Resource partitioning in buds and insect induced galls in the biocontrol of *Acacia pycnantha*.

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Abstract

Two *Trichilogaster* sp. (gall-forming wasps) have been introduced against *Acacia longifolia* and *Acacia pycnantha* in the Cape. *Trichilogaster acaciaelongifoliae* has reduced both seed production (by between 85 and 100% in the Cape) and vegetative growth in *Acacia longifolia*. The aim of this project was to determine whether the galls and buds of *Acacia pycnantha* have significantly different energy values, and to determine what affect this has on the plant.

There was no significant difference between gall and bud energy values (p=0.029, df=7, alpha=0.05) but gall energy values were, on average, 9.35% lower than those of bud material. The mass of galls increased steadily through out the year while bud material remains at the same weight. The resource loss to the plant caused by the galls is dependant on the size and number of galls present on the tree. Even with similar energy values per unit dry mass there were many more grams of gall material on a tree than bud material and thus they would demand more resources to be invested in the gall material. An important factor concerning the effect galling has on *A. pycnantha* is the time during the trees reproductive cycle that galling occurs. It has been shown that when galling coincides with the season when the most energy is channelled into reproduction that the damage to the tree is heaviest.

Introduction

Alien plants, which invade native vegetation, are known as environmental weeds (Adair, 1993). Often these weeds are intentionally introduced for economic or ornamental reasons and only become problems when they begin to dominate the native vegetation. There are several situations that predispose a taxon to becoming a weed; a broad ecological range, lack of natural enemies (often natural enemies are not present in the country where the plant is introduced), being difficult to control with conventional methods, long-distance dispersal and high biomass production (Adair, 1993).

Biological control can offer a solution to problems caused by alien invasive plants. This generally involves the use of natural enemies of the plants to stop their spread and reduce their abundance (Andres & Goeden, 1969). These natural enemies also have to be host-specific so that there is no danger of them attacking non-target plants. Long-term control of weeds is most often achieved through reduction of the weed's competitive ability and reduction of energy reserves as well preventing or reducing reproductive ability (Andres & Goeden, 1969).

Although gall-forming insects have not been used extensively in biological control, they have played a prominent role in some programmes (Myers & Harris, 1980; Brown & McGavin, 1982). Gall producing biocontrol agents can be very damaging to their hosts because they use up the energy reserves of plants in producing the galls as well as reducing the reproductive ability, either by direct means such as galling of reproductive structures or by limiting the amount of resources the plant has available for reproduction

(Andres & Goeden, 1969). Galls are abnormal vegetative structures that are induced by insects and other organisms (Porter Felt, 1940). Another advantage is that gall-forming insects tend to be highly host specific.

Biological control offers benefits over the other control methods because agents are host specific, can self disperse, are persistent and are self sustaining so that they are cost effective in the long term (Adair, 1993). A number of Australian *Acacia* species have become weeds in South Africa and biological control is playing an important part in an effort to control them (Dennil *et al*, 1999). *Acacia cyclops* (rooikrans), *A. dealbata* (silver wattle), *A. decurrens* (Green Wattle), *A. longifolia* (long-leaved wattle), *A.mearnsii* (black wattle), *A. melanoxylon* (Australian blackwood), *A. pycnantha* (golden wattle) and *A. saligna* (Port Jackson willow) are some of the species which have become weeds in South Africa. Many of these plants are economically useful but they have invaded a number of vegetation types, such as cultivated forests (*A. longifolia* and *A. pycnantha*) coastal habitats (*A. cyclops* and *A. saligna*), lowland fynbos (*A. cyclops*, *A. longifolia*, *A. pycnantha* and *A. saligna*), mountain fynbos *(A. longifolia*) and natural forests (*A. dealbata*, *A. mearnsii* and *A. melanoxylon)* (Dennill *et al*, 1999).

One of the main problems that Australian *Acacias* cause in South Africa is a reduction in streamflow and soil water retention due to the high evapotranspiration rates of the plants (Dennill *et al*, 1999; Neser, 1985). Due to the economic importance of these trees biocontrol is limited in its options of the kinds of agents that can be used. Generally, only agents, which reduce the flower and seed set, are used, so that the desirable economic attributes of the plants are not affected (Dennill *et al*, 1999). These types of agents are particularly effective when used against acacias because they cause a reduction in the number of seeds produced and hence restrict the capacity for huge seed banks to accumulate in the soil (sometimes as many as 45 800 seeds per meter squared) (Milton, 1980; Dennill *et al*, 1999). To date biological control agents have been released against six of the invasive Australian Acacia species in South Africa (Table 1).

Table 1: - Acacia Species and the biological control agents used against them (Dennill *et al*, 1999).

Acacia species	**Biocontrol agents**
A. longifolia	*Trichilogaster acaciaelongifoliae* (bud-galling wasp) *Melanterius ventralis* (seed-feeding weevil)
A. melanoxylon	*Melanterius acaciae* (seed-feeding weevil)
A. cyclops	*Melanterius servulus* (seed-feeding weevil)
A. pycnantha	*Trichilogaster* sp. (bud-galling wasp)
A. dealbata	*Melanterius* sp. (seed-feeding weevil)
A. mearnsii	*Melanterius maculatus* (seed-feeding weevil)

Bud-galling wasps (*Trichilogaster* spp.) have been used against both *A. longifolia* and *A. pycnantha*. *Trichilogaster* spp. are pteromalid wasps that deform the buds of Australian Acacia species (Dennill, 1985; Dennill, 1988; Dennill *et al*, 1999; Neser, 1985). The larvae develop entirely within the galled buds of the plant. Females lay their eggs in the immature flower buds. The eggs hatch in spring after a period of dormancy as the flower buds start to increase in size. The wasp larvae grow and the flower bud distorts to form a swollen, hardened gall. The galls contain chambers where the larvae complete their development (Dennill *et al*, 1999; Dennill, 1987). *Trichilogaster acaciaelongifoliae* was the first biocontrol agent to be used against an alien acacia in South Africa.

It was released in 1981 in the Cape and has reduced seed production of *A. longifolia* by 85 to 100% (Dennill, 1985; Dennill, 1987; Dennill, 1988; Dennill *et al*, 1999; Neser, 1985; Kluge, 1992). *Trichilogaster acaciaelongifoliae* has also been released in Natal against *Acacia longifolia*, although it has not been as successful as in the Cape, probably due to climatic factors (Kluge, 1992)

Acacia pycnantha (Golden wattle) is a shrub or a small tree up to 8m. It has leathery, smooth leaves with a single prominent mid vein. In Australia it is abundant in Victoria and adjacent areas of South Australia and New South Wales. It is the floral symbol of Australia and is economically useful for it's very high tannin content (Dennill & Gordon, 1991; www.lycaeum.org/drugs/Tryptamines/DMT/ acacia/pycan). In South Africa *Acacia pycnantha* grows on shales and alluvium and especially moist sites in the Cape (Milton, 1980) (Fig. 1).

In it's natural habitat at least 75% of *Acacia pycnantha* seed is buried or destroyed by ants (Milton, 1980). Fungi, birds, wasps, bugs and lepidopteran larvae damage even more seed (Milton, 1980). In the Cape less than a quarter of the seed produced is destroyed by naturally occurrances. This means that there are many more viable seeds in the seed bank in South Africa than in Australia (Milton, 1980). This contributes to the abundance of *Acacia pycnantha* in the Cape and the difficulty of controlling the problem and highlights the necessity for the introduction of a biocontrol agent that targets seed production.

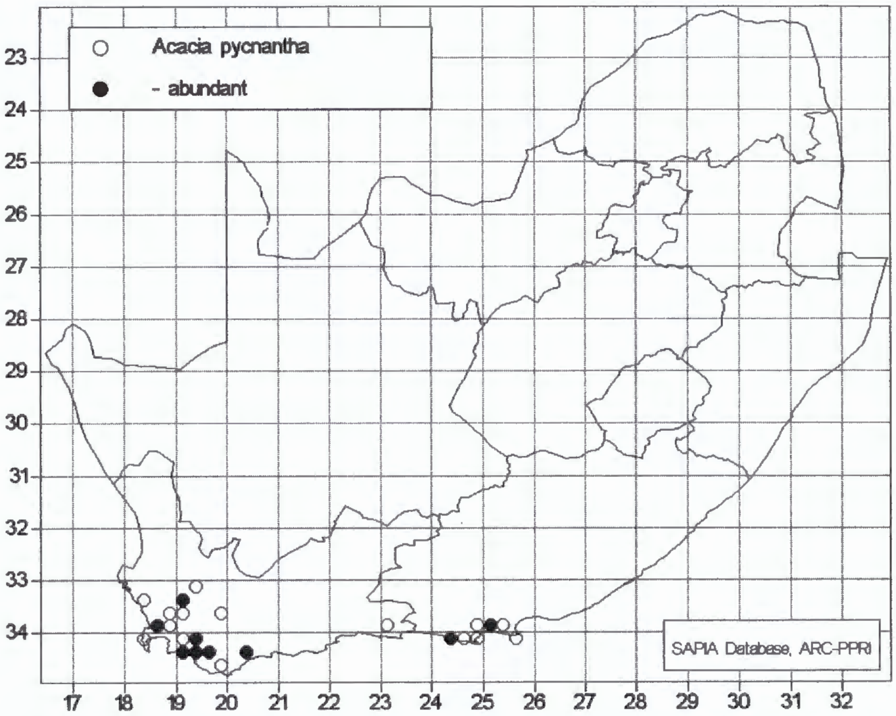

Figure 1:- Map showing areas where *Acacia pycnantha* grows in South Africa and where it is most abundant (black dots).

An undescribed *Trichilogaster* species was introduced to combat *A. pycnantha* in the Western Cape (Dennill & Gordon, 1991). Host specificity testing was done using wasps imported from Australia in January 1985 and January 1987 (Dennill& Gordon, 1991; Dennill *et al*, 1999). These wasps failed to establish when they were sleeved on *A. pycnantha* in Stellenbosch and it was assumed that they were the incorrect strain of wasp and were not compatible with the South African strain of *A. pycnantha*. Another strain of *Trichilogaster* sp. was imported in January 1992 and after a time lag the wasps started to establish themselves on *Acacia pycnantha* trees. In laboratory studies *Trichilogaster* sp. was found to gall plants but not to as great an extensively as *T. acaciaelongifoliae* does *A. longifolia* (Dennill & Gordon, 1991). Since 1996 there has been a substantial increase in the wasp populations and manual redistribution of the insects was started in 1997.

Trichilogaster sp. has now been established at 16 sites in the South-western Cape (Dennill *et al,* 1999).

The aims of this project were to determine the amount of resources that galled plants were being deprived of by the activities of the insects as part of an effort to assess the indirect effects of galling. The study contributes to an overall assessment of the effectiveness of the insects as biological control agents.

Methods

All material used in this project was collected from Bouchard Findlayson Wine Farm (Hemel and Arde Valley) by John Hoffmann during the course of 1999. Five samples were taken at approximately monthly intervals, between July and December. Branches from *A. pycnantha* trees were stored in black plastic bags in a freezer at –18 °C until processing.

From a previous study it was determined at which time of year buds, galls and pods were present on the trees (Fig. 1) (Hoffmann, unpublished data). Buds were collected from July, August, September and October and galls were collected from all the samples.

100 buds and 5 galls were removed from the branches of each sample. The buds and galls were dried in an oven at 80°C for 2 days and then ground into a fine powder. A mortar and pestle were used to grind the buds and a grinding mill was used to grind the galls.

Energetic values for the bud and gall samples were then obtained using a bomb calorimeter. The minimum weight required for processing in the calorimeter was 0.5g. This meant that several the buds had to be combined in each sample due to their small individual mass. One sample of bud material (made up of 80-90 dried buds to get a mass of approximately 0.5g) and three samples of gall material (made up of one dried gall each) were run for each month. Before being placed in the bomb calorimeter the weight of each sample was recorded.

After energetic values had been obtained for the bud and gall samples an f-test was performed to check that the variances of the data were equal (Zar, 1999). Then a two-sample t-test assuming equal variances was performed to determine whether there was a significant difference in the mean energy values for buds and galls. Statistical analyses were done using Excel.

The maximum number of inflorescences, buds, pods and galls was recorded on each of 40, tagged branches in 1998, 1999 and between January and October 2000 at the site at Bouchard Findlayson Wine Farm (Hoffmann, unpublished data).

Samples were collected in October 2000 from a site in De Hoop Nature Reserve where the wasp population was fully established. 82 racemes were collected from branches with no galling and lots of pods. The inflorescences on these ungalled branches were classified according to how many pods they formed (from none to 8 pods). Six branches were collected from heavily galled trees and the number of galls and number of inflorescences was counted for each branch to determine the percentage of reproductive structures that form galls.

Results

The mass values for galls, buds, pods and flowers throughout the year were obtained (Hoffmann, unpublished data) (Fig. 2). The dry mass of galls increased steadily throughout the year from June to December 1999 and then remained constant until samples were discontinued in February 2000. The mass of pods also increased between October and January while the mass of the buds stayed constant through out the period that they are present (January- November). Flowers were present from August to November and they had a constant mass that is slightly higher than that of buds. Galls reach a peak weight of 907.6mg (average) in February; this is 152.02 times greater than the mass of buds in the same month (average of 5.97mg)

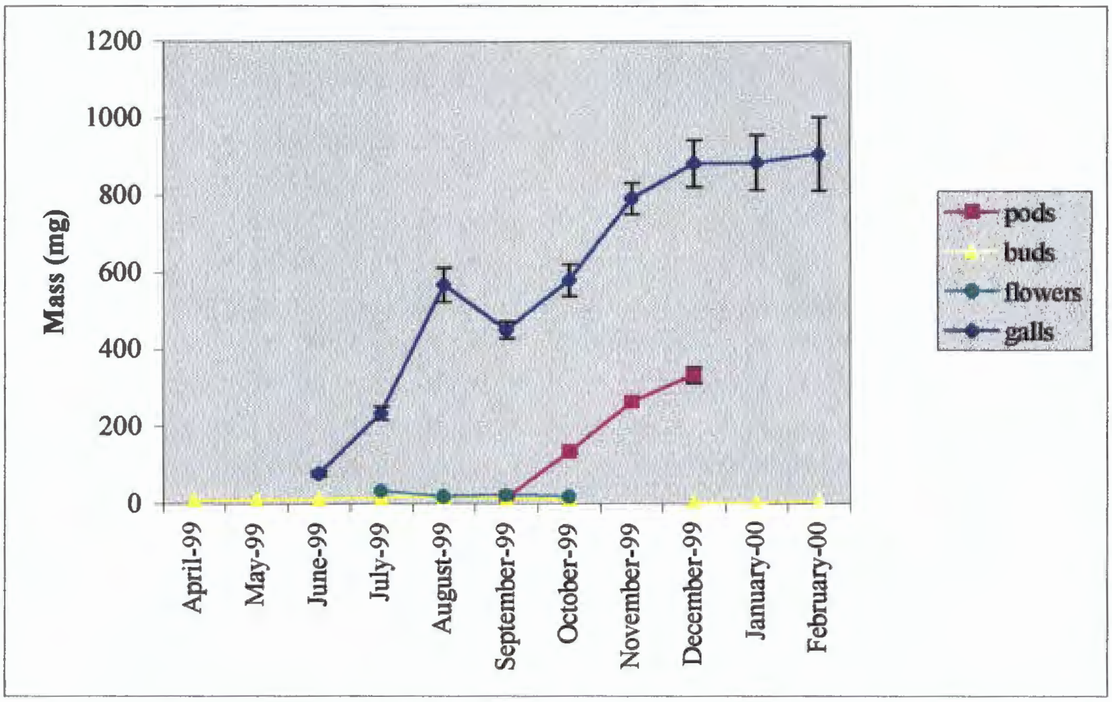

Figure 2: - Graph showing the mass values for buds, galls, pods and flowers of *Acacia pycnantha* in the months that they occur (Hoffmann, unpublished data).

The average energy values for buds and galls did not vary considerably from month to month. The energy values for galls were lower than those for buds for all the months sampled (Fig. 3). The highest energy value for bud tissue was found in the September sample and the lowest was found in the October sample. The highest energy value for gall tissue was found for the December sample and the energy value was at its lowest in the July sample. Gall energy values were, on average, 9.4% lower than bud energy values.

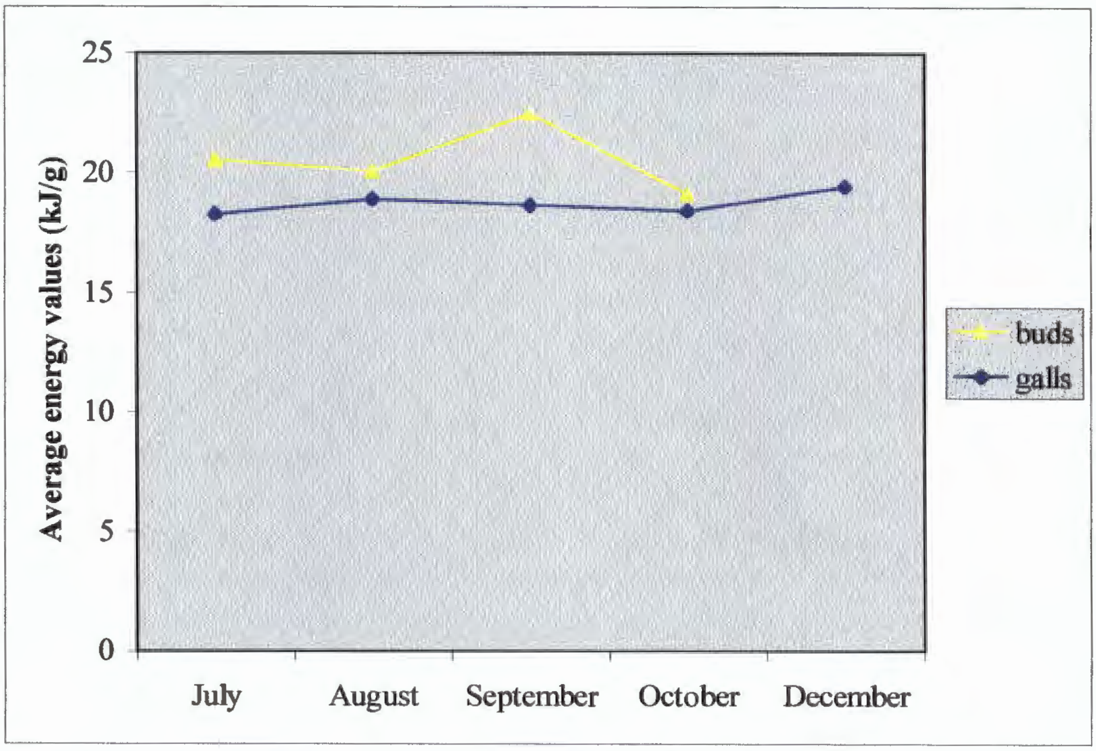

Figure 3: - Graph showing the average energy values for buds and gall material sampled.

Statistical tests to determine whether there was a significant difference between the energy values of galls and buds in *A. pycnantha* showed that the variances were equal and that there was no significant difference between the mean energy values of buds and galls (Table 2).

Table 2: - Table showing the results of the statistical tests performed on the data.

F test	f statistic	9.309
	P	0.028
	Confidence level	95%
t test	t statistic	2.741
	p (two tailed)	0.029
	Degrees of freedom	7
	Confidence level	95%

The figures for the maximum number of inflorescences, buds, pods and galls are shown in Table 3. The numbers of buds decreased from 1998 to 2000, as did the number of inflorescences. There are a similar number of pods and galls on the branches and very few buds survive from year to year.

Table 3: - maximum numbers of inflorescences, buds, pods and galls for 1998, 1999 and 2000 (January-October) (Hoffmann, unpublished data).

	1998	**1999**	**2000 (Jan.-Oct.)**
Raceme	479	321	235
Inflorescence	4163	2623	1074
Pods	82	130	-
Galls	103	106	102

The number of pods formed from inflorescences was counted using the samples collected from De Hoop Nature Reserve (Fig.4). 643 inflorescences were counted from the racemes collected. It was calculated that only 17.1% of inflorescences formed pods.

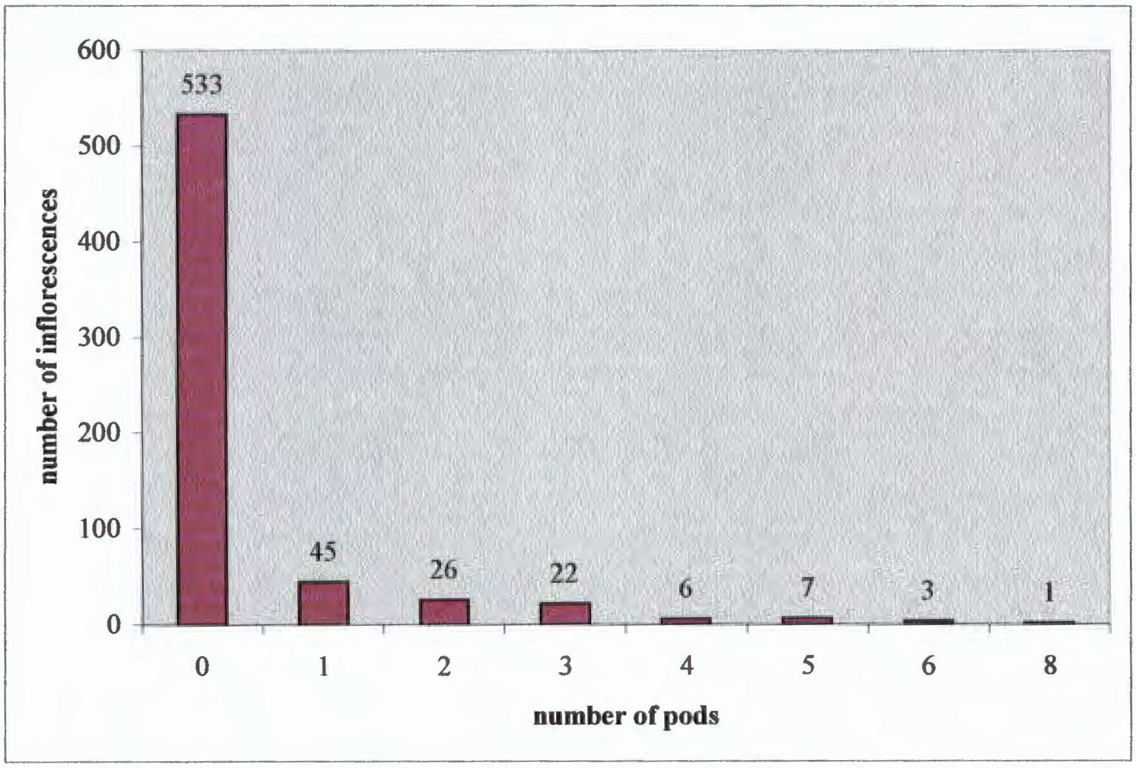

Figure 4: - Graph showing the categories for number of pods formed and the number of inflorescences that fall into each category.

The numbers of galls and buds on the heavily galled branches from De Hoop Nature reserve was counted and the total number of galls and buds was calculated (Fig.5). It was determined that 50.4% of buds form galls on heavily galled trees.

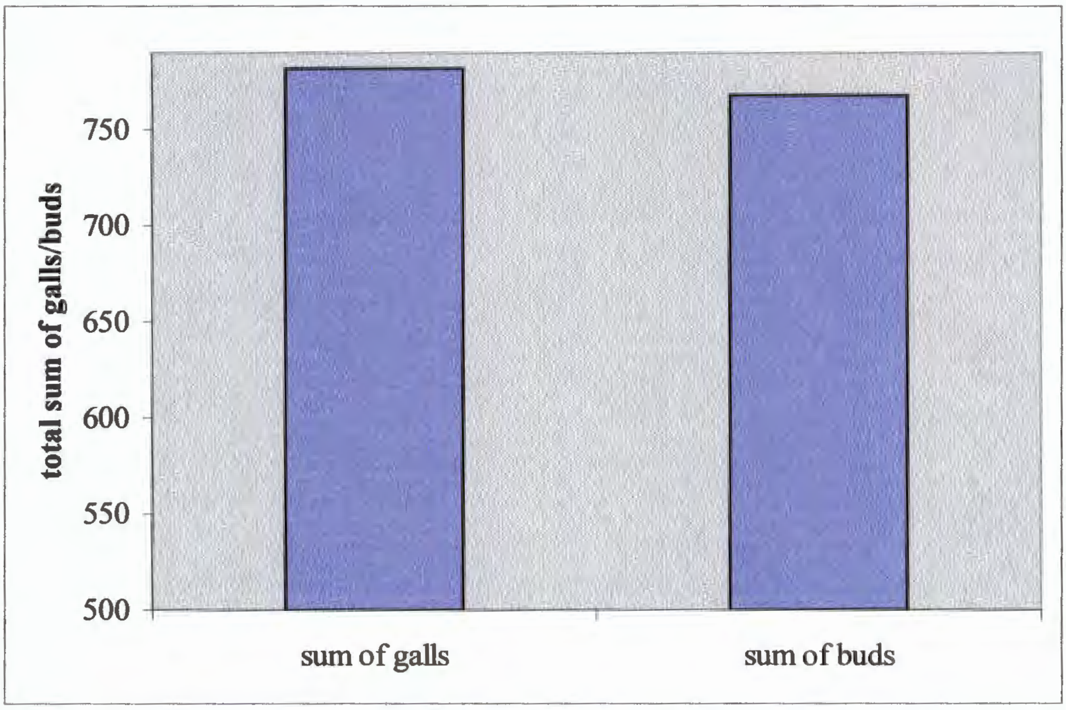

Figure5: - Graph showing the total number of buds and galls counted on 6 heavily galled branches of *Acacia pycnantha* from De Hoop Nature Reserve.

Discussion

The most important consequences of the galls formed by *Trichilogaster* sp. are the resource drain and the loss of seeds by the plant. If a wasp galls an inflorescence then no flowers and pods or seeds are produced from that inflorescence. The mass of each gall increases as the season progresses and increasingly more resources are invested in the galls. This could have an indirect draining effect by reducing the number of pods set by adjacent inflorescences because the plants have fewer resources to allocate to seed formation.

There was no significant difference between the mean energy values for buds and galls of *A. pycnantha* ($p=0.029$, $df=7$, $\alpha=0.05$). This would indicate that there is not an energy drain on the plant by the gall material. However a resource drain could be caused by the greater mass of galls found on a plant (compared to the mass of buds found on a plant), resulting in a forced increase in energy allocation to gall material by the plant. The extent of the resource drain eventually depends on the size and number of the galls present on the tree.

When one looks at the number of pods and galls produced from the buds on the *A. pycnantha* trees sampled it is evident that there is little difference between the number of galls and the number of pods produced. This indicates that the wasps are galling at least as many buds as there are producing pods. The area where the samples were taken does not have a fully established wasp population and this may account for the relatively low galling rate. The samples taken from De Hoop Nature Reserve come from an area where the wasps are fully established. 50.4% of the inflorescences on the heavily galled trees in De Hoop develop galls and only 17.1% of inflorescences on branches with no galls develop pods. The figures obtained from these samples are somewhat biased because the samples were intentionally taken from the branches with the heaviest galling, however they give an indication of the percentage galling that can be expected when the wasps are fully established at a site. The figures indicate that when the wasps are fully established they have a significant impact on the reproductive potential of the trees.

Dennill (1987) did similar research to this project looking at the relationship between *T. acaciaelongifoliae* and *A. longifolia*. He concluded that there was no significant difference between the energy values of galls and buds with galls having, on average, a 7% lower biomass diversion than that of buds. In our study we observed a similar trend with an average 9.4% difference between gall and bud energy values per gram dry mass. An increase in gall size was reported for *A. longifolia*, with a peak in September of 25 times the mass of unfertilised bud material (Dennill, 1987; Dennill, 1988). *A. pycnantha* shows a similar increase with a peak in February of 152.03 times the mass of bud material.

An important factor is that the growth of the galls on both *A. longifolia* and *A. pycnantha* spans the entire reproductive period of the tree. On *A. pycnantha*, galls begin developing before the flowers become mature (in June) and their mass increases at the same time as pods are produced. This means that energy used for gall growth is effectively "stolen" from the reproductive structures of the plant (Dennill, 1987; Dennill, 1988).

The importance of the time of gall development relative to the phenology of the host plant is illustrated by a study done on the effects of *Urophora affinis* and *U. quadrifasciata* (gall-forming flies) on the diffuse and spotted Knapweeds (Myers & Harris, 1980; Dennill, 1987). Both species of fly attack both species of plant but with differing results. Galled diffuse knapweed was found to have many fewer seed heads and was 71% lighter than ungalled plants at the same stage of development (Myers & Harris, 1980; Dennill, 1987). On the other hand, the spotted knapweed when exposed to the same amount of galling by both fly species had no significant difference in either weight or seed set compared to ungalled plants. The difference in phenology of these two plants was concluded to be the factor affecting these results. Spotted knapweed completes its growth and flower head development early relative to the larvae in the galls and so was less affected by the galls, however production of flower heads and growth in diffuse knapweed continues after the gall flies have begun to gall the plants. This means that the gall larvae divert resources, which the plant would have used for growth and flower production (Dennill, 1987).

Dennill (1987) observed that biomass diversion to gall production on *A. longifolia* was greater during the early part of the reproductive season when buds had just started to develop. This places an enhanced stress upon the plant. A similar effect is seen in *A. pycnantha* where the galls reach their highest mass at the time of year when new buds are being produced (December-February). This would place a large amount of stress on the plant and limits the number of new buds that can be produced with the resources available. In fact, it has been observed that during the period from 1998 to 2000 the trees produced fewer buds each year.

It has been shown that *T. acaciaelongifoliae* reduces seed production as well as vegetative growth of *A. longifolia* (Dennill, 1987; Neser, 1985). This illustrates the effects of gall production on plants even if the energy per gram is similar to, and even slightly lower than the normally occurring reproductive tissue. It also clearly illustrates the usefulness of gall-forming insects as biocontrol agents.

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