on the tree after all of the *D. dielsii* insects have matured and emerged. Gall clusters from the previous season can be identified by their greyish colour; as opposed to the brown of this seasons gall clusters. Identification of the previous seasons gall clusters is also made based on their positioning on older branches with last season's growth.

Once all five collections had been made and frozen, randomly chosen sub samples were set aside for dissection. Sub samples varied in size from 5 to 20 gall clusters.

The gall clusters were dismantled and the individual galls were dissected to determine occupancy. Occupancy was divided between parasitoids and non-parasitized *D.dielsii* larvae. Initial findings showed that gall clusters in the final sample had the highest occupancy overall. Hence, an additional sub sample (consisting of five clusters) was made. These gall clusters were dismantled and the individual galls were dissected as done previously, to determine occupancy for individual galls as well as for the whole gall cluster.

Patterns of emergence

It is unknown at present what stimuli cause the overwintering insects to proceed with development and emerge as adults in the new season. As they enter their state of dormancy in autumn and winter and only emerge in early spring and summer, it is thought that an environmental factor could play a substantial role. This is because dormancy commences when daylength and temperature decrease, while adult emergence recommences when daylength and temperature increase. Changes in temperature and daylength have been shown to determine emergence in other Cecidomyiidae insects and my study focused on the influence of these two parameters on *D. dielsii*.

Temperature treatments.

In order to study the patterns in emergence, an additional collection of gall clusters was made in late May, to ensure live insects were present. Brown gall clusters were separated into individual galls, which were placed individually in the wells of 16 microtitre trays. A total of 1536 individual galls were retained, divided into four sets of four trays each. Each tray was covered with a lid to prevent any emerging insects from escaping.

A group of four trays was designated as a control group and was placed at ambient temperature, in the shade of the tree from which the galls were collected. A second group of four trays was placed in a temperature-controlled fridge at 15°C. A third group was placed in an incubator at 21°C, and a fourth group was placed at 27°C in an environmental room. 21°C has been shown to be the optimal temperature for the emergence of other insect species in the Cecidomyiidae (Wellso 1991). The other two

temperatures were chosen because they represent below optimal conditions (15°C) and above optimal conditions (27°C). The trays were retained under these constant treatment temperatures for 3 weeks. Galls were checked daily for emergences, which were noted.

After three weeks, some of the trays were moved to different temperature treatments. This was an attempt to simulate different temperature changes that might occur naturally e.g. a gradual increase in temperature as the seasons change from winter to spring, or fluctuations in temperature, from a warm spell to cold snaps. Table 3 summarises the temperature treatments that the trays of galls were subjected to.

Table 3: Summary of temperature treatments that trays of individual galls were subjected to, to determine emergence patterns.

Initial temp.	**Temp. 2**	**Temp. 3**
15 °C	15 °C	15 °C
21 °C	21 °C	21 °C
27 °C	27 °C	27 °C
15 °C	21 °C	21 °C
15 °C	27 °C	27 °C
15 °C	4 °C	4 °C
21 °C	27 °C	27 °C
21 °C	15 °C	15 °C
27 °C	15 °C	15 °C
21 °C	27 °C	21 °C
15 °C	21 °C	27 °C
15 °C	4 °C	21 °C
21 °C	15 °C	21 °C

Day length treatment.

Temperature and day length treatments ran concurrently for the first two weeks of the investigation. Initially, within each temperature treatment of four trays, two trays were subjected to an alternating light cycle and two trays were subject to constant light. The trays subject to an alternating light cycle were exposed to 16 hours of light to simulate day lengths of summer.

Statistical treatment

All statistical analyses were done using Statsoft® Statistica version 6.1. ANOVA tests and regression analyses were performed on the data.

RESULTS

Patterns of parasitism

A small percentage (1.4%) of galls from the previous year was still occupied at the beginning of the study period. Over the period between harvest one and harvest four, there was an increase in the mean percentage of galls occupied by both parasitoids and *D. dielsii* (Fig.1). This indicates that more insects were entering a dormant state as the seasons progressed from autumn to early winter. Some insects remained in a dormant state from the previous year, in effect remaining dormant over both winter and summer.

Galls were occupied by both parasitoids and *D. dielsii*. Most of the galls had parasitoids and the percentage increased over time (Fig. 2). The percentage of galls occupied by parasitoids ranged from 6.8% in harvest one to 18.5% in harvest four. At the same time, the percentage of galls occupied by non-parasitized *D. dielsii* remained low, ranging between 0.8% and 4.5% for all harvests. This showed that as the number of *D. dielsii* entering a dormant state over time increased, more were being parasitized.

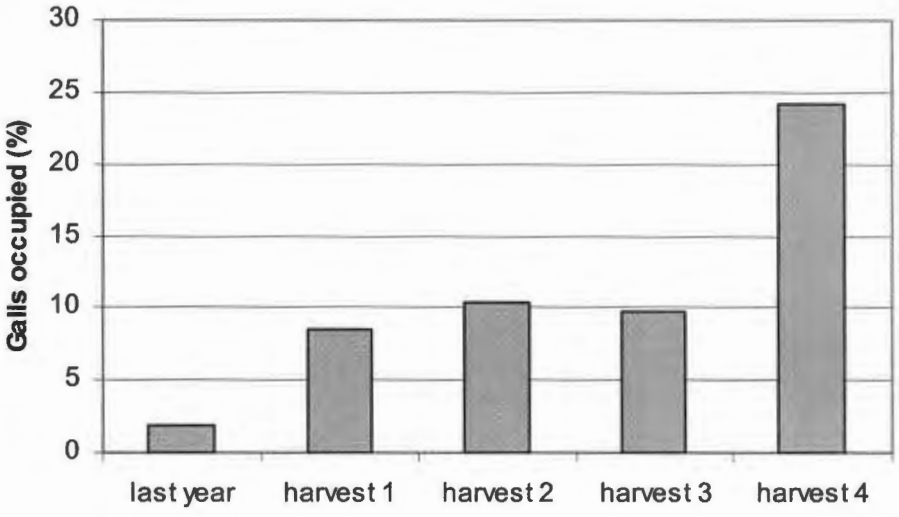

Fig. 1: Mean percentages of galls occupied by both parasitoids and non-parasitized *D. dielsii* per gall cluster, for each harvest.

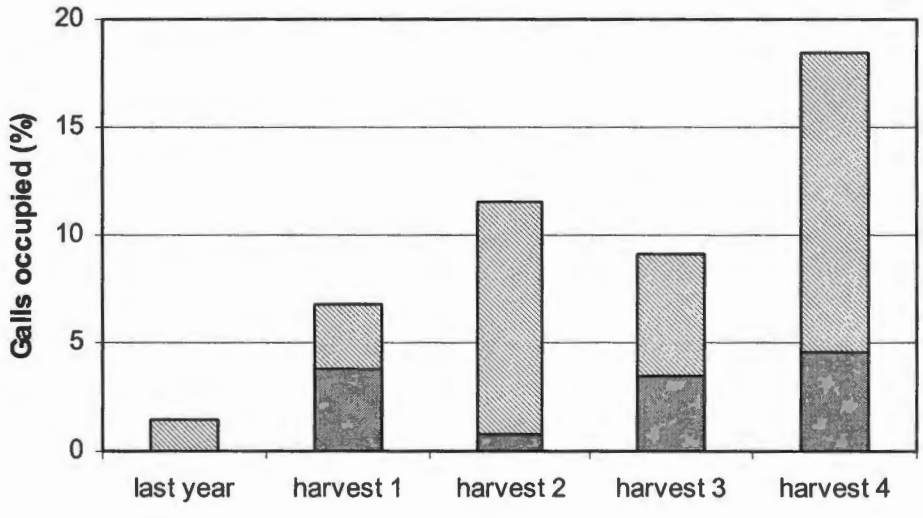

Fig. 2: Percentage of galls occupied by parasitoids (striped bars) as opposed to non-parasitized *D. dielsii* (solid bars) alone.

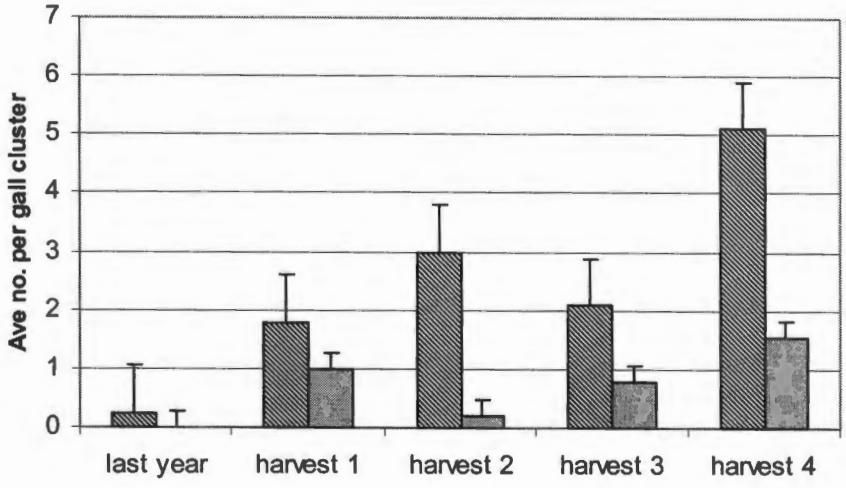

Fig. 3: Mean number of parasitoids (striped bars) and *D. dielsii* (solid bars) per gall cluster.

This pattern of increasing parasitism is also reflected by the average number of parasitoids and non-parasitized *D. dielsii* larvae per gall cluster (Fig. 3). The average number of parasitoids per gall cluster increased over time, while the number of non-parasitized *D. dielsii* larvae per gall cluster remained almost constant. However, there was no significant difference between the number of parasitoids per cluster among harvests ($dF = 4$, $F = 2.33$, $p = 0.07248$) nor between the number of *D. dielsii* per cluster among harvests ($dF = 4$, $F = 2.49$, $p = 0.0588$).

Overall, as the total number of occupants (*D. dielsii* and parasitoids) per gall cluster increased, so did the percentage of the gall clusters occupied by parasitoids (Fig. 4). There was a significant correlation between these two parameters ($p = 0.003$). The mean number of occupants per gall cluster explained 96.4% of the variance observed in the percentage of the gall clusters occupied by parasitoids. This result supports the previous results in showing that as more *D. dielsii* enter dormancy, more hosts become available and a greater proportion of *D. dielsii* larvae were parasitized.

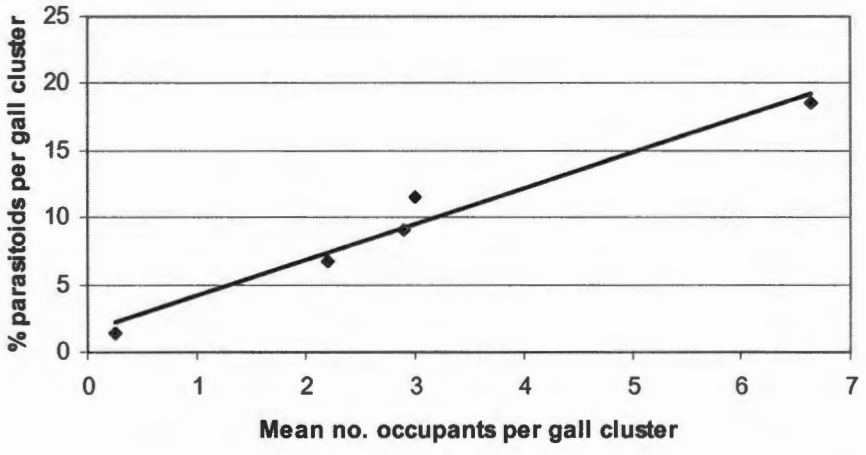

Fig. 4: Relationship between the mean number of occupants (*D. dielsii* and parasitoids) per gall cluster per harvest and the percentage of parasitoids per gall cluster per harvest ($r^2 = 0.964$, $p = 0.003$, $n = 5$).

There were positive relationships between cluster weight and the number of galls per cluster for all harvests (Fig. 5). However, none of the harvests showed significant results. The weight of the gall clusters explained 33% of the variance found in the number of galls per cluster in harvest 1, 64% in harvest two, 71% in harvest four and only 25% of the variance in the harvest of the previous season's clusters. The average size of each gall declines with overall gall cluster mass. Hence, as gall clusters get bigger, they consist of more galls, not that individual galls increase in size.

In addition to the number of galls per gall cluster increasing with cluster weight, the total number of chambers found in the gall clusters also increased with cluster weight (Fig. 6). There were positive relationships between cluster weight and the number of chambers per cluster for all harvests. However, only the relationship for harvest 1 was significant ($p = 0.006$). The weight of the gall clusters explained 94% of the variance found in the number of chambers per cluster in harvest 1, 48.2% in harvest two, 64.7% in harvest four and 86.9% of the variance in the harvest of the previous season's clusters. This, combined with the previous result, shows that as gall clusters get bigger, they consist of more individual galls, which contain more chambers. This means that bigger galls harbour more *D. dielsii* and, therefore, can harbour more parasitoids.

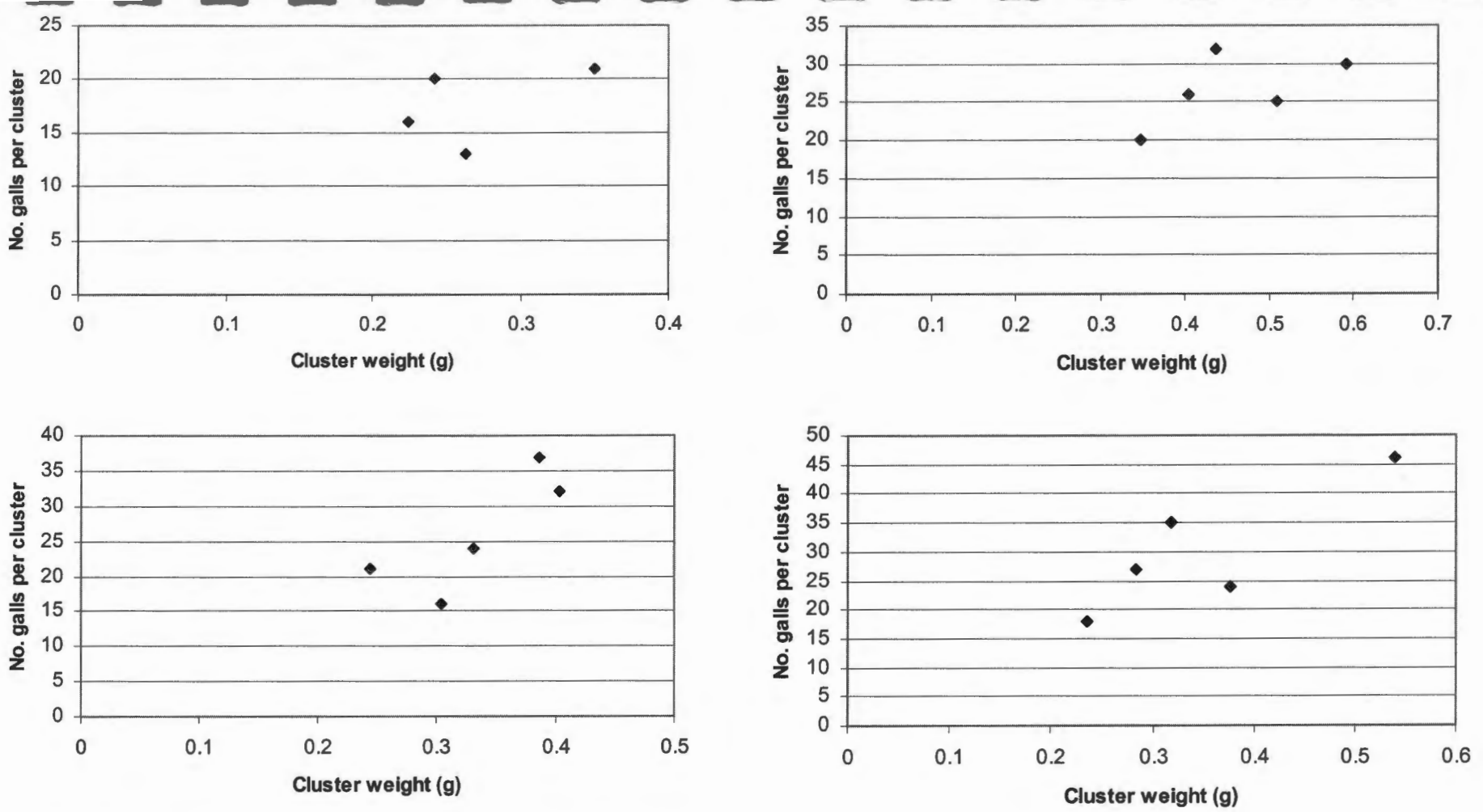

Fig. 5: Four graphs representing the relationship between gall cluster weight and number of galls per gall cluster for last year's harvest, top left ($r^2 = 0.25$, $p = 0.498$, $n = 4$); harvest 1, top right ($r^2 = 0.33$, $p = 0.312$, $n = 5$); harvest 2, bottom left ($r^2 = 0.64$, $p = 0.102$, $n = 5$) and harvest 4, bottom right ($r^2 = 0.71$, $p = 0.073$, $n = 5$).

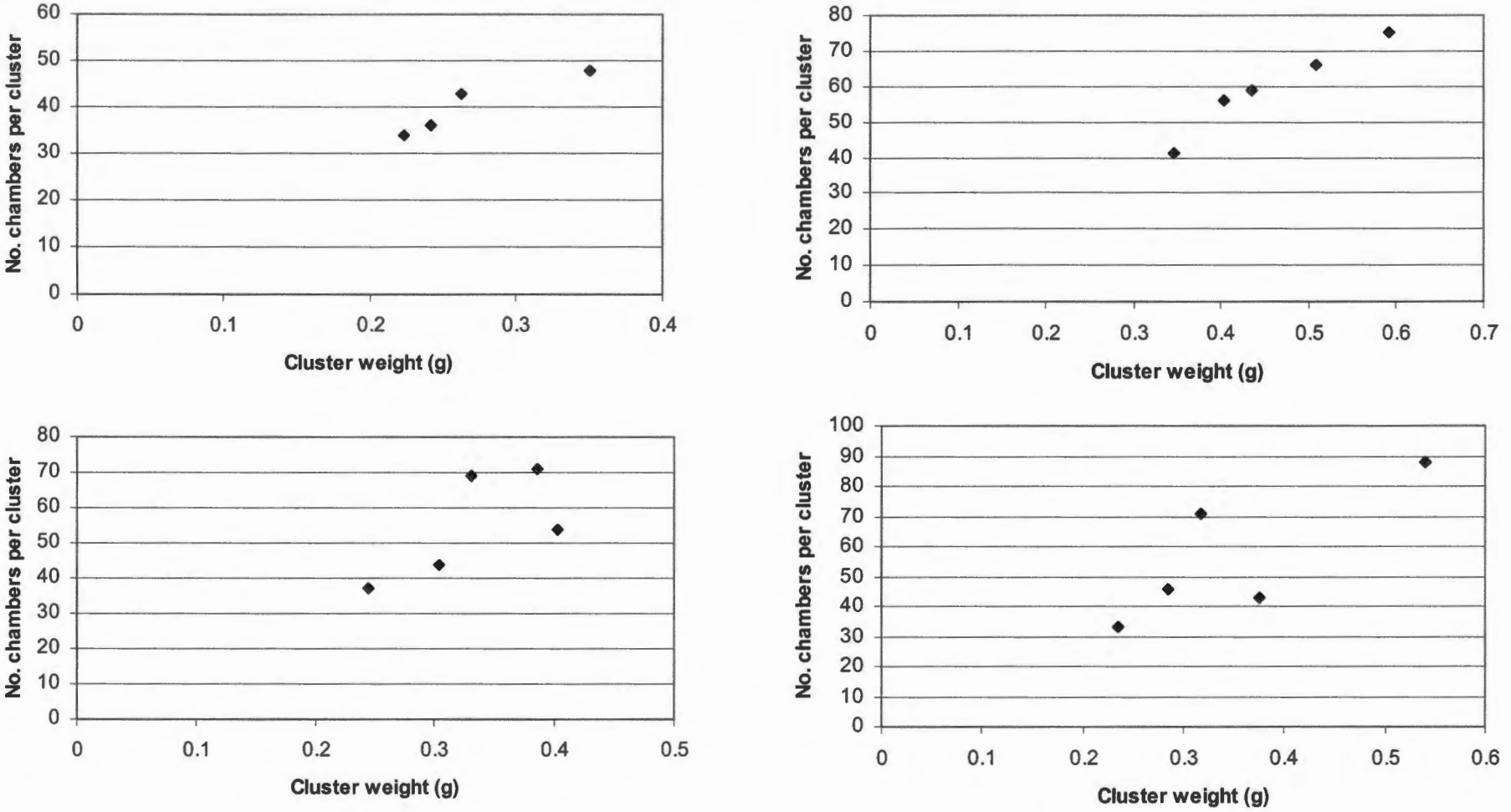

Fig. 6: Four graphs representing the relationship between gall cluster weight and total number of chambers per gall cluster for last year's harvest, top left ($r^2 = 0.8699$, p =0.067, n = 4); harvest 1, top right ($r^2 = 0.9409$, p = 0.006, n = 5); harvest 2, bottom left ($r^2 = 0.4823$, p = 0.193, n = 5) and harvest 4, bottom right ($r^2 = 0.647$, p = 0.101, n = 5).

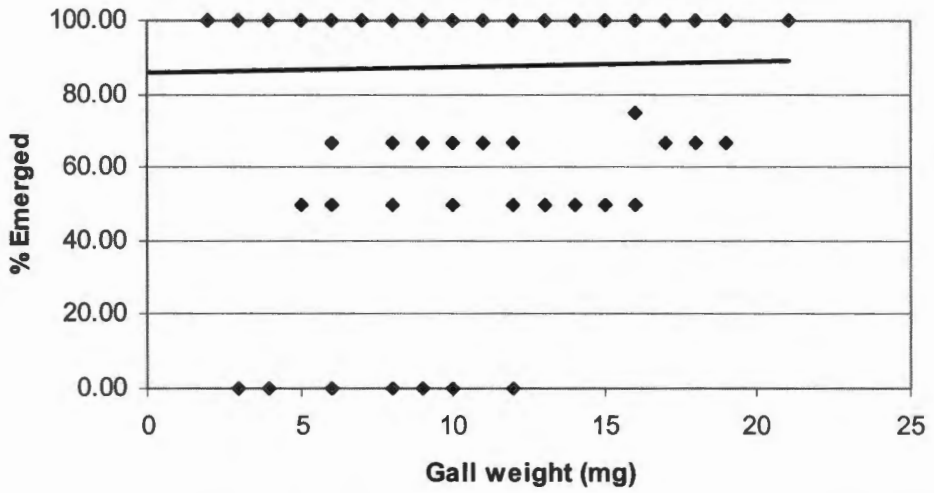

Fig. 7: The relationship between gall weight and the percentage of insects emerged (r^2 = 0.0007, p = 0.784, n = 150).

There was no relationship between gall weight and the percentage of insects emerged (Fig. 7). Virtually none of the variance in the percentage of insects emerged is explained by gall weight (r^2 = 0.0007).

Patterns of emergence

Temperature treatments.

After three weeks at constant temperature treatments of 15 °C, 21 °C and 27 °C, no midges emerged in either the 15 °C or the 27 °C treatments. Only one midge and one parasitoid emerged in the 21°C treatment. No midges emerged from the control group at ambient temperature. After further temperature manipulation, no more midges or parasitoids emerged in any of the treatments. This included the trays for which temperatures were increased, decreased and fluctuated.

Daylength treatment.

Two trays from each temperature treatment were subjected to a 8:16 light/dark cycle. The other two trays from each temperature treatment were subjected to constant light. After two weeks, there were no emergences in either light treatment.

DISCUSSION

This study examined the extent of parasitism on *D. dielsii* and addressed two questions: 1) At what level are dormant *D. dielsii* subject to parasitism by native parasitoids and is parasitism reducing the effectiveness of biological control of *D. dielsii* on *A. cyclops*? and 2) What induces overwintering or dormant *D. dielsii* to emerge in spring? Is this stimulus seasonal e.g. temperature or photoperiod? These questions were asked in an attempt to determine whether the biological control exerted by *D. dielsii* on *A. cyclops* is affected by parasitism or insect dormancy. Can *A. cyclops* set seed while *D. dielsii* remains dormant and do enough non-parasitized *D. dielsii* survive to adulthood to maintain an effective population?

The "triggers" that cause the onset and subsequent break of diapause in *D. dielsii* are, at present, unknown. This study attempted to identify these triggers by using temperature and light manipulations. However, these were unsuccessful in breaking dormancy and causing emergences. It must be concluded from this, that emergences are independent of the daylength and temperatures used here. Previous studies have also found that daylength does not trigger emergences (Wellso 1991). Even though a wide variety of temperature treatments were used, temperature, or possibly temperature combined with another unknown factor, must not be excluded as the trigger of emergence.

It is possible that temperature alone is not enough to trigger emergence, and further study on this topic could examine the combined effects of temperature, daylength and humidity. Perhaps the experimental period of this study was too short to allow for emergence of adult *D. dielsii*, and larvae must be subjected to "trigger" temperatures for longer than they were subjected to in the course of this study. It must be recalled that *D. dielsii* enters arrested development when dormant. Hence, the larvae must be subjected to the trigger for long enough to begin further development, as well as for the time taken for final development. Only after this length of time will they emerge as adults. Future studies of *D. dielsii* should determine the trigger that causes them to emerge from dormancy and the length of time required before emergence. This would allow study on a much larger scale than is currently possible.

Overwintering is an important method of surviving adverse seasonal conditions for many insects. Overwintering and diapause are also mechanisms of ensuring genetic variation within the population (Corley et. al. 2004). If the surviving population that emerges in spring is small, it could lead to inbreeding depression and other negative effects associated with very small populations. This could eventually lead to a reduction in the efficacy of *D. dielsii* as a biocontrol agent. Since about 5% of all *D. dielsii* enter dormancy and survive parasitism, there is a very slight chance of a bottleneck occurring. If the surviving population remains at this level each year, there is little worry that *D. dielsii* will experience any threat from inbreeding depression.
As the seasons progressed from autumn to early winter, more galls were occupied by both parasitoids and non-parasitized *D. dielsii* combined. Over the study period, there was an increase in the number of parasitoids per gall cluster. The number of dormant *D. dielsii* remained low, but almost stable, over the same time. Hence, as the season progressed, more *D. dielsii* entered a dormant state. However, this meant that there were more available hosts for parasitoids, and more *D. dielsii* became parasitized. There was a positive relationship between the numbers of *D. dielsii* and the number of parasitoids per gall cluster. Similar positive insect/parasitoid relationships have been established for other insects of the order Cecidomyiidae (Baxendall et. al. 1983; Gahukar 1984; Kausalya et. al 1997), confirming that this is a common trend.

The highest level of parasitism was reached at the end of the study period, with nearly 20% of galls being occupied by parasitoids. This is in comparison to the nearly 5% occupied by non-parasitized *D. dielsii*. If none of the dormant *D. dielsii* were parasitized, nearly 25% of the population would have entered dormancy and survived to the following season. Instead, a midge population a fifth of the size would survive to spring. Even though the surviving population is small, there are some surviving *D. dielsii* to emerge in spring and reproduce.

It has been suggested that parasitism levels of 30 – 40% of the host population will severely limit its biocontrol potential. At levels higher than 40%, the biocontrol agent will be ineffective (Harris & Shorthouse 1996). At 20%, parasitism of *D. dielsii* is high, but not high enough to render it ineffective as a biocontrol agent. The trend in increasing levels of parasitism as the season progresses to winter was also found in other insects of the order Cecidomyiidae (Thompson et. al 1998; Wehling & Piper

1988). *Cystiphora schmidti* (Diptera: Cecidomyiidae), an insect used for biocontrol of Rush Skeletonweed in America, was found to have 10% parasitism in summer, which rose to 65% in autumn and 95% by winter (Wehling & Piper 1988). This rendered *C. schmidti* almost totally ineffective as a biocontrol agent. Although *D. dielsii* is not subject to such extensive parasitism, this indicates what could happen if parasitism increases, i.e. there may be a reduction in the efficacy of *D. dielsii* as a biocontrol agent.

This study also found that heavier and bigger gall clusters contained more individual galls, consisting of more chambers. Thus, bigger gall clusters have the potential to harbour more dormant *D. dielsii* larvae and, therefore, host more parasitoids. However, there was no relationship between individual gall weight and the percentage of *D. dielsii* that emerged. Thus, heavier gall clusters may contain more galls, more chambers and more *D. dielsii.* However, the weight of the individual galls cannot be used to determine whether the occupant midges enter dormancy or are parasitized. This is contrast to other studies, which found a relationship between survival (emergence) of a midge, *Rhopalomyia californica* (Diptera:Cecidomyiidae), and gall size (Ehler & Kinsey 1990).

Since larger gall clusters harbour more midges and these, in turn, can host more parasitoids, it seems that there is no advantage to be gained by *D. dielsii* by laying more eggs on the same inflorescence. In fact there appears to be an advantage in laying fewer eggs on more inflorescences. In this way, there is a greater chance for survival of offspring to the following season.

The efficacy of *D. dielsii* as a biocontrol agent may be increased if there is an advantage to be had by dispersing eggs over several inflorescences. The offspring of those midges with tendencies to spread their eggs over a few inflorescences are more likely to survive parasitism and emerge as adults the following season. This may result in more midges with the same tendency. If this happens, *D. dielsii* will be capable of destroying more *A. cyclops* inflorescences with the same number of eggs. This could result in a much better rate of control than is currently achieved.

However, during the study period, when all *D. dielsii* were dormant, some aseasonal flowering of *A. cyclops* was observed. This allowed the tree to set seed while the two biocontrol agents used (*Melanterius servulus and D. dielsii*) were both in a dormant state. Hence, even if *D. dielsii* does destroy 100% of *A. cyclops* inflorescences while it is active, there may be some that it cannot destroy. This could be a problem if total control of *A. cyclops* is to be achieved.

So, *D. dielsii* is subject to parasitism by native parasitoids. The level of parasitism is high but I do not believe that it is too limiting to the biocontrol efficacy of the midges. If parasitism levels increase any further, there may be a reduction in the efficacy of *D. dielsii* as a biocontrol agent.

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