EFFECTS OF THE BIOCONTROL AGENT, *COELOCEPHALAPION CAMARAE* KISSINGER, GALLING ON PETIOLE TISSUES, PLANT GROWTH AND STORED RESERVES IN PLANT PARTS OF TWO *LANTANA CAMARA* L. (VERBENACEAE) VARIETIES

THESIS
Submitted in fulfilment of the
requirements for the degree of
Master of Science *Ad Eundem Gradum* (Science)
of Rhodes University

By

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December 2015

ABSTRACT

The agent evaluated in this study, *Coelocephalapion camarae* Kissinger (Brentidae), a petiole galling apionid, was released against *Lantana camara* L. (Verbenaceae) in South Africa and has the potential to significantly contribute to the control of this noxious weed. An important aspect of evaluating the effectiveness of a biocontrol agent; include the understanding of the mechanism in which the biocontrol agent causes damage to the plant and the plant's response to this damage. Another aspect that was considered in evaluating the effects of the apionid was the varietal differences that exist within the weedy *L. camara* complex.

Larval feeding of *C. camarae* damaged the tissue in the petioles causing gall formation and this was quantified on two common South African *L. camara* varieties (017 and 018). Up to 100% of the vascular tissue (xylem and phloem) of effected petioles was eaten by larvae, 25 days after ovipostion in both the *L. camara* varieties, effectively severing the vascular connection from the leaves.

The effect of feeding and galling by different population densities of *C. camarae*, on biomass and total soluble sugar and starch concentrations of different plant parts was measured on these two varieties during autumn, and compared to a similar study, done during summer. Relatively low galling percentages were achieved in the experiments of this study. Plant growth was less affected by apionid feeding at similar galling levels, as higher galling densities in these trials were similar to those at low exposures in trials by Baars (2002). The dry weights of all individual and combined plant parts for lantana variety 017 was less for both adult density exposures after 70 days compared to the weights of its controls, although not statistically significant at $P > 0.05$ The opposite effect, though barely noticeable was recorded for lantana variety 018; here, the dry weights of individual plant parts and as whole plants weighed more in the plants exposed to both densities of weevil feeding and galling after 70 days, compared to its controls. *Coelocephalapion camarae* herbivory may thus be more effective in inflicting damage on some *L. camara* varieties compared to others.

The effects of late season carbohydrate storage revealed that, from early- to mid-autumn starch concentrations increased significantly in stems of both *L. camara* varieties and *L. camara* var. 018 had larger starch reserves for winter. In *L. camara* var. 018 stems, starch increased 52 times and concentrations doubled in stems of *L. camara* var 017. The increase in the sugar and decrease in starch concentrations in leaves of plants of both varieties exposed to apionids in this study was attributed to a possible reduction of available nitrogen and

phosphorus, due to apionid feeding, whilst starch was reallocated within the plants to stems and roots. The increase in starch concentrations in stems of plants that were exposed to apionids may have been expected, as compensation for herbivory has been associated with, increases in photosynthetic rates and the mobilization of stored resources.

Herbivory by the apionid early in the growing season may be easily compensated for by *L. camara* as there are nutrient flushes experienced by plants that allows the maximum uptake of nutrients facilitating recovery, whereas later in the season plants suffer lower nutrient availability and don’t recover so readily. Both early and late in the season the accumulated effects of *C. camarae* feeding over time will undoubtedly decrease fitness of most lantana varieties.

ACKNOWLEDGEMENTS

I am grateful to Prof M.P. Hill (Rhodes University, supervisor) for having me as a student, for guidance, advice and comments.

I appreciate all the time and effort Fritz Heystek (ARC-PPRI) has put into providing advice, reviewing this thesis, and for his suggestions and criticisms.

Thanks are also due to Prof Marcus Byrne and Dr Danica Marlin (both University of Witwatersrand) for arranging the use of facilities at the university; Innocent Rabohale (Ecological Engineering and Phytotechnology Programme, University of the Witwatersrand) for the use of her laboratory, assistance, advice and support during the sugar and starch analysis work; Prof Patricia M. Tilney (University of Johannesburg) for her advice on the making of the petiole cross sections; Anthony King, Hildegard Klein, Lulu Madire and Liame van der Westhuizen (colleagues at PPRI, Weeds Division) for their advice, discussions, support and encouragement.

I would like to acknowledge financial support from the Agricultural Research Council and the *Working for Water* Programme, of the National Resource Management Programme, Department of Environmental Affairs.

CONTENTS

CHAPTER 1
GENERAL INTRODUCTION

1.1 BACKGROUND ON *LANTANA CAMARA* L. (*SENSU LATO*)

The weedy, woody shrub that is most often referred to as *Lantana camara* L. (Verbenaceae) is probably more correctly described as *Lantana camara* L. (*sensu lato*) (Urban *et al*. 2011). The plant has its origins in tropical and subtropical South and Central America. Various species of the genus *Lantana* were taken to Europe in the 17th century and subjected to considerable plant breeding efforts to produce several ornamental varieties (Stirton 1977). It is therefore an extremely variable entity that presents a complex taxonomic problem (Munir 1996). The complexity and confusion around the taxonomy of the weedy *Lantana camara* species lies in its convoluted history of cultivation, hybridisation and artificial selection that begun 60 years before Linnaeus described the genus *Lantana* in 1753. (Sanders 2006; Munir 1996).

The weedy *Lantana camara* has become naturalised in about 50 countries after its introduction in the tropical, subtropical and temperate regions of the world (Holm *et al.* 1977). The plant invades a wide range of climatic and geographical regions around the world, and is considered one of the world’s worst weeds (Muniappan *et al.* 1992). Invasions of exotic species, and in particular *L. camara,* are amongst the most important global-scale problems experienced by natural ecosystems (Sharma *et al.* 2005) and one of the top threats to biodiversity (Chornesky *et al*. 2003).

In South Africa *L. camara* has become invasive in the warm, moist subtropical and temperate regions (Oosthuizen 1964; Stirton 1977) of the Northern Province, Gauteng, Mpumalanga and KwaZulu-Natal, as well as the southern coastal regions of the Eastern and Western Cape provinces (Baars & Neser 1999) (Figure1.1). It forms dense impenetrable thickets that reduce the biodiversity of natural ecosystems through allelopathic suppression of indigenous plant species (Gentle & Duggin 1997). In the same manner it also out-competes desirable vegetation in commercial plantations and orchards as well as pastures, thereby reducing the carrying capacity and productivity of the land. The leaves, stems and fruits of *L. camara* contains toxic compounds (Kellerman *et al.* 1996), that may cause photosensitivity, liver and kidney damage, intestinal haemorrhaging and death if consumed by cattle, sheep and horses.

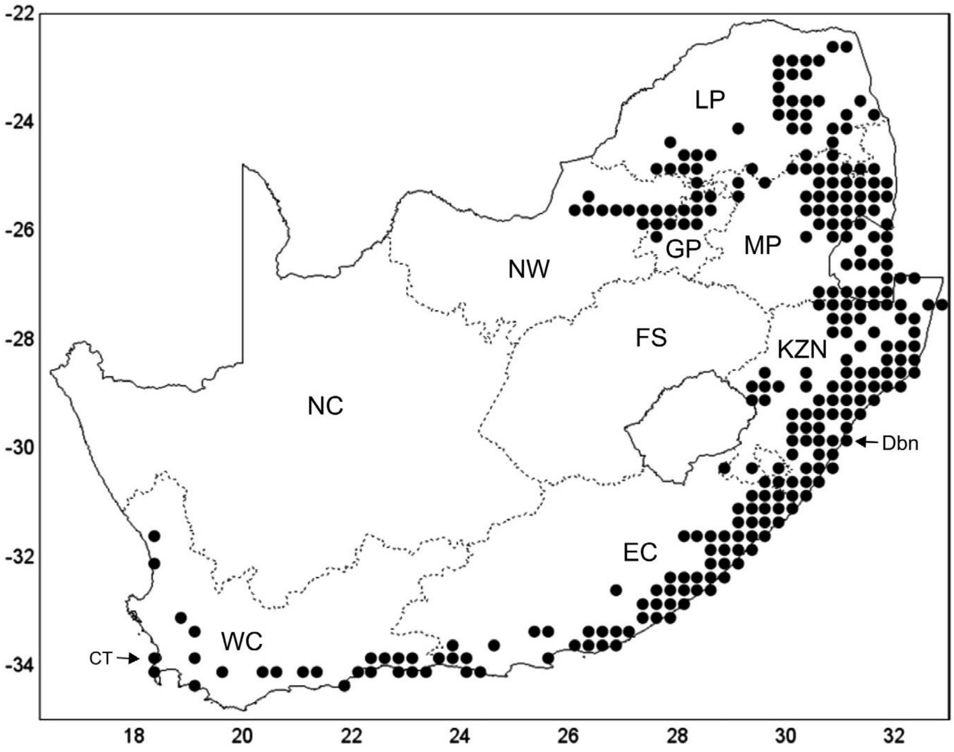


Figure 1.1: Distribution of *Lantana camara* L. (*sensu lato*) in South Africa, dots represent quarter degree squares in the provinces of Limpopo (LP), North West (NW), Gauteng (GP), Mpumalanga (MP), Northern Cape (NC), Free state (FS), Kwa-Zulu Natal (KZN), Western Cape (WC) and Eastern Cape (EC) CT (Capetown), DBN (Durban). (L. Henderson, SAPIA database, ARC-PPRI).

1.1.1 Lantana control

Lantana camara is a declared weed in South Africa and by law landowners are compelled to controlling it (National Environmental Management: Biodiversity Act No. 10 of 2004) (NEM:BA) (Henderson 2001). Controlling lantana mechanically and chemically, can be effective but is labour intensive and expensive (Cilliers & Neser 1991), especially when dealing with an invader like lantana which is aggressive, vigorously growing, can tolerate a wide variety of environmental conditions (Baars & Neser 1999) and by the early 1990's had invaded approximately 400 000 ha in Kwa Zulu-Natal and 75 000 ha in the Northern Province, Gauteng and Mpumalanga (Cilliers & Neser 1991). For control methods to be effective, follow up programmes are imperative. Often lantana plants re-coppice if they are slashed or if the chemical application was not adequate to kill the plants. There is also a seed bank in and on the soil, and germination of these seeds results in re-infestation of cleared areas (Erasmus 1991). The expenses incurred by mechanical and chemical control have fostered the hope that a biological control programme may provide a solution for the lantana problem (Baars & Neser 1999).

1.1.2 Biological control of lantana

Lantana camara being a man-made polypoid complex (Stirton 1977) makes the weed very variable. This variability combined with the aggressive qualities of an invader, which are superior growth by aggressive competition for resources, efficient dispersal and rapid establishment (Sharma *et al.* 2005) makes controlling it difficult. Even successful biological control will not eradicate a weed and is influenced by interactions with the environment, and thus dynamic and variable, with levels of control varying between seasons and years (Julien 1997).

The biological control programme against *L. camara* was first initiated in South Africa in 1961 (Oosthuizen 1964). Candidate biological control agents were collected off lantana-like plants from the countries where *Lantana camara* is considered to be indigenous and therefore differ from the weedy lantana entities (Urban *et al*. 2011). Various biological control agents released against lantana over a period of more than 50 years, that have become established, have only made a small contribution to the management of lantana. However, there has been notable and impressive damage to some cultivars of the plant in certain areas, but generally the levels of control achieved have been disappointing (Zimmermann *et al*. 2004). The benefit to cost ratio for lantana biocontrol in South Africa was calculated at 22:1 from the initiation of the biocontrol research up to the year 2000 (van Wilgen *et al.*,2004), indicating that the benefit of biological control might have been underestimated and is probably most noticeable in the reduction in spread of the weed.

The effective implementation of the biological control programme against lantana therefore depends on the release of a suite of natural enemies that have the ability to cope with the factors that limit the impact of the agents already established (Baars & Heystek 2003). Twenty four agents have been introduced against the weedy *L. camara* between 1961 and 2010 (Table1.1). Twenty two of the agents were insect species, one a fungus and the other a mite. Of these, 13 have become established. Eleven agents were released prior to 2007 and have had various degrees of impact on the weed. The three agents that were released in 2007 have established in localised areas and their effectiveness and impact have yet to be quantified in the field.

Table 1.1: Natural enemies of *Lantana camara* established in South Africa, year of first introduction, mode of feeding, current status and relative perceived damage to the weed (Heystek 2005; Klein 2011).

Biocontrol agent	**Main release**	**Feeding niche**	**Field establishment**	**Biocontrol contribution**
Coleoptera:				
Coelocephalapion camarae (Kissinger) (Brentidae)	2007	Petiole gall	Preliminary	Unknown
Longitarsus bethae (Savini & Escalona) (Chrysomelidae)	2007	Root chew (Larvae), Leaf chew (Adults)	Preliminary	Unknown
Octotoma scabripennis (Guérin-Méneville) (Chrysomelidae)	1971 1974 1981	Leaf mine	Abundant , localised inland in the warm, moist eastern range of lantana	Extensive localised defoliation
Uroplata girardi (Pic) (Chrysomelidae)	1974 1983	Leaf mine	Abundant on KwaZulu Natal coast, and rare in warm, moist inland range of *L. camara*	Extensive localised defoliation
Diptera:				
Calycomyza lantanae (Frick) (Agromyzidae)	1982 1989	Blotch leaf mine	Widely, in low numbers across the weed range	Limited damage
Ophiomyia camarae (Spencer) (Agromyzidae)	2001	Herring-bone leaf mine	Widely, along KwaZulu Natal coast	Extensive defoliation
Ophiomyia lantanae (Froggatt) (Agromyzidae)	1961	Fruit mine	Widely abundant across weed range	Low impact on seed viability
Hemiptera:				
Falconia intermedia (Distant) (Miridae)	1999	Leaf sap sucking	At sites in the Eastern Cape and Mpumalanga	Locally high, but limited by climate and population fluctuations
Teleonemia scrupulosa (Stål) **(**Tingdae)	1961 1971 1984 1981	Flower and leaf sap sucking	Widely, across the entire range of *L.camara*	Serve damage sporadically - complete defoliation and flower abortion

(Continued)

Biocontrol agent	Main release	Feeding niche	Field establishment	Biocontrol contribution
Lepidoptera:				
*Hypena laceratalis** (Walker) (Noctuidae)	1961	Leaf chewing	Widely. - Larvae only active during late summer and autumn	Considerable damage
Lepidoptera:				
Lantanophaga pusillidactyla (Walker) (Pterophoridae)	Pre-1961 1984	Flower feeding	Widely - in low numbers.	Unknown
Salbia haemorrhoidalis (Guenée) (Pyralidae)	1962	Leaf feeding	Widely - in low numbers	Unknown
Crocidosema lantana (Busck) *(*formerly in *Epinotia)* * (Tortricidae)	1961 1984	Flower-peduncle and shoot-tip borer	Widely - in low numbers	Unknown
Acari:				
Aceria lantanae (Cook) (Eriophyidae)	2007	Distorts flower buds into inflorescence galls	Well established at frost free sites in South Africa	Extensive galling of flowers

*Insect species already present in South Africa prior to deliberate introduction; the origin of *H. laceratalis* is considered to be Africa, Indo-Malaysia and Australia (Broughton 2000). Its origins is unclear, as it was present in South Africa and Australia prior to it being deliberately introduced (Baars & Neser 1999; Day *et al.* 2003) and is thought to have been introduced with the plant, or it is indigenous or has become naturalised; *Epinotia lantana* naturally occurs in Mexico and was probably inadvertently introduced with the weed (Baars & Neser 1999).

1.2 THE BIOLOGICAL CONTROL AGENT USED IN THIS STUDY

1.2.1 Introduction

Coelocephalapion camarae (Kissinger) in the family Brentidae is a leaf petiole galling apionid weevil that was first collected from a lantana species, probably *L. camara* during surveys in Mexico in 1997 at Cárdenas in Tabasco Province. It was subsequently collected at a number of sites in Veracruz and at six sites along the Gulf coast of Mexico in 1998 and again on the East coast between Bocca del Rio and Anton Larzado in 2007 (Baars 2007, Baars &

Neser1999, Baars *et al*. 2007, Heystek pers.com.). Gall formers are favoured as biological control agents because they tend to have a narrow host range and threaten few non-target plants, are usually a nutrient sink, and are well protected against generalist parasitoids (Harris & Shorthouse 1996; Hill & Hulley 1995). Therefore *C. camarae* was considered a potentially good biological control agent. In 1998 a once-off field survey was conducted in Mexico to determine the distribution of the apion. This along with other locality records shows *C. camarae* to occur at altitudes between sea level and 1500 m and to cover a wide geographic area (Baars *et al*. 2007). This geographic range suggests that it might potentially be pre-adapted to cover a wider geographic area in South Africa (Baars *et al*. 2007), which has been one of the limiting factors of some of the other established agents (Baars & Heystek 2003). However, the apion that was cultured in the quarantine laboratories at ARC (Plant Protection Research Institute) in Pretoria originates from two collections, the 1997 collection at Cárdenas which consisted of six individuals (Baars *et al.* 2007) and the other from the collection made in 2007 between Bocca del Rio and Anton Larzado consisting of five individuals (Heystek pers.com). These two collections are both from the east coast and at low altitudes and may therefore not be representative of the native field population (Baars *et al.* 2007).

The life history, laboratory impact, varietal preference and host specificity studies on *C. camarae* were reported by Baars (2002). These are summarized below (1.2.2 to 1.2.5).

1.2.2 Life history

Adults of *C. camarae* are about 2mm in length and mostly black. There are no distinct external morphological difference between the males and females; however the females generally have a wider abdomen and a longer rostrum. Adults feed small shot holes in the leaves and live four to six months in the laboratory. They overwinter in the dried leaf litter at the base of the plant. This diapause behaviour along with being long-lived may allow them to establish in the drier parts of South Africa as they can potentially survive defoliation and can recover earlier in the season. This possible early recovery should enable the apion to build sufficient population numbers, preventing plants recovering from the previous season's damage. The female pre-ovipostion period is approximately ten days and she lays about one egg per day. The female inserts her eggs into the leaf-petiole and also into the inflorescence-peduncle at the base of the receptacle (Baars *et al.* 2007). However, ovipostion occurred two to four times more frequently on the leaf-petiole than the inflorescence-peduncle. Ovipostion is restricted to leaf-petioles with a diameter range of 0.15- 0.30 mm and to leaves with length range of 3.27- 9.80cm. The eggs are pale white, approximately 0.5mm in length and takes about six days to hatch. The larva is small and white, with a light brown head-capsule. There are three larval instars and development from egg to adult takes 35 days. The larva feeds on the vascular tissue, stimulating the plant to produce proliferated tissue, initiating the gall. The larva feeds predominantly on the vascular and proliferated tissue.

1.2.3 Laboratory impact

During larval development, in excess of 90% of the petiole tissue is damaged across the galled area and was assumed to cause a disruption to the vascular system and therefore preventing the transport of solutes to and from the leaves (the focus of this thesis). The larval development has a significant effect on the plant, causing a significant reduction in the root biomass (Baars *et al.* 2007). At low levels of galling (18% of petioles), root growth ceased. The plant compensates for the loss of photosynthetic area by a flush of new leaves; however, at high galling densities (40%) this leaf flush is prevented (Baars *et al.* 2007)

1.2.4 Varietal preference

Varietal performance and preference studies were conducted on *L. camara* in the laboratory. No-choice varietal performance trials were conducted using three naturalised South African lantana varieties and the natural host *Lantana* plant, probably *camara* from Mexico. This was done to determine the suitability of the South African lantana varieties for *C. camarae*. These trials found that the adult preference and reproductive performance was similar on all varieties tested.

Simultaneous-multiple choice trails were conducted using four South African lantana varieties and a non-target indigenous *Lippia* species (*Li*. sp. B - yet un-described, distinctly different *Lippia* sp., with the largest petioles), to establish if there was preference between any of the South African varieties. It was found that ovipostion per plant was not significantly different between the four lantana varieties (Baars 2002). The *Lippia* species was however considered an unsuitable host plant. This indicates that *C. camarae* can be expected to establish and impact equally on all lantana varieties in the field in South Africa. *Lantana camara* biological control agents that have displayed varietal preference in the laboratory have shown to have similar preferences in the field (Heystek 2006).

1.2.5 Host specificity

Host specificity testing provides the primary information for making decisions on whether to release a biological control agent (Heard 1999). To determine host specificity various trials were conducted. These were adult feeding and ovipostion no-choice trials, adult preference pair-choice trials and host preference multiple-choice trials. In these trials, 55 plant species from 14 plant families were tested. The test plant species were chosen based on their taxonomic relatedness to *L. camara*, using the centrifugal phylogenetic method (Wapshere 1974; 1989).

The no-choice trials indicated that 11 non-target species to be suitable hosts, however these trials are restrictive and disrupt the host selection process (Shepherd 1990; Harris & McEvoy 1995). The pair-choice trials indicated a clear preference for *L. camara*, however three *Lippia* species were utilized as alternate host plants, but low numbers of progeny was recorded on these species and was considered an inferior host (Baars 2002). The weevils showed an almost exclusive preference for *L. camara* in multiple-choice walk-in cage trials which better mimicked a field situation (Baars 2002).

1.2.6 Release and establishment

Governing departments approved the release of this biological control agent in 2007. Releases of *C. camarae* were initially made only in subtropical coastal (Richards Bay) and inland (Nelspruit) areas and its establishment and spread was monitored (Heystek 2007). In October 2007, 300 adults were released and a further 300 in January 2008 at a site at Richards Bay onto a single plant in a long ‘hedge’ of lantana. In January 2008, 500 adults were release at each of three Nelspruit sites (two of these sites were however lost soon after releases). At the site that remained, the release was onto a single plant on the edge of an approximately 5 hectare dense stand of lantana. A second release was made at this same site in December 2009, as there was no evidence of the weevil, after initial establishment at this site. On subsequent visits to the Richards Bay site, the presence of ‘shot-holes’ caused by adult feeding found alongside leaf veins on the release plant made it evident that the adults had survived. The first petiole galls were detected on sampled branches of the release plant in July 2008 and by December 2008, between one and three galls were found on nearly every sampled branch on plants up to 20 m from the release plant. By June 2009, up to seven galls were found per sampled branch, and the insect had spread approximately 200 m. In total, from all the test branches sampled, 9% of the petioles were galled (Heystek & Kistensamy 2009). However, the site was mechanically and chemically cleared by October 2010.

There was no evidence of persistent presence of *C. camarae* at any of the sites in Nelspruit to date. Subsequent releases at various other sites were made, but to date C. *camarae* has only established at sites in Northern KZN at Richards Bay and Empangeni.

1.3 AIM OF THIS STUDY

The aim of this study was to quantify the extent of damage to petiole tissues of two common South African *L. camara* varieties, in particular the vascular tissue, of galled petioles, and is discussed in chapter two. A further aim was to determine the effect of different population densities of *C. camarae* feeding and galling, on biomass of different plant parts on these two different varieties, which is discussed in chapter three, and lastly to measure the effects of adult feeding and galling of the different population densities of the weevils on concentrations of total soluble sugars and starch in different plant parts of these two *L. camara* varieties and how the concentration differences affect these varieties and is discussed in chapter four.

These two varieties, were used so comparisons could be made to the biomass studies conducted by Baars (2002), on which, the experiments for the biomass study was modelled. The biomass and total soluble sugar and starch concentration experiments were done by exposing different densities of weevils to the two *L. camara* varieties. These adult exposures were also done late in the season (late summer to autumn) to determine how season effects apionid performance and plant response to herbivory by the insect. Since the biomass experiments by Baars (2002), was conducted in mid-summer, seasonal comparisons of apionid performance and plant response to apionid herbivory could be made and is discussed in Chapter 5 of this thesis.

CHAPTER 2

EFFECT OF *COELOCEPHALAPION CAMARAE* LARVAL FEEDING AND GALLING ON PETIOLE TISSUES OF TWO LANTANA VARIETIES

2.1 INTRODUCTION

Insect induced galls are specialised feeding and sheltering structures used by some arthropods and act as nutrient sinks to the plant (Samsone *et al.* 2012). The fundamental reason that gall inducing insects are dependent on plants is nutrition (Raman & Ananthakrishnan 1983), as the galling herbivores tap into the host vascular system and feed on photoassimilates and nutrients moving through the plant from sites of production to sites of active use or growth (Larson & Whitham 1997).

The developing larvae of *Coelocephalapion camarae* may therefore be strategically located to use photoassimilates and the damage their feeding causes to the vascular tissue prevents the transport of sucrose and carbohydrates to the stems, and roots, therefore having the ability to severely affect the growth and development of the plant.

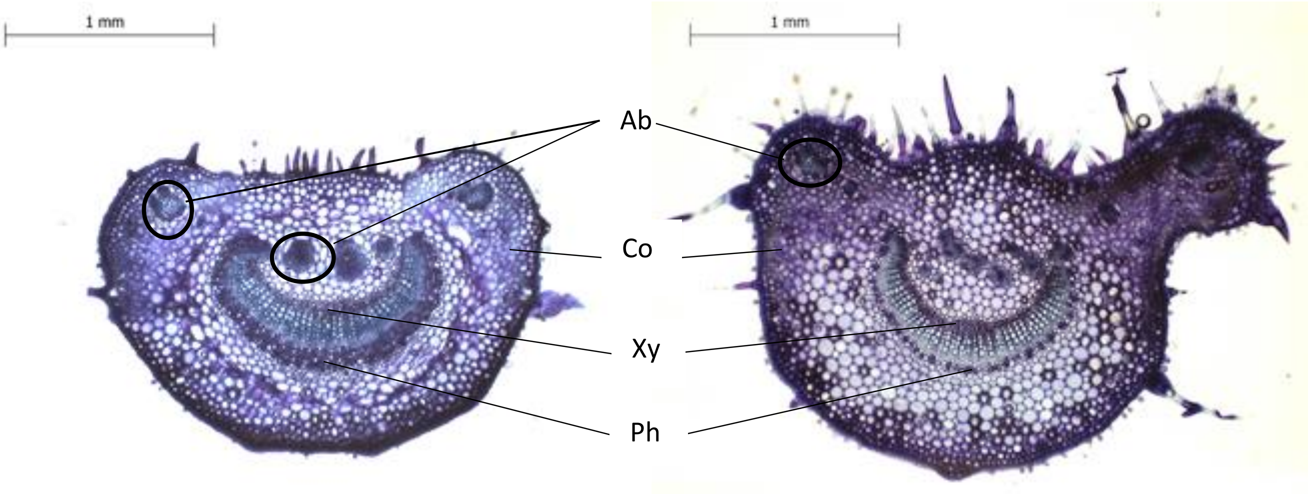
Laboratory impact studies on *C. camarae* that were reported by Baars (2002) showed that during larval development, in excess of 90% of the petiole tissue is damaged in the galled region, causing a disruption to the vascular system. However, varietal differences have been shown to affect petiole size, and may also affect structure and proportions of vascular bundles and other tissues in petioles, which may be affected directly by larval feeding. Therefore, the aim of this chapter was to investigate if there were any differences in the structure and proportion of the vascular bundles in the two *L. camara* varieties and to quantify the damage done to the vascular tissue from ovipostion to adult eclosion.

2.2 MATERIALS AND METHODS

The varieties of *L. camara* used in this study, 017 orange and 018 dark pink, are morphologically very different, and commonly occur in South Africa. *Lantana camara* variety 017 tended to be a low growing plant, with orange coloured mature flowers, and variety 018 normally grew as an upright shrub, with a dark pink flower (Heystek 2006). Both were collected around the Sabie-area in Mpumalanga, and they compared equally in thorniness and stem and leaf hairiness. In addition, Baars (2002) used these varieties for *C. camarae* impact studies, and this study aimed to expand on that work. *Lantana camara* variety 017 was reported to be a slower growing variety than *L. camara* var.018 (Baars 2002). Plants used in the experiment were propagated from similar sized cuttings and grown for twelve months before being used in the experiment. Plants were grown in 10 litre pots, under 50% shade-net, with overhead irrigation in an outdoor nursery. Six plants of each variety of approximately the same size were used. Three plants of each variety were exposed to 200 adult apionids for a day (24 hours). The remaining three plants of each variety served as controls. Three petioles that had evidence of ovipostion were selected from each of the plants exposed to apionids at five day intervals, starting from day one, up to day forty. Three petioles from each control plant were also removed at the same intervals and placed into vials containing Formalin-acetic acid – alcohol preservative (F.A.A.). Hand cross sections of these petioles were made under a dissection microscope. To maintain consistency the cross sections for the test petioles with evidence of larval feeding and when galls became visible was done across the centre of the damaged area or gall. The cross sections were stained with toluidine blue for about a minute and then rinsed in distilled water before mounting onto a slide. The sections were each mounted on a slide, in a small drop of glycerine, and covered with a slide cover slip of which the edges were sealed with natural nail varnish to make temporary slides. The slides were photographed with a scale bar under a Leica EZ4 HD microscope. These scaled photographs were used to measure the actual area of each of the tissue types in the control petioles and to measure the area damaged by larval feeding on the tests. Data was described, using basic descriptive statistics, and means were analysed for differences using an ANOVA.

2.3 RESULTS

The distribution of the tissue types that were present in the petioles of *Lantana camara* varieties 017 orange and 018 dark pink were identified and shown in Figure 2.1. Both these varieties had the same tissue types and approximate layout. The average area of the tissue types are shown in Figure 2.2. Petiole sizes were similar, but vascular tissues of *L. camara* var. 017 was significantly larger on average, measuring approximately 0.35 (± 0.01) mm^2 (Mean ± SE) and that of variety 018 measuring approximately 0.28 (± 0.01) mm^2 ($F(1,160) = 19.9222$, $p = 0.00002$) (ANOVA) ($n = 72$) (Figure 2.3.).



a) **b)**

Figure 2.1: Cross sections of healthy *Lantana camara* petioles of varieties 017(a) and 018 (b), where Ab = accessory bundles, Co = collenchyma, Xy = xylem, Ph = phloem.

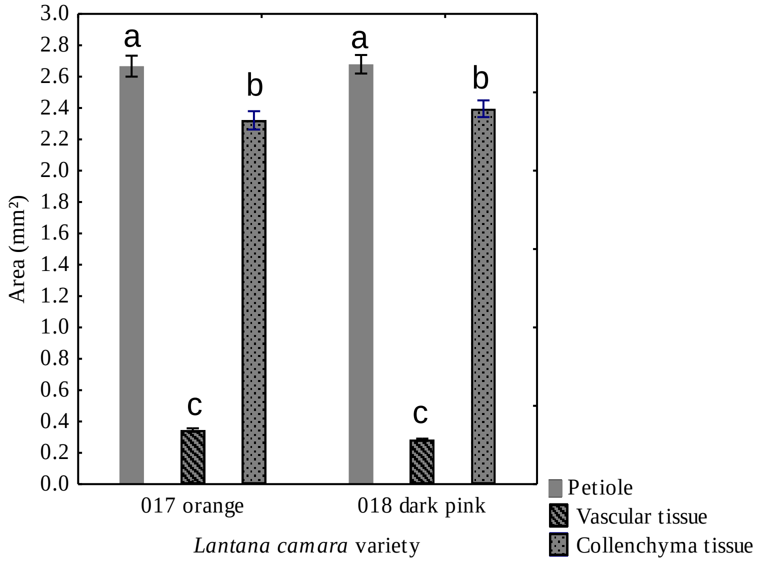


Figure 2.2: The average area (mm²) of the different tissues in petioles of *Lantana camara* in varieties 017 and 018. Different letters for corresponding tissues above columns indicate significant differences between means.

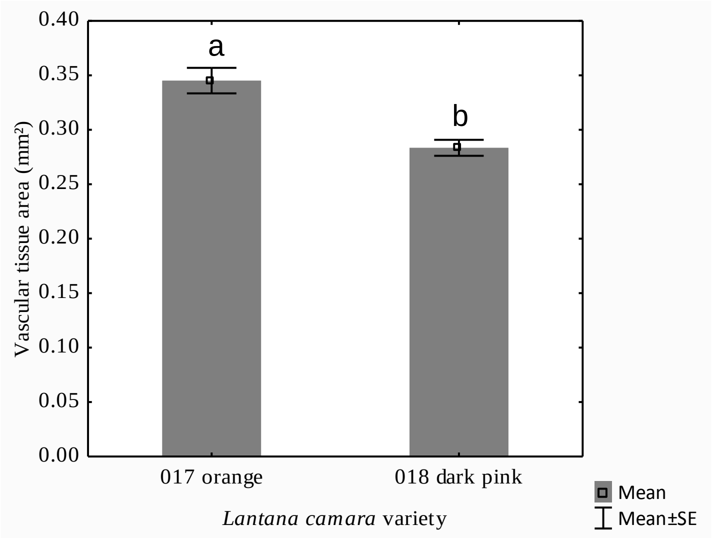
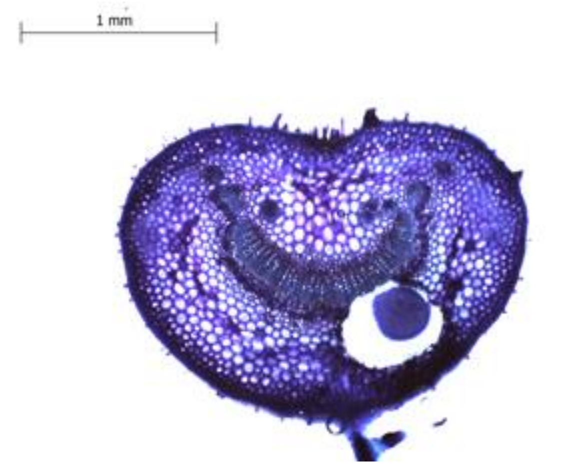


Figure 2.3: Average vascular tissue area (mm²) in petioles of *Lantana camara* variety 017 and 018. Different letters above columns indicate significant differences between means.

Figure 2.4 shows damaged petioles of both *L. camara* varieties 017 and 018, from oviposition at day one, up to day 40, after eclosure of adults. The area damaged by larval feeding appeared relatively unchanged for the first 10 to 15 days from the petiole cross sections. This was caused by the way the larvae fed; initially larvae fed by tunnelling from the oviposition site, along the length of the petiole toward the plant stem. Tissue differentiation, or proliferating of tissue, occurred in both varieties between day 15 and 20. The exact day that proliferation occurred is not known, as sections were made at five day intervals, but were observed for the first time in sections on day 20.

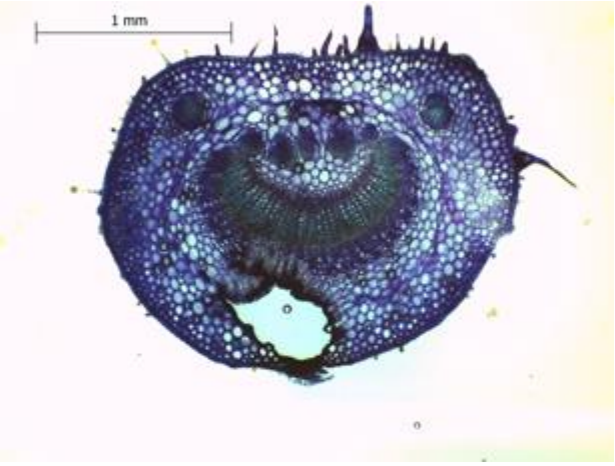
Using the day one cross sections (n = 9 for each variety) the tissue that the eggs were placed into by the female was recorded and the frequency was calculated and expressed as percentage. In petioles of *L. camara* var. 017, 89% of eggs were placed across both the vascular and the collenchyma tissues, and 11% of eggs oviposited only in the collenchyma tissue. Whereas for *L. camara* var. 018 fewer eggs, 67%, were placed across both vascular and collenchyma tissue, and, 33% in the collenchyma tissue only. This is probably because the vascular tissue area is significantly larger in *L. camara* var. 017 than in *L. camara* var. 018 (Figure 2.3). A larger percentage of vascular tissue was consumed by day 15 and 20 in *L. camara* var. 017, approximately 50% and 100% respectively, than that of variety 018, were vascular tissue consumed by day 15 and 20 was approximately 22% and 54% respectively (Figures 2.5 & 2.6). However, 100% of vascular tissue in both *L. camara* varieties was consumed by larval feeding by day 25.

Lantana camara variety 017



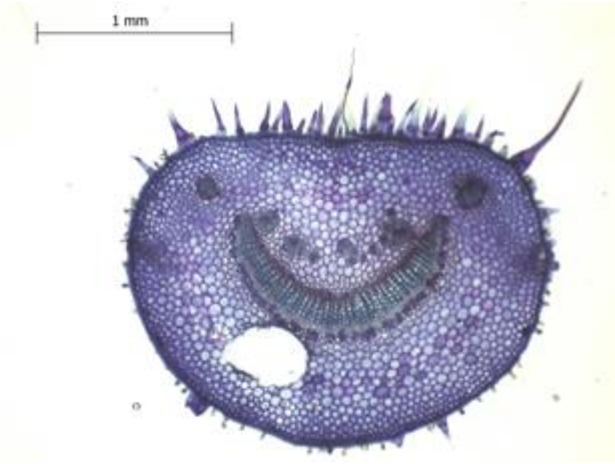
Adult feeding damage and egg placement, day 1

Larval feeding damage day 5

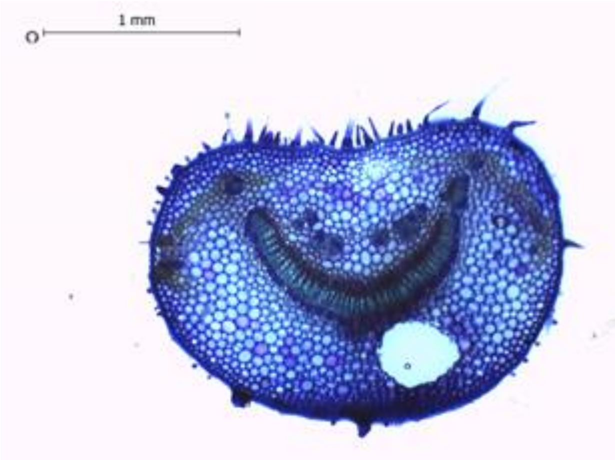


Larval feeding damage day 10

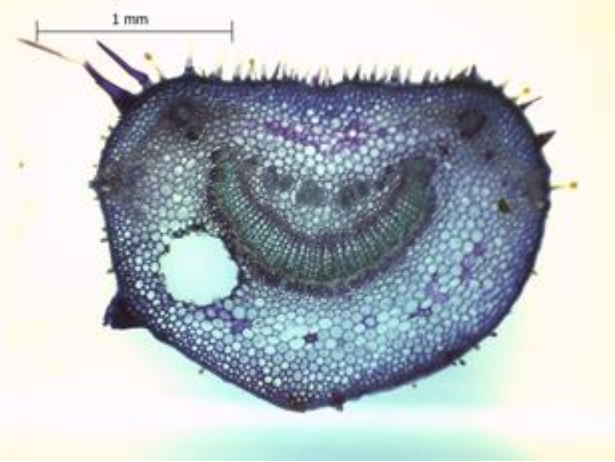
Lantana camara variety 018



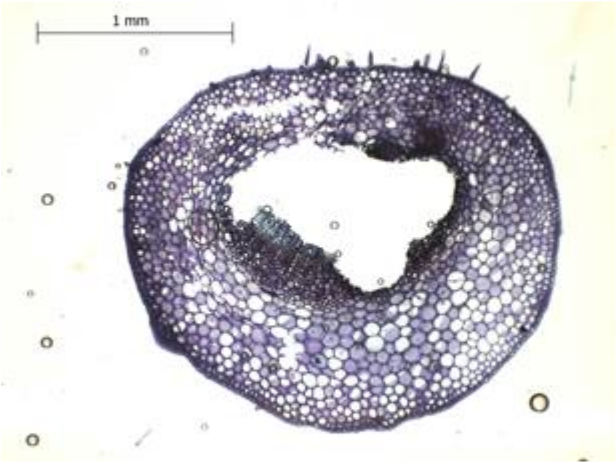
Adult feeding damage and egg placement, day 1



Larval feeding damage day 5

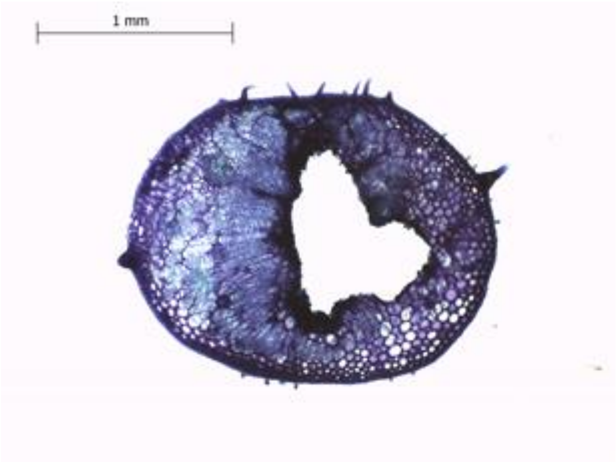


Larval feeding damage day 10

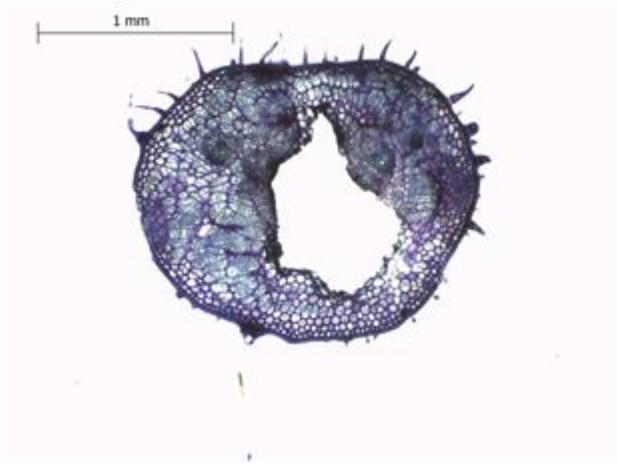


Larval feeding damage day 15

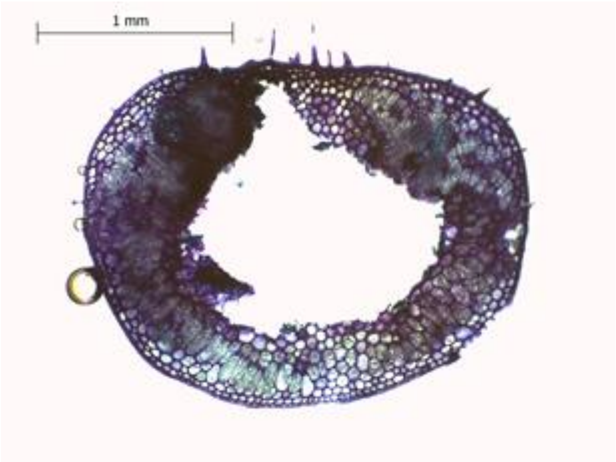
Larval feeding damage day 15



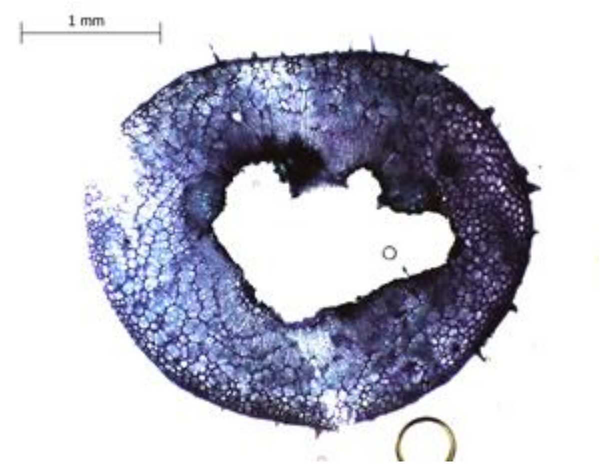
Larval feeding damage day 20



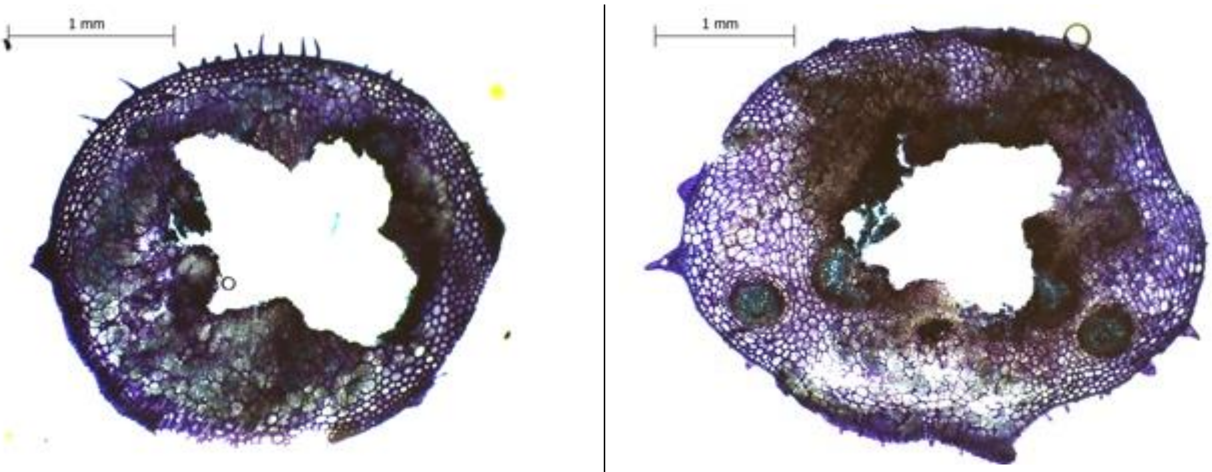
Larval feeding damage day 20



Larval feeding damage day 25

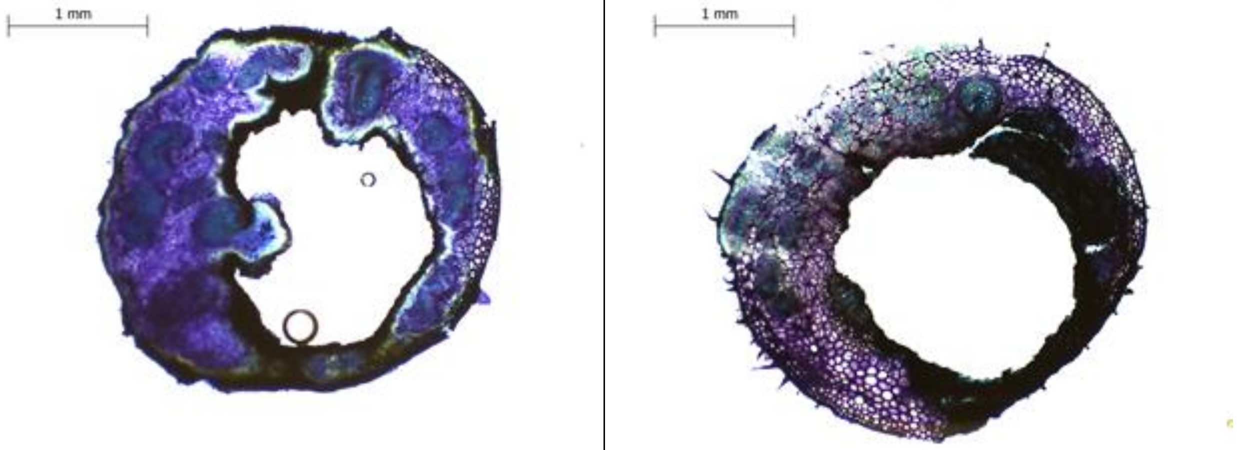


Larval feeding damage day 25



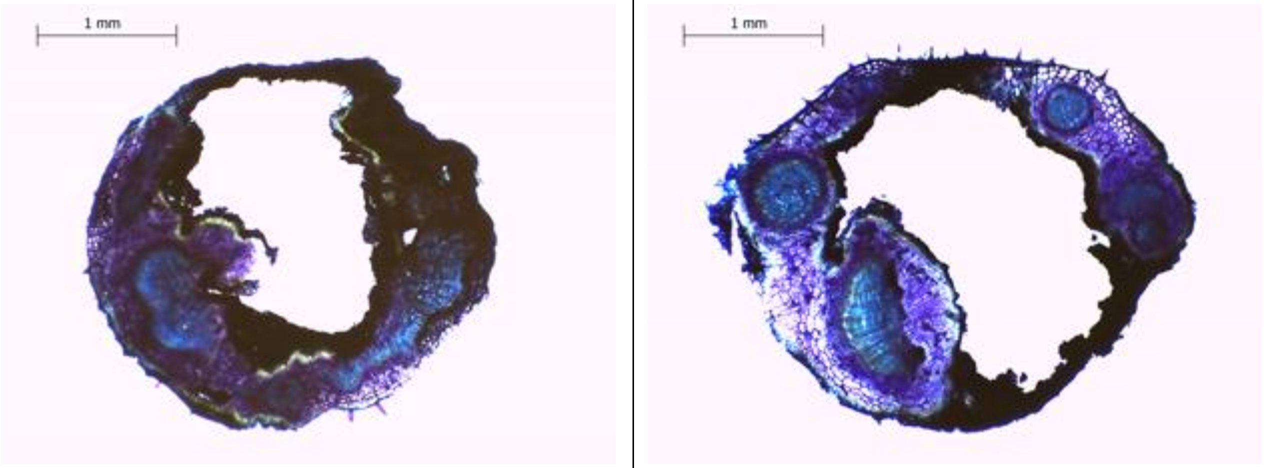
Larval feeding damage day 30

Larval feeding damage day 30



Larval feeding damage day 35

Larval feeding damage day 35



Larval feeding damage day 40

Larval feeding damage day 40

Figure 2.4: Oviposition and feeding damage of *Coelocephalapion camarae* larvae on two varieties of *Lantana camara*, as observed from petiole cross sections at five-day intervals.

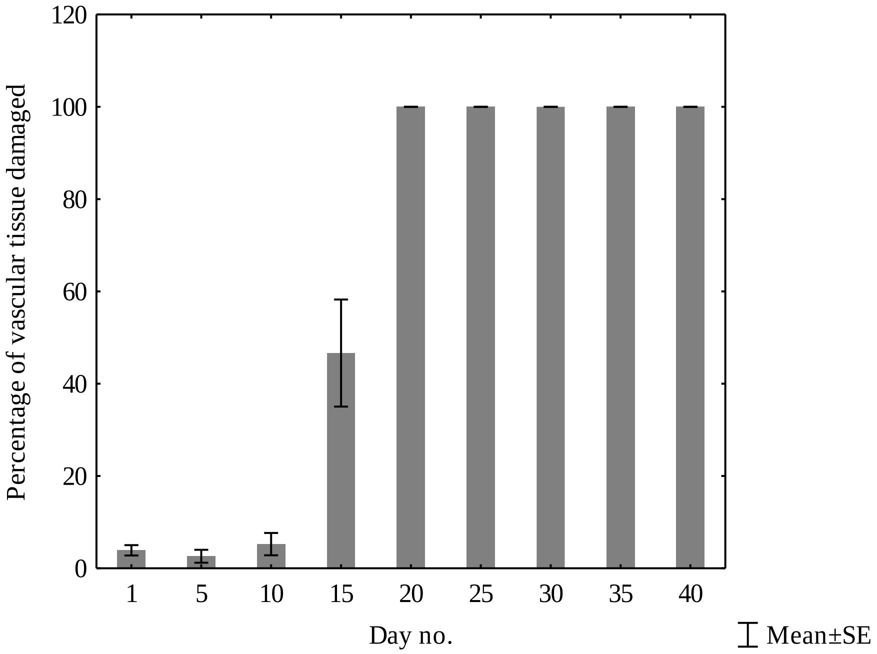


Figure 2.5: Percentage of vascular tissue damaged in *Lantana camara* variety 017 at different five-day intervals.

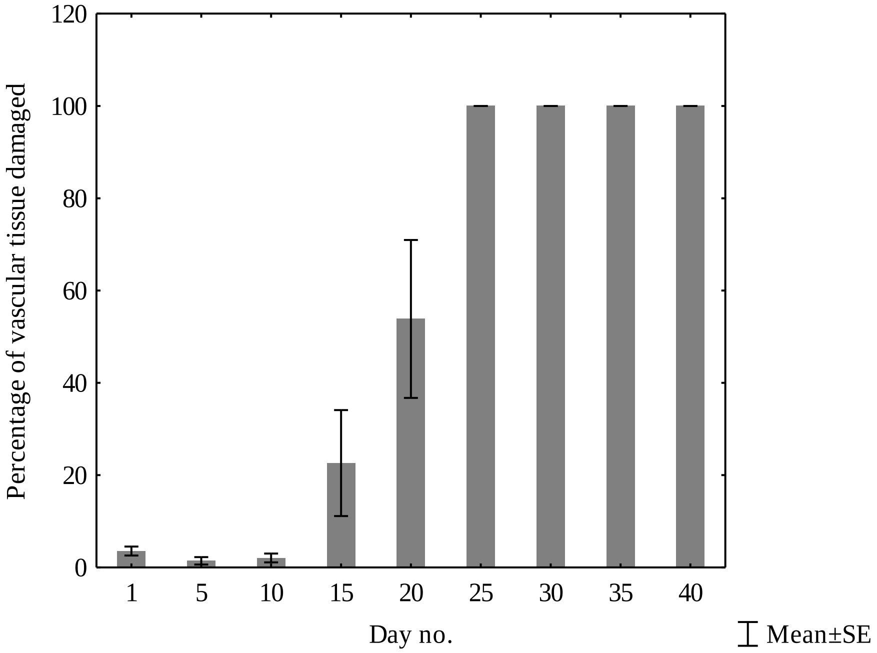


Figure 2.6: Percentage of vascular tissue damaged in *Lantana camara* variety 018 at different day-intervals.

2.4 DISCUSSION

The larval feeding of *Coelocephalapion camarae* damaged the vascular tissue in the petiole. Up to 100% of the vascular tissue of effected petioles was removed by larval feeding, by day 25 in both the *L. camara* varieties, effectively severing the vascular connection from the leaves (Figures 2.5 and 2.6). Since the leaf petiole is the organ that connects the leaf blade with the stem and the transport of organic and inorganic molecules between the blade and the stem is its main function (Maksymowych *et. al*. 1983), the damage caused by the larva of *C. camarae* should have a significant effect on other plant parts as photosynthates are mainly produced in mature leaves (Inbar *et al.* 1995; Iglesias & Podesta 2005). A plant's leaves are therefore its main source of nutrition, and it may affect the rest of the plant. *Coelocephalapion camarae* select the larger petioles for oviposition (Baars 2002), and these are connecting the larger, mature leaves to the plant and thus target the most productive leaves on the plant. In addition, herbivory induces changes in plant responses, and the degree of plant response is affected by the degree of vascular connectivity that links the damaged and undamaged plant tissue (Kaplan *et al*. 2008). The feeding and chemical secretions of galling insects cause considerable stress to attacked plant tissues, and the plant responds by translocating photoassimilates and growth promoters to the areas under stress (Raman 2007), therefore using resources from other healthy plant tissue.

Various studies on the effect of insect herbivory on plants have been done and account for varied responses from plants (Baldwin & Preston 1999; Kosola *et al*. 2001; Lestienne *et al*. 2006). Studies by Kosola *et al*. (2001), found that relatively little growth to trees occurred after a period of peak defoliation because re-foliation probably occurred too late in the season for the trees to make up the difference in growth. This same study also observed different responses in nitrogen uptake that may be affected by differences in defoliation intensities. There was also a delay in starch accumulation in defoliated plots until leaf regrowth was completed. Studies by Lestienne *et al.* (2006) also looked at nitrogen uptake and found that plants that were repeatedly defoliated took up significantly more nitrogen if it was available, which translated into a greater root mass.

Baldwin and Preston (1999), however, found that different ecological and physiological factors affected insects, herbivory and plant response, and all these complex factors needs to be considered when evaluating plant response to insect herbivory. The effect of *C. camarae* will be dependent on various factors and include insect densities achieved on plants and the season when the damage occurs, amongst others. Unpublished field data by Heystek and

Kistensamy, collected over a four year period, from a site were insects established, indicates that *C. camarae* numbers increased relatively slowly. Three hundred adults were released at a point on a 200 m *L. camara* hedge, and galls were detected for the first time ten months later, and a year later the apionid had spread 200 m and 9 % of petioles were galled. No further data was collected from this site as it was destroyed. The responses of plants to damage by *C. camarae* will probably be similar, if not more severe, to that of plants suffering defoliation, as galled leaves may remain a sink to the plant. However, the faster consumption of vascular tissue in one variety compared to another, as observed from the trials, may significantly affect plant response. The effect from damage from galling will, however be greatly affected by the percentage of galling on a plant. The effect on growth and carbohydrate resources of lantana varieties under varying levels of *C. camarae* populations is investigated in Chapters 3 and 4.

CHAPTER 3

THE EFFECT OF LATE SEASON EXPOSURE OF *COELOCEPHALAPION CAMARAE* ON THE GROWTH OF TWO *LANTANA CAMARA* VARIETIES

3.1 INTRODUCTION

The biomass that plants invest in shoots and roots are generally balanced and there is co-ordination in the growth of their organs and biomass partitioning among various plant organs to optimise the capture of nutrients, light, water and carbon dioxide so plant growth is maximised (Poorter & Nagel 2000).

Insect herbivory generally has a negative effect on the fitness of plants, but the magnitude of herbivory, for example insect densities and seasonal timing may create variation in the average effects on plant fitness (Knight 2003). The ability of plants to recover will be affected by the availability of resources such as light, water and nutrients (Poorter & Nagel 2000). The galling of *C. camara* will have similar effects to plants that have been defoliated, as the damage to the vascular tissue in the leaf petiole prevents the translocation of photosyntates, as shown in chapter two of this study. Studies have shown that the most common response to herbivory is increased plant performance by compensatory growth (Maschinski & Whitham 1989; Agrawal 1998). However, this compensatory growth decreases in probability when competition with other plants increase, nutrient availability decreases and herbivory occurs late in the season (Maschinski & Whitham 1989).

Two varieties of *L. camara*, variety 017 and 018 were used in these biomass impact studies. They were chosen so comparison could be made with the study done by Baars (2002), on which these experiments were modelled. This study however, was conducted during summer-autumn, whereas the study by Baars (2002) was conducted in midsummer. This was conducted to establish late season insect performance and impact on plant growth.

3.2 MATERIALS AND METHODS

The plants of the two *Lantana camara* varieties, 017 and 018 (as used by Baars (2002)) used in the experiment were propagated from similar sized cuttings and grown for five months (September to February) before being used in the experiment. Plants were grown in 2.5 litre pots, under 50% shade-nett, with overhead irrigation in an outdoor nursery, and were fertilized once with 3.6 grams of 3:2:1 (25) (N:P:K, 25%) during this period. Forty plants of approximately equal size of each variety were selected for use in the experiment. The experiment was conducted in a quarantine glasshouse at ARC, PPRI, Pretoria, South Africa, in cages with dimensions of 90cm x 55cm x 55 cm. The experiment was done during summer-autumn (February- May) to determine the effect the apionid may have on plant growth approaching winter. This was considered important, as plants are largely dormant in winter in most of South Africa, but rely on stored resources to sprout in spring. The average temperatures in the glasshouse during the duration of the trial was 23°C (day) and 21°C (night) and the average humidity was 55% (day) and 71% (night). The natural day length at the start of the experiment was 13 hours and it decreased to 11 hours by the end of the experiment.

Test plants were exposed to, 20 and 40 experienced adults (1:1 sex ratio was assumed) per each of five treatments per insect density for a period of five days to achieve different galling intensities (the same apionid densities and exposure times were used by Baars (2002)), and thereafter plants were maintained for 30 days in the glasshouse, before being destructively sampled. Additional treatments with controls were added. The additional treatments exposed another five plants of each variety, to the same levels of insects as described above, but were not destructively sampled after 35 days as for the previous treatment, but ten adult apionids were added onto each of the plants and they remained on the plants for a further 35 days. The adults were removed after the 35 days and the plants destructively sampled. This set of replicates with its control was therefore destructively sampled 70 days after the start of the trial. This was done to measure the effect of prolonged adult and larval feeding, and galling, similar to what may be expected to occur in reality in the field, where most adults may disperse, while some will likely remain on the plants.

During destructive sampling, the number of leaves, flowers, and in the case of the treatment plants, the eggs and galls in petioles were counted. The wet weights of leaves, flowers, stems and roots were determined on a laboratory scale, accurate to 1 mg. To measure dry weight, the separated plant parts were dried in paper bags for 3 days at 68°C. Samples were then weighed. Thereafter it was milled in preparation to measure the concentrations of total soluble sugar and starch in the different plant parts (See Chapter 4).

The dry weights of the different plant parts (leaves, flowers, stems and roots) were used for analysis. This data was not normally distributed and the non-parametric test, Kruskal Wallis was therefore used to compare means. Because the Kruskal Wallis test was used to compare means, post hoc analysis cannot be performed were significant differences existed between the means for treatments and controls. To compare the means between the different plant parts of the control plants of the two *L. camara* varieties at the start of the experiment (Control T_o) T-tests were used because comparison was being made between means of two samples.

3.3 RESULTS

3.3.1 Plant part growth of two lantana varieties

The controls to determine plant size before exposure to different treatments (Control T_o) for both varieties showed little variation in mean biomass of leaves, stems and roots, respectively, varied little within each variety (var. 017: 8.8 %, 9.6% and 16 %) and (var. 018: 6.2%, 6.7% and 13%). Both *L. camara* varieties 017 and 018 were grown under the same conditions, but differed from each other in size and weights of their different plant parts (Figure3.1). The dry weight of leaves for *L. camara* var. 018 was significantly higher than that of *L. camara* var. 017, *P* = 0.003275. The dry weight of roots for *L. camara* var. 018 was also significantly higher than that of *L. camara* var. 017, *P* = 0.000035.

Lantana camara var. 017 initially appeared to be a slower growing variety, as all plants were grown from equally sized cuttings, and the dry weights of the T_o plants were less than that of plants from variety 018 (Figure 3.2). Similar results were reported from studies by Baars (2002). The dry weight of *L. camara* var. 017 however increased by 85% in 35 days and that of *L. camara* var. 018 by 63% (Figure 3.2). Between day 35 and 70, *L. camara* var. 017 had a biomass increase of 24%, were as *L. camara* var.018 had an increase of only 4% (Figure3.2). It is evident that there was a slowing down of growth rate of both varieties approaching winter, however, *L. camara* var. 018 growth rates appeared to have slowed down sooner than that of *L. camara* var. 017. This was a notable reduction in the growth rate of *L. camara* var. 018 as it enabled *L. camara* var. 017 to be almost equal in biomass after 70 days, differing by only 2.6 g compared with the difference in weight between the varieties at the start of the experiment, 6.2 g (Figure 3.2).

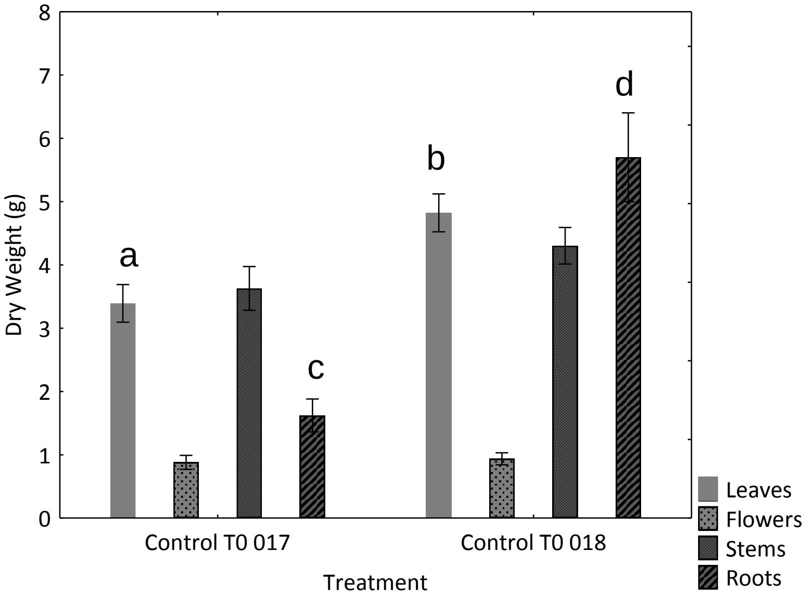


Figure 3.1: The mean (± SE) dry weight (g) of different plant parts at the start of the trial (Control T_o) for the two *Lantana. camara* varieties 017 and 018. Means of bars of same plant parts with different letters are significantly different, *P* < 0.005, T-test).

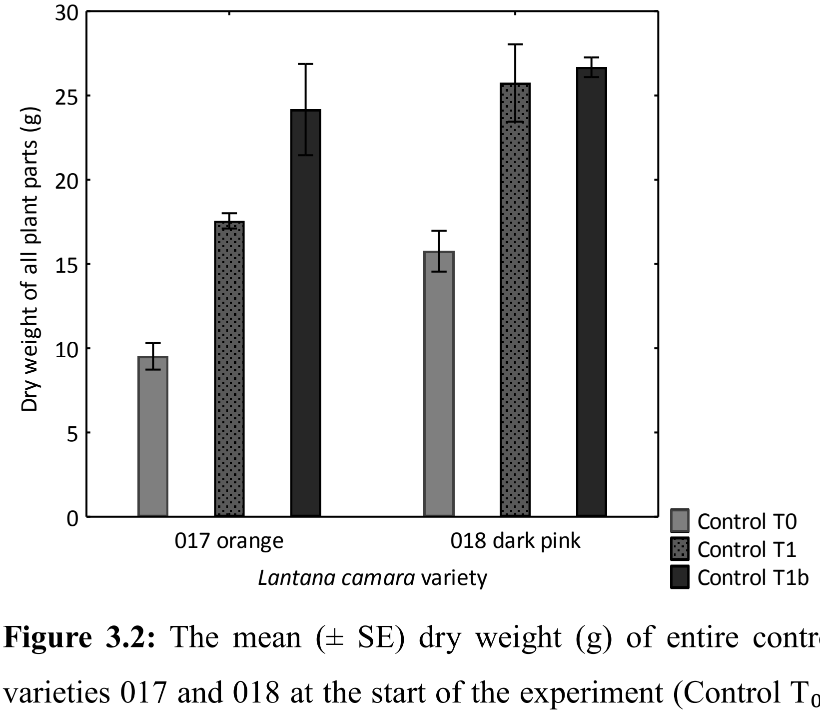


Figure 3.2: The mean (± SE) dry weight (g) of entire control plants of *Lantana camara* varieties 017 and 018 at the start of the experiment (Control T_0 - sacrificed at the start of the trial), after 35 days (Control T_1 - sacrificed after 35 days) and after 70 days of growth (Control T_1b – sacrificed after 70 days) without insects.

3.3.2 Galling levels on lantana varieties from exposure to apionids

Plants at the two adult exposure densities of 20 adults and 40 adults exposed for 5 days to both lantana varieties recorded low galling percentages, 4 % and 5 % respectively for variety 017 and did not differ statistically, and 7 % at both exposure densities for variety 018, when counted on day 35 (Table 3.1). A higher galling density was achieved on the test plants of both varieties after the second exposure, to ten additional insects, for a period of an, additional 35 days (and was thus counted at day 70). The galling densities recorded were 15 % and 13 % respectively for variety 017 and 25 % and 27 % respectively for variety 018, and neither differed statistically (Table 3.1). The proportion of galling achieved during this experiment conducted late in the season were low in comparison to the experiment conducted by Baars (2002), which were done in summer, and may be due to the insects entering a period of overwintering, or plant ques that discourage oviposition this late in the season. Higher galling percentages were recorded on lantana variety 018 in this experiment, and similarly in the one conducted by Baars (2002).

Table 3.1: Mean numbers (± SE) of leaves and galls obtained from plants of *Lantana camara* varieties 017 and 018, at different densities and exposure periods to adults of *Coelocephalapion camarae* and resultant galling proportions.

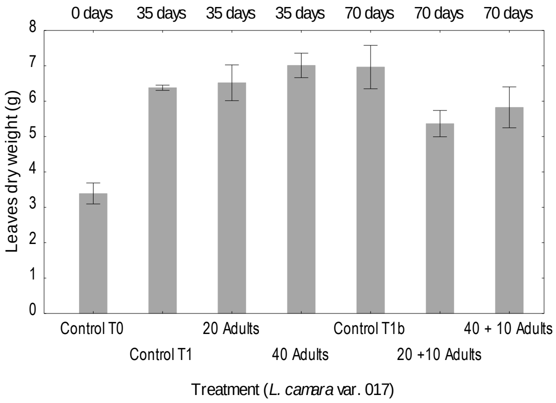
Treatments*				**Leaves per plant at day 0**	**Leaves on sampling day**	**Galls per plant**	**% galling**
Name	**Adults exposed**	**Days exposed**	**Days to sampling**				
Lantana camara variety 017							
T_0	0	0	0	85.1 ± 6.9	-	0.0 ± 0.0	0
T_1	0	0	35	97.8 ± 3.9	181.2 ± 17.5	0.0 ± 0.0	0
20A	20	5	35	111.6 ± 12.6	205.0 ± 13.4	7.8 ± 0.3	4
40A	40	5	35	110.2 ± 10.6	233.2 ± 12.3	10.8 ± 0.4	5
T_1b	0	0	70	98.0 ± 17.2	178.0 ± 10.0	0.0 ± 0.0	0
20+10A	20+10	5 + 35	70	81.0 ± 3.2	144.2 ± 15.0	22.0 ± 0.9	15
40+10A	40+10	5 + 35	70	87.6 ± 14.8	157.2 ± 10.0	20.2 ± 1.2	13
Lantana camara variety 018							
T_0	0	0	0	96.8 ± 4.1	-	0.0 ± 0.0	0
T_1	0	0	35	105.0 ± 11.7	164.2 ± 8.5	0.0 ± 0.0	0
20A	20	5	35	120.2 ± 12.1	156.6 ± 21.1	11.6 ± 0.8	7
40A	40	5	35	92.2 ± 15.7	162.8 ± 9.4	11.4 ± 0.6	7
T_1b	0	0	70	96.8 ± 3.0	119.4 ± 10.3	0.0 ± 0.0	0
20+10A	20+10	5 + 35	70	115.0 ± 13.0	128.0 ± 9.0	32.2 ± 5.6	25
40+10A	40+10	5 + 35	70	128.6 ± 17.0	133.4 ± 8.6	36.4 ± 0.9	27

*All treatments were replicated five times (n=5), except control T0, where n=10.

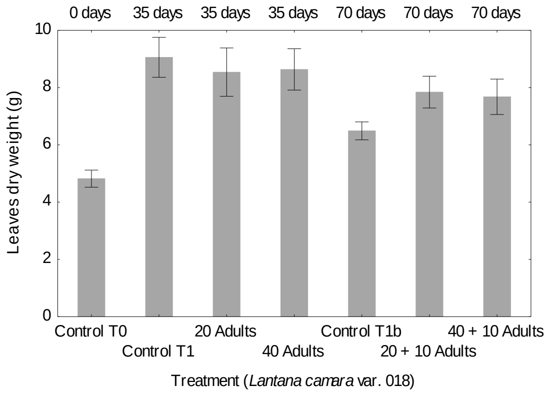
3.3.3 Effect of feeding and galling of apionids on growth of two lantana varieties

There were no statistically significant differences between any of the dry weights of respective plant parts (excluding flowers in variety 018 after 70 days that differed statistically) and their controls at any of the population density or exposure periods in either variety, where $P > 0.05$ (Figure 3.3 (a-j)). Although the dry weights of flowers for treatment 40 + 10 Adults on *L. camara* variety 018 was significantly higher than that of the control, KW-H (2, 15) = 6.151, $P = 0.0462$ (Figure 3.3 (d)), it may be an anomaly, and possibly due to the small proportion of the total weight of the plant material comprised by flowers, and an increased likelihood of chance, and is further elaborated on in the discussion. Seasonality had the greatest effect on flower production, as it reduced in both varieties when measured at 70 days compared to 35 (Figure 3.3 (c, d)).

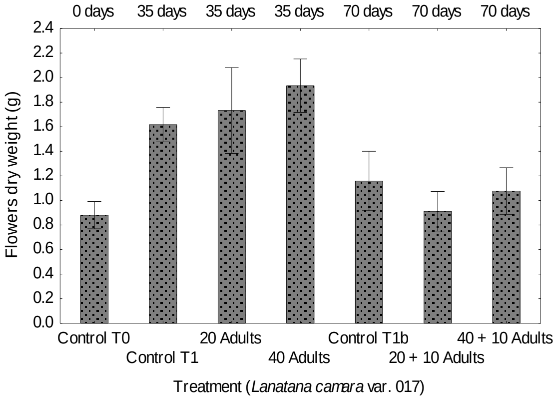
The dry weights of all individual and combined plant parts for lantana variety 017 was noticeably less for both adult density exposures after 70 days compared to the weights of its controls, although not statistically significant at $P > 0.05$ (Figure 3.3 (a, c, e, g & i)). The opposite effect, though barely noticeable can be seen for lantana variety 018; here, the dry weights of individual plant parts (excluding flowers at the lower adult exposure) and as whole plants weighed more in the plants exposed to both densities of apionid feeding and galling after 70 days, compared to its controls (Figure 3.3 (b, d, f, h & j)).


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Leaves dry weight (g)
0
1
2
3
4
5
6
7
8
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 +10 Adults
40 + 10 Adults
Treatment (*L. camara* var. 017)

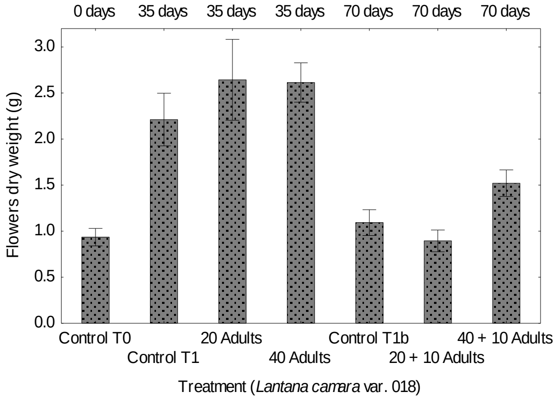
a)


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Leaves dry weight (g)
0
2
4
6
8
10
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 + 10 Adults
40 + 10 Adults
Treatment (*Lantana camara* var. 018)

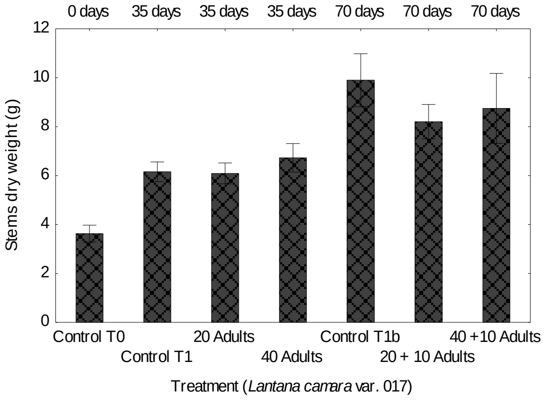
b)


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Flowers dry weight (g)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
2.4
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 + 10 Adults
40 + 10 Adults
Treatment (*Lanatana camara* var. 017)

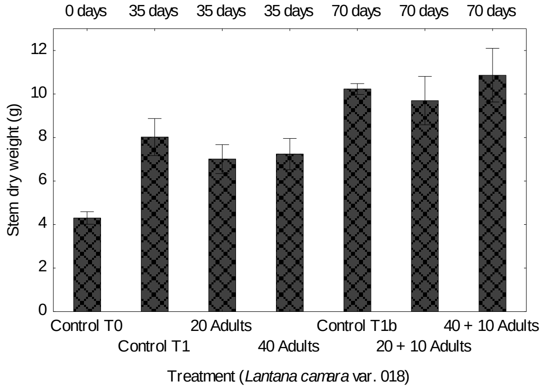
c)


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Flowers dry weight (g)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 + 10 Adults
40 + 10 Adults
Treatment (*Lantana camara* var. 018)

d)


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Stems dry weight (g)
0
2
4
6
8
10
12
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 + 10 Adults
40 +10 Adults
Treatment (*Lantana camara* var. 017)

e)


0 days
35 days
35 days
35 days
70 days
70 days
70 days
Stem dry weight (g)
0
2
4
6
8
10
12
Control T0
Control T1
20 Adults
40 Adults
Control T1b
20 + 10 Adults
40 + 10 Adults
Treatment (*Lantana camara* var. 018)

f)

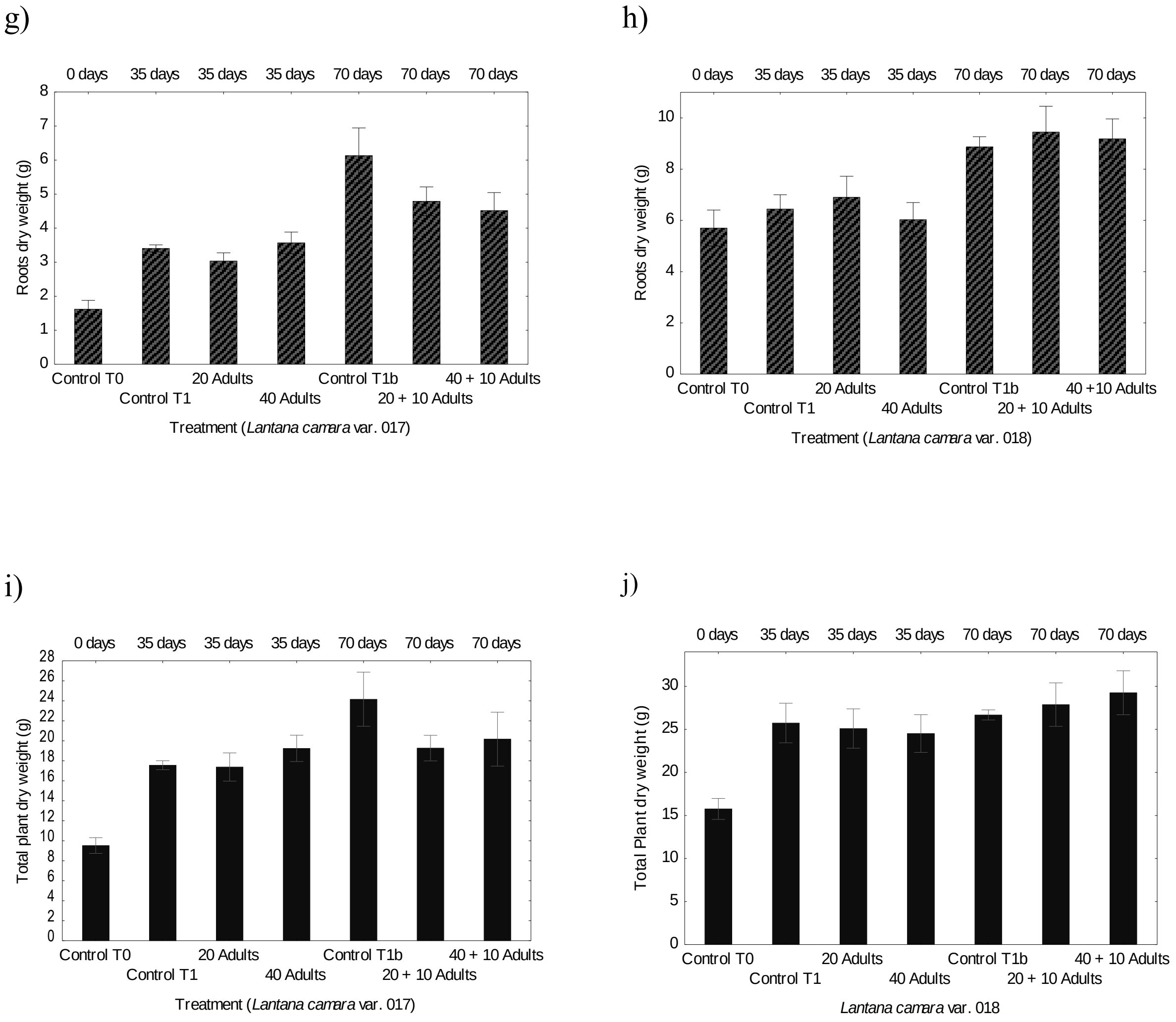


Figure 3.3: The effect of feeding and galling by different population densities and exposure periods to *Coelocephalapion camarae* on mean (± SE) dry weights (g) of plant parts and whole plants on *Lantana camara* varieties, 017 and 018 sampled at either 0, 35 or 70 days: leaves, var. 017 (a) and 018 (b); flowers (including flower stalk and seeds), var. 017 (c) and 018 (d); stems, var. 017 (e) and 018 (f) and roots (material below where the first root arises), var. 017 (g) and 018 (h) and total plant weight (combining leaves, flowers, stems and roots), var. 017 (i) and 018 (j).

3.4 DISCUSSION

Relatively low galling percentages were achieved in the experiments of this study, which was possibly an indication that the apionids were slowing down as they were going into an overwintering phase and therefore laid fewer eggs. *Coelocephalapion camarae* was previously reported to dramatically reduce egg laying in winter (Baars *et al.* 2007). In addition, the seasonal change affected the growth of the plants, and therefore likely indirectly insect performance as well. A review done by Cornelissen *et al.* (2008) of studies on the effects of plant vigour on insect herbivory, observed that vigorously growing plants attracted a higher percentage of insect herbivory.

Lantana camara var. 018 had a greater biomass at T_0 (start of the experiment), than that of *L. camara* var. 017, however, as the season progressed from late summer to mid-autumn (end of the experiment), *L. camara* var. 018 growth rate had slowed down more than that of *L. camara* var. 017 so that both had a similar biomass at that time.

There were various aspects of insect behaviour and plant parameters that differed between the experiments conducted here, and those of Baars (2002) that was probably influenced by the seasonal differences between these trials.

Although plants of the *Lantana camara* varieties were grown for the same duration, prior to the start of the experiment, plants of both varieties were about half the biomass of those used by Baars (2002). The plants in this experiment were grown a bit later in the year and fertilized only once with 3.6 grams of 3:2:1 (25), as opposed to twice, as by Baars (2002). When nitrogen is supplemented, it often enhances health, growth, reproduction, and survival (Mattson 1980). It is therefore possible that growth was enhanced more by the time difference and additional fertilizers in the trial of Baars (2002). This should however not have affected the results obtained, as it would have been relative to the biomass of control plants used in the experiment and galling was proportional to its relative plant size.

Plant growth was less affected by apionid feeding at similar galling levels, as higher galling densities in these trials were similar to those at low exposures in trials by Baars (2002). This may likely be due to the timing of the experiments, as those conducted by Baars (2002) was in mid-summer, and these were between late summer and mid-autumn and may possibly be ascribed to the reduced growth rate experienced later in the season, as a reduced growth was observed in the control plants as well. Crawley (1984) reported that the ability of a plant to compensate for herbivory is regulated by, the physiological changes within the plant and the amount of time remaining in the growing season, as herbivory early in the growing season will be easily compensated for as there are nutrient flushes experienced by most plants that allows the maximum uptake of nutrients facilitating recovery, whereas later in the season plants suffer lower nutrient availability and don’t recover so readily. Thus the season itself as factor played a large role in these experiments. Maschinski and Whitham (1989) similarly reported that the factors that generally limit plant growth are also the factors that will limit the compensatory response in plants.

The dry weight of the flowers in the treatment that were exposed to 40 and then a further 10 adults on *L. camara* var. 018 after 70 days was significantly higher than that of the control, and may have been attributed to the number of adults the plants were exposed to. Both insect density treatments achieved similar galling percentages on this variety after 70 days, however, the adult feeding may have influenced the spike in flowers as this treatment had been exposed to 40 adults initially, where the other treatment were exposed to 20.

Although there was no statistical significant differences in biomass of plant parts under different herbivory treatments (excluding flowers as discussed above), and the two *L. camara* varieties responded very differently to *C. camarae* feeding and galling in this study. Similarly, the apionid responded differently to the varieties. *L. camara* var. 017, was growing more vigorously during the duration of this experiment, and had more leaves after 70 days than *L. camara* var. 018, but lower galling ratios were achieved on *L. camara* var. 017, than on *L. camara* var. 018 for the same treatment. This may be related to one or a combination of several factors; plant chemicals, including nitrogen levels may affect this interaction. It was reported that when plant tissue or organs are rapidly expanding during seasonal growth cycles, there is a peak in nitrogen concentration and as soon as growth tissue starts to wane the nitrogen concentrations drop sharply (Mattson 1980). Vascular tissue of *L. camara* var. 017 was consumed sooner than that of *L. camara* var. 018 (Chapter 2). The rate at which the larvae of *C. camarae* consume vascular tissue in different varieties may also influence the plant response in varieties.

The genus *Lantana* is extremely variable (Munir 1996), and was subject to considerable plant breeding efforts to produce several ornamental varieties (Stirton 1977) and there is thus a great deal of variation between the varieties. The effectiveness of *C. camarae* on *L. camara* will probably be largely dependent on the variation in varieties, and the response plants, as this will affect the level of damage that can be inflicted. *Coelocephalapion camarae* herbivory may be more effective in inflicting damage on some *L. camara* varieties compared to others.

CHAPTER 4

THE EFFECT OF *COELOCEPHALAPION CAMARAE* ON TOTAL SOLUBLE SUGARS AND STARCH IN PLANTS OF TWO *LANTANA CAMARA* VARIETIES

4.1 INTRODUCTION

Photosynthesis is the process by which atmospheric CO_2 is fixed into sucrose and other carbohydrates (photosynthates), by radiation from the sun, using moisture and nutrients from the soil (Kooke & Keurentjies 2012), and mainly occur in the plants leaves (Iglesias and Podesta 2005; Inbar *et al*. 1995). Plants are dependent on photosynthesis to acquire energy and resources for metabolism which is an integral part of growth, development and performance (Kooke & Keurentjies 2012). A plant's sucrose and carbohydrates, and photoassimilated carbon moves from sources (mostly leaves) to sinks (e.g. areas of growth, or plant parts for storage for regrowth) in a plant, using the pressure flow mechanism through the phloem (Inbar *et al*. 1995; Iglesias & Podesta 2005). Lantana becomes leafless in winter in large areas in South Africa, and may rely on accumulated carbohydrates for regrowth in spring. Starch and sugars are stored in all perennial parts (stems and roots) of a plant and these reserves are used when growth resumes in spring (Loescher *et al*. 1990).

The leaf petiole is the organ that connects the leaf blade with the plants stem, and its main function is the transport of organic and inorganic molecules between the blade and the stem (Maksymowych *et al*. 1983). Studies conducted on cyclamen and celery leaves showed that transient carbohydrate accumulation occurred in petioles and axial organs (Keller & Matile 1989; Rothe *et al.* 1999). It may therefore be that *C. camarae* galls are formed in the leaf petioles of *L. camara* to use the accumulated photoassimilates. In Chapter two of this study, it was shown that the larval feeding of *C. camarae* severed both phloem and xylem bundles in petioles, which may prevent the transport of sucrose and carbohydrates to the stems and roots of *L. camara*. It may also hamper the translocation of solutes from the roots to the effected leaves.

Therefore the aim of this chapter was to measure the effect of galling by different population levels of *Coelocephalapion camarae* on the production of sugar and starch, and their redistribution to different plant parts, in two varieties of lantana, particularly late in the growth season, when healthy plants were expected to accumulate carbohydrate reserves in stems and roots.

4.2 MATERIALS AND METHODS

The materials and methods in chapter three of this study described the exposure levels and times of the two lantana varieties to *C. camarae*. The sugar and starch analysis was performed using the same dried plant samples (leaves, stems and roots) obtained from the biomass experiments, with the exception of flowers, which was excluded as it comprised a very small sample weight, (under the 50 mg sample that was required for analysis). The Control T_0 samples were also not analysed for total soluble sugars and starch concentrations as the substances used in the analysis were expensive and the Control T_0 samples were not required as comparisons were being made in the experiments between the plants exposed to apionids and their controls. The plant samples were ground up in a micro hammer mill (Culatti) to pass 40-mesh, which gave samples a maximum particle size of 0.42 mm diameter. The hammer mill was cleaned with compressed air after the milling of each sample to prevent contamination of the next sample. Each sample was stored in an airtight container in the dark, at room temperature until being used for analysis. Chow and Landhäusser (2004) described a method to determine total soluble sugar and starch concentrations in woody plants. This method was used in this study and was employed as follows:

4.2.1 Sugar and Starch extraction from plant samples

50 mg of each dry ground up tissue sample was weighed off and mixed with 5 ml of 80% ethanol, placed in a 95°C water bath for 10 minutes to extract the soluble sugars and thereafter centrifuged at 2500 rpm for 5 minutes. This ethanol extraction and centrifugation process was performed twice. The supernatants (clear fluid above the sediment) of the two extractions then contained the total soluble sugars (TSS) and was combined and used for TSS analysis. The pellet (the sediment) contained the starch and was used for the starch analysis. The pellets were stored at -20°C until analysis.

4.2.2 Determination of the relationship between light absorption and total soluble sugar in samples

Following Chow and Landhäusser (2004), a mixture of Glucose (30%), Fructose (50%) and Galactose (20%) (GFG) was used as a standard for the colorimetric determination of TSS. The concentrated GFG solution was prepared by mixing, 60mg glucose, 100mg fructose and 40mg galactose into 1 litre of deionized distilled water (ddH$_2$O). This concentrated solution was used to prepare a GFG dilution range from 25 to 200 mg/l at 25 mg/l increments. For the

TSS assay, 1 ml of 2% phenol and 2.5 ml of 95.5% sulphuric acid was added to 1 ml of solution for each concentration. A parallel sample was prepared at the same time, adding 1 ml of ddH_2O instead of phenol for the sample without phenol. All samples were incubated at room temperature in the dark for 10 minutes and then placed into a 22°C water bath for 30 minutes. Light absorbance of the different concentrations were read at 490nm using a spectrometer (Spectroquant Pharo 300). Regression graphs were plotted of light absorbance against sugar concentration for assays with phenol and ones without, to obtain the regression equation ($y = a + b(x)$). Using the absorbance coefficients obtained from these equations, the corrected total soluble sugar concentrations could be calculated. The regression equations for the GFG assay with phenol was; $y = 0.0034 + 0.004x;\ r^2 = 0.9957$, and the regression for the GFG assay without phenol was; $y = -0.0003 + 0.0004x;\ r^2 = 0.8587$.

4.2.3 Determination of total soluble sugar from plant samples

For the TSS assay of the plant samples being analysed, 1ml of 2% phenol and 2.5ml of 95.5% sulphuric acid was added to 1ml of supernatant from the extracted tissue sample. A parallel sample was prepared at the same time, adding 1 ml of ddH_2O instead of phenol for the sample without phenol. These parallel assays with and without phenol were performed to correct the interfering effects of other substances present in the plant tissue. All samples were incubated at room temperature in the dark for 10 minutes and then placed into a 22°C water bath for 30 minutes. The absorbance was read at 490nm using a spectrometer (Spectroquant Pharo 300). The corrected total soluble sugars was calculated, using the following equation:

$$[sugar]\ corrected = \frac{\mathrm{A} - \mathrm{A}'}{as - a's}$$

Where:

A: absorbance with phenol

A': absorbance without phenol

as: absorbance co-efficient of GFG standard with phenol (0.004)

a's: absorbance co-efficient of GFG standard without phenol (0.0004)

to convert total soluble sugars to mg/g sample = *Dilution* X *0.1*

Calculation for dilution:

Dilution = [sugar] corrected X *dilution factor*

Dilution factor = volume of phenol + sulphuric acid/volume of sample

which is *2 + 2.5/1 = 4.5 ml*

4.2.4 Determination of the relationship between light absorption and starch in samples

Starch was converted into glucose by enzymatic digestion before colorimetric analysis. A 1% glucose solution was used as a standard for the colorimetric determination of starch. The 1% glucose solution was mixed by dissolving 100mg of anhydrous glucose in 10ml of 0.05 M NaOAc (sodium acetate) buffer solution.

The 0.05 M Sodium acetate buffer, pH 5.1 was made by adding 2.84ml of glacial acetic acid to 900ml of ddH2O. The pH was adjusted to 5.1 with the addition of 30% NaOH (sodium hydroxide). It was then topped up, to reach 1000 ml using ddH_2O.

The 1% glucose solution and the 0.005 M sodium acetate buffer was used to prepare the glucose dilution range from 0, 0.5, 1, 2, 4, 8 and 10mg of glucose/100ml of solution. 0.2ml of each concentration was used to run the starch assay. Absorbance's of the different concentrations were read at 525nm using a spectrometer (Spectroquant Pharo 300). A regression graph, was plotted of light absorbance against glucose concentration to obtain the regression equation ($y = 0.0365 + 0.0677x; r^2 = 0.9959$).

4.2.5 Determination of starch in plant samples

For the starch assay 0.5 ml digestive enzyme mixture (the digestive enzyme mixture contains 1000 Uml^{-1} of Alpha amylase (from *Bacillus licheniformis*) and 5 Uml^{-1} of Amyloglucosidase (from *Aspergillus niger*) in 0.05 M sodium acetate buffer (pH 5.1)) was added to the pellet (residue containing starch after ethanol extraction) of each sample and incubated in a 50°C water bath for 20 hours. Thereafter the digest was centrifuged at 2500 rpm for 10 minutes. To the supernatant (clear fluid above the sediment) 2.5 ml sodium acetate buffer was added; this is a 1:5 dilution which is suitable for tissue with 1 - 4% starch. Of this, 0.2 ml is used, to which 2 ml of peroxidase-glucose oxidase reagent (PGO) was added. This was incubated in the dark at room temperature for 45 minutes. After incubation, 0.4 ml of 75% sulphuric acid was added and absorbance read at 525nm. The glucose concentrations of the samples were obtained by substituting the absorbance readings into the x variable to get the glucose concentrations. The milligrams of starch per milligram of sample was calculated using the equation:

$$mg\ of\ starch\ /\ mg\ of\ sample = y_g d_f v h_f / dw$$

where:

y_g is the glucose concentration (mg/100 ml)

d_f is the dilution factor (6 for a 1:5 dilution)

v is the original volume of starch extract (3 ml)

h_f is the starch hydrolysis factor 0.9 (Volenec 1986)

dw is the original dry weight of the sample (50 mg)

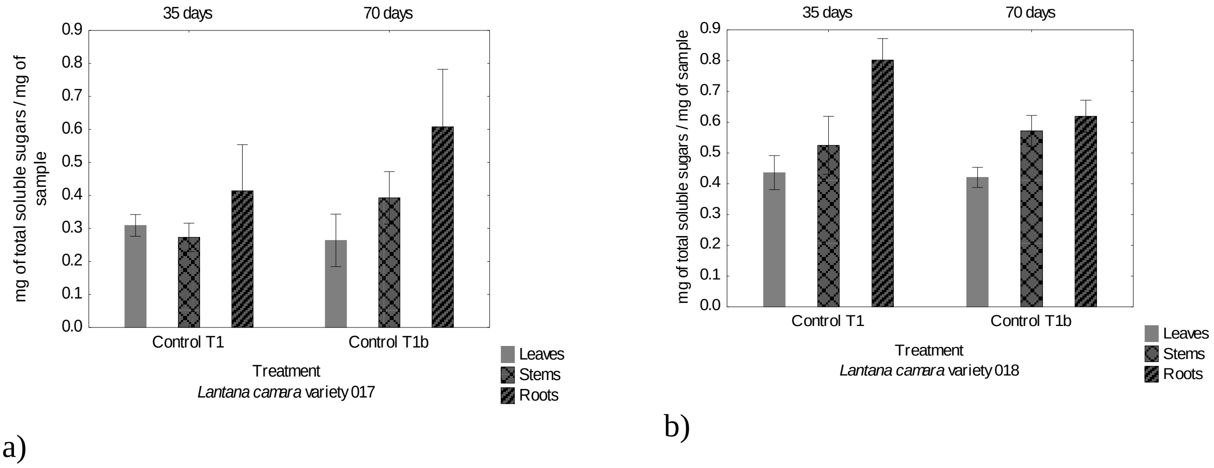
4.2.6 Statistical analysis

The data was normally distributed and an ANOVA was used to compare means. Where there were significant differences between tests and controls, either the Tukey or Fisher's LSD post hoc tests were used and are stated where relevant. To compare the means between starch concentrations (mg/mg) of Control T_1 (35 day) and Control T_1b (70 day) between the different plant parts of the control plants of the two *L. camara* varieties T-tests were used because comparison was being made between means of two samples.

4.3 RESULTS

4.3.1 Effect on sugar concentrations by growth and seasonal changes in L. camara *varieties*

The TSS concentrations for *L. camara* var.017 increased over 35 days in stems and roots from Control T_1 to Control T_1b, and decreased for leaves (Figure 4.1 a). *Lantana camara* var.018 had an increase in the TSS concentrations of stems, and a decrease in the leaves and roots over the same period (Control T_1 to Control T_1b), there was a notable decrease in roots although not statistically significant (Figure 4.1 b). The TSS concentrations was consistently the highest in roots, followed by stems for both lantana varieties (Figures 4.1 a & b).



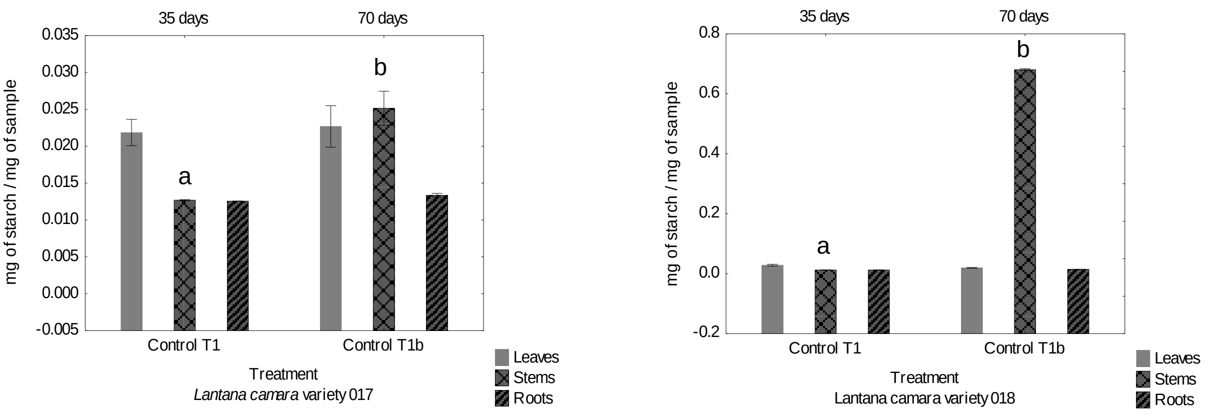
a)

b)

Figure 4.1: The mean (± SE) total soluble sugar concentrations (mg/mg) of different plant parts of Control T_1 (35 day) and Control T_1b (70 day) for; a) *Lantana camara* var. 017, and b) *Lantana camara* var. 018

Starch concentrations in leaves, stems and roots in *L. camara* var. 017 increased from Control T_1 to Control T_1b, but the increase in the concentration in the stems from Control T_1 to Control T_1b was statistically significant, $P = 0.000648$, almost doubling its concentration from 0.013 (± 0.000) mg to 0.025 (± 0.002) mg (Figure 4.2 a). Similarly, the starch concentrations in *L. camara* var.018 increased in stems and roots, however, it decreased in leaves from Control T_1 to Control T_1b (Figure 4.1b). The stem starch concentration in this variety however increased significantly, $P = 0.000168$ (T-test), (by 52 times, from 0.013 (± 0.000) mg to 0.681 (± 0.003) mg) (Figure 4.2 b). The increases in root starch in both lantana varieties from Control T_1 to Control T_1b, was relatively small. It increased from 0.0126 (± 0.0000) mg to 0.0133 (± 0.0002) mg in *L. camara* var. 017 and from 0.0124 (± 0.0000) mg to 0.0146 (± 0.0006) mg in *L. camara* var. 018.

The most substantial increase in the concentration of starch were in the stems of both varieties of *L. camara* and may indicate stems to be the main plant part where starch reserves are stored before winter. However, the increase in starch concentrations in *L. camara* var. 018 was much more substantial, compared to *L. camara* var. 017 (Figures 4.2 a & b).



a) b)

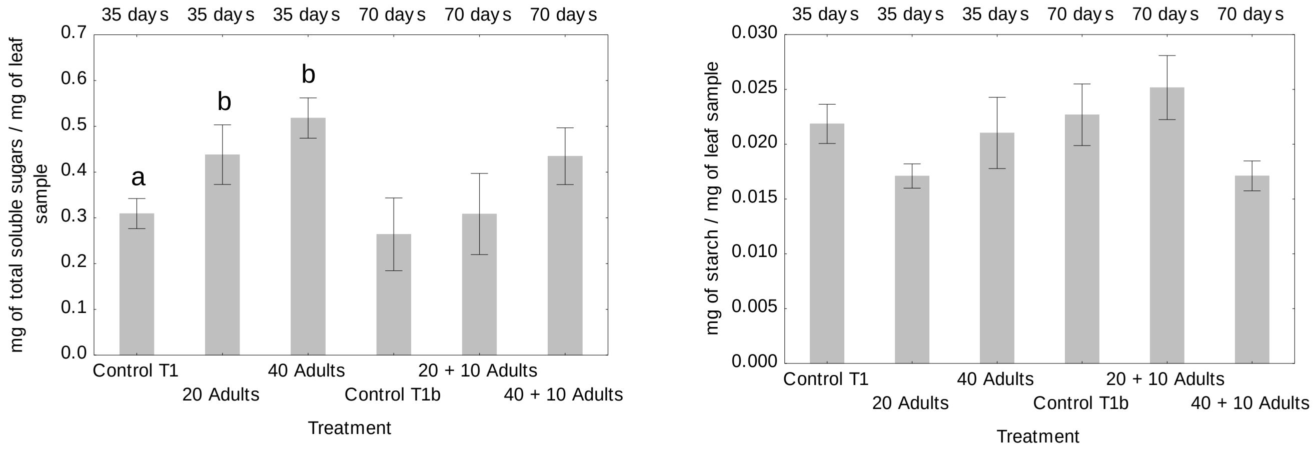
Figure 4.2: The mean (± SE) starch concentrations (mg/mg) of Control T_1 (35 day) and Control T_1b (70 day) for; a) *Lantana camara* var. 017, and b) *Lantana camara* var. 018. Means of bars with different letters are significantly different, $P < 0.001$, T-test).

4.3.2 Effect of the apionid on sugar and starch concentrations of Lantana camara *variety 017*

- *Leaves*

The TSS concentrations in leaves were higher in the treatments (exposed to apionids) compared to the control, for both experiments, destructively sampled after 35 days and after70 days for *L. camara* var. 017 (Figure 4.3 a). The experiment, that was destructively sampled after 35 days and had galling percentages of 4 % and 5 %, for the 20 and 40 adult exposure densities showed statistically significant differences, F (2, 12) = 4, 5979, $P = 0.0329$ (Figure 4.3a). A Tukey post hoc test was performed for significant differences between means and showed that the concentration of the 40 adult density exposure was significantly higher than the concentration of the control, $P = 0.028$. The total soluble sugar concentrations increased with the increased apionid densities although galling percentages achieved were very similar in both adult exposure densities for the respective experiments destructively sampled at 35 and 70 days. The soluble sugars did not move from the leaves to the rest of the plant or the apionids affect the allocation of the sugars. This is possibly due to the damage caused to vascular tissue by larval feeding in the petiole. The adult and larval feeding maybe also be stimulating increased sugar production.

The starch concentration in the leaves for both adult exposure densities after 35 days was lower than the control, although not statistically significant (Figure 4.3 b). The starch concentration after 70 days increased at the low adult exposure density and decreased at the high adult exposure density compared to the control (Figure 4.3 b).



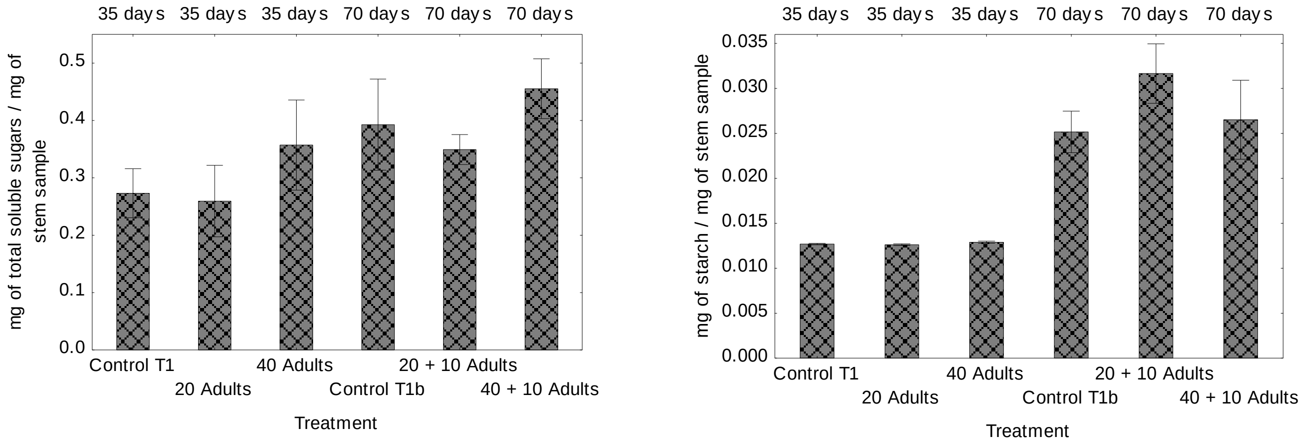
a) b)

Figure 4.3: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of leaves of *Lantana camara* variety 017, sampled at either 35 or 70 days: a) total soluble sugar concentration in leaves; b) starch concentration in leaves. Means of bars with the same letter are not significantly different at $P > 0.05$, (Tukey post hoc test).

- *Stems*

The total soluble sugar concentrations in the stems after 35 and 70 days for both adult exposure densities were not statistically significantly different from its respective controls (Figure 4.4 a). There was however a slight decrease in the concentration of the total soluble sugars for plants exposed to 20 adults (35 days) and 20 + 10 adults (70 days) densities in comparison to their respective controls, but a slight increase in the TSS concentration to plants exposed to the 40 adult (35 days) and 40 +10 adult (70 days) densities compared to their respective controls (Figure 4.4 a).

There were no significant differences between means of treatments and corresponding controls in starch concentrations in lantana variety 017. The starch concentration in stems after 35 days was the same for the control as well as both adult exposure densities (Figure 4.4 b). Starch concentrations in stems after 70 days for both adult density exposures, was noticeably higher than the control (Figure 4.4 b). There was however, a general increase in starch concentrations in the stems, as indicated by the increase from Control T_1 (35 day) and Control T_1b (70 day) and is probably influenced more by seasonal changes. Although not statistically significant, starch concentrations after 70 days was generally higher, and plants exposed to the apionids had higher starch concentrations compared to the control.



a)

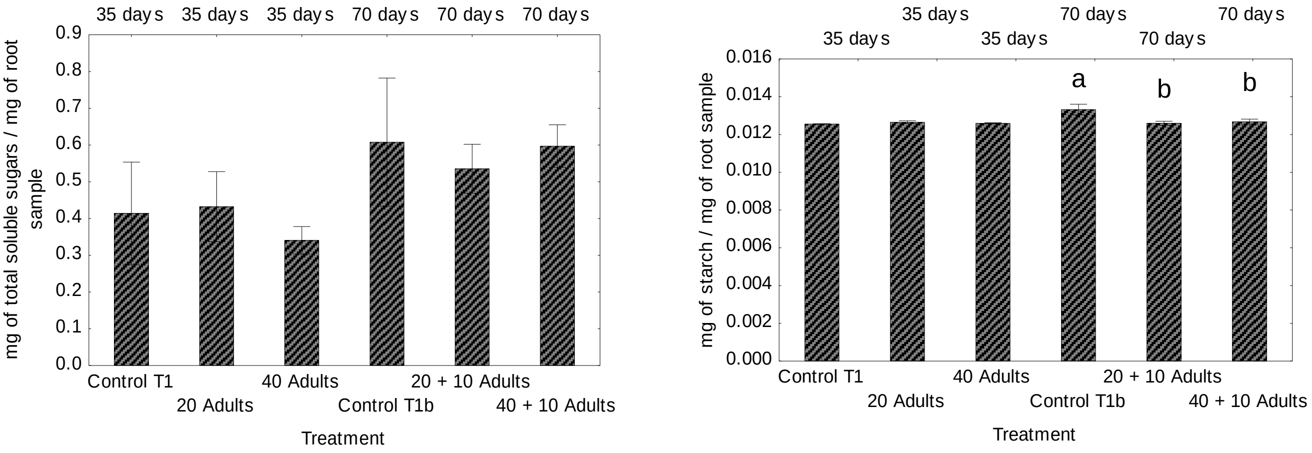
b)

Figure 4.4: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of stems of *Lantana camara* variety 017, sampled at either 35 or 70 days: a) total soluble sugar concentration in stems; b) starch concentration in stems

- *Roots*

There was a decrease, although not statistically significant, in the TSS concentration after 35 days in plants exposed to the 40 adult density and to both the adult densities after 70 days, compared to their respective controls (Figure 4.5 a).

The starch concentrations in the roots were the same for both adult exposure densities as well as their control after 35 days (Figure 4.5 b). There was however, a statistically significant difference in sampled after 70 days between the tests and control, F (2, 12) = 4.2909, *P* = 0.0393 (Figure 4.5 b). The Fisher's LSD post hoc test was performed for the significant differences between means and showed that the starch concentrations in the test with 20 + 10 adults, was significantly lower, than the control, *P* = 0.0207. The concentrations of starch in the test with 40 + 10 adults were also significantly lower than the control, *P* = 0.0342.



b)

a)

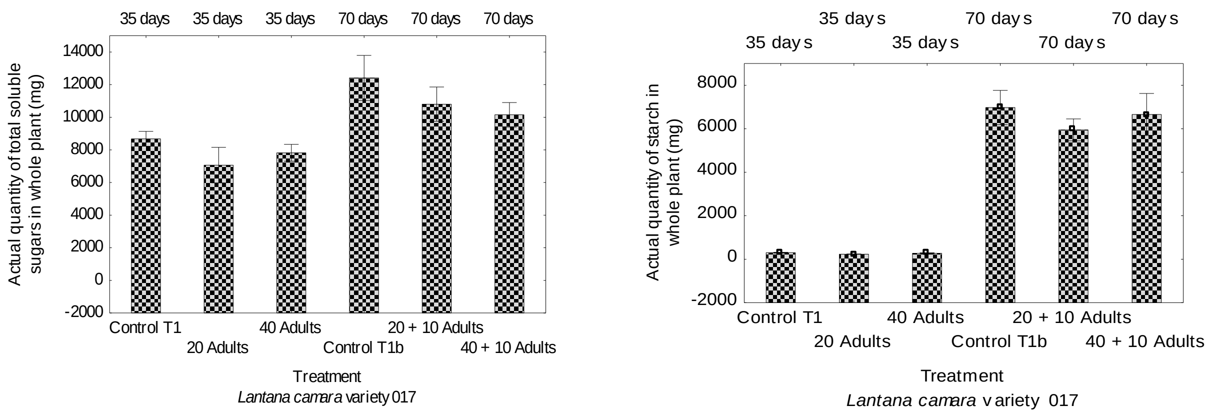
Figure 4.5: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of roots of *Lantana camara* variety 017, sampled at either 35 or 70 days: a) total soluble sugar concentration in roots; b) starch concentration in roots. Means of bars with the same letter are not significantly different at *P* > 0.05, (Fisher's LSD post hoc test).

- *Whole plants*

The effects on actual total soluble sugars and starch quantities in whole plants, with and without apionids was calculated by multiplying the dry weights of leaves, stems or roots, in milligrams (Chapter 3), to its relevant concentration of total soluble sugar or starch.

The quantity of total soluble sugars in plants of *L. camara* var. 017, although not statistically significant, was lower in the plants exposed to both adult exposure densities, and in both experiments destructively sampled at 35 and 70 days (Figure 4.6 a).

The starch quantities between the tests and the controls after 35 and 70 days were not statistically significant. The starch concentration after 35 days, although not clearly visible on the graph, due to scaling, was lower for both adult exposure densities than in the control (Figure 4.6 b). Plants exposed to the lower adult density after both 35 and 70 days had lower quantities of starch than the higher adult exposure density (Figure 4.6 b).



a) b)

Figure 4.6: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) actual quantities of total soluble sugars and starch of whole plants of *Lantana camara* variety 017, sampled at either 35 or 70 days: a) total soluble sugar in plants; b) starch in plants.

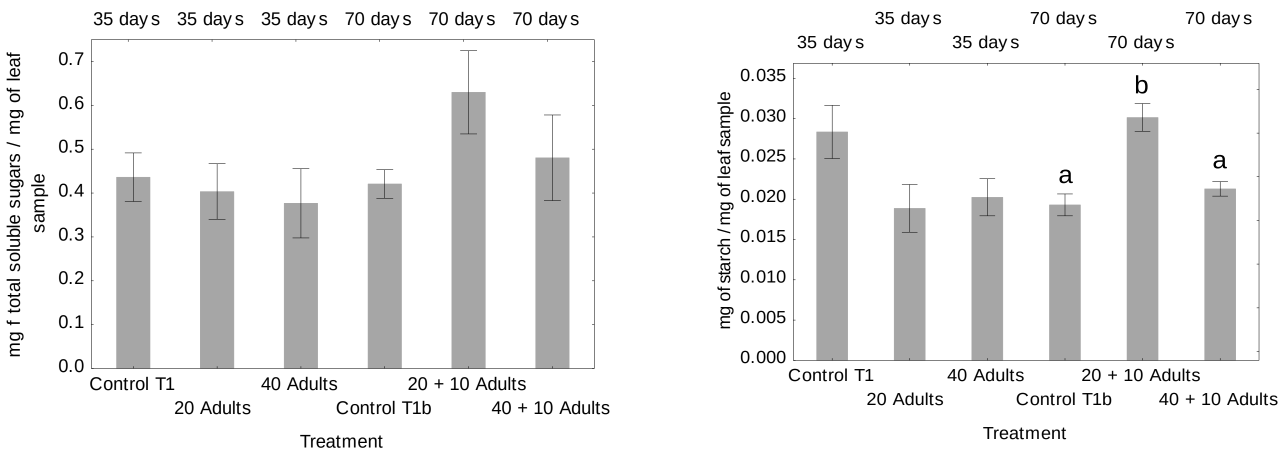
4.3.3 Effect of the apionid on sugar and starch concentrations of Lantana camara *variety 018*

- *Leaves*

The concentration of TSS in leaves of *L. camara* var. 018, was lower in both adult exposure densities after 35 days compared to the control (Figure 4.7 a). The concentrations after 70 days were, however higher in the leaves, for both the adult exposure densities than the control, and the 20 +10 adult exposure density was higher than the 40 + 10 adult exposure density (Figure 4.7 a).

The starch concentrations were lower after 35 days for the both adult exposure densities than for the control. However, the starch concentrations differed significantly after 70 days between tests and control, F (2, 12) = 17.8329, *P* = 0.0003 (Figure 4.7 b). The Tukey post hoc test was performed for significant differences between means and showed that the starch concentrations were significantly higher for the 20 + 10 adult exposure density than the control, *P* = 0.000469 as well as than the 40 + 10 adult exposure density, *P* = 0.001828 (Figure 4.7 b).

The concentrations of both total soluble sugars and starch in leaves of *L. camara* var. 018 were higher for the 20 + 10 adult exposure density, although they had very similar galling percentages, and may have been affected by adult feeding.



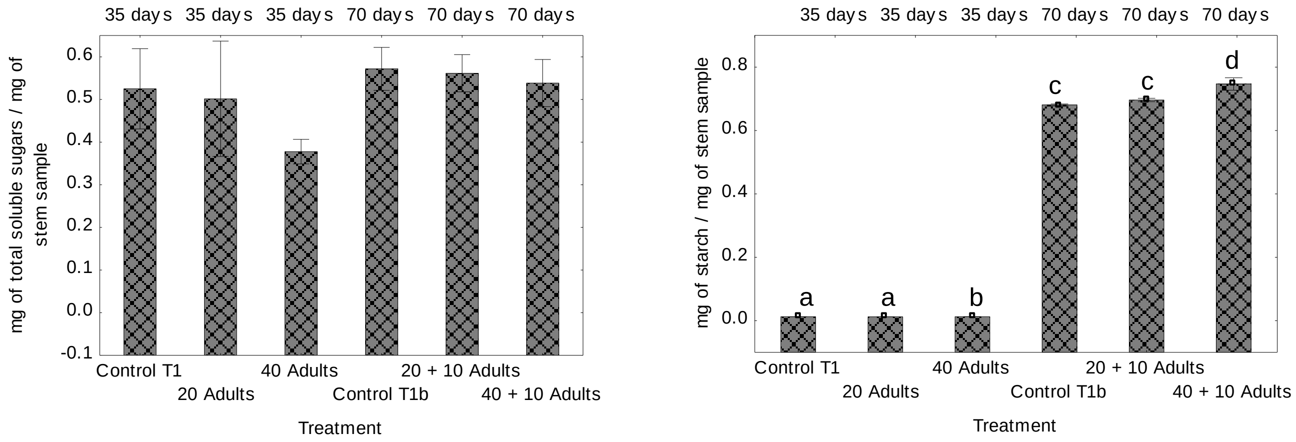
a) b)

Figure 4.7: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of leaves of *Lantana camara* variety 018, sampled at either 35 or 70 days: a) total soluble sugar concentration in leaves; b) starch concentration in leaves. Means of bars with the same letter are not significantly different at *P* > 0.005, (Tukey post hoc test).

- *Stems*

The TSS concentration in stems of *L. camara* var. 018 after 35 days for both adult exposure densities was lower than the control (Figure 4.8 a). The plants exposed to 40 adults, had a much lower TSS concentrations than the control (Figure 4.8 a). Although very slight, the TSS concentration in stems for both adult exposure densities after 70 days was lower than that of the control (Figure 4.8 a).

There was a statistically significant difference in starch concentrations in stems of *L. camara* var. 018 between plants for both apionid density exposures after 35 days, F (2, 12) = 4.4816, *P* = 0.0352 (Figure 4.8 b). The Fishers LSD post hoc test was performed for the significant differences between means and showed that the 40 adults exposure density had a significantly higher starch concentration than both, the control, *P* = 0.035 and the 20 adult exposure density, *P* = 0.017. Although, these differences are barely visible on the graph, the concentrations for the control being, 0.0125 (± 0.0001) mg starch / mg stem sample and that for the 20 and 40 adult exposure density being, 0.0124 (± 0.0001) mg and 0.0127 (± 0.0001) mg starch / mg stem sample respectively varied significantly (Figure 4.8 b).The starch concentration in the stems after 70 days for both adult exposure densities are statistically significantly higher than the concentration in the control, F (2, 12) = 8.5032, *P* = 0.0050 (Figure 4.8 b). The Tukey post hoc test was performed for significant differences between means and showed that the high adult exposure density had a significantly higher starch concentration than the control, *P* = 0.0053, and, than that of the low adult exposure density, *P* = 0.027, after 70 days.



a) b)

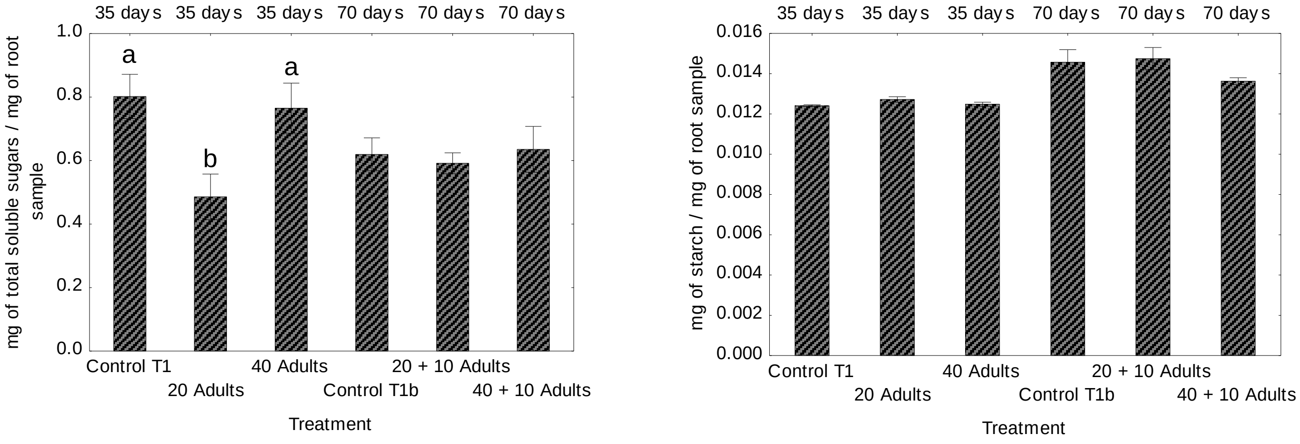
Figure 4.8: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of stems of *Lantana camara* variety 018, sampled at either 35 or 70 days: a) total soluble sugar concentration in stems; b) starch concentration in stems. Means of bars for graph (b) with the same letter are not significantly different, *P* > 0.05, (Fisher's LSD post hoc test). Means of bars for graph (a) with the same letter are not significantly different *P* > 0.05, (Tukey post hoc test).

- *Roots*

There was a statistically significant difference in concentrations of total soluble sugar in roots of *L. camara* var. 018 for treatments and controls destructively sampled after 35 days, F (2, 12) = 5.515, *P* = 0.020 (Figure 4.9 a). The Tukey post hoc test was performed to identify significant differences between means and showed that the total soluble sugar concentration in the control was significantly higher than for the 20 adult exposure density, *P* = 0.026 and that the TSS concentration for the 40 adult exposure density was also significantly higher than in the 20 adults exposure density, *P* = 0.049. The 70 day experiment, although not statistically significant, showed the same trends as the 35 day experiment, with the TSS concentration in the control being higher than that of the 20 adult exposure density and the concentration of the 40 adult exposure density being higher than in the 20 adult exposure density (Figure 4.9 a).

The starch concentrations after 35 days were virtually the same for both adult exposure densities and control (Figure 4.9 b). The starch concentration was lower after 70 days for the

40 + 10 adult exposure density compared to the control (Figure 4.9 b). The 20 + 10 adult exposure density concentration was the same as that of the control (Figure 49 b).

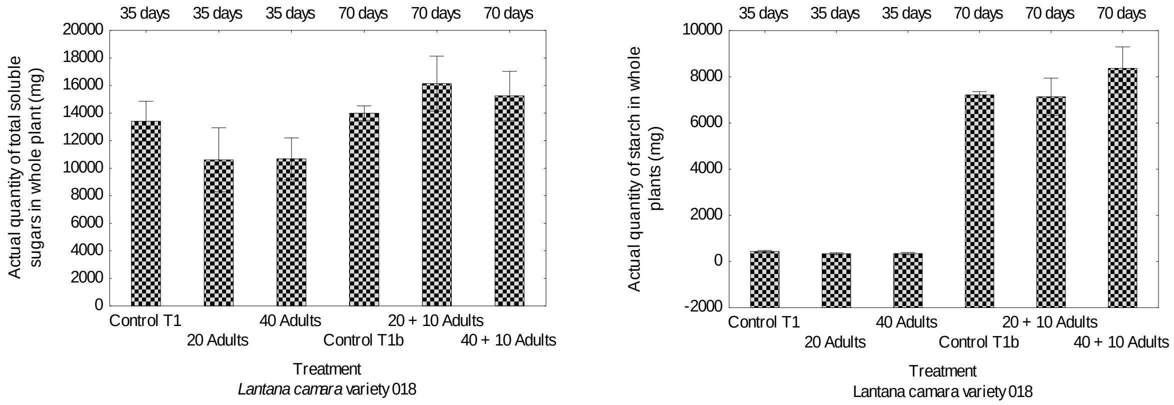


a) b)

Figure 4.9: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) total soluble sugars and starch of roots of *Lantana camara* variety 018, sampled at either 35 or 70 days: a) total soluble sugar concentration in roots; b) starch concentration in roots. Means of bars with the same letter are not significantly different at $P > 0.05$, (Tukey post hoc test).

- *Whole plants*

The means of actual quantities of total soluble sugars and starch were not statistically significant different between test and control plants after 35 or 70 days (Figure 4.10 a & b). The actual quantity of TSS in plants of *L. camara* var. 018, although not statistically significant, was lower in the plants exposed to both adult densities after 35 days than the control (Figure 4.10 a). The actual TSS quantities after 70 days, was opposite of that of those after 35 days, in that the quantities were higher for both adult exposure densities than the control. The actual quantity of starch after 35 days, although not readily visible on the graph, was lower at both the low and high adult exposure densities than the control (Figure 4.10 b). The actual starch quantities were; Control T_1 = 436.41 (± 42.14) mg, 20 Adults = 337.78 (± 43.14) mg and 40 Adults = 347.88 (± 52.94) mg. The high (40 adult) exposure density starch quantity was slightly lower than that of the low (20 adult) exposure density. The quantities after 70 days were similar for the control and the low adult exposure density. The starch quantity for the high adult exposure density was higher than that of the control.



a) b)

Figure 4.10: The effect of feeding and galling by different population densities and exposure periods of *Coelocephalapion camarae* on mean (± SE) actual quantities of total soluble sugars and starch of whole plants of *Lantana camara* variety 018, sampled at either 35 or 70 days: a) total soluble sugar in plants; b) starch in plants.

4.4 DISCUSSION

The TSS and starch concentrations were measured in this chapter late in the growing season to determine the effects of *Coelocephalapion camarae* on these important resources during this time of year as it was assumed to affect the ability of the plant to recover after the dormant season. Other authors showed that plants that are consumed early in the growing season had higher fitness than those consumed later in the growing season (Maschinski & Whitham 1989; Tiffin 2000). Carbohydrate reserves change dramatically throughout the year within plants, but increase late in the growing season for storage in all perennial organs of woody plants, and the highest concentrations are usually found in root tissue (Loescher *et al.* 1990). The storage organ for *L. camara* is most likely the stems as the starch in stems doubled in *L. camara* var. 017 and increased 52 times in *L. camara* var. 018 between early-autumn to mid-autumn (Figure 4.2 b of this chapter). The translocation of assimilates from photosynthesis from the leaves, to growing tissue, reproductive parts and storage organs is the basis of plant performance, as it affects plant metabolism (Komor 2000). The translocation of reserves may have been enhanced by the apionid feeding and galling, as it was shown that feeding and chemical secretions of galling insects cause considerable stress to attacked plant tissues, to which the plant responds by translocating photoassimilates and growth promoters to the areas under stress (Raman 2007).

The total soluble sugars and starch concentrations varied in the different plant parts and between the two *L. camara* varieties. The galling percentages that were achieved at the low and high adult exposure densities were similar, for each of the lantana varieties, in each experiment destructively sampled after 35 and 70 days and differences noted at the different apionid exposure densities are therefore more likely due to differences in levels of adult feeding rather than the larval galling. The number of adults feeding on the plant seems to influence the TSS and starch concentrations in different plant parts. Adults feeding caused small 'shot holes' in the leaves, which also removed plant tissue containing photoassimilates and may therefore also have reduce nutrient quantities, may have caused higher water loss and reduced photosynthetic area.

Phytophagous insects have high nitrogen and phosphorus requirements, which they need to obtain from their hosts (Huberty 2005). Damage by herbivores often affects the concentrations of available nitrogen and other important nutrients in the leaves of plants (Tuomi *et al.* 1984). The increased sugars in leaves of plants are early physiological effects of nitrogen and phosphorus deficiencies which causes the accumulation of sugars and starch in leaves and higher levels of carbon allocation to the roots or storage organs (Scheible *et al.* 2004; Hermans *et al.* 2006). Higher carbon availability equates to higher levels of sugars (Smith & Stitt 2007).

The increase in the TSS concentration in leaves of plants exposed to apionids in this study may thus be attributed to a possible reduction of available nitrogen and phosphorus, due to apionid feeding. The generally lower starch concentrations in leaves of plants exposed to apionids may be due to starch being reallocated within the plants to stems and roots. Compensation for herbivory has been associated with, increases in photosynthetic rates and the mobilization of stored resources (Chapin & McNaughton 1989). Since increased sugars in leaves are physiological response to nitrogen and phosphorus deficiencies (caused by insect feeding), in turn stimulates an increase in carbon allocation to roots or sink organs (roots and stems) (Chapin & McNaughton 1989; Scheible *et al.* 2004; Hermans *et al.* 2006; Smith & Stitt 2007) an increase in sugars in stems and /or roots of plants that were exposed to apionids was thus to be expected.

McNaughton (1983) defined compensation in plants for herbivory as the replacement of lost biomass. *Lantana camara* var. 018 appeared to be able to compensate for apionid damage, much better, as both treatment and control plants had massive (in excess of 52 times) increases in starch concentrations in stems, and an increase in biomass in the latter part of the experiments, whereas the ability of *L. camara* var. 017 to compensate, was much less pronounced.

CHAPTER 5

GERNERAL DISCUSSION

5.1 INTRODUCTION

There is increasing criticism of classical weed biological control programmes for their lack of rigorous evaluation of the outcomes of deliberate introductions of exotic organisms (Louda *et al*. 2003; Pearson & Callaway 2003). The predictions made of agent efficacy prior to release have seldom been clearly demonstrated in the field after release; however, demonstrating the effectiveness of an introduced agent, especially in reducing populations of its target weed in its new geographical range over the long-term is challenging (Morin *et al.* 2009). Researchers need to determine whether the agent has adversely affected the weed, whilst considering the biotic and abiotic environmental factors that will also influence effects observed in the target weed. Certain types of data assist in the evaluation of agent effectiveness, for example quantifying feeding damage, loss of photosynthetic tissue, severity of the damage per unit biomass, distribution of galls on plants, or galling densities (Morin *et al.* 2009). Another factor that also needs to be considered when evaluating the effectiveness of agents, especially in the case of lantana, is varying levels of agent performance on different varieties. Studies done by Heystek (2006) showed that there is variability in *L. camara* plants, exhibited by differences in various characteristics (flower colour, leaf and stem hairiness and thorniness) and these characteristics occur in various combinations in different varieties, and affected the population densities of established biocontrol agents. This variability has affected performance of biocontrol agents and has been argued to have prevented establishment in some cases (Day & Neser 2000). This study, as well as that of Baars (2002), found that the two *L. camara* varieties (017 & 018) influenced agent performance, and therefore influenced the level of damage inflicted on plants of the different varieties. The level of damage inflicted on the different varieties by the apionids were in turn influenced by seasonal changes, as these changes influenced the allocation of plant resources that affect plant fitness and therefore influenced insect performance (Chapters 3 & 4).

An important aspect of evaluating effectiveness of an agent includes the understanding of the mechanism in which the biocontrol agent causes damage to the plant and the plants response to this damage. Most often the plant response involves reallocation of resources within the plant to compensate for losses (Chapin & McNaughton 1989).

This study tested a method in the laboratory to evaluate effectiveness of a biocontrol agent, to potentially apply as a tool to measure agent impact in the field. Additionally, in this chapter the effect of damage to the vascular tissue in petioles, the relationships and interactions following larval feeding and galling at different exposure densities of *C .camarae* on varieties of *L. camara* plants, its effect on plant growth and resources, and potential implications for biocontrol are discussed.

5.2 IMPACT OF THE AGENT

The larval feeding of *C. camarae* removed up to 100% of the vascular tissue of affected petioles 25 days after egg hatch in both the *L. camara* varieties, effectively severing the vascular connection in the leaves of galled petioles (Chapter 2). This damage is substantial as 80 % of carbon acquired in photosynthesis is transported via the plants vascular system to the sink or import dependant organs (Chiou & Bush 1998). Vascular architecture controls the transport of photosynthates, signal molecules and hormones among leaves (Murray *et al.* 1982). Healthy leaves, that have a strong vascular connection to damaged leaves, will therefore also be affected, as leaves are differentially interconnected via the plants vascular system (Orians 2005). The vascular tissue of *L. camara* var. 017 was consumed faster, although petiole sizes were similar for both varieties, the vascular tissues of *L. camara* var. 017 were significantly larger on average than that of *L. camara* var. 018 which resulted in the female oviposting eggs more frequently into the vascular tissue of *L. camara* var. 017. The larvae started feeding in the vascular tissue sooner and therefore consumed it sooner (Chapter 2). The effects on whole plants from galling damage will, however be greatly affected by the percentage of galling achieved on a plant. In this study, the galling percentages reached were similar for a given lantana variety at the low and high adult exposure densities, and in both exposure periods, but differences were noted in the effect that different apionid exposure densities had on biomass, and on total soluble sugars and starch concentrations of plants, and were therefore likely due to differences in levels of ‘shot hole’ feeding in the leaves by the adults, rather than the larval galling. This indicated that adult feeding of *C. camarae* also had an effect on plant resources, and could have been due to the removal of leaf tissue. Kosola *et al*. (2001) noted that leaf feeders removed plant resources, of which the most notable was nitrogen (which is an important component for plant growth and carbohydrates), and damage by adult feeding of *C. camarae* should not be underestimated. It was shown in this study as well as that of Baars (2002), that galling rates were higher on *L. camara* var. 018 than *L. camara* var. 017, irrespective of season, indicating that the one variety was more suitable to *C. camarae* than the other. *Lantana camara* var. 017 was shown in this study to be a slower growing variety compared to *L. camara* var. 018, and was probably one of the factors that contributed to it being the less suited variety.

The proportion of galling achieved during this experiment conducted late in the season was low in comparison to the numbers of galls achieved in experiments by Baars (2002), which were done in summer. This could have been an effect of reduced oviposition rates by the apionids approaching a period of overwintering, or may have been a consequence of plant cues and condition that discourage oviposition this late in the season, as it was observed that reallocation of resources to storage were increased as time passed. Starch concentrations in stems of healthy plants of both lantana varieties in this study increased substantially during the seasonal shift from early-autumn to mid-autumn, whereas the increase in root starch in both lantana varieties during this period was relatively small. The most substantial increase in the concentration of starch were in the stems of both varieties of *L. camara* which indicates stems to be the main plant part where lantana starch reserves are stored before winter. However, the increase in starch concentrations in *L. camara* var. 018 was much more substantial, compared to *L. camara* var. 017 and it may be better able to compensate for damage caused by phytophagous insects approaching winter as it has larger reserves. This appeared to be the case, as *L. camara* var. 018, which had the opposite response in these experiment compared to experiments by Baars (2002). There were increases in stem and root biomass in experiments exposed to apionids in this study, but those of Baars (2002) showed no increase in biomass for stems, and root biomass decreased in comparison to control plants, when experiments with similar galling levels were compared. The differences in plant responses may likely be due to the difference in season when experiments were conducted.

The quantities of TSS and starch were lower in whole plants exposed to apionids than control plants for *L. camara* var. 017 and these plants also had a smaller biomass than the controls. *Lantana camara* var. 017 thus suffered overall negative effects from *C. camarae* damage, both early and late in the season and the accumulated effects over time will undoubtedly decrease fitness of this variety. In *Lantana camara* var. 018, however, quantities of TSS and starch in whole plants generally increased in plants exposed to apionids and these plants also had a greater biomass than the control plants during the late season experiments. Compensation for herbivory has been associated with, increases in photosynthetic rates and the mobilization of stored resources (Chapin & McNaughton 1989) and the increase experienced in the biomass of *L. camara* var.018, is a strong indication of the adequate stored resources it probably has during mid-autumn, that allowed the plant to compensate for damage by *C.*

camarae. Although the experiments in this study showed an increase in plant biomass of leaves, stems and roots in *L. camara* var. 018, this compensatory growth so late in the season may affect this varieties ability to recover after winter as it has used some of its stored reserves. Winter survival is dependent on adequate reserves as there is often some bud growth during the dormant season and reserves are needed when growth resumes in spring (Loescher *et al*. 1990).

Plants have to cope with the damage inflected by herbivores and have therefore developed sophisticated means of defending themselves, which likely use aspects of their metabolism (Schwachtjie & Baldwin 2008). And, as observed in this study as well as others, tolerance to herbivores depends on the plant growth rate, post-damage allocation patterns, photosynthetic rates and reserves of carbohydrates and nutrients (Rosenthal & Kotanen 1994).

This study used the control plants destructively sampled after 35 and 70 days to observe growth rate and allocation patterns of TSS and starch concentrations, which yielded valuable information regarding the rate of reduction of growth and the differences in starch allocation to stems in the two different lantana varieties. These differences in growth rate and starch quantities between the varieties tested indicate that this may probably be the case among other *L. camara* varieties.

The control T_0 samples were not analysed in this study for total soluble sugars and starch and had it been, it would have been useful in obtaining allocation patterns for the concentration of TSS and starch in healthy plants before the experiment commenced as this would have given a better idea of the changes in resource allocation over a longer space of time encompassing the changes occurring from late-summer (start of experiment) to mid-autumn (end of experiment).

The agent evaluated in this study, *Coelocephalapion camarae* was expected to establish over a wide geographical range in South Africa as it was found to occur in its native range at altitudes between sea level and 1500 m covering a wide geographic area (Baars *et al*. 2007). Releases at various sites in South Africa were made, but to date *C. camarae* has only established at sites in Northern KZN at Richards Bay and Empangeni.

This study as well as those of Baars (2002) indicated that *Coelocephalapion camarae* have attributes of a good biocontrol agent with the potential to significantly contribute to the control of *L. camara* in South Africa. Both studies demonstrate significant damage done over very short periods of time in two different varieties over two different seasons which reflected in some negative effects on biomass and TSS and starch resource allocation. Even where plants are able to compensate for losses, the prolonged effect of persistent and accumulative damage over time will undoubtedly have a negative effect on plant fitness. The effect of adult feeding appeared to have been underestimated, and contribute greatly to affecting plants.

The establishment achieved at a limited number of sites, although disappointing, gives an indication of the ability of the agent to establish, spread and persist and, with greater release effort establishment may improve. The limited success in establishment in different areas also indicates that establishment is possibly being restricted by some environmental factor/s and this aspect needs further investigation.

5.3 THE FUTURE OF *LANTANA. CAMARA* BIOCONTROL

The genus *Lantana* is extremely variable and has presented a complex taxonomic problem (Munir 1996) and is one of the exotic species whose invasion of natural ecosystems has been experienced globally (Sharma *et al.* 2005). There has been an increase in requests from the public in recent years for advice on *L. camara*, which is an indication that it is spreading (Urban *et al.* 2011).

A review by Urban *et al*. (2011), discussed ongoing studies examining morphology and DNA of the weedy lantana complex which may in future offer some clarity about this weed that will assist by giving a clearer indication of the countries of origin of the weedy hybrids enabling the collection of agents that may be more suitable. The review also discussed the current state of the performance of some agents released in recent years in South Africa has being variable because some agents performed better than predicted, whilst others that were expected to perform well did not. The possible reasons for those agents that did not perform as expected is that candidate biological control agents were collected off lantana-like plants from the countries where *L. camara* is considered to be indigenous and therefore differ from the weedy lantana entities. Another reason as shown in various studies that the performance and establishment of agents released against *L. camara* differed on the different varieties (Urban & Simelane 1999; Urban *et al*. 2004; Heystek 2006; Baars *et al*. 2007). Therefore the introduction of many agents and biotypes, will overcome the varietal preferences and achieve adequate biocontrol of the whole array of weedy lantana taxa (Urban *et. al.* 2011). However whilst this is true, without the effective evaluation of the currently established agents it will be difficult to determine their effects. Agent damage is also probably not effectively measured as researches lack the ability to determine the effects on important plant resources that are usually affected by insect herbivory as this study has demonstrated, the resources herbivores exploit in plants has effects that are expressed in the sugar and starch levels in plants.

Since sugars and starches are the resource that the insect agents are exploiting from plants, this important resource should be studied in healthy lantana plants of different *L. camara* varieties under different climatic conditions in different seasons considering varietal differences in the areas where *L. camara* grows. This should be done to establish pre-release baseline data. The method used in this study can be applied to plants growing in the field, enabling researchers to evaluate levels of control that may vary between varieties, climatic conditions, seasons and years. Since *L. camara* plants have a great deal of variation which seems to be the biggest problem in controlling it, a study of how the different varieties of this complex species grows, allocates resources and biomass, responds to seasonal and climate change will provide an affective reference point to be able to evaluate how agents that are released against this variable weed performs.

The recent focus to approach the control of *L. camara* encourages the study of the target to better understand plants morphology and DNA to establish its origins (Urban *et al*. 2011). To further expand this concept, the study of the plants growth habits, resource allocation of sugars and starches, and their effects on biomass on the different varieties whilst considering seasonal changes and the climatic regions they grow in should add greatly in determining weaknesses of the target. This may enable clearer goals, a better understanding and improved evaluation of the impact of agents that were and are to be released against *L. camara*.

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