Population and behavioural studies on *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae), a biological control agent of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) in South Africa

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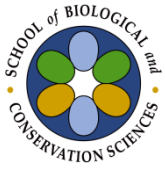

PREFACE

The laboratory-based experimental work described in this dissertation was carried out at the Agricultural Research Council, Plant Protection Research Institute (ARC-PPRI), and the field-based survey work was conducted at Sappi Cannonbrae plantation, Umkomaas, from February 2009 to February 2011, under the supervision of Drs Terry Olckers (University of KwaZulu-Natal) and Costas Zachariades (ARC-PPRI).

This dissertation represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where there is use of work done by others, this is duly acknowledged in the text.

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ABSTRACT

Chromolaena odorata (L.) King and Robinson (chromolaena, triffid weed) (Asteraceae: Eupatorieae) is one of the most problematic weeds in the subtropical northeastern parts of South Africa. *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae) was introduced as a biological control agent for the control of this weed. No study has yet been done to quantify field populations of *C. eupatorivora* since its establishment in 2003. The aim of this study was therefore to measure aspects of the field population and laboratory behaviour of *C. eupatorivora* on *C. odorata*.

The first objective was to determine the percentage leaf area mined by larvae of *C. eupatorivora* on *C. odorata* plants exposed to three densities of mated flies, and also to determine the number of mines produced by these different densities, and their distribution on the plant. It also attempts to determine the relationship between chromolaena leaf quality and usage by *C. eupatorivora*. The maximum percentage of leaf area damaged was 37.5% for one of the trials involving five pairs of flies. Mean percentage leaf area damaged was slightly higher with five (28.5%) than ten pairs (22.0%) of adults and was lowest with one pair (6.5%), but these differences were not significant. In relation to the mean number of mines per plant, five and ten pairs of flies caused slightly more mines than one pair. The other significantly different parameter was number of leaves mined per plant, which was higher for five pairs. Within a plant, *C. eupatorivora* probably selects a subset of leaves with certain chemical and physical characteristics for oviposition since certain leaves were left unmined while others received multiple eggs. Percentage water content did not differ between mined and unmined leaves, but clear patterns were shown by acid detergent lignin which was higher in unmined leaves and nonstructural carbohydrates which were much higher in mined leaves. It is likely that leaf age plays a role in its suitability.

The second objective was to quantify *C. eupatorivora* infestation levels, by counting and examining larval leaf mines, on *C. odorata* in the field at four times (‘seasons’ - September, December, March and July) over a 12-month period, and at three study sites that each included two habitats, viz. open and shady. At each of these six sampling sites, line transects were laid out and plants/branches sampled along them. Both plant/branch height and the number of leaves increased between September and March, and plants in the open habitats were taller and had

more leaves than those in the shaded habitats. At the third site, the shady habitat supported taller plants with more leaves compared to the same habitat at the other sites. There was a steep increase in the number of *C. eupatorivora* mines from December to March. The mean number of mines, both total and in relation to leaves available, was highest in March, and was higher in the shaded habitats compared to the open habitats. The mean number of mines per damaged leaf was slightly higher in December compared to the other seasons, and was also higher in the open than the shaded habitats. Mean larval mortality was high (70%) in September but decreased to 32% in December, and increased again in late summer. The overall levels of mining by *C. eupatorivora* were low, with less than 5% of leaves sampled having mines.

Taken together, the laboratory and field trials suggest that *C. eupatorivora* is restricted to a subset of the leaves of *C. odorata* for its development; that the field population is unable to make full use of the resource of young, palatable leaves that develop in early- to mid-summer because it only becomes large in late summer; and that the high mortality rate of young larvae negatively affects both the population of the fly and the level of damage to the plant. Given that these results were obtained in an area where the population of *C. eupatorivora* is relatively high, it is unlikely that the fly is having anything more than a negligible effect on *C. odorata* in South Africa at present.

Key words: *Chromolaena odorata*, *Calycomyza eupatorivora*, open and shaded habitats, mine density, larval mortality, leaf quality, field population, biological control, oviposition behaviour

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CHAPTER 1: General introduction

1.1 Invasive alien plants in South Africa

An invasive alien species is an organism that is non-native to an ecosystem, which may pose threats or cause harm to existing indigenous species or cause economic and environmental harm (Richardson and van Wilgen, 2004). These non-native organisms become invasive in new areas mainly because of their competitive ability, and also because they have no natural enemies. Natural ecosystems worldwide are threatened by a wide diversity of invasive alien species that include diseases, weeds, and insect pests (Richardson and van Wilgen, 2004). Invasive alien plants (weeds) present a major threat since they are able to spread over considerable distances into new, undisturbed, natural areas and replace the indigenous vegetation. Invasive plants also negatively affect agriculture, forestry, animal and human health, and economic productivity (Bryson and Carter, 2004; Richardson and van Wilgen, 2004). Invasive alien plants have been introduced into South Africa since the mid-1600s when sailors to and from the Spice Islands were using the Cape of Good Hope as a transit point (Wells *et al.*, 1986; Richardson *et al.*, 1997; Zimmermann *et al.*, 2004). From that time onwards, South Africa became a suitable arena for the establishment of alien plants from many countries, particularly Australia and the Americas, with some having been deliberately introduced as ornamentals and crops and others accidentally as contaminants of agricultural produce (Mack, 1995; Zimmermann *et al.*, 2004).

Invasive alien species often coexist with native species for extended periods, before their superior competitive ability becomes apparent as their populations grow larger and denser and they become better adapted to their new locations (Kolar and Lodge, 2001). Invasive alien plants possess a variety of characteristics that enable them to disperse rapidly into new areas and out-compete crops and natural vegetation for light, water, nutrients, and space (Kolar and Lodge, 2001; Bryson and Carter, 2004). These features may include their ability to reproduce rapidly, fast growth rates, high dispersal ability, phenotypic plasticity (their ability to alter their growth form to suit current conditions) and ability to tolerate a wide range of environmental conditions (Thebaud *et al.*, 1996; Reichard and Hamilton, 1997; Kolar and Lodge, 2001; Bryson and Carter,

2004). Moreover, invasive plant species might be able to exploit resources previously unavailable to native species, such as deep water sources that can only be accessed by a long taproot, or an ability to live on previously uninhabited soil types (Huenneke *et al.*, 1990; Reichard and Hamilton, 1997). For example, barbed goatgrass *Aegilops triuncialis* (L.) Raspail (Poaceae) is invasive in California (USA) on serpentine soils, which have low water-retention properties, low nutrient levels and possible heavy metal toxicity. Native plant populations on these soils tend to show low density, but goatgrass can form dense stands on these soils crowding out native species that have not adapted well to growing on serpentine soil (Huenneke *et al.*, 1990; Reichard and Hamilton, 1997).

Many alien plant species have become highly invasive in South Africa, probably because they were introduced from their region of origin without any of their own natural enemies that feed on them or cause them to develop diseases (Mack, 1995). This mechanism is known as the Enemy Release Hypothesis (ERH) or herbivore/predator escape, which states that plant species, on introduction to a new region, should experience a decrease in regulation by herbivores and other natural enemies resulting in an increase in their abundance and distribution (Mack, 1995; Keane and Crawley, 2002). This hypothesis argues that natural enemies are important regulators of plant populations and that they have a much greater impact on native than on exotic plants (Keane and Crawley, 2002). Although the ERH might play a role in many plant invasions, there are several alternative hypotheses that may also explain exotic plant invasiveness. Some focus on how native plant species, through competitive exclusion, prevent exotics from establishing and increasing and how disruption of these processes leads to invasion (Shea and Chesson, 2002). Other hypotheses state that plants become invasive in the absence of other regulatory factors such as unfavourable soil and climatic conditions that were present in their native region (Mack, 1995).

1.2 Control of invasive alien plants in South Africa

Effective management of invasive alien plants is important if we are to prevent their considerable negative impacts (Blossey, 1999; Myers *et al.*, 2000). An integrated approach involving the combined use of a range of methods is usually necessary to control invasive alien plants

effectively (Blossey, 1999). The various methods that are available are usually classified as mechanical, chemical or biological control. Mechanical control refers to the physical removal of plants through felling, slashing or uprooting and is largely labour intensive (Williamson, 1996; Blossey, 1999). Chemical control involves the use of herbicides and like mechanical control is more effective in the short-term and often relies on rigorous follow-up efforts and sometimes rehabilitation (Myers *et al.*, 2000). Biological control (biocontrol) refers to a wide range of interventions aimed at reducing the population density of the target plant (Wiedenmann, 2000) and is characterized by the use of living organisms that are natural enemies of the plant (Cory and Myers, 2000; Wiedenmann, 2000), chiefly host-specific insects, mites and fungal pathogens. Such natural enemies are introduced to a new area (where they did not originate or do not occur naturally) where the weed has invaded and this is known as classical biocontrol. It is a preferred method for managing invasive plants as it is often the only really sustainable solution in the longer term (Myers *et al.*, 2000).

During the past 150 years, until the end of 1996, about 365 species of invertebrates and fungi were deliberately released on 133 weed species in 75 countries (Julien and Griffiths, 1998; McEvoy and Coombs, 1999). In about 25% of these cases, biocontrol has been completely successful, i.e. the target plants have been suppressed for decades without requiring further management interventions (Zimmermann *et al.*, 2004). When the benefits of weed biological control are calculated, they are measured in hundreds of millions of dollars, and the benefit to cost ratios are highly favourable (Le Maitre *et al.*, 2001; McConnachie *et al.*, 2003; Van Wilgen *et al.*, 2004). In South Africa, biological control of weeds was initiated in 1913 and since then, 63 species of biological control agents have been successfully established on 44 invasive weed species, in many cases reducing the costs of management (Moran *et al.*, 2005). Eleven (25%) of the 44 target weeds have been completely controlled in that no other control methods are needed to maintain the weed populations at acceptable levels (Zimmermann *et al.*, 2004). Examples of complete control include red water fern, *Azolla filiculoides* Lam. (Azollaceae) (McConnachie *et al.*, 2003), and red sesbania, *Sesbania punicea* (Cav.) Benth. (Fabaceae) (Hoffmann and Moran, 1998). In other cases (38%), the target weeds have been substantially controlled in that the need for and costs of additional control efforts has been reduced, while in other cases the outcomes of biocontrol have been negligible or are still uncertain (Zimmermann *et al.*, 2004). In general, the

best results are obtained when two or more of the above methods (chemical, mechanical, biological) are combined as part of an integrated control strategy.

1.3 Chromolaena odorata in South Africa

Of the many invasive weeds present in South Africa, *Chromolaena odorata* (L.) King and Robinson (chromolaena, triffid weed) (Asteraceae: Eupatorieae) is one of the most problematic and has hence been targeted for a number of control measures, including chemical, mechanical and biological control (Holm *et al.*, 1977; Goodall and Erasmus, 1996; Zachariades *et al.*, 1999).

Chromolaena odorata, previously known as *Eupatorium odoratum* L., is a fast-growing, perennial shrub originating in the neotropics (King and Robinson, 1970) that has become a major weed of crops, plantations, savannas and natural forests in many parts of the world. In South Africa, it was first recorded as naturalized near Durban, KwaZulu-Natal province in the mid 1940s, and may have been introduced accidentally or as a garden plant (Henderson and Anderson, 1966; Pickworth, 1976; Goodall and Erasmus, 1996). Since then, it has reached alarming proportions in South Africa and in neighbouring Swaziland, making it one of the sub-region's worst weeds (Goodall and Erasmus, 1996). Chromolaena is a declared noxious weed and falls under Category 1 of the Conservation of Agricultural Resources Act (CARA) (Goodall and Erasmus, 1996).

1.3.1 Description of *C. odorata*

Within its wide native distribution, from northern Argentina to the southern United States, chromolaena shows marked morphological variability in terms of leaf shape and hairiness, flower colour, smell of crushed leaves, and plant architecture within this range (Erasmus, 1988; Zachariades *et al*., 2004). The form of chromolaena invading southern Africa is morphologically different from that invading Asia and West Africa (Zachariades *et al.*, 2004). The two forms also differ from one another in biology and ecology, and there is little variation within each form (Scott *et al.*, 1998; Von Senger *et al.*, 2002; Ye *et al.*, 2004); thus, they are functionally distinct

entities, and have been characterized as biotypes (Zachariades *et al.*, 2004). The biotype that is invasive in Asia and West Africa has pale blue–lilac flowers, dull-green leaves and stems. The southern African biotype has glabrous stems and leaves, its flowers are white (Vanderwoude *et al.*, 2005), and the smell emitted by the crushed leaves is sharp when compared with that of the Asian/West African biotype (Zachariades *et al.*, 2004). The southern African biotype also has an upright habit. Both biotypes have a fibrous root system that does not penetrate beyond 20-30 cm in most soils.

Chromolaena forms dense tangled bushes that range between 1.5 and 3.0 m in height and occasionally reaches its maximum height of 10 m as a scrambler over other plants (McFadyen, 1989, 1991; McFadyen and Skarratt, 1996). Its stems branch freely, with lateral branches developing in pairs from the axillary buds (Vanderwoude *et al.*, 2005).

1.3.2 Ecology of *C. odorata*

The habitats in which the plant grows are similar in both the native and introduced ranges (McFadyen, 1991). It occurs in agricultural areas, natural forests, planted forests, grasslands, riparian zones, disturbed areas and in shrublands (Goodall and Erasmus, 1996). Chromolaena grows on a wide range of soils but has a short life span in poor soils with frequent waterlogging, and hence prefers well-drained soils (McFadyen, 1988, 1989; Goodall and Erasmus, 1996). In South Africa it grows in a range of vegetation types, ranging from grasslands to arid bushveld (Goodall and Erasmus 1996). However, in arid areas chromolaena is restricted to riverbanks and will only become invasive in the frost-free areas of mesic to dry bushveld, which are not water-stressed in the growing season (Erasmus, 1988).

The morphology, physiology, biochemistry and seed production of chromolaena can differ even between close localities because of differences in soil moisture, relative humidity, temperature, sunlight and precipitation (Muniappan and Marutani, 1988). Studies have shown that chromolaena is not a serious weed in its area of origin, probably due to competition with many other *Eupatorium* species and to attack by natural enemies, including insects and diseases that are absent in invaded areas (McFadyen, 1989, 1991). It acts as a pioneer plant, growing at high

densities in recently disturbed areas, but it is soon outcompeted by successional vegetation and disappears after a few years (Cruttwell, 1972; McFadyen, 1988, 1989).

There is usually better performance in terms of higher relative growth rate, reproductive potential and nutrient uptake efficiency of chromolaena populations in open environments (Sivagnanam and Swamya, 2010). Thus, chromolaena exhibits an exploitative strategy (Grime, 1974) and is able to attain dominance in open environments that are temporarily enriched with nutrients and radiant energy (Swamy and Ramakrishnan, 1987). The plants do not tolerate deep shade (McFadyen, 1988) and thus thrive in open areas (Kluge, 1990; McFadyen, 1991). Chromolaena takes advantage of the flush of soil nitrogen that becomes available after disturbances like fire or land clearing for agriculture and exhibits relatively high foliar nitrogen, phosphorus and potassium contents (Saxena and Ramakrishnan, 1983). Chemicals with allelopathic properties that are produced by chromolaena have been shown to prevent the germination of adjacent plants (Holm *et al.*, 1977; Waterhouse, 1994).

For optimal growth, chromolaena requires a relative humidity in the range of 60 – 70%; at values higher than 80% the growth performance becomes poor (Ambika, 2002). Studies have shown that chromolaena seedlings grow well at 30°C and even better on mulched soils at 25°C (Ambika, 2002). Chromolaena plants flower at the end of the growing season, with flowering triggered at least partly by shorter day lengths in both the native and introduced ranges (Liggitt, 1983). In Trinidad (northern hemisphere), flowering occurs from late December until the end of March whereas in the KwaZulu-Natal (KZN) province of South Africa (southern hemisphere), flowering takes place from June with a peak in July and August (Liggitt, 1983; McFadyen, 1991).

In its invasive range, chromolaena often produces a phenomenal number of seeds that have tiny barbs that cause them to adhere to clothes, fur, feathers and other objects, especially when these are wet (Waterhouse, 1994). Seeds are dispersed by wind, as well as via animal fur, clothing, and vehicles (Gautier, 1992, 1993; Blackmore, 1998). In South Africa, chromolaena may have been spread in the same way or along railways and roads, which may have provided reservoirs of seeds for infesting the surrounding countryside (Von Senger *et al.*, 2002). At the beginning of

the rainy season, the plants regenerate rapidly from seeds and through re-sprouting of established plants (Holm *et al.*, 1977; McFadyen, 1991).

The Asian/West African biotype does not thrive in areas with extremely high rainfall (Kriticos *et al.*, 2005) and was predicted to invade areas with a minimum annual rainfall of 1200 mm (McFadyen, 1989). The southern African biotype is more cold tolerant (Kriticos *et al.*, 2005), occurring in frost-free zones with an annual rainfall of 500–1500 mm (Goodall and Erasmus, 1996).

1.3.3 Harmful impacts of *C. odorata*

In its invasive range, chromolaena grows rapidly and forms dense scrambling thickets that grow through and over the existing vegetation and prevent the establishment of other species, both due to competition and allelopathic effects (Sahid and Sugau, 1993), thus reducing biodiversity and the carrying capacity of native ecosystems (McFadyen, 1989; Kluge, 1990). It also has an impact on livestock and grazing (Goodall and Erasmus, 1996) and on water usage by other plants including agricultural crops (Meijninger and Jarmain, 2011). In the Old World, chromolaena is an invasive transformer species, at least partly because it lacks natural enemies (Richardson *et al.*, 2000). It readily invades areas of natural or human-induced disturbance, but can also invade undisturbed land. Chromolaena interferes with natural ecosystem processes as evidenced in the Greater St. Lucia Wetland Park in South Africa (Leslie and Spotila, 2001). Leslie & Spotila (2001) showed that Lake St. Lucia's nesting Nile crocodiles, *Crocodylus niloticus* Laurenti (Reptilia: Crocodilidae), require open sunny, sandy areas in which to deposit and incubate their eggs. Chromolaena plants shade and overtake these nesting sites creating fibrous root mats that are unsuitable for egg chamber and nest construction. Furthermore, shading by chromolaena led to female-biased sex ratios in the offspring and the nesting sites were often abandoned (Leslie and Spotila, 2001).

Wildfires fuelled by chromolaena, when it is dry, often kill native plant species (Goodall and Erasmus, 1996), particularly since these are hotter than normal because chromolaena forms a higher fuel load than the vegetation that it replaces and contains essential oils (Cock and

Holloway, 1982; Liggitt, 1983). These fires can also lead to soil erosion as they are capable of sterilizing the soil and killing the roots that keep the soil particles together.

Chromolaena displaces grassland which is used as pasture for domestic livestock by both resource-poor and commercial farmers (Zachariades and Goodall, 2002). The invasion of pasture lands by chromolaena causes cattle to avoid these lands, and subsequently to overgraze non-infested lands. This is particularly a problem for nomadic and semi-nomadic livestock systems where free grazing is practiced around the settlements during the rainy season (Goodall and Erasmus, 1996). This weed also has ecotourism implications in that it reduces tourism potential by obstructing game and bird viewing in recreation areas (Goodall and Erasmus, 1996).

1.3.4 Control of *C. odorata*

Chromolaena control strategies include chemical, mechanical and biological control. For chemical control, several foliar- and stump-treatment herbicides, such as glyphosate and triclopyr are registered for chromolaena in South Africa (Goodall and Erasmus, 1996) and no further research on chemical control is currently justified (Zachariades and Goodall, 2002). These herbicides are either applied to the cut stumps of slashed plants or to the leaves of seedlings and coppice growth (Zachariades and Goodall, 2002). Mechanical control involves burning and slashing of chromolaena by means of brush cutters, hoes or tractor-drawn implements (Goodall and Erasmus, 1996). However, slashing causes regeneration and therefore needs to be followed by chemical control to be effective (Goodall and Erasmus, 1996). Manual weeding is tedious and labour intensive, and the use of tractor-drawn equipment is limited to accessible areas (Goodall and Erasmus, 1996). Mechanical methods may also lead to soil disturbance or erosion and may damage untargeted species that are mistakenly cleared in dense infestations of the weed.

1.3.4.1 Biological control of C. odorata

Biocontrol is potentially the only sustainable and permanent control method for chromolaena in South Africa, and is therefore considered to provide the only feasible long-term approach, as part of an integrated control strategy. The weed is considered to be a good target for biocontrol because it does not reproduce vegetatively, it is a homogenous taxon in South Africa and also because there are vulnerable stages in its life cycle (Kluge, 1991). Biocontrol research on chromolaena was initiated in the late 1960s internationally, and in the late 1980s in South Africa; and although initial progress was slow, this has improved over the past few decades. Kluge (1991) attributed the slow progress initially experienced more to the geographic distribution of its invasion than to any inherent inability to control it biologically; most countries in the tropics and subtropics are developing nations with few resources to conduct the initial, expensive phases of biocontrol research (Cruttwell, 1974; Kluge, 1991).

A few agents have been successfully released worldwide in attempts to control chromolaena: *Pareuchaetes pseudoinsulata* Rego Barros (Lepidoptera: Arctiidae), a moth with defoliating larvae, *Pareuchaetes insulata* Walker, another moth with defoliating larvae, *Cecidochares connexa* Macquart (Diptera: Tephritidae), a stem-galling fly and *Calycomyza eupatorivora* Spencer (Diptera: Agromyzidae), a fly with leaf-mining larvae (Zachariades *et al.*, 1999). In South Africa, releases of *P. pseudoinsulata* did not establish possibly because of extensive egg predation by ants (Cock and Holloway, 1982). *Pareuchaetes aurata aurata* Butler (Lepidoptera: Arctiidae), was introduced into South Africa following the unsuccessful early attempts to establish *P. pseudoinsulata* and was thought to be a good candidate because of its oviposition habit (scattering its eggs on the ground) that made it less susceptible to predators (Kluge 1991). However, extensive releases (148 000 individuals) also failed to achieve establishment, possibly because the culture was contaminated with microsporidian disease, and efforts to collect disease-free cultures did not succeed (Kluge and Caldwell, 1993, 1996; Zachariades *et al.*, 1999). *Cecidochares connexa*, which has become established elsewhere in the world, failed to develop on the southern African biotype of chromolaena (Horner, 2002) and no releases were ever conducted. *Calycomyza eupatorivora*, originally from Jamaica, was the fourth agent to have been released, and the second to have been established (after *P. insulata*), against chromolaena in

South Africa and is the subject of this study. The larvae of this fly form blotch mines on the upper surfaces of the leaves which could retard the weed's growth and reproduction, thereby contributing to a decrease in its invasive potential (Zachariades *et al.*, 2002).

1.4 Leaf-mining insects and their ecology

Leaf miners form a functional rather than a taxonomic grouping of insect species that are adapted to a special type of environment. Leaf mining is a form of endophagous herbivory in which the insect larvae inhabit and consume the leaf tissue of plants (Sinclair and Hughes, 2010). Leaf miners are most numerous in the tropics but are still widely distributed in the temperate zones. The vast majority of leaf-mining insects are moths (Lepidoptera), sawflies (Hymenoptera: Symphyta) and flies (Diptera), though some beetles and wasps also exhibit this behaviour. The Agromyzidae (Diptera) is a well-known family that has many species of leaf miners.

The larvae of all leaf miners have an advantage in that they are protected from predators and plant defenses by feeding within the tissues of the leaves, selectively eating only the layers that have the least amount of cellulose (Sinclair and Hughes, 2010). The eggs of leaf-mining insects are either laid on the leaf surface, whereupon the larvae penetrate the leaf, or the adult females insert the eggs into the leaf tissues (Bultman and Faeth, 1986; Sinclair and Hughes, 2010). The larvae may spend their entire life cycle in the leaf or feed there for only a few instars. Some miners feed internally for the first few instars and then feed externally during the later stages. Leaf miners are known to attack nearly all plant families and many are forestry, agricultural or horticultural pests. Unlike most phytophagous insects, leaf miners normally feed on a single leaf during larval development and larval emigration between leaves does not occur. Hence, in general, females alone determine host leaf selection, based on leaf quality (Bultman and Faeth, 1986).

Although some leaf-mining insects are pests, others have been used as weed biological control agents, for example *Hydrellia pakistanae* Deonier (Diptera: Ephydridae) used as a control agent for *Hydrilla verticillata* (L.f) Royle (Hydrocharitaceae). Leaf miners are potentially good biological control agents even though they often need to be integrated with other agents. Since it

was introduced in Florida (USA), the population density of *H. pakistanae* has never exceeded more than 15 adults per m^2 of hydrilla stands and the level of damage has not been more than one-fifth the level estimated to be necessary to produce a significant impact on the plant (Wheeler and Center, 2001). *Ophiomya camarae* Spencer (Diptera: Agromyzidae) (for the control of *Lantana camara*) has had some impact on the target weed (Simelane, 2002). Other leaf-mining biocontrol agents include *Aristaea onychota* (a moth for *L. camara*), *Calycomyza lantanae* (a fly for *L. camara*) and *Uroplata girardi* (a beetle for *L. camara*), all of which have had minor impacts on their target weeds (Harley, 1969; Simelane, 2002).

1.4.1 *Calycomyza eupatorivora*, a leaf-mining agent for *C. odorata*

Calycomyza eupatorivora was described in Spencer and Stegmaier (1973). The adult is predominantly black with a broad yellow-white area laterally on the thorax that extends above the wing base. It is a relatively large species, with a wing length of 2.3-2.4 mm (Spencer and Stegmaier, 1973). Although it was previously confused with *Calycomyza flavinotum* Frick, 1956, a Nearctic species, *C. eupatorivora* has a Neotropical distribution, with holotype and paratypes from Jamaica, where it was reared from leaf blotch mines on chromolaena (Spencer and Stegmaier, 1973). The fly has also been recorded on chromolaena in Venezuela (Spencer and Stegmaier, 1973). Specimens identified as *C. flavinotum*, that were reared from chromolaena leaves in Trinidad (Cruttwell, 1972), are thus likely to be *C. eupatorivora.*

Calycomyza eupatorivora is host specific in both its physiological and behavioural host range, attacking only *C. odorata* (Zachariades *et al.*, 2002; Gareeb and Zachariades, 2003). Reasons for considering this fly as a biocontrol agent for chromolaena were that in Jamaica it is abundant and widespread, and causes significant visible damage. Moreover, the region of origin of the southern African *C. odorata* biotype has been shown to include Jamaica (I. Paterson & C. Zachariades, unpubl.); therefore, there should be no incompatibility problems. The fly also multiplies rapidly due to its high fecundity and short life cycle. It takes about two weeks for the larva to reach maturity (final instar), then it exits the leaf and pupates on the ground. The pupation period is about two weeks. Female adults start to lay eggs after 1-2 days and live up to two weeks. *Calycomyza eupatorivora* was first imported into quarantine in South Africa from

Jamaica in 1997 (Zachariades *et al*., 1999). Culturing methods were developed and the species was partially tested for host-specificity before this culture was lost in 1999. However, a new culture was successfully re-imported in 1999 and host-specificity tests were completed in 2001.

The female lays a single egg each time, by inserting her ovipositor into the under-surface (adaxial surface) of the leaf, usually between the central and lateral veins. Two days later the egg hatches, and a transparent to whitish larva emerges and feeds on the mesophyll tissues of the leaf. First instar larval feeding damage begins as a short linear mine, and increases to a large blotch mine (created from the second instar on) as feeding progresses. By the time the larva reaches the dark yellow final instar, it will have mined approximately 50-100% of the leaf surface, depending on the size of the leaf. It takes about two weeks for the larva to reach the final instar. At this stage, it exits the leaf to pupate on the ground. The adult that emerges about two weeks later has a lifespan of approximately two weeks. Mating occurs within two days of emergence. As mentioned above, *C. eupatorivora* has the potential to increase the size of its population quite rapidly, due to its short generation time and high fecundity (Gareeb and Zachariades, 2003).

Following clearance for its release, *C. eupatorivora* was released at several sites in KZN in 2003 and 2004. The fly initially established at one coastal site from which it spread along the coast (Zachariades and Strathie, 2006) and has since been recorded up to 200 km from the original release site. The fly appears to be more abundant in shaded and semi-shaded habitats and high infestation levels have been observed occasionally. It is likely that the eventual distribution of the fly in South Africa will be largely restricted to the coastal region and to habitats where the plants remain in reasonable condition during the dry season. In Jamaica, at least four species of hymenopteran parasitoids attack the larvae at two developmental stages, but probably account for less than 25% mortality (Zachariades *et al*., 2002). Establishment success and population size in the field in South Africa could thus be negatively affected by parasitoids of native leaf-mining Agromyzidae, as was the case with *Calycomyza lantanae* released on *Lantana camara* (Baars and Neser, 1999).

1.5 Aims and objectives of the study

Subsequent to the establishment of *C. eupatorivora*, there have been no studies to investigate its impact on *C. odorata*, either in the laboratory or in the field. Also unknown is the fly's seasonal pattern of abundance in the field and whether there are any biotic (e.g. parasitoids, predators) or other mortality factors that affect population densities. The aim of this study was thus to elucidate aspects of the field ecology of *C. eupatorivora* in the KZN coastal region where the insect has been established for several years. In addition, a laboratory study was undertaken to assess the oviposition and larval development patterns resulting from different densities of adult flies.

The specific objectives of this study were to:

1. Investigate the density and distribution of *C. eupatorivora* mines on *C. odorata* at three field sites and determine: (i) larval infestation levels in shady versus sunny environments; (ii) larval infestation levels over the course of a year, measured at 3-monthly intervals; and (iii) levels of larval mortality.
2. Investigate the oviposition and larval development patterns resulting from different densities of *C. eupatorivora* adult flies on *C. odorata* plants in the laboratory and determine: (i) the incidence of mining and amount of leaf damage inflicted; and (ii) the relationship between leaf quality and usage by the flies.

CHAPTER 2: Oviposition and larval development patterns resulting from different densities of *C. eupatorivora* adult flies on *C. odorata* plants

2.1 Introduction

The larvae of *Calycomyza eupatorivora* form blotch mines on the upper sides of leaves of chromolaena, presumably reducing photosynthesis. *Calycomyza eupatorivora* was chosen as a biocontrol agent because its leaf-mining activity was expected to result in a reduction in the growth of chromolaena plants and therefore contribute to a decrease in the weed's invasive potential (Zachariades *et al.*, 2002). Undoubtedly the amount of damage caused by the fly is density dependent, i.e. varies as a function of the fly's population size, because the latter determines the number of eggs laid in the plant tissues. This relationship may, however, not be linear. If plants are exposed to very high numbers of damaging insects, this might lead to intraspecific competition thus leading to decreased damage by individual insects, and therefore a non-linear curve (Reitz and Trumble, 2002). A study on the golden loosestrife beetle, *Galerucella pusilla* Duftschmidt (Coleoptera: Chrysomelidae) which feeds on purple loosestrife, *Lythrum salicaria* L. (Myrtales: Lythraceae) revealed a positive linear relationship between insect density and damage at lower insect densities, but at increasing insect populations (nearing carrying capacity), this was replaced with a nonlinear, asymptotic relationship (Schooler and McEvoy, 2006). If this competition leads to mortality of feeding stages or a disproportionate decrease in oviposition, the total damage inflicted on the plant may even decrease at high insect densities (Reitz and Trumble, 2002).

The damage caused by an herbivorous insect species to a plant may be limited by its preference for, and performance on, a subset of the available resource. For example, *C. eupatorivora* females may only select leaves of a certain quality for oviposition, and/or larvae may develop better in such leaves. In this case, even if the population density is high, the proportion of

suitable leaves may be low and thus the damage caused by the insect is limited. Thus even in the presence of many undamaged leaves, there may be high intra-specific competition, leading to decreased oviposition per female, higher mortality of immature stages and/or the production of undersized adult progeny with lower fecundity. Other studies have demonstrated that conspecific larvae of leaf miners tend to co-occur on a single leaf, regardless of the fact that the increased oviposition would result in interference or exploitative competition between larvae (Stiling *et al.*, 1984; Faeth, 1990). Selection of oviposition sites by adults of leaf-mining insects is largely influenced by variation in leaf structure (Reavey and Gaston, 1991), leaf size (Faeth, 1991) and leaf chemistry (Minkenberg and Offenheim, 1990; Kagata and Ohgushi, 2001). Some of these parameters can in turn be affected by leaf age and exposure of the leaf to other physical conditions, such as light levels. The value of a leaf to developing offspring may change with leaf age because of changes in the chemical or physical properties of that particular leaf (Raupp and Denno, 1983; King *et al.*, 1998). Young leaves are soft and usually contain higher levels of nitrogen and moisture than older leaves (Raupp and Denno, 1983; Raupp, 1985; Denno *et al.*, 1990), but possibly also contain more defensive chemicals. Leaf suitability for the insect may also be affected in similar ways by the physical conditions that the plant is exposed to (Faeth, 1991). Furthermore, not only leaf quality but also other factors such as predation and mechanical factors may determine the suitability of a leaf for an insect (Raupp and Denno, 1983).

This study was carried out to determine the percentage leaf area mined by *C. eupatorivora* larvae on plants exposed to different densities of mated flies (1, 5 and 10 pairs), and also to determine the number of mines produced by these different densities of mated flies, and on patterns of oviposition by females. Since leaf miners appear to be selective regarding the leaves in which they oviposit and larvae tend to co-occur in a single leaf (because adult females lay more than one egg on a leaf) (Stiling *et al*., 1984), this study also attempted to determine the relationship between aspects of chromolaena leaf chemistry and usage by *C. eupatorivora* flies. Determining whether there are differences in the quality of leaves selected by *C. eupatorivora* for oviposition will allow predictions on the proportion of leaves that are vulnerable to mining, and this may change according to season and the growth conditions of the plant. If few leaves, and only leaves of a certain quality, are preferred for oviposition, this can have important consequences for the

effectiveness of *C. eupatorivora* as a biological control agent. Such consequences would be negative if the criteria for leaf suitability for oviposition are narrow.

2.2 Materials and methods

Laboratory experiments were carried out in the Controlled Environment room (25^{o}C, 60% relative humidity) at the Agricultural Research Council – Plant Protection Research Institute, Cedara, KwaZulu-Natal, South Africa (29°32'45.5"S, 30°16'17.7"E). These experiments were carried out from September 2009 to April 2010.

2.2.1 Plants and insect cultures

Chromolaena cuttings were rooted in a mistbed, planted in 18 cm diameter pots and were left to grow in the ARC-PPRI shade house. Cuttings were grown at the same time to avoid inconsistency in plant size. The *C. eupatorivora* flies used in this study were reared in a large walk-in cage in the ARC-PPRI glasshouse. Chromolaena leaves with mature *C. eupatorivora* larvae were collected from the glasshouse and at times also from the shadehouse where they occurred as pests on chromolaena stock plants. These were layered between slightly dampened tissue paper in a 2 litre plastic container with a gauze lid, to allow pupation. After about 5 days, most of the larvae had pupated and they were then counted and placed in emergence boxes. Eclosing *C. eupatorivora* males and females were used in this study. Adults were sexed by examining the terminal segments of the abdomen, where an ovipositor was apparent on the female**.**

2.2.2 Experimental design and data collection

2.2.2.1 Relationship between leaf area and leaf length and width

A pre-trial was done to determine if leaf length or width could be used as a predictor of leaf area, within the context of determining the percentage leaf area available to, and mined by, *C.*

eupatorivora. Sixty leaves were selected haphazardly from a number of plants and photocopied onto paper. Leaves less than 1 cm in length were not selected. The copies were cut out and weighed individually using a Sartorius BP61 scale with a range from 0.1mg – 61g, and the length and width of each paper leaf was measured. To obtain the area of each leaf, the mean mass (0.03118 ± 0.0027g, n = 10 of a known area (4 cm^2) of the same paper was measured. The mass of each paper leaf was then divided by 0.03118g to determine its area in cm^2.

2.2.2.2 Relationship between number of adult flies and leaf area damaged by their progeny

Fairly small chromolaena plants (15-20 cm tall) with single main stems were selected, and plant size was kept as consistent as possible. Plants were not trimmed, nor were leaves removed beforehand, because this may have affected the chemistry of the plants and therefore the outcome of the trials. Each plant was placed in a steel-framed cage (0.4 x 0.4 x 1 m) with gauze panels. Cages had hinged doors and a transparent plastic flap over the entrance to prevent the highly active insects from escaping when the cage was opened. Plants were then exposed to 1, 5 or 10 pairs of newly eclosed flies and three replicates were conducted for each of the three fly densities. Flies were left inside the cages for two weeks to allow each female to lay a full (lifetime) complement of eggs and to allow larvae to develop through to pupation, i.e. maximizing mine size. All leaves (mined and unmined) were then harvested from each plant and their lengths were measured in order to calculate approximate leaf areas (available and damaged). For leaves containing mines, the area damaged was placed into one of five categories as follows: 1 = 1-10%; 2 = 11-25%; 3 = 26-50%; 4 = 51-75%; and 5 = 76-100% with a median percentage of 5%, 17.5%, 37.5%, 62.5% and 87.5% for category 1-5, respectively. The number of undamaged leaves was also recorded, with each being scored as category 0. The damage category of each leaf was then expressed as its corresponding median % damage, and converted to a proportion by dividing this value by 100. For each leaf, the approximate area damaged (cm^2) was calculated by multiplying this proportion by the calculated area of the leaf. The total leaf area available on the plant, and the total leaf area damaged, were then calculated as the sum of the total and damaged area of each leaf. The percentage leaf area damaged per plant could then be calculated from these two quantities.

2.2.2.3 Relationship between number of adult flies and number of leaf mines produced

Plants of similar size to those used in the trial described above were selected and exposed to 1, 5 and 10 pairs of mated flies as before. However, for this trial the larvae in the mines were squashed as soon as mines became visible; this was done to make it possible to count individual mines before they develop into full mines that coalesce with others. An assumption was made that the number of mines initiated reflected the oviposition preference of the female i.e. that the same proportion of eggs hatched regardless of where they were laid. This was done because it is not possible to detect unhatched eggs without removing leaves from the plant and examining them under a microscope. After two weeks, the plant was removed from the cage and set aside in a fly-free area for a few days to allow any newly-laid eggs to hatch. All leaves were then removed from the plant as previously and the total number of mines per leaf and per plant was counted.

2.2.2.4 Leaf quality in relation to selection by C. eupatorivora females for oviposition

Measures of leaf quality in the literature include percentage water content which is measured by leaf wet weight – leaf dry weight / leaf wet weight *100 (Steinbauer, 2001). Leaf toughness, which is measured by Specific Leaf Area (SLA) = area (mm^2) / dry weight (mg) is another measure. The inverse of this has also been used, and is called Specific Leaf Weight (SLW). Leaf discs of a fixed size can be used to measure SLA or SLW (Steinbauer, 2001). Finally, nutrient analysis of leaves (Scheirs *et al.*, 2002) has also been used to determine their quality. In this study, leaf quality was similarly analyzed, although different methods from those above were used.

Chromolaena plants were placed in cages as in the two trials described above. Each plant was exposed to what was estimated to be a "saturation" level of adult *C. eupatorivora* (± 20 pairs) in order to be reasonably certain that all suitable leaves should have been selected for oviposition. Plants that were used were actively growing and therefore had a range of leaf ages. Plants were exposed to as many females as possible over a short period of time so that leaves could be

harvested as soon as possible after oviposition; their chemical properties may have changed if they were harvested and processed long after females had selected them to oviposit in. Larvae in mines were squashed as soon as mines appeared; the assumption here was that the initiated leaf mines did not significantly affect the quality of the leaf. After 14 days, all leaves were removed from the plant and immediately placed in Ziploc bags to minimize water loss. Mined and unmined leaves were then counted, divided into two groups and weighed (wet weight), oven dried at 70^{o}C for 3 days and weighed again (dry weight). This procedure was repeated until there was enough material for analysis.

Two sets of leaf samples (one each of mined and unmined chromolaena leaves) were taken for milling and analysis at the Feed and Plant Laboratories of the KZN Department of Agriculture, Environment and Rural Development (DAERD), which conduct standard analyses for quality of livestock feed and the chemical composition of crop plants, respectively. Each set weighed about 2 g when milled and were from approximately 60 mined and 63 unmined leaves. The two samples were analyzed for several standard elements (Ca, Mg, Na, K, P, Fe, Cu, Zn, Mn, Cl) in the Plant Laboratory. In the Feed Laboratory, the "National Research Council Pasture Package" was selected. This included analysis of ash, fat, fibre (Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF), crude protein, acid detergent lignin, acid detergent insoluble protein and nonstructural carbohydrates.

2.2.3 Data analysis

Linear regression (in Microsoft Excel) was used to determine the relationship between leaf area and leaf length/width. GENSTAT was used for the other statistical analyses. One-way ANOVA was used to compare the percentage leaf areas damaged and number of mines on plants that were exposed to varying adult fly densities. A square root-arcsine transformation was performed on the percentage data before running the ANOVA; this was done to normalize the percentage data. The Least Significant Differences (LSD) Post Hoc test was performed to test for differences between the three fly-density categories; provided there was an overall significant difference. No statistical analyses of nutrient and element analysis of mined versus unmined leaves were

possible because the unmined leaves were combined into one sample for analysis, as were the mined leaves.

2.3 Results

2.3.1 Relationship between leaf area and leaf length/width

Leaf length was selected as the most accurate measure of leaf area because it showed a slightly stronger relationship ($Y = 4.7421x - 13.48$, $R^2 = 0.8975$, $p = 0.0022$) with leaf area than did leaf width ($Y = 6.8635x - 8.1695$, $R^2 = 0.8732$, $p = 0.0023$) (Fig. 2.1).

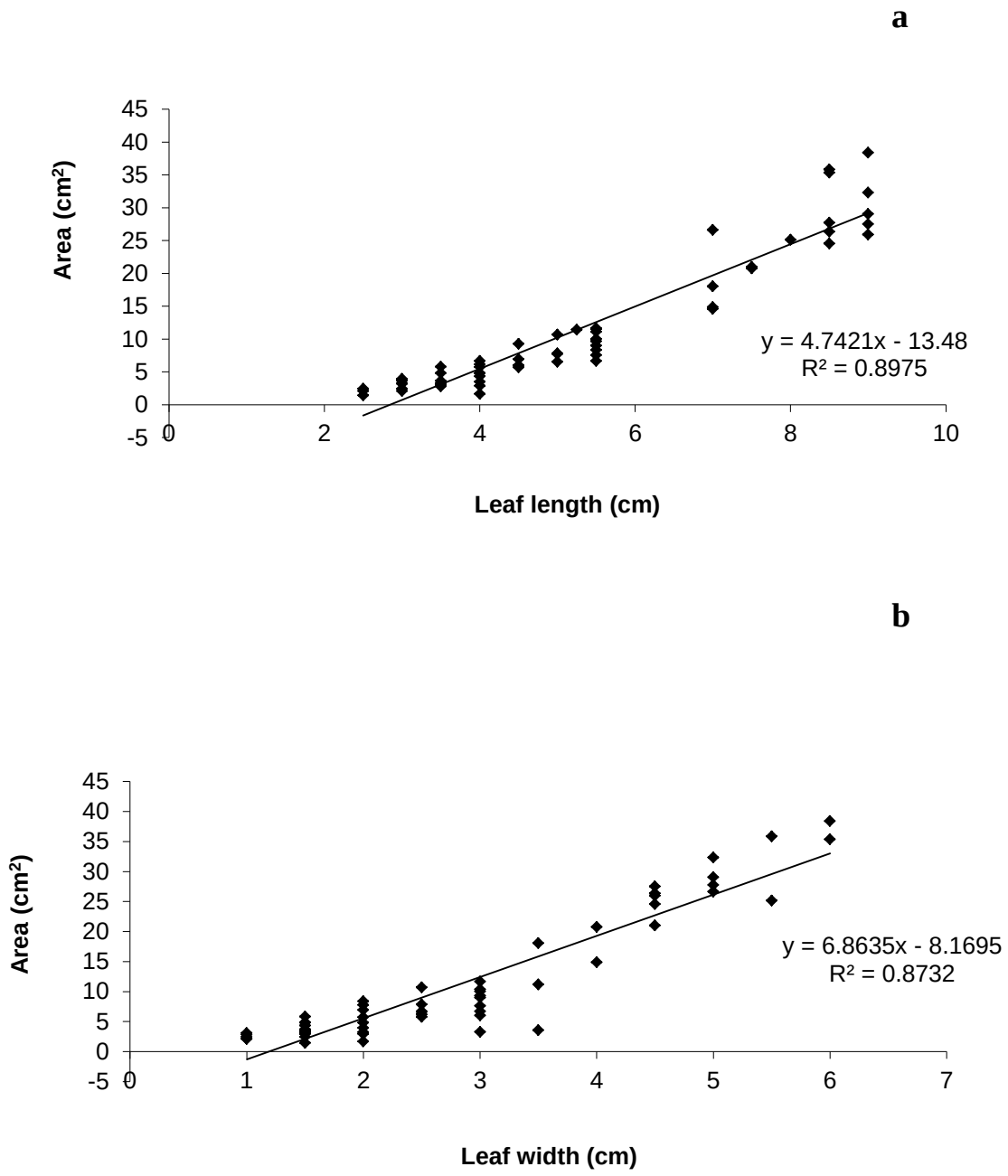

Fig.2.1. Relationship between the area of 60 *C. odorata* leaves and the (a) leaf length and (b) leaf width of each of these leaves.

2.3.2 Leaf area damaged by *C. eupatorivora* larvae

The mean leaf area of plants did not differ significantly between the treatments ($F_{(2: 8)} = 0.66$, $p = 0.549$, Fig. 2.2a), indicating that plants were of similar size between treatments. Neither mean leaf area damaged nor percentage leaf area damaged differed significantly between the three fly densities, although the differences were nearly significant for both ($F_{(2: 8)} = 4.72$, $p = 0.059$, Fig. 2.2a; $F_{(2: 8)} = 4.47$, $p = 0.065$, Fig. 2.2b respectively). Percentage leaf area damaged was slightly higher with five (28.5%) than with 10 pairs (22%) of adults and was lowest with one pair (6.5%). The maximum percentage of leaf area damaged during the trials was 37.5% for one of the trials involving five pairs of flies.

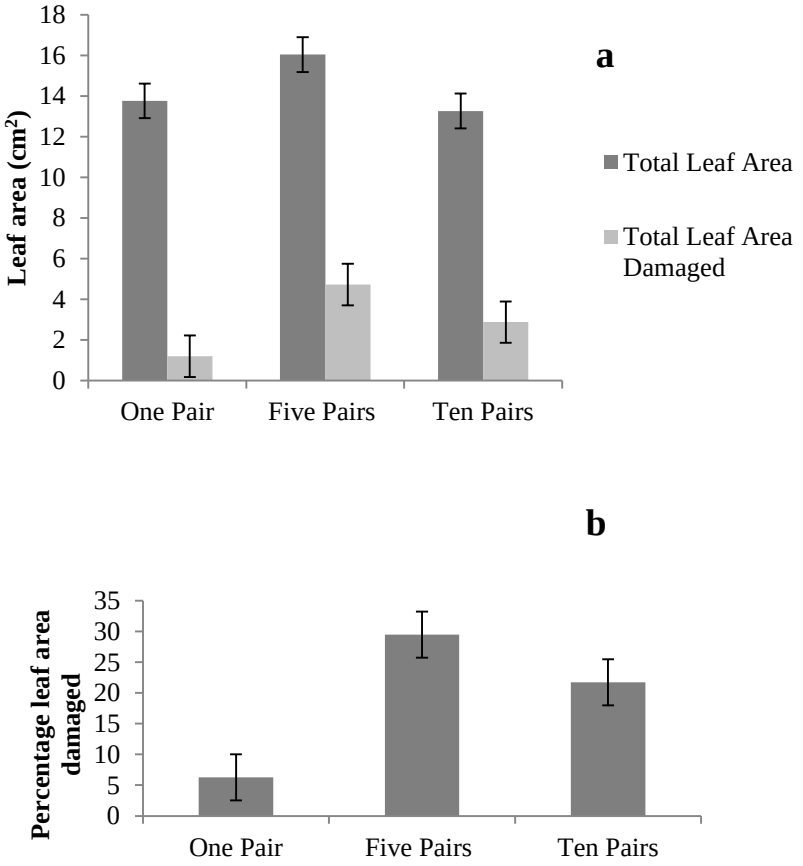

Fig. 2.2. Leaf area of *C. odorata* plants available and damaged following exposure to one, five and ten pairs of *C. eupatorivora* flies in the laboratory. (a) Mean (± 1 SE) leaf area available and leaf area damaged per plant; (b) Mean (± 1 SE) percentage leaf area damaged per plant. There were no significant differences between any categories. Three replicates were carried out in all cases.

2.3.3 Number of leaves damaged and mines per damaged leaf

The mean number of leaves did not differ significantly between the treatments ($F_{(2: 8)} = 1.92$, $p = 0.226$, Fig. 2.3a), again confirming that plants were similar-sized. However, the mean number of leaves damaged differed significantly between the three fly densities ($F_{(2: 8)} = 6.42$, $p = 0.032$, Fig. 2.3a) with five adult pairs damaging slightly more leaves than one and 10 pairs, but with no significant difference between one and 10 pairs. There was no significant difference in percentage of leaves damaged between any of the fly densities ($F_{(2:98)} = 0.54$, $p = 0.585$, Fig. 2.3b). Overall, five pairs caused significantly more mines in each plant ($F_{(2: 8)} = 4.40$, $p = 0.042$, Fig. 2.3c). In relation to the mean number of mines per damaged leaf on each plant, five and 10 pairs of flies caused slightly more mines than one pair, but again the differences were not significant ($F_{(2: 98)} = 2.39$, $p = 0.097$, Fig. 2.4).

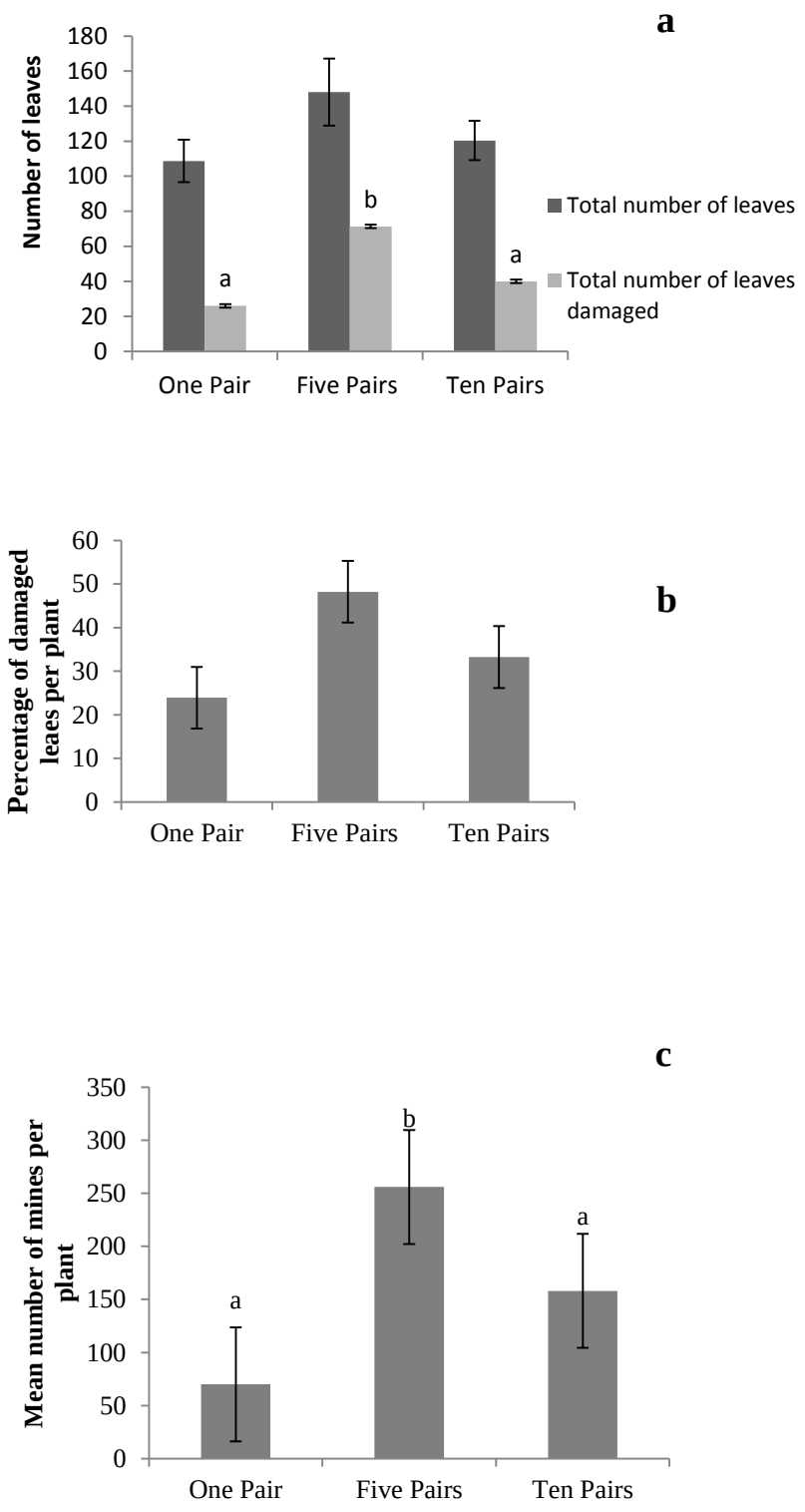

Fig. 2.3. Number of *C. odorata* leaves damaged following exposure to one, five and ten pairs of *C. eupatorivora* flies in the laboratory. (a) Mean (± 1 SE) number of leaves available and number of leaves damaged per plant; (b) Mean (± 1 SE) percentage of damaged leaves per plant; (c) Mean (± 1 SE) number of mines per plant. Three replicates were carried out in all cases.

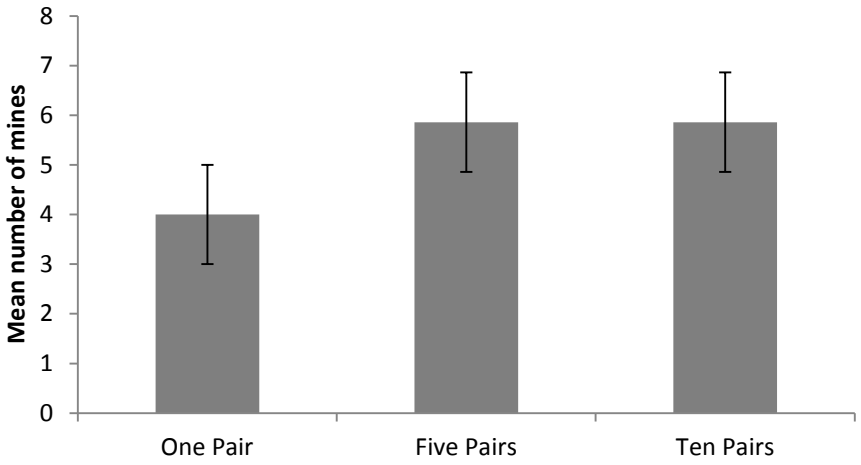

Fig. 2.4. Mean (± 1 SE) number of larval mines per damaged leaf on *C. odorata* plants following exposure to one, five and ten pairs of *C. eupatorivora* flies in the laboratory. Three replicates were carried out in all cases.

2.3.4 Leaf quality in relation to usage by *C. eupatorivora*

Mean percentage water content was almost identical for mined and unmined leaves. For mined leaves the mean (± SE) was 78.11 ± 3.97 % and for unmined leaves it was 78.27 ± 3.60 %. There was thus no significant difference between the two groups ($F_{(1:15)} = 0.01$, $p = 0.0934$, $n = 16$). The nutritional composition results are presented in Table 2.1. There were clear differences with Acid Detergent Lignin which was 62% higher in unmined leaves and Nonstructural Carbohydrates which were 55% higher in mined leaves. However, these were only biological differences; statistical analysis could not be undertaken because samples were pooled within leaf category (mined versus unmined) in order to obtain sufficient material for analysis (Table 2.1). The rest of the measured components showed no major differences, but Ca, Na, Cl and Cu were slightly higher in unmined leaves, while K/Ca+Mg and P was slightly higher in mined leaves.

Table 2.1. Nutritional composition of mined and unmined *C. odorata* leaves.

Nutrients assessed	Mined	Unmined	Unit	%mined: unmined
Ash	14.17	14.82	% dry matter	95.6
Fat	5.71	5.81	% dry matter	98.3
Acid detergent fibre (ADF)	19.37	19.92	% dry matter	97.2
Neutral detergent fibre (NDF)	38.56	36.98	% dry matter	104.3
Acid detergent lignin (ADL)	4.62	7.49	% dry matter	61.7
Crude protein	27.8	25.41	% dry matter	109.4
Nonstructural carbohydrates (NSC)	7.05	4.55	% dry matter	154.9
Acid detergent insoluble nitrogen (ADIN)	3.08	2.92	% dry matter	105.5
Neutral detergent insoluble nitrogen (NDIN)	5.57	5.56	% dry matter	100.2
Ca	1.89	2.28	% dry matter	82.9
Mg	0.64	0.7	% dry matter	91.4
K	3.31	2.96	% dry matter	111.8
Na	0.03	0.04	% dry matter	75.0
K/Ca+Mg	0.58	0.44	% dry matter	131.8
Cl	0.77	0.95	% dry matter	81.1
P	0.59	0.48	% dry matter	122.9
Zn	61	66	mg/kg or ppm	92.4
Cu	5	14	mg/kg or ppm	35.7
Mn	110	103	mg/kg or ppm	106.8
Fe	188	209	mg/kg or ppm	90.0

2.4 Discussion

From the plants that were exposed to different numbers of pairs of flies in the trial to determine the relationship between fly density and percentage leaf area damaged, I observed that *C. eupatorivora* flies often oviposited more than once on selected leaves while avoiding other leaves. Therefore, mines would co-occur on these leaves and damage them extensively (category 5). This suggests that leaves of the same plant differ with regard to quality, toughness or nutritional composition. These results showed that five pairs of flies caused more damage than one pair, but that 10 pairs did not cause more damage than five pairs, and that the flies rarely damaged more than 30% of the leaves. This is probably caused by a combination of there being a

small proportion of suitable leaves and larval saturation and competition when plants are exposed to high densities of insects, as was demonstrated (Schooler and McEvoy, 2006) with the golden loosestrife beetle *G. pusilla*, on purple loosestrife *L. salicaria*. It could also, in theory, be because females each lay fewer eggs when overcrowded. At extremely high densities, insect performance is probably reduced by competition as the insects will be competing with each other for the same resource; adults could be competing for oviposition sites and larvae for food within a leaf. In this study, even though differences were not statistically significant, 10 pairs of flies resulted in larval progeny which damaged a slightly lower leaf area than five pairs. One adult pair per plant is probably a rather low density and therefore causes relatively low levels of damage. There was considerable variability in the data within each treatment and this was probably caused by variability in mating success, longevity and fertile egg production between the pairs of flies. Higher numbers of replicates would probably have led to lower variances and standard errors, and thus an increased likelihood of statistically significant differences being detected. Counting the number of larvae successfully pupated and eclosed would also have given an indication of the density of larvae in a leaf, above which larvae cannot complete their life cycle properly.

The mean number of mines per plant did not differ significantly between the three fly densities although it was slightly lower for the one pair treatment compared to the other treatments. The proportion of damaged leaves per plant also did not differ between the treatments but there was variability within each treatment that was probably caused by the same factors as above, namely stochastic variability. However, plants exposed to five adult pairs had significantly more mined leaves than the other treatments.

In the leaf quality analysis, there was no significant difference in percentage water content between mined and unmined leaves. I expected water content to be higher in the mined leaves as it is a measure of leaf quality, and *C. eupatorivora* presumably mines leaves of better quality. Water content is also a leaf characteristic that is crucial to the nutrition of herbivorous insects (Steinbauer, 2001).

High leaf quality is also indicative of high nutritional value (Barnes *et al.*, 2007). Fibre (Neutral Detergent Fibre, Acid Detergent Fibre, Acid Detergent Lignin) is recognized as a vital quality-related attribute of plants (Scheirs *et al.*, 2002; Barnes *et al.*, 2007). Fibre includes lignin and cellulose; these two substances form the plant cell wall and constitute a large proportion of the solid parts of plants, so the higher the levels these substances, the tougher the plant will be (Scheirs *et al.*, 2001; Barr, 2009). Lignin resists attack by herbivores as it is not digestible (Scheirs *et al.*, 2001; Barr, 2009). This study showed that unmined leaves were substantially higher in ADL, which suggests that *C. eupatorivora* might have preferred certain leaves because they were younger and softer.

Mined leaves were higher in Nonstructural Carbohydrates (NSC) than unmined leaves. This suggests that *C. eupatorivora* adults chose leaves with higher levels of carbohydrates. NSC provide a key survival strategy for perennial plants like chromolaena, because they enable plants to survive disturbance and winter (Kozlowski, 1992; Ikuenobe and Ayeni, 1998). NSC reserves also influence the plant's response to stress, which can affect plant health (Ikuenobe and Ayeni, 1998). Carbohydrate metabolism is important for the replenishment of reserves and for regulating energy available for plant tolerance to environmental stress (Kozlowski, 1992). This shows that the higher the levels of NSC, the healthier the plant. It is often assumed that mature plants/leaves are higher in fibre and lower in NSC content than immature plants. This suggests that *C. eupatorivora* chose leaves with high NSC because they were immature and therefore, softer. NSC content may vary between leaves of the same plants because some leaves are more mature (older) than others. For chromolaena, young leaves at the top of the plant will be higher in NSC content than those at the bottom of the plant. This might influence oviposition site choice by *C. eupatorivora*. The consequences of the selectivity of *C. eupatorivora* females in terms of oviposition preference are that some leaves will remain unmined if they are only exposed to the flies once they are older. This implies that *C. eupatorivora* will perform better in actively growing plants or seedlings than in mature plants. My observation (non-quantified) was that the flies preferred younger fully-expanded leaves, and therefore I would expect that actively growing plants with many young leaves would experience a higher % leaf area, and a higher proportion of leaves, mined. The highest mean leaf area damaged was 30% (for 5 fly pairs, Fig. 2.2b), while the highest proportion of leaves mined was 48 (Fig. 2.3b).

In conclusion, the relationship between the number of *C. eupatorivora* adults and the damage caused by their progeny, like several other insects, appears to be non-linear because the females are selective as to the quality of the leaves they use for egg laying (they seem to prefer leaves, for example, with lower acid detergent lignin and higher nonstructural carbohydrate levels), in combination with density-dependent competition. Therefore, at higher fly densities the same number of leaves receive eggs but these have increased egg loads, resulting in little increase in leaf area damaged. It is likely that at high densities of adults, fewer progeny are able to reach maturity, or result in smaller less fecund adults, due to an increase in intraspecific competition. Finally, due to the females' selectiveness, only a relatively low percentage of leaves on a chromolaena plant may be mined, and as a biocontrol agent the fly is thus limited in its efficacy. This may vary depending on how the situation of the plants (e.g. growth phase, light intensity) may affect leaf quality.

CHAPTER 3: Abundance of *C. eupatorivora* larval mines on *C. odorata* in the field

3.1 Introduction

Chromolaena odorata (chromolaena) is a perennial species that is well adapted to a wet–dry tropical and subtropical climate because its aboveground foliage can die off during the dry season (May–September in South Africa) when little rain falls (Liggitt, 1983; McFadyen, 1991). However, the roots remain alive and the above-ground parts of the plant grow back vigorously during the wet season (November–April in South Africa). In South Africa, seeds germinate at the beginning of the wet season and flowering occurs in June–July, and because flowering is triggered by a decrease in day length, all plants in an area flower at much the same time of the year (Liggitt, 1983; McFadyen, 1991). Studies have shown that there is usually better performance in terms of higher relative growth rate, nett assimilation rates, reproductive potential and nutrient uptake efficiency of chromolaena populations in open, sunny environments (McFadyen, 1989; Muniappan and Marutani, 1988; Sivagnanam and Swamya, 2010). In established forestry plantations with a completely closed canopy, chromolaena is usually restricted to the edges (Ambika, 2007) and any plants growing in the interior eventually develop a straggling habit depending on the height of the tree. In essence, chromolaena does not tolerate deep shade (Ambika, 2007) but it can grow in light shade.

Since its release and establishment as a biological control agent in South Africa in 2003, *Calycomyza eupatorivora* has spread and been distributed widely. However, the fly has been observed to not exploit leaves equally between habitats, geographical regions, and plants and even within a single plant. For example, it has been observed to be possibly more abundant in shaded areas, and high infestation levels have been seen occasionally in such habitats. In the laboratory, *C. eupatorivora* was sensitive to low humidity levels, suggesting that the insect might not establish in drier areas. Also, the leaves of chromolaena lose condition in winter in these areas (Zachariades *et al.*, 1999), and the lack of an obvious diapause period also makes it unlikely that *C. eupatorivora* would establish in these areas. Leaves of chromolaena appear

larger and softer under plantations (shady environments) than those growing in open sunny habitats (personal observation). Studies on other systems have shown that the suitability of a leaf to developing offspring of the fly may change with leaf age because of changes in the chemical or physical properties of that particular leaf (Raupp and Denno, 1983; King *et al.*, 1998). Similarly, leaves growing in shady areas are physically softer and usually contain more nitrogen and moisture than those in full sun (Raupp and Denno, 1983; Raupp, 1985; Denno *et al.*, 1990).

This study was carried out to quantify *C. eupatorivora* infestation levels and damage at four distinct times over a 12-month period encompassing four consecutive seasons, and at three closely-placed study sites that each included two habitats, namely open and shaded (under eucalyptus plantations). In addition, larval mortality levels were also recorded.

3.2 Materials and methods

Field surveys were carried out in the Sappi Cannonbrae eucalyptus plantation near uMkomaas, on the South Coast of KwaZulu-Natal province, South Africa [30° 12' 6.98" S, 30° 47' 6.90" E]. Sampling was done over four consecutive seasons: September 2009 (spring), December 2009 (early summer), March 2010 (late summer/autumn) and July 2010 (winter).

3.2.1 Experimental design and data collection

Data were collected from three sites, within 1km of one another, within the study area. Each site consisted of a planted eucalyptus compartment under which chromolaena was growing, adjacent to a 'conservation unit' which had not been planted to eucalyptus but consisted largely of a dense chromolaena infestation. This allowed data to be collected at each site from the two habitats in question, viz. shaded (under eucalyptus) and open. At each of these six sampling sites, a 50 m line transect was laid out using a tape measure. The beginning and end of each line transect was marked with a wooden stake. The chromolaena plant closest to each 5m interval along the tape-measure (starting at 0m and continuing up to 45m) was selected for sampling, ensuring 10 plants per transect. For small plants with a single stem, the whole plant was selected for sampling, but for large plants with many stems, a representative branch was selected. The selected

plant/branch was marked with an individually numbered plastic tag and the following data were recorded at each sampling interval: plant/branch height (to the tip of the tallest live stem) from the ground (for large sprawling plants measured from the point on the ground directly below the base of the selected branch); total number of leaves (>0.5cm long); number of mined leaves; number of leaf mines; number of live larvae. Mines were inspected to determine if they were 'complete' or 'incomplete'; 'complete' mines being judged to be large enough to have supported larval development through to pupation and 'incomplete' mines being all mines smaller than this. Mines were also scored as being 'old' or 'young'; the 'young' mines being those over which the leaf epidermis was still white and fresh-looking and could contain live larvae, and 'old' mines being those that had developed a few weeks back, over which the epidermis had turned brown or peeled away. The same marked individual plants/branches were measured on each of the four sampling occasions. Where plants/branches had died, a new plant/branch in close proximity was selected for subsequent measurements. The 50m transect was replicated three times in each habitat, with transects placed parallel from each other, ensuring that a total of 30 plants were sampled in each habitat, and consequently 60 plants at each site, during each of the four sampling occasions.

3.2.2 Data analysis

Data obtained were analyzed using GENSTAT, Repeated Measures Analysis of Variance (Generalized Linear Model) to take into account the fact that the same plants were sampled over the four seasons. Individual plants were used as data points/sampling units, without regard to transect number; that is, transects were pooled because we did not want to compare different transects within the same habitat sampled. Season was treated as data at successive times because the same chromolaena plants were sampled repeatedly over the four seasons. The treatment structures were the season and site. GLM was used so that the assumptions of normality of data distribution and homogeneity of variances were met.

Post Hoc tests, namely Least Significant Difference (LSD) tests, were performed to determine if there were significant differences between the three sites, two habitats and the four seasons in relation to the recorded variables. From the data collected, the mean number of mines per

damaged leaf was calculated by dividing the total number of mines counted on the plant/branch by the number of damaged leaves on the same plant/branch, where a damaged leaf was a leaf with at least one initiated *C. eupatorivora* mine. The mean number of mines in relation to leaves available was calculated by dividing the total number of mines by the number of leaves on the plant/branch. Two estimates of larval mortality ('minimum' and 'higher') were also determined. Both models assume that all complete mines have produced pupae. 'Minimum' larval mortality was calculated by dividing the number of old incomplete mines (i.e. the larva did not complete development and thus presumably died) by the total number of mines. It thus assumes that all young incomplete mines still contain larvae, and is therefore an underestimate of mortality. The alternative, 'higher larval mortality' was calculated using the following formula: $\sum$ (1- (number of live larvae + young complete mines + old complete mines) / total number of mines). This formula assumes that young incomplete mines are empty and therefore represent mortality unless live larvae are visible inside them. This provides an over-estimate of mortality because we certainly missed some very small live larvae still in the mines.

Regression analysis was performed to determine the strength of the relationship between plant height and the number of leaves.

3.3 Results

3.3.1 Plant/branch height

Mean plant/branch height increased slightly during the growing season, from September to March. Overall there was a significant difference between seasons ($F_{(3:522)} = 51.62$, $p < 0.001$, Fig. 3.1a) but March and July were not significantly different from one another. There was also a significant difference between habitats ($F_{(1:174)} = 38.52$, $p < 0.001$, Fig. 3.1b) and sites ($F_{(2:174)} = 44.23$, $p < 0.001$, Fig 3.1c), with plants/branches being taller in the open habitat and at site 1 and 3. The interaction between habitat and site was also significant ($F_{(2:174)} = 25.78$, $p < 0.001$). The rest of the interactions were not significant, namely season and site ($F_{(3:522)} = 0.59$, $p = 0.661$), season and habitat ($F_{(3:522)} = 1.69$, $p = 0.188$) and season, habitat and site ($F_{(6:522)} = 3.33$, $p = 0.13$).

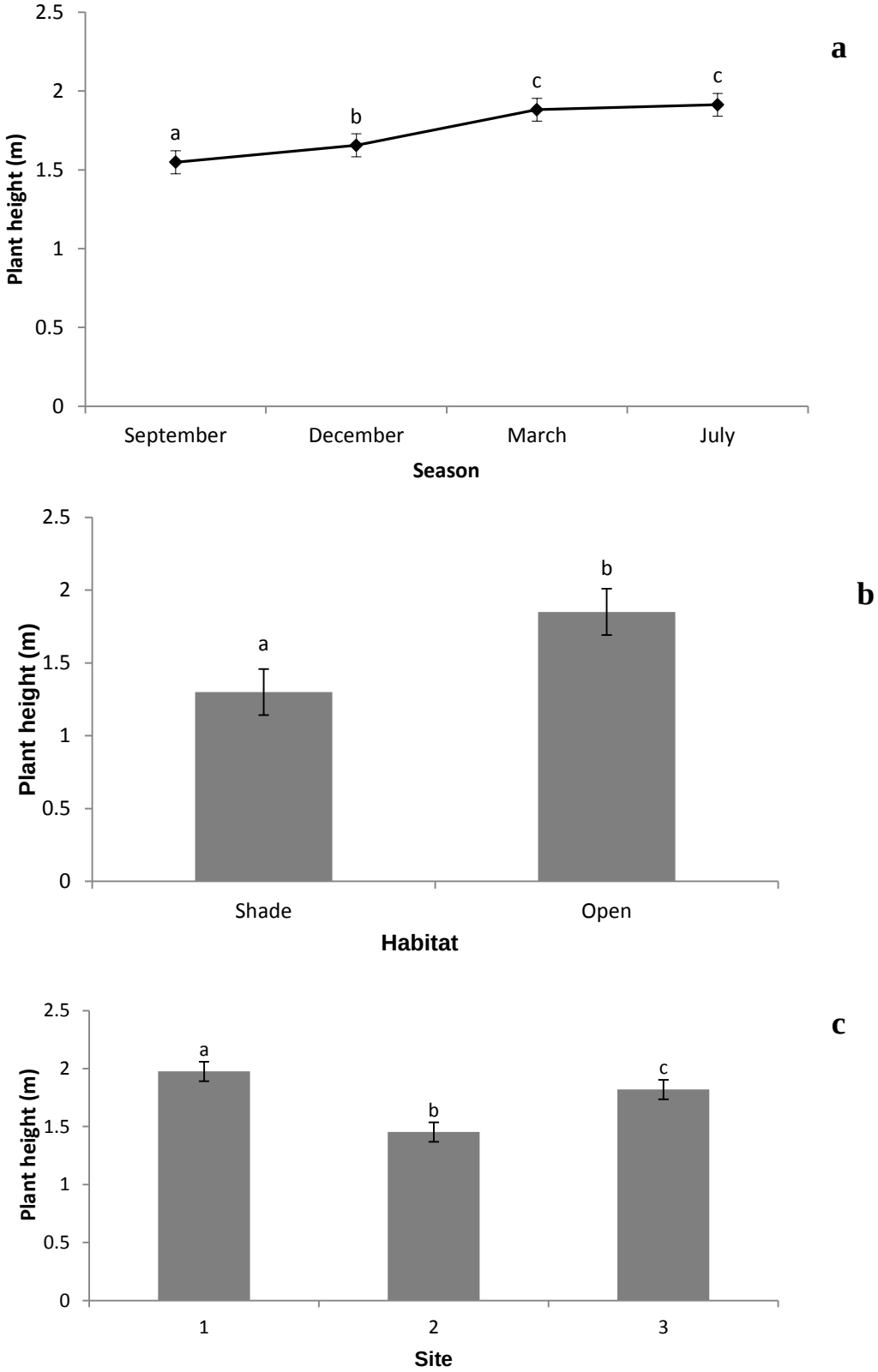

Fig. 3.1. Mean (± 1 SE) plant/branch height between (a) seasons, (b) habitats and (c) sites. Different letters above points and bars indicate significant differences between them.

3.3.2 Number of leaves

The mean number of leaves per plant/branch was low in spring, September 2009, but increased as the next season, summer (December) approached and was highest in March 2010. However, leaf density decreased as winter (July 2010) approached (Fig. 3.2a). Overall, there was a significant difference between the four seasons ($F_{(3: 522)} = 84.88$, $p < 0.001$), but there was no significant difference between December and March. The number of leaves was significantly higher in the open habitats than in the shaded habitats ($F_{(1: 174)} = 11.70$, $p < 0.001$, Fig. 3.2b). Overall, there were also significant differences between the three sites ($F_{(2:174)} = 10.46$, $p < 0.001$, Fig. 3.2c), even though sites 1 and 2 were not significantly different.

There was no significant interaction between season and habitat ($F_{(3: 522)} = 2.84$, $p = 0.059$) - although this was almost significant - or between season, habitat and site ($F_{(6: 522)} = 1.07$, $p = 0.369$) in relation to the mean number of leaves. However, the interactions between season and site ($F_{(6: 522)} = 18.99$, $p < 0.001$) and between site and habitat ($F_{(2: 174)} = 11.40$, $p < 0.001$) were significant.

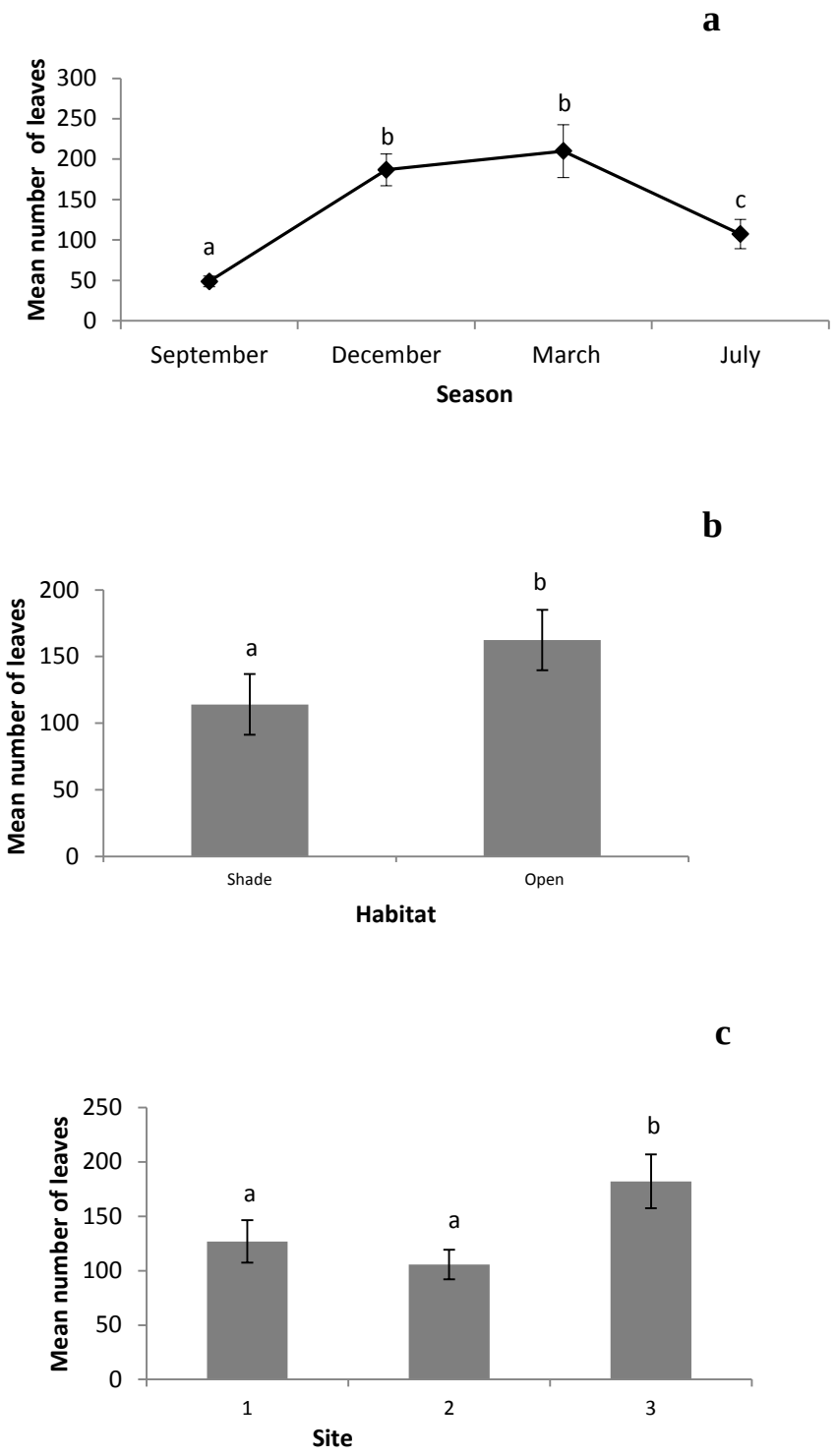

Fig. 3.2. Mean (± 1 SE) number of chromolaena leaves per plant/branch between (a) seasons, (b) habitats and (c) study sites. Different letters above points and bars indicate significant differences between them.

3.3.3 Relationship between number of leaves per plant/branch and plant height

Number of leaves and height showed a significant positive linear relationship although the relationship was slightly stronger ($R^2 = 0.4488$) for the shaded habitats but weaker ($R^2 = 0.0389$) for the open habitats (Fig. 3.3).

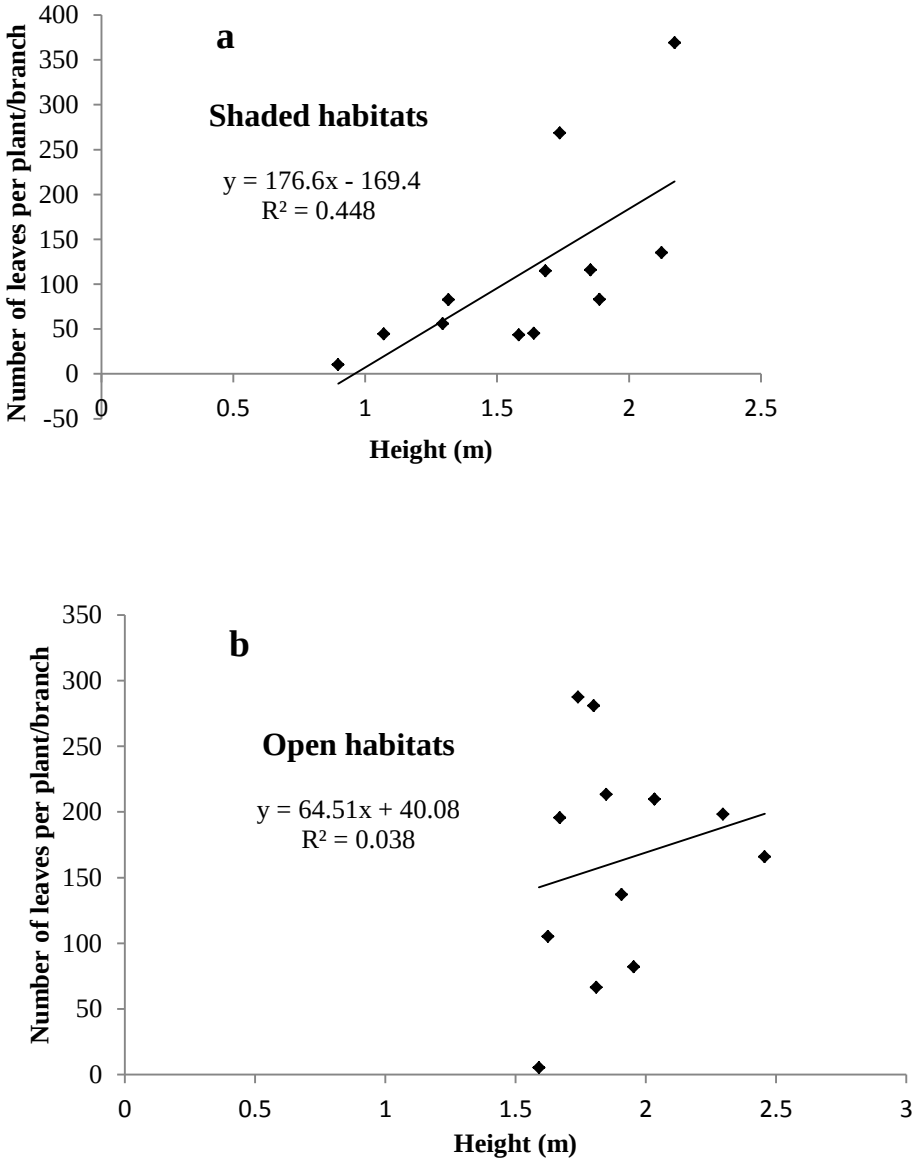

Fig. 3.3. Relationship between the number of leaves and plant/branch height in (a) the shaded habitats ($R^2 = 0.4488$, $p = 0.056$) and (b) the open habitats ($R^2 = 0.0389$, $p = 0.084$). Each point represents an overall mean for each site at each sampling occasion (i.e. 3 sites x 4 seasons; n= 12).

3.3.4 Numbers of leaf mines per plant/branch

The mean number of mines (for both all mines and complete mines only) per plant/branch was highest in March and very low in December. There was a substantial increase from December to March, and numbers decreased again as July approached (Fig. 3.4a). There were significant differences in numbers of leaf mines (all mines and complete mines) between the four seasons ($F_{(3:522)} = 54.56$, $p < 0.001$, and $F_{(3:522)} = 67.64$, $p < 0.001$ respectively). The overall number of mines (i.e. all mines) did not differ significantly ($F_{(1:174)} = 2.52$, $p = 0.113$) between the two habitats, although it was slightly higher in the shade ($F_{(1:\ 174)} = 10.70$, $p = 0.91$, Fig. 3.4b). Overall, there were significant differences between the three sites for both all mines and complete mines ($F_{(2:174)} = 8.24$, $p < 0.001$ and $F_{(2:174)} = 6.76$, $p < 0.001$, respectively, Fig. 3.4c). The number of both all mines and complete mines per plant/branch was significantly higher at site 1 than at the other two sites.

There was a significant interaction between season, habitat and site in relation to both the total number of mines ($F_{(6:\ 522)} = 5.47$, $p < 0.001$) and the number of complete mines ($F_{(6:\ 522)} = 3.73$, $p < 0.014$) per plant/branch. There was a significant interaction between season and habitat ($F_{(3:\ 522)} = 5.67$, $p = 0.010$) in relation to the number of complete mines but not in relation to the total number of mines ($F_{(3:\ 522)} = 1.14$, $p = 0.319$). In relation to the total number of mines, there was a significant interaction between habitat and site ($F_{(2:\ 174)} = 9.78$, $p < 0.001$) but not between season and site ($F_{(6:\ 522)} = 2.33$, $p = 0.059$). In relation to the number of complete mines, there were significant interactions between season and site and between habitat and site ($F_{(6:\ 522)} = 2.77$, $p = 0.047$ and $F_{(2:\ 174)} = 4.99$, $p = 0.008$).

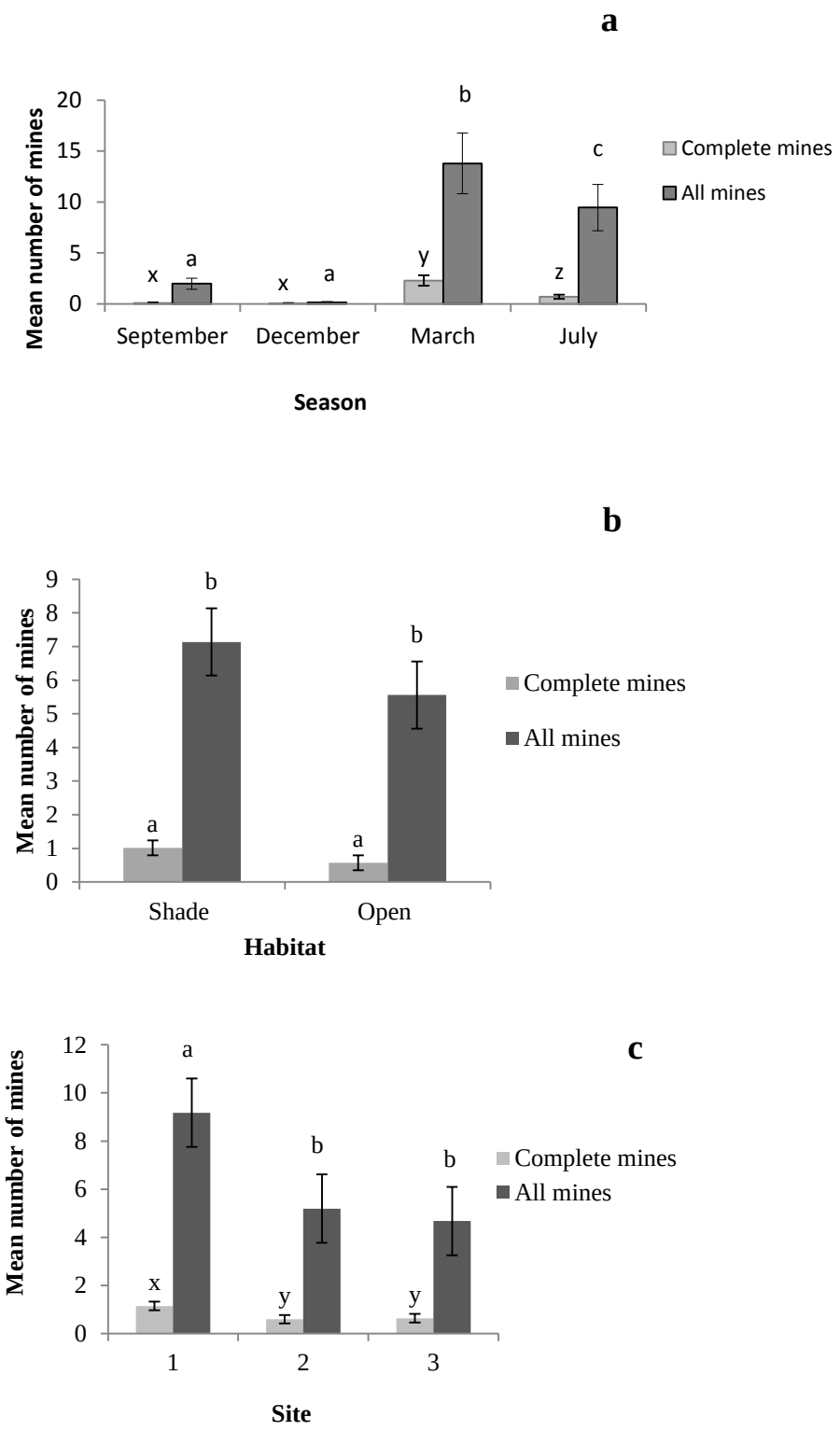

Fig. 3.4. Mean (± 1 SE) number of leaf mines (all mines and complete mines) of *C. eupatorivora* per plant/branch between (a) the four different seasons, (b) the two habitats and (c) the three sites. Different letters above bars indicate significant differences between them – statistical comparisons are made only between seasons, habitats and sites within a category of mine.

3.3.5 Mean proportion of mines in relation to leaves available

The mean proportion of mines in relation to leaves available per plant/branch showed a similar pattern to that of the total number of mines and number of complete mines. Relative numbers (proportions) decreased between September and December but increased dramatically thereafter and were at their highest in March and July. Overall, the difference between the four seasons was significant ($F_{(3:500)} = 29.01$, $p < 0.001$, Fig. 3.5a), but March and July were not significantly different from one another. There was also a significant difference between habitats ($F_{(1:174)} = 22.31$, $p < 0.001$) with the number of mines in relation to leaves available being higher in the shade (Fig. 3.5b), and there was a significant difference ($F_{(1:174)} = 16.74$, $p < 0.001$, Fig. 3.5c) between sites as well. At all of the three sites, the mean number of mines in relation to leaves available was always lower in the open habitats (Fig. 3.5d). There were significant interactions between season and habitat ($F_{(3:500)} = 4.52$, $p = 0.010$), season and site ($F_{(6:500)} = 2.65$, $p = 0.030$), site and habitat ($F_{(2:174)} = 5.59$, $p = 0.004$), and season, habitat and site ($F_{(6:500)} = 3.54$, $p = 0.006$).

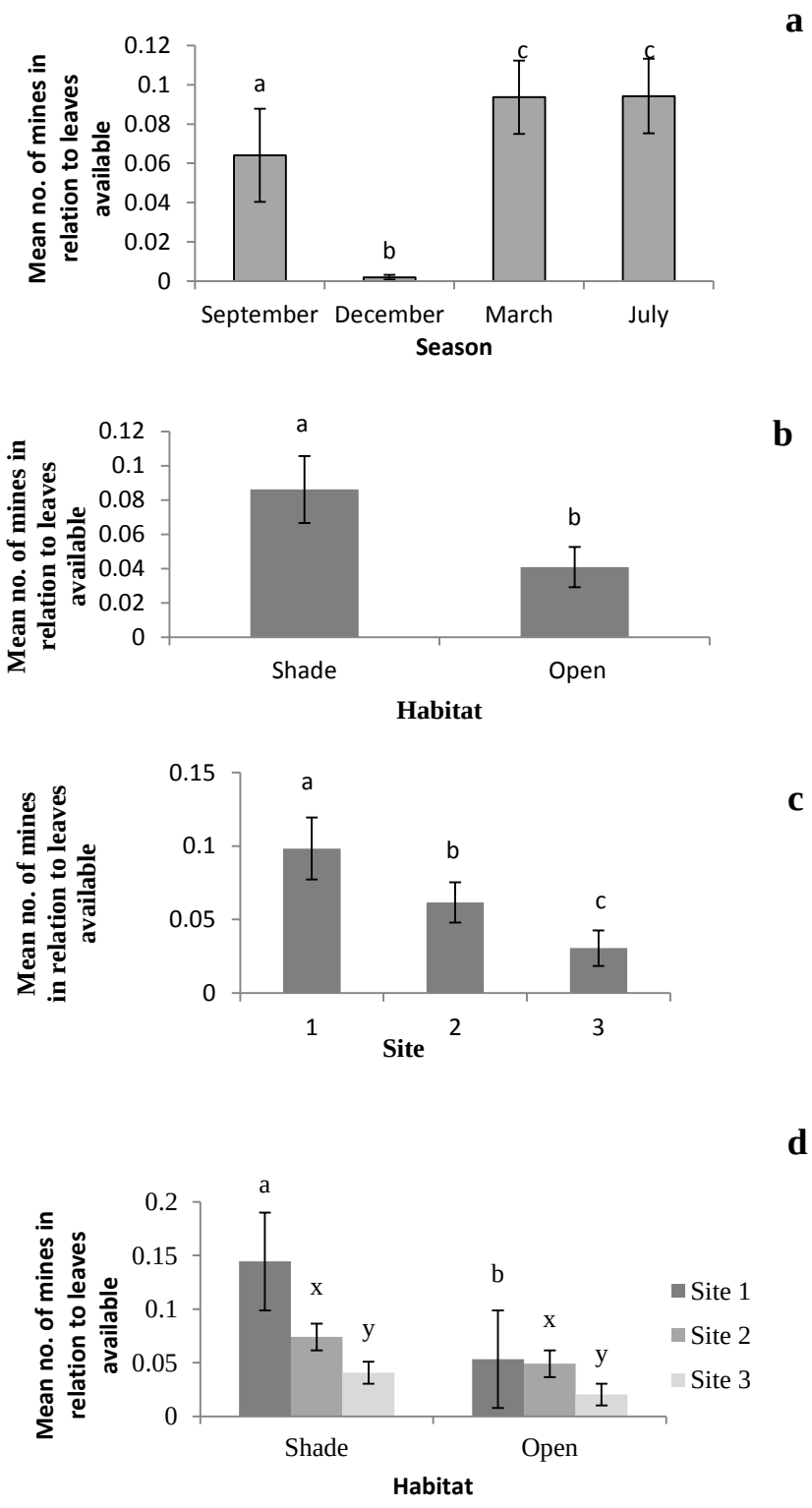

Fig. 3.5. Mean (± 1 SE) proportion of leaf mines in relation to leaves available between (a) the four seasons, (b) the open and shaded habitats, (c) the three study sites and (d) the habitats between sites. Different letters above bars indicate significant differences between them.

3.3.6 Mean number of mines per damaged leaf

There was a significant increase in the mean number of mines per damaged leaf, calculated on a per plant/branch basis, from approximately 1.2 mines per leaf in September to 1.7 mines per leaf in December, with around 1.4 mines per leaf in March and July (Fig. 3.6a). Overall, the difference in mines per damaged leaf between the four seasons was significant ($F_{(3:171)} = 34.64$, $p < 0.001$), but March and July were not significantly different from one another. There was also a significant difference in numbers of mines per damaged leaf between the habitats ($F_{(1:164)} = 5.54$, $p = 0.020$, Fig. 3.6b), but there was no significant difference between the sites ($F_{(2:164)} = 1.11$, $p = 0.330$, Fig. 3.6c).

There were no significant interactions between season, habitat and site ($F_{(3:171)} = 0.17$, $p = 0.740$) and between site and habitat ($F_{(2:164)} = 0.79$, $p = 0.457$), but there were significant interactions between season and habitat ($F_{(3:171)} = 10.13$, $p < 0.001$) and between season and site ($F_{(6:171)} = 3.08$, $p = 0.041$).

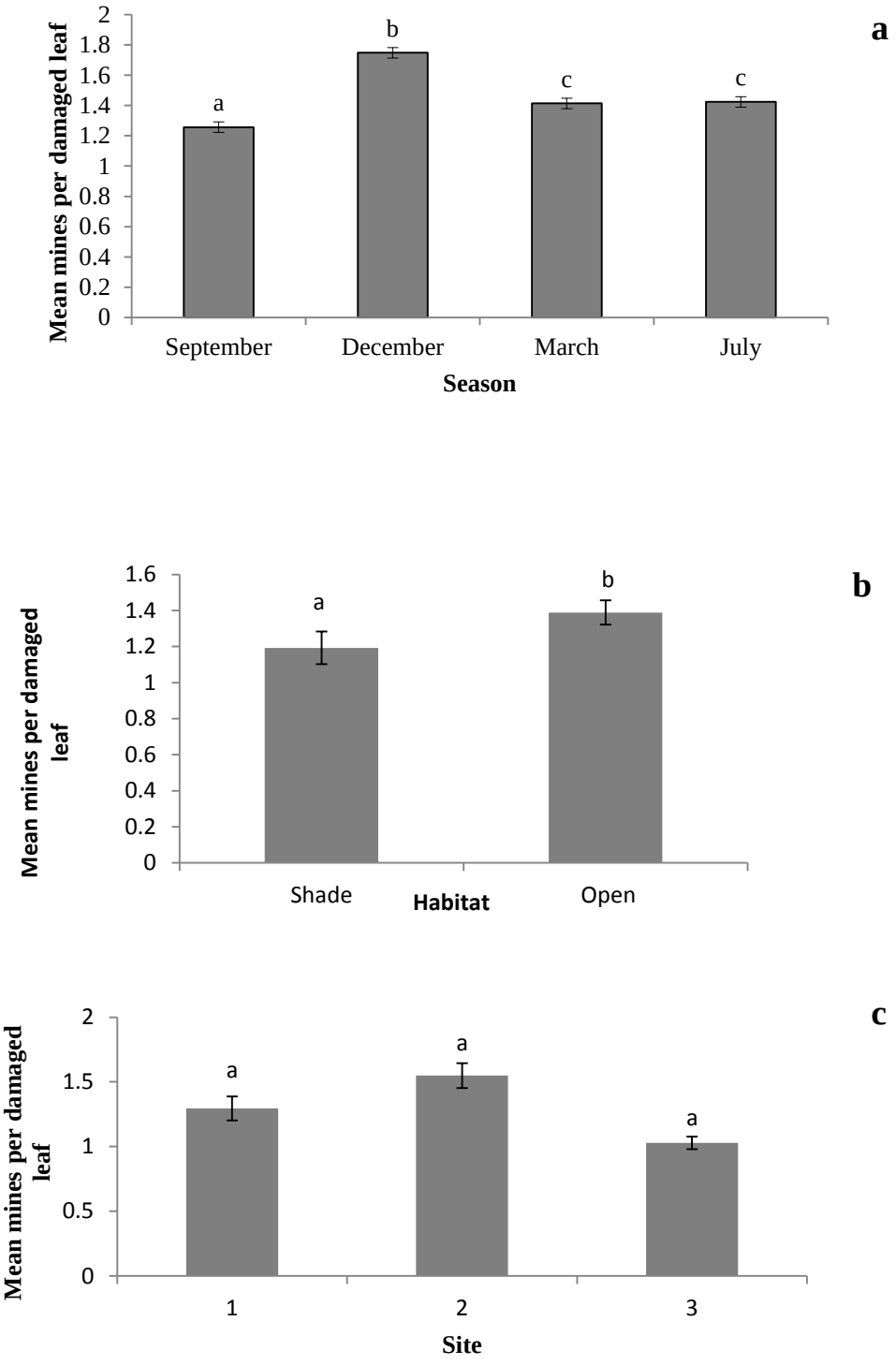

Fig. 3.6. Mean (± 1 SE) number of mines per damaged leaf (a) between the four seasons, (b) in shaded and open habitats and (c) between the three sites. Means followed by different letters are significantly different.

3.3.7 Mean proportional larval mortality ('minimum')

Larval mortality was high in September and July (around 70-75%), slightly lower in March (about 50%) and at its lowest in December (about 35%) (Fig. 3.7a). The difference in larval mortality between the four seasons was significant overall ($F_{(3:171)} = 113.31$, $p < 0.001$). There was significantly lower mortality in the shaded versus the open habitats ($F_{(1:164)} = 4.57$, $p = 0.034$, Fig. 3.7b). There were no significant differences in mortality between the sites ($F_{(2:164)} = 0.96$, $p = 0.384$, Fig. 3.7c).

There were significant interactions between season and habitat ($F_{(3:171)} = 11.92$, $p < 0.001$) and between season and site ($F_{(6:171)} = 9.45$, $p < 0.001$). However, there were no significant interactions between season, habitat and site ($F_{(3:171)} = 1.52$, $p = 0.221$) and between habitat and site ($F_{(2:164)} = 2.29$, $p = 0.104$). The calculation of 'higher' mortality (see section 3.2.2) was only slightly higher than the 'minimum' mortality, but since the difference between the two was very low the former results are not presented here.

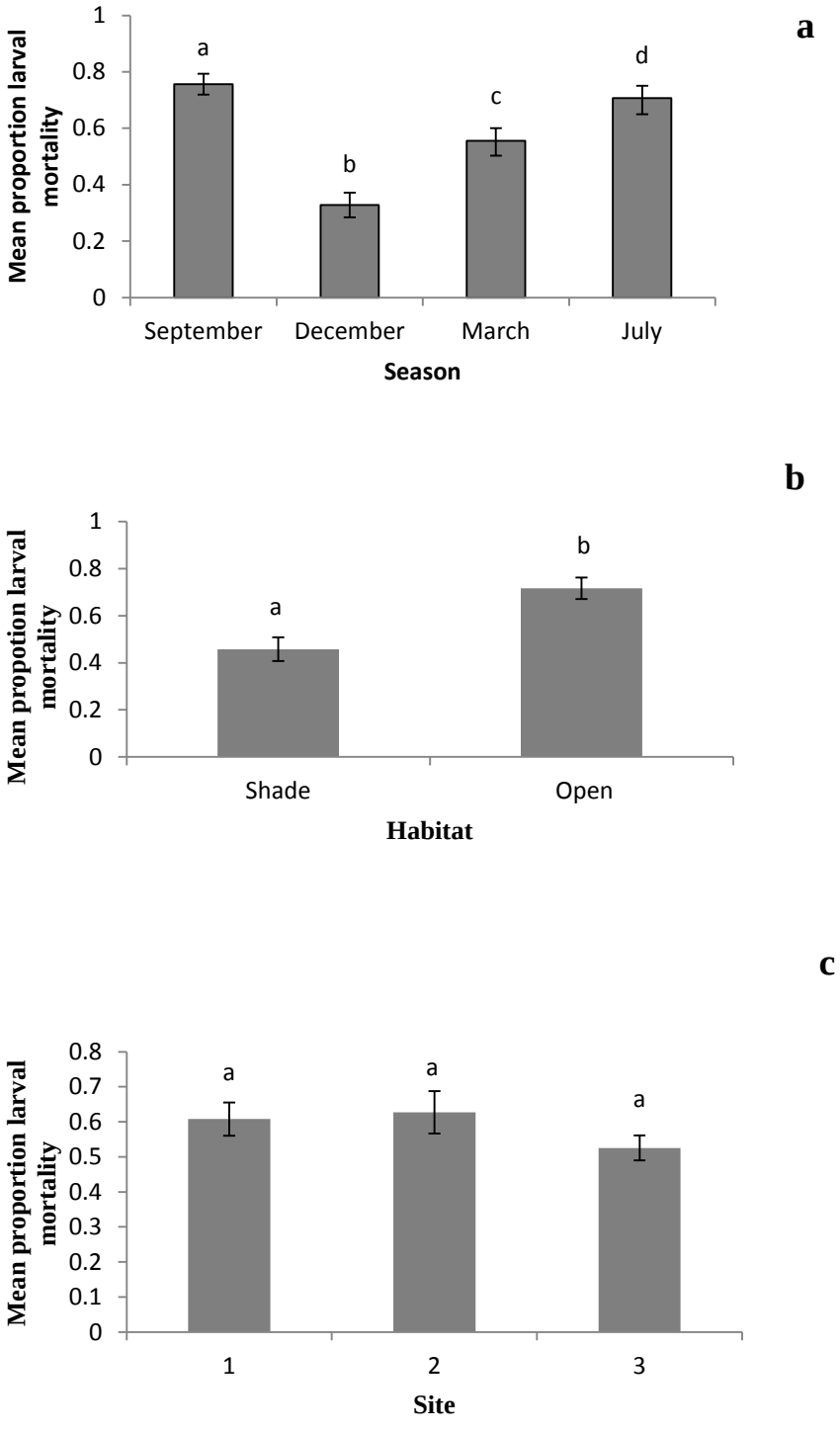

Fig. 3.7. Mean (± 1 SE) proportional larval mortality ('minimum') between (a) the four seasons, (b) shaded and open habitats and (c) the three sites. Means followed by different letters are significantly different.

3.3.8 Overall levels of mining

Over the entire study, less than 5% of the leaves sampled contained mines. Of all the mines counted, only 12% were complete (i.e. developed through to pupation) while 88% were incomplete, mostly due to larval mortality rather than having developing larvae inside them. Overall, there were approximately 1.4 mines per damaged leaf.

3.4 Discussion

3.4.1 Number of leaves and height of chromolaena plants

The growing season for chromolaena is the wet season (November to March in South Africa) (Liggitt, 1983; McFadyen, 1991). This is when it produces numerous young leaves and also increases in height through stem growth. In this study, the mean number of leaves per plant increased from the first sampling event in September to a high number in December and March, while plant height showed a similar, although weaker, trend. This was partly because established plants were selected before the beginning of the growing season, and new branches were largely initiated from the lower parts of the plants. In fact, some of the selected branches were outcompeted during the growing season by new branches, and became moribund or died. Chromolaena produces flowers between June and July, and therefore invests its resources in reproduction instead of growth at that time of the year; this corresponds to a decrease in the number of new leaves produced as July approaches. The overall decrease in the number of leaves per plant/branch implies that between March and July, the rate of leaf production is lower than the rate of leaf mortality.

In this study, the mean number of leaves per plant/branch and plant/branch height was significantly higher in the open habitats compared to the shaded habitats, i.e. plants were generally denser in the open habitats although for some seasons (September and July) there were minimal growth and die backs. The plants were far more robust and larger (taller and covering a greater surface area) in the open habitat at all of the three sites. As shown by other studies, precipitation, temperature and light intensity control the distribution and spread of this species (Muniappan and Marutani, 1988; McFadyen, 1989; Sivagnanam and Swamya, 2010). The

differences in measured parameters presented in my study, particularly for numbers of leaves, certainly under-represent differences between whole plants in shaded and open habitats because, in general, whole plants were selected in the former but only single branches of a bigger plant were selected in the latter. These results are consistent with those of Ambika and Jayachandra (1980) who showed that light has a great influence on the vegetative phase of growth and plant establishment which explains the low plant densities they found in closed plantations.

There was also a significant difference in growth between the three sites sampled, and this could be explained by the fact that site 3 was north facing and therefore received more sunlight and was hotter and drier than the other sites. In the open habitat of this site, *C. odorata* plants generally had smaller, tougher leaves than plants in the open habitats of sites 1 and 2, and also lost condition more in the dry season. In the shaded habitat of site 3, *C. odorata* plants were larger than those in the shaded habitat of sites 1 and 2. It is possible that the larger size of *C. odorata* plants in the shade at this site was an indirect effect of site 3 being on a north-facing slope: it appeared that the eucalyptus trees at site 3 were smaller and less dense, probably because it was hotter, drier and more sunny, allowing more light, heat and/or rain to penetrate the understorey. The plants in the shaded habitat (under eucalyptus) contributed more to the difference in number of leaves and height between the three sites; hence a significant interaction between habitat and site, but at sites 1 and 2 plants in the open were taller and had more leaves. There is also a significant interaction in leaf numbers between season and site, which means that leaf numbers at each site do not vary independently from season (in September (first sampling season), site 3 had very few leaves especially in the open habitat compared to site 1 and 2).

3.4.2 Relationship between number of leaves and plant/branch height

In most plants, especially those with a single stem, there is a positive relationship between number of leaves and plant height. Robertson (1994) showed that the height of individual maize plants can be related throughout growth to leaf appearance and final leaf number. In this study, there was also a positive relationship between the number of leaves and height, although this was fairly strong only in the shaded habitats, but weak in the open habitats. This is possibly because existing branches on the plants generally do not grow much longer in the new season, even

though they grow many new leaves. Instead, the most vigorous stem growth in the new season often branches from near the base of the plant, so that as the bushes age they develop multiple stems of similar lengths (personal observations). Therefore, the number of leaves on branches selected before the start of the growing season is unlikely to be strongly related to plant/branch height. Also, for the most part whole plants were sampled in the shaded habitats whereas single branches on larger shrubs were sampled in the open habitats, which could explain the differences in the strength of the leaf-height relationships between the habitats.

3.4.3 Overall number of mines (total and complete) and number of mines in relation to leaves available

The overall number of mines and the number of mines in relation to leaves available show a similar pattern to one another. Mine numbers were very low in December, when plants had already produced many new leaves. This may have been because the fly population had started off from a low baseline at the beginning of the season due to one or more of several reasons (lack of suitable leaves for larval development the previous winter, high rates of mortality at the end of the previous summer), and therefore the available resource (young leaves – see Chapter 2) could not be fully exploited. By March and July, the fly population had increased and was more fully exploiting the available resource. It is possible that the plant puts a lot of resources into growth early in the season to escape for a period of time from its natural enemies whose populations increase more slowly.

There was also a large difference between the total number of mines and the number of complete mines, which was due to the high number of old, incomplete mines (i.e. that had not developed through to pupation) and which reflected high larval mortality. Possible causes of larval mortality are discussed below. Even though the total numbers of *C. eupatorivora* mines were slightly higher in the shady habitats, there were no significant differences in either the total number of mines or the number of complete mines.

However, the number of mines in relation to the number of leaves available was significantly higher in the shade. In Jamaica, from which the population of *C. eupatorivora* released in South

Africa originates, mines have been observed at fairly high densities on young plants (seedlings or regrowth) mainly in shady habitats (Zachariades *et al.*, 1999, 2002). Both the total number of mines and the mean number of mines in relation to leaves available were the lowest at site 3. This could be because site 3 was a drier site and although plants growing there had the highest number of leaves, (i) plants growing in the open habitat had leaves that were small in size and tough in texture, possibly less suitable for oviposition and/or (ii) the climate of this habitat could have been less suitable for adults (hotter, drier, lower humidity), thus population levels were lower. There was a significant interaction between habitat and season, indicating that the number of mines in relation to leaves available in the two habitats did not show a constant pattern across the four seasons. A significant interaction between season and site also showed that the three sites did not show a constant pattern across the four sampling seasons. Although at all three sites, the number of mines in relation to leaves available appeared to be slightly higher in the shaded habitat, there is a significant interaction between habitat and site which means that the pattern is not consistent across the three sites.

3.4.4 Mean number of mines per damaged leaf

If only a small proportion of the leaves on a plant are suitable for mining and the population of the leaf-mining insect is high, then mines would tend to co-occur in those suitable leaves, while the unsuitable leaves would remain unmined, regardless of the competitive consequences of a female laying multiple eggs in a leaf or several females laying singly in the same leaf (such behaviour should also decrease a female's genetic fitness by decreasing the number or fecundity of her offspring) (Stiling *et al.*, 1984; Faeth, 1990). In this study, the overall degree of competition between larvae is likely to be low since less than two mines (1.7 mines was the highest mean value) co-occurred in a single leaf. Although no trials have been conducted, it is likely that for many chromolaena leaves, two *C. eupatorivora* larvae can complete development without reducing their fitness. Comparing between the two habitats, the number of mines per damaged leaf was slightly higher in the open, and this is possibly because there were fewer suitable leaves in relation to the number of eggs laid.

Even though there was little apparent competition between larvae, the fact that the number of mines per damaged leaf increased over the growing season, while the percentage of leaves mined remained low, may imply that suitable leaves are a limiting resource for *C. eupatorivora*. However, if this is the case, the higher numbers of mines per damaged leaf in December are hard to explain, since there were a lot of young leaves available in December and therefore leaves should have not been a limiting factor.

There are significant interactions in mean number of mines per damaged leaf between season and habitat, and season and site, indicating that the mean number of mines across the four seasons did not vary consistently across habitats or sites.

3.4.5 Mean proportional larval mortality ('minimum')

Although *C. eupatorivora* was observed to be well established in the field, there was high larval mortality. The larvae of *Calycomyza lantanae* Frick (Diptera: Agromyzidae), a biocontrol agent released on *Lantana camara* L. (Verbenaceae) in South Africa, which causes similar blotch mines on the leaves, were attacked by several parasitoid species (Baars and Neser, 1999). In Jamaica, at least four species of hymenopteran parasitoids attack *C. eupatorivora* larvae at two developmental stages, but probably accounted for less than 25% mortality (C. Zachariades, unpubl.). The population size of *C. eupatorivora* in the field in South Africa could thus also be negatively affected by parasitoids of native leaf-mining Agromyzidae, as was the case with *C. lantanae*. Furthermore, because most *C. eupatorivora* larvae were killed at an early stage of their development, mines remained small and therefore the proportion of the leaf damaged was very small. However, although hymenopteran parasitoids were reared from field-collected mines, they were very few in comparison to the amount of larval mortality found in this study. Presumably therefore, generalist predators (including ants, wasps and predatory bugs) and possibly leaf-suitability factors were responsible for much of the larval mortality. Over the four seasons, mortality was high in spring (possibly a reflection of high predator pressure at the end of the previous summer), was low in December, then increased in summer to another high level in winter (July); this may be caused by a seasonal lag in predator build-up, tracking the herbivore build-up. This may suggest that larval mortality was due to predation rather than other factors

such as UV or environmental conditions, but I cannot be certain of this since I did not examine all mortality factors.

The proportion of *C. eupatorivora* that died as larvae also differed between the two habitats; it was significantly higher in the open habitat. There is a possibility that parasitoids and/or predators of *C. eupatorivora* were more abundant in the open habitats, but other factors could also be the cause of this. Larval mortality did not vary significantly across sites. There is a significant interaction between season and site, and season and habitat, which means that across the four seasons, the proportional larval mortality did not vary consistently across the two habitats or the three sites.

CHAPTER 4: General discussion and conclusions

Weed biocontrol using insects can be very successful, leading to high damage rates and decreases in growth and/or reproductive potential of the target weed. It is also the most cost-effective weed management method available. However, predicting the success of an agent, even once it has been shown to be host specific, is difficult. One cannot be certain whether the agent is likely to establish, become abundant and cause any meaningful damage to the weed. Abundance levels in the country of origin are not a good predictor; they do not guarantee the agent's establishment in the new country. *Calycomyza eupatorivora* has several potentially positive attributes, e.g. Jamaica has been shown to be within the region of origin of the South Africa chromolaena biotype, therefore there should be no compatibility problems between insect and host plant. Flies are generally easy to establish and are good dispersers. *Calycomyza eupatorivora* has high fecundity and a short lifecycle, and therefore should multiply quickly. However, potentially negative attributes include the fact that Jamaica is not climatically very similar to the regions invaded by chromolaena in South Africa. Also, *C. eupatorivora* is a leaf feeder and does not have an obvious diapause and might thus have a restricted distribution range in South Africa, while the larvae form exposed blotch mines which could be vulnerable to attack by predators and parasitoids. Jamaica is also an island ecosystem which often has lower levels of predation/parasitism compared with mainland ecosystems (e.g. Venezuela on the South American mainland has generally low levels of *C. eupatorivora*). Because of these issues, it is essential to conduct post-release monitoring. After establishment is confirmed, a good first step is to quantify abundance, mortality, and try to look for patterns in population dynamics (seasonally, between habitats, between sites). This is what the field survey in this study (Chapter 3) has done. Laboratory trials (Chapter 2) to determine oviposition patterns and damage levels as a function of population size are also useful in understanding the ecology of the fly in the field and what factors may limit its effectiveness.

Two chromolaena biocontrol agents have been established in the field, namely *C. eupatorivora* and the moth *P. insulata.* The role of these two agents in the integrated control of chromolaena has not yet been determined. *Calycomyza eupatorivora* has become established and has spread along the coastal region of KZN since 2003 (Zachariades *et al.*, 2011). The six-year period

between establishment and the current study should have provided sufficient time for the population to increase to optimal levels, and also to have accrued predators and parasitoids. Leaf mines of the fly were observed to be widely dispersed at the Cannonbrae study area, which was chosen because: (i) it is fairly close to the initial site of establishment at Amanzimtoti, and therefore the insect has been present for several years here; (ii) it is on the coast, believed to be climatically optimal for the insect in South Africa; (iii) leaf mines of the fly had previously been observed to be abundant at this site, relative to others; and (iv) it offers a large area of dense chromolaena in both shaded and open habitats.

This study consisted of two parts, namely laboratory and field trials. The laboratory trials were initiated to investigate the oviposition and larval development patterns resulting from different densities of *C. eupatorivora* adult flies on *C. odorata* plants. The second part of the laboratory trials aimed to obtain some information on whether ovipositing adults are selective with regard to leaf quality. The field trials were conducted to determine the fly's abundance and mortality rates, measured by examining and counting larval leaf mines, in the field (at a site which had been observed to support a good population of the fly), as well as any seasonal and habitat differences. Sampling of three sites within the same area provided additional information on variation that can be expected between sites.

Overall, it appears that adult females are selective with regard to the leaves that they lay eggs in. Laboratory studies (Chapter 2) showed a non-linear relationship between the number of flies and the leaf area damaged, even though the percentage leaf area damaged was still low (maximum ±30%) at higher adult fly densities, and nutrient analysis also provided some evidence of selective oviposition. With regard to the relationship between fly density and the amount of damage inflicted on the leaves (Chapter 2), average fly densities (5 pairs) caused more damage than low densities (1 pair) but not significantly less than high densities (10 pairs). However, for some parameters, there was no significant difference between the three fly densities. This suggests that fly populations are constrained by a lack of leaves of suitable quality and that higher fly numbers leads to higher levels of oviposition only in leaves that are suitable, and hence increased larval competition. Leaf quality analyses (Chapter 2) showed that, within a single plant, *C. eupatorivora* mines are more likely to occur in leaves that are less tough (lower

in lignin) and higher in other nutrients, such as nonstructural carbohydrates. From the plants that were exposed to 20 pairs of flies (for leaf quality analysis), I observed that *C. eupatorivora* selected certain leaves to oviposit in and therefore larvae would co-occur in these leaves and damage/mine them extensively, whereas other leaves were left unmined. This was also observed in the actual laboratory trials and also in the field. Although I did not measure this, the absence of leaf mines in leaves with higher lignin and lower carbohydrate levels probably correlates with leaf age, with older leaves being tougher and less nutritious. I also did not demonstrate whether the absence of mines in these leaves was as a result of the adult females not laying eggs in them, or the eggs not hatching. Although it is more likely to be a matter of adult female choice, it is likely that the larvae would perform less well in older, lignified, low nutrient leaves. The fly's potential to inflict consistently high levels of damage on *C. odorata* populations in the field is therefore limited. If *C. eupatorivora* flies were present in good numbers consistently, from the time of germination of the seed, the young leaves would probably always be targeted and therefore all leaves on the plant would be attacked as the plant grew. This would probably have a significant negative effect on the plant's fitness. However, as shown in Chapter 3, fly densities are only high in late summer, at the end of chromolaena's vegetative growing period. Therefore, in the field, all available leaves will not be attacked.

Field studies (Chapter 3) indicated that the majority of leaves were not utilized, even when the fly population was at its peak in March. Over the entire study period, fewer than 5% of the leaves sampled contained mines. That this may have been due to selection of certain leaves over others by the adult *C. eupatorivora* females is implied by: (i) the larger proportion of leaves that were mined in the shade adjacent to open habitats and; (ii) the presence of multiple mines per damaged leaf in the presence of many unmined leaves. Although a female laying multiple eggs in a leaf or several females laying singly in the same leaf may have negative consequences in terms of competition between larval progeny (such behaviour should also decrease a female's genetic fitness by decreasing the number or fecundity of her offspring) (Stiling *et al.*, 1984; Faeth, 1990), in this study competition between larvae was probably low. This is because the mean number of mines per damaged leaf was only 1.4, and many mines remained small due to early-instar larval mortality. There is a likelihood that leaf quality, as affected by leaf age and position (with respect to e.g. light intensity), was probably a factor in females choosing certain

leaves to oviposit in. Leaf age is a crucial oviposition site characteristic among herbivorous insects (King *et al.*, 1998). The value of a leaf to developing offspring may change with leaf age because of changes in the chemical or physical properties of that particular leaf (Raupp and Denno, 1983; King *et al.*, 1998). Young leaves are soft and usually contain more nitrogen and moisture than older leaves (Raupp and Denno, 1983; Raupp, 1985; Denno *et al.*, 1990), and are often more suitable for the developing immature stages of insects. Oviposition site selection by adults of leaf-mining insects is also largely influenced by variation in leaf structure (Reavey and Gaston, 1991), leaf size (Faeth, 1991) and leaf chemistry (Minkenberg and Offenheim, 1990; Kagata and Ohgushi, 2001). However, it is also possible that the female's choice does not always result in optimal larval survival (King *et al.*, 1998).

Although the number of chromolaena leaves increased greatly between September and December, the number of larval leaf mines of *Calycomyza eupatorivora* showed a 3-month lag, only increasing between December and March (Chapter 3). This lag in the increase in mines is probably due to a low starting point for the fly population at the end of the previous winter. KZN lies in a summer-rainfall region, with dry, cool winters. As a result, few suitable leaves are available for larvae during this period, and the winter weather probably also results in faster adult mortality due to low humidity, and slower larval development due to low temperatures. *Calycomyza eupatorivora* has no obvious diapause, therefore the population probably decreases dramatically during winter, and the fly needs more than one generation to build up population levels. Larval mortality was also highest in late summer, further decreasing the winter population. Certainly this lag cannot be due to a lack of young palatable leaves.

The high larval mortality in the field (Chapter 3) also has negative implications for the efficacy of the insect as a biological control agent, not only because the populations are reduced, but also because most mortality occurs when the larvae are young and therefore have not caused much damage to the plants. Over the entire study, more than 80% of the mines were incomplete (i.e. did not develop through to pupation). This shows that the mortality rate was very high, which could be due to high levels of predation, some parasitism, and possibly other factors.

In conclusion, my study suggests that the effectiveness of *C. eupatorivora* is mainly reduced by its preference for high quality leaves for oviposition (Chapter 2) in combination with a slow build-up of the population during the summer, and high levels of larval mortality (Chapter 3). Given that the *C. eupatorivora* population levels in the study area (KZN South Coast) appear to be some of the highest within the introduced range of this biocontrol agent (C. Zachariades, unpubl.) it seems unlikely that the fly is currently providing anything more than negligible biological control of *C. odorata* at a landscape level in South Africa.

Despite these negative conclusions, there are several opportunities for additional research. Future research on leaf status (i.e. those that are mined versus unmined) is recommended, to directly measure leaf toughness (Specific Leaf Area/Specific Leaf Weight) and its probable implications for selection for oviposition. This could incorporate a leaf-age study in the laboratory to determine whether *C. eupatorivora* prefers leaves of a certain age, and whether leaf age correlates to toughness, lignin content and nutrient levels. One could then extrapolate the laboratory results to the field to see if this can predict which leaves will be exploited by the fly. Although 30% leaf damage may seem low, it may still reduce the vigour of plants, resulting in decreased growth rates and reproductive output. This could be examined in the laboratory by exposing plants to herbivory and measuring growth and reproductive parameters. A study by Crawley (1989) showed that a mere 5% herbivory in oak trees led to reduction in acorn production. More studies (e.g. life table studies) could also be carried out to determine the causes of larval mortality in the field, and maybe consider larval mortality under laboratory conditions and try to link this to leaf quality. Finally, one could also conduct manipulative field trials (e.g. chemical exclusions), especially on plants in shade and on seedlings, to determine whether *C. eupatorivora* has any measurable impact on some chromolaena plants within a population.

REFERENCES

Ambika, S.R. (2002). Allelopathic plants. *Chromolaena odorata* (L.) King & Robinson. *Allelopathy Journal* 9: 35-41.

Ambika, S.R. (2007). Effect of light quality and intensity on emergence, growth and reproduction in *Chromolaena odorata*. In: *Proceedings of the Seventh International Workshop on Biological Control and Management of Chromolaena odorata and Mikania micrantha*, Taiwan, September 12 to 15, 2006, Po-Yung Lai, G.V.P. Reddy & R. Muniappan (eds). ROC Bureau of Forestry: 56-62.

Ambika, S.R. & Jayachandra, S. (1980). Influence of light on seed germination in *Eupatorium odoratum L. Indian Forester* 106: 637- 640.

Baars, J.R. & Neser, S. (1999). Past and present initiatives on the biological control of *Lantana camara* (Verbenaceae) in South Africa. *African Entomology Memoir* 1: 21-33.

Barnes, R.F., Nelson, C.J., Moore, K.C. & Collins, M. (2007). *Forages: The Science of Grassland Agriculture*. Wiley - Blackwell. Iowa State University Press.

Barr, G.E. (2009). The structure and function of lignin in plant cells. *Annals of Botany* 3: 23-28.

Blackmore, A.C. (1998). Seed dispersal of *Chromolaena odorata* reconsidered. In: *Proceedings of the Fourth International Workshop on the Biological Control and Management of Chromolaena odorata*, P. Ferrar, R. Muniappan & K.P. Jayanth (eds). Agricultural Experiment Station, University of Guam, Publication 216: 16–21.

Blossey, B. (1999). Before, during and after: the need for long-term monitoring in invasive plant species management. *Biological Invasions* 1: 301-311.

Bryson, C.T.& Carter, R. (2004). Biology pathways for invasive weeds. *Weed Technology* 18: 1216-1220.

Bultman, T.L. & Faeth, S.H. (1986). Selective oviposition by a leaf miner in response to temporal variation in abscission. *Oecologia* 69: 117-120.

Cock, M.J.W. & Holloway, J.D. (1982). The history of, and prospects for, the biological control of *Chromolaena odorata* (Conpositae) by *Pareuchaetes pseudoinsulata* Rego Barros and allies (Lepidoptera: Arctiidae). *Bulletin of Entomological Research* 72: 193-205.

Cory, J. & Myers, J.H. (2000). Direct and indirect ecological effects of biological control. *Trends in Ecology & Evolution* 15: 137-139.

Crawley, M.J. (1989). Insect herbivores and plant population dynamics. *Annual Review of Entomology* 34: 531-562.

Cruttwell, R.E. (1972). The insects of *Eupatorium odoratum* L. in Trinidad and their potential as agents for biological control. Ph.D. Thesis, University of the West Indies.

Cruttwell, R.E., (1974). Insects and mites attacking *Eupatorium odoratum* in the neotropics: An annotated list of the insects and mites recorded from *Eupatorium odoratum* L., with a key to the types of damage found in Trinidad. *Technical Bulletin of the Commonwealth Institute for Biological Control* 17: 87-125.

Denno, R.F., Larsson, S. & Olmstead, K.L. (1990). Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology* 71: 124-137.

Erasmus, D.J. (1988). A review of mechanical and chemical control of *Chromolaena odorata* in South Africa. In: *Proceedings of the First International Workshop of Biological Control of Chromolaena odorata*, R. Muniappan (ed.). Agricultural Experiment Station, Guam, 34-39.

Faeth, S.H. (1990). Aggregation of a leafminer, *Cameraria* sp. nov. (Davis): Consequences and causes. *Journal of Animal Ecology* 59: 569–586.

Faeth, S.H. (1991). Effect of oak leaf size on abundance, dispersion, and survival of the leafminer *Cameraria sp.* (Lepidoptera: Gracillariidae*). Environmental Entomology* 20: 196-204.

Gareeb, M. & Zachariades, C. (2003). *Calycomyza eupatorivora*, a new agent for biological control of *Chromolaena odorata* in South Africa. *Proceedings of the South African Sugar Technologists Association* 77: 622-623.

Gautier, L. (1992). Taxonomy and distribution of a tropical weed, *Chromolaena odorata* (L.) R. King & H. Robinson. *Candollea* 47: 645–662.

Gautier, L. (1993). Reproduction of a pantropical weed: *Chromolaena odorata* (L.) R. King & H. Robinson. *Candollea* 48: 179–193.

Goodall, J.M. & Erasmus, D.J. (1996). Review of the status and integrated control of the invasive alien, *Chromolaena odorata*, in South Africa. *Agriculture, Ecosystems and Environment* 56: 151-164.

Grime, J.P. (1974). Vegetation classification by reference to strategies. *Nature* 250: 26-31.

Harley, K.L.S. (1969). The suitability of *Octotoma scabripennis* Guer. and *Uroplata giradi* Pic. (Coleoptera: Chrysomelidae) for the control of *Lantana camara* (Verbenaceae) in Australia. *Bulletin of Entomological Research* 58 (4): 835-843.

Henderson, M. & Anderson, J.G. (1966). Common weeds in South Africa. *Memoirs of the Botanical Survey of South Africa* 37: 50-54.

Hoffmann, J.H. & Moran, V.C. (1998). The population dynamics of an introduced tree, *Sesbania punicea*, in South Africa, in response to long-term damage caused by different combinations of three species of biological control agents. *Oecologia* 114: 343-348.

Holm, L.G., Plucknett, D.L., Pancho, J.V. & Herberger, J.P. (1977). *The World's Worst Weeds: Distribution and Biology*. Honolulu, Hawaii, USA: University Press of Hawaii.

Horner, T.A. (2002). Field release of *Cecidochare*s (*Procecidochares*) *connexa* Macquart (Diptera: Tephritidae), a non-indigenous, gall-making fly for control of Siam weed, *Chromolaena odorata* (L.) King & Robinson (Asteraceae) in Guam and the Northern Mariana Islands. Environmental Assessment. USDA-Animal and Plant Health Inspection Service, Permits and Risk Assessment, Riverdale, Maryland: 26-30.

Huenneke, L., Hamburg, S., Koide, R., Mooney, H. & Vitousek, P. (1990). Effects of soil resources on plant invasion and community structure in California (USA) serpentine grassland. *Ecology* 71: 478-491.

Ikuenobe, C.E. & Ayeni, A.O. (1998). Herbicidal control of *Chromolaena odorata* in oil palm. *Weed Research* 38: 397-404.

Julien, M.H. & Griffiths, M.W. (1998). *Biological Control of Weeds. A World Catalogue of Agents and their Target Weeds*. 4th ed.Wallingford, UK: CAB International Publishing.

Kagata, H. & Ohgushi, T. (2001). Preference and performance linkage of a leaf-mining moth (*Paraleucoptera sinuella*) on different Salicaceae species. *Population Ecology* 43: 141–147.

Keane, R.M. & Crawley, M.J. (2002). Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17: 164-170.

King, B.H., Crowe, M.L. & Blackmore, M.D. (1998). Effects of leaf age on oviposition and on offspring fitness in the imported willow leaf beetle, *Plagiodera versicolora* (Coleoptera: Chrysomelidae). *Journal of Insect Behavior* 11(1): 23-36.

King, R.M. & Robinson, H. (1970). Studies in the Eupatorieae (Compositae). XXIX: The genus *Chromolaena*. *Phytologia* 20: 196-209.

Kluge, R.L. (1990). Prospects for the biological control of triffid weed *Chromolaena odorata*, in southern Africa. *South African Journal of Science* 86: 22-23.

Kluge, R.L. (1991). Biological control of triffid weed, *Chromolaena odorata* (Asteraceae) in South Africa. *Agriculture, Ecosystems and Environment* 37: 193-197.

Kluge, R.L. & Caldwell, P.M. (1993). The biology and host specificity of *Pareuchaetes aurata aurata* (Lepidoptera: Arctiidae), a "new association" biological control agent for *Chromolaena odorata* (Compositae). *Bulletin of Entomological Research* 83: 87-94.

Kluge, R.L. & Caldwell, P.M. (1996). Failure and frustration of biocontrol of *Chromolaena odorata* in South Africa. In: UK Prasad, R Muniappan, P Ferrar, J.P. Aeschliman & H. De Foresta (eds) *Proceedings of the Third International Workshop on Biological Control and Management of Chromolaena odorata*. Mangilao, Guam, Agricultural Experiment Station, University of Guam: 202-210.

Kolar, C.S. & Lodge, D.M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology & Evolution* 16: 199–204.

Kozlowski, T.T. (1992). Carbohydrate sources and sinks in woody plants. *The Botanical Review* 58: 107-222.

Kriticos, D.J., Yonow, T. & McFadyen, R.E. (2005). The potential distribution of *Chromolaena odorata* (Siam weed) in relation to climate. *Weed Research* 45: 246–254.

Le Maitre, D.C., van Wilgen, B.W. & Gelderblom, C.M. (2001). Invasive alien trees and water resources in South Africa: Case studies of the costs and benefits of management. *Forest Ecological Management* 160: 143-150.

Leslie, A.J. & Spotila, J.R. (2001). Alien plants threatens Nile Crocodile (*Crocodylus niloticus*) breeding in St. Lucia, South Africa. *Biological Conservation* 98: 347-355.

Liggitt, B. (1983). The invasive alien plant *Chromolaena odorata*, with regard to its status and control in Natal. University of Natal Monograph 2: 41-45.

Mack, R.N. (1995). Understanding the processes of weed invasions: the influence of environmental stochasticity. In: *Proceedings of BCPC Symposium No. 64. Weeds in a Changing World*: 65-74.

McConnachie, A.J., Hill, M.P., Byrne, M.J. & de Wit, M.P. (2003). Economic evaluation of the successful biological control of *Azolla filiculoides* in South Africa. *Biological Control* 28: 25-32.

McEvoy, P.B. & Coombs, E.M. (1999). Biological control of plant invaders: Regional patterns, field experiments, and structured population models. *Ecological Applications* 9: 387-401.

McFadyen, R.E.C. (1988). Phytophagous insects recorded from *C. odorata*. *Chromolaena odorata Newsletter* 2: 5-23.

McFadyen, R.E.C. (1989). Siam weed: a new threat to Australia's North. *Plant Protection Quarterly* 4: 1-7.

McFadyen, R.E.C. (1991). The ecology of *Chromolaena odorata* in the Neotropics. In: *Proceedings of the Second International Workshop on Biological Control of Chromolaena odorata*, R. Muniappan & P. Ferrar (eds.). BIOTROP Special Publication: 1-9.

McFadyen, C.R. & Skarratt, B. (1996). Potential distribution of *Chromolaena odorata* (Siam weed) in Australia and Oceania. *Agriculture, Ecosystems and Environment* 51: 89-96.

Meijninger, W.M.L. & Jarmain, C. (2011). Satelite based ET of invasive alien plants in South Africa. *Journal of Hydrology*: in press.

Minkenberg, O.P.J.M. & Offenheim, J.J.G.W. (1990). Effects of leaf nitrogen content of tomato plants on preference and performance of a leaf mining fly. *Oecologia* 83: 291-298.

Moran, V.C., Hoffmann, J.H. & Zimmermann, H.G. (2005). Biological control of invasive alien plants in South Africa: necessity, circumspection, and success. *Frontiers in Ecology and the Environment* 3: 77-83.

Muniappan, R. & Marutani, M. (1988). Assessment of *Chromolaena odorata* before release and after establishment of natural enemies. In: *Proccedings of the First International Workshop on Biological Control of Chromolaena odorata*, R. Muniappan (ed), Mangilao Guam: Agricultural Experiment Station, University of Guam: 21-24.

Myers, J.H., Simberloff, D., Kuris, A.M. & Carey, J.R. (2000). Eradication revisited: dealing with exotic species. *Trends in Ecology & Evolution* 15: 316–320.

Pickworth, G. (1976). Triffid weed (*Eupatorium odoratum*). An address to the Lower Tugela Farmer's Conservation Committee. Unpublished report, Department of Agriculture and Water Supply.

Raupp, M.J. (1985). Effects of leaf toughness on mandibular wear of the leaf beetle *Plagiodera versicolora*. *Ecological Entomology* 10: 73-79.

Raupp, M.J. & Denno, R.F. (1983). Leaf age as a predictor of herbivore distribution and abundance. In: *Variable Plants and Herbivores in Natural and Managed Systems*, R.F. Denno & M.S.McClure (eds), National Pingtung University, Taiwan: 68-80.

Reavey, D. & Gaston, K.J. (1991). The importance of leaf structure in oviposition by leaf-mining microlepidoptera. *Oikos* 61: 19–28.

Reichard, S.H. & Hamilton, C.W. (1997). Predicting invasions of woody plants introduced into North America. *Conservation Biology* 11: 193-203.

Reitz, S.R. & Trumble, J.T. (2002). Competitive displacement among insects and arachnids. *Annual Review of Entomology* 47: 435–465.

Richardson, D.M., Macdonald, I.A.W., Hoffmann, J.H. & Henderson, L. (1997). Alien plant invasions. In: *Vegetation of Southern Africa*, R.M. Cowling, D.M. Richardson & S.M. Pierce (eds). Cambridge University Press.

Richardson, D.M., Pysek, P. & Rejmanek, M. (2000). Naturalization and invasion of alien plants: concepts and definitions. *Diversity and Distributions* 6: 93-107.

Richardson, D.M. & van Wilgen, B.W. (2004). Invasive alien plants in South Africa: how well do we understand the ecological impacts? *South African Journal of Science* 100: 45-52.

Robertson, M.J. (1994). Relationships between internode elongation, plant height and leaf appearance in maize. *Field Crops Research* 38 (3):135-145.

Sahid, I.B. & Sugau, J.B. (1993). Allelopathic effects of lantana (*Lantana camara*) and Siam weed (*Chromolaena odorata*) on selected crops. *Weed Science* 41: 303-308.

Saxena, K.G. & Ramakrishnan, P.S. (1983). Growth, resource allocation patterns and nutritional status of some dominant annual weeds of shifting agriculture (Jhum) in north-eastern India. *Acta Oecologica* 4: 323-333.

Scheirs, J., De Bruyn, L. & Verhagen, R. (2001). Nutritional benefits of the leaf-mining behaviour of two grass miners: a test of the selective feeding hypothesis. *Ecological Entomology* 26: 509-516.

Scheirs, J., De Bruyn, L. & Verhagen, R. (2002). Seasonal changes in leaf nutritional quality influence grass miner performance. *Ecological Entomology* 27(1): 84–93.

Schooler, S.S. & McEvoy, P.B. (2006). Relationship between insect density and plant damage for the golden loosestrife beetle, *Galerucella pusilla*, on purple loosestrife (*Lythrum salicaria*). *Biological Control* 36: 100–105.

Scott, L.J., Lange, C.L., Graham, G.C. & Yeates, D.K. (1998). Genetic diversity and origin of Siam weed (*Chromolaena odorata*) in Australia. *Weed Technology* 12: 27-31.

Shea, K. & Chesson, P. (2002). Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17: 15-18.

Simelane, D.O. (2002). Biology and host range of *Ophiomyia camarae*, a biological control agent for *Lantana camara* in South Africa. *BioControl* 47:575-585.

Sinclair, R.J. & Hughes, L. (2010). Leaf miners: the hidden herbivory. *Austral Ecology* 35: 300-313.

Sivagnanam, C. & Swamya, P.S. (2010). Growth patterns of *Chromolaena odorata* in varied ecosystems at Kodayar in the Western Ghats, India. *Acta Oecologica* 36: 383-392.

Spencer, K.A. & Stegmaier, C.E. (1973). Agromyzidae of Florida, with a supplement on species from the Caribbean. Arthropods of Florida and neighbouring land areas. *Bureau of Entomology, Florida Department of Agriculture and Consumer Services* 7 : 171-174.

Steinbauer, M.J. (2001). Specific leaf weight as an indivator of juvenile leaf toughness in Tasmanian bluegum (*Eucalyptus globulus* ssp. *globulus*): implications for insect defoliation. *Australian Forestry* 64(1): 32-37.

Stiling, P.D., Brodbeck, B.V. & Strong, D.R. (1984). Intraspecific competition in *Hydrellia valida*, (Diptera: Ephydridae), a leaf miner of *Spartina alterniflora*. *Ecology* 65: 660-662.

Swamy, P.S. & Ramakrishnan, P.S. (1987). Weed potential of *Mikania micrantha* H.B.K., and its control in fallows after shifting agriculture (Jhum) in North-East India. *Agriculture, Ecosystems and Environment* 18: 195-204.

Thebaud, C., Finzi, A.C., Affre, L., Debussche, M. & Escarre, J. (1996). Assessing why two introduced *Conyza* differ in their ability to invade Mediterranean old fields. *Ecology* 77: 791-804.

Vanderwoude, C., Scanlam, J.C., Davis, B. & Funkhouser, S. (2005). Plan for National Delimiting Survey for Siam weed. Department of Natural Resources and Mines, Land Protection Services: Queensland Government.

Van Wilgen, B.W., de Wit, M.P. & Anderson, H.J. (2004). Costs and benefits of biological control of invasive alien plants: case studies from South Africa. *South African Journal of Science* 100: 113-122.

Von Senger, I., Barker, N.P. & Zachariades, C. (2002). Preliminary phylogeography of *Chromolaena odorata*: finding the origin of a South African weed. In: *Proceedings of the Fifth International Workshop on Biological Control and Management of Chromolaena odorata*, C. Zachariades, R. Muniappan & L.W. Strathie (eds). Pretoria, South Africa: ARC-PPRI: 91-99.

Waterhouse, D.F. (1994). Biological control of weeds: Southeast Asian prospects. ACIAR, Canberra 320.

Wells, M.J., Poynton, R.J., Balsinhas, A.A., Musil, K.J., Joffe, H. & Van Hoepen, E. (1986). The history of introduction of invasive alien plants to southern Africa. In: *The Ecology and Management of Biological Invasions in Southern Africa*, I.A.W. Macdonald, F.J. Kruger & A.A. Ferrar (eds), 21-35.

Wheeler, G.S. & Center, T.D. (2001). Impact of the biological control agent *Hydrellia pakistanae* (Diptera: Ephydridae) on the submersed aquatic weed *Hydrilla verticillata* (Hydrocharitaceae). *Biological Control* 21: 168-181.

Wiedenmann, R. (2000). Introduction to biological control. *Weed Research* 25: 129-135.

Williamson, M. (1996). Biological invasions. *Biocontrol News and Information* 26: 2–8.

Ye, W.H., Mu, H.P., Cao, H.L. & Ge, X.J. (2004). Genetic structure of the invasive *Chromolaena odorata* in China. *Weed Research* 44: 129-135.

Zachariades, C. & Goodall, J.M. (2002). Distribution, impact and management of *Chromolaena odorata* in southern Africa. In: *Proceedings of the Fifth International Workshop on Biological Control and Management of Chromolaena odorata*, Durban, South Africa, October 2000, C. Zachariades, R. Muniappan & L.W. Strathie (eds), 34-39.

Zachariades, C., Strathie-Korrubel, L.W. & Kluge, R.L. (1999). The South African programme on the biological control of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) using insects. In T. Olckers & M.P. Hill (eds.). Biological control of weeds in South Africa. *African Entomology Memoir* 1: 89-102.

Zachariades, C. & Strathie, L.W. (2006). Biocontrol of chromolaena in South Africa: recent activities in research and implementation. *Biocontrol News and Information* 27: 10–15.

Zachariades, C., Strathie, L.W. & Kluge, R.L. (2002). Biology, host specificity and effectiveness of insects for the biocontrol of *Chromolaena odorata* in South Africa. In:

Proceedings of the Fifth International Workshop on Biological Control and Management of Chromolaena odorata, C. Zachariades, R. Muniappan & L.W. Strathie (eds), 160-166.

Zachariades, C., von Senger, I. & Barker, N.P. (2004). Evidence for a northern Caribbean origin for the southern African biotype of *Chromolaena odorata*. In: *Proceedings of the Sixth International Workshop on Biological Control and Management of Chromolaena*, M.D. Day & R.E. McFadyen (eds). ACIAR Technical Reports 55: 25–27.

Zachariades, C., Strathie, L., Retief, E. & Dube, N. (2011). Progress towards the biological control of *Chromolaena odorata* (L.) R.M. King & H. Robinson (Asteraceae) in South Africa. *African Entomology* 19 (2): in press.

Zimmermann, H.G., Moran, V.C. & Hoffman, J.G. (2004). Biological control in the management of invasive alien plants in South Africa, and the role of the Working for Water programme. *South African Journal of Science* 100: 34-40.