“Is more, less?” Insect – insect interactions in a biological control context using water hyacinth as a model

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Abstract

Interactions between insects have been shown to be important regulators of population abundances and dynamics as well as drivers of spatial segregation and distribution. These are important aspects of the ecology of insects used in biological control and may have implications for the overall success of a particular programme. In the history of biological control there has been a tendency to release a suite of agents against a weed, which in some cases has increased the level of success, while in others little change has been observed. In most of these cases the implications of increasing the level of complexity of the system is not taken into account and there is little research on the effect of releasing another agent into the system.

A brief meta-analysis was done on all the biological control programmes initiated in South Africa. Emphasis was placed on multi-species releases and the effects that overlapping niches were having on the number of agents responsible for the success of a programme. Where overlapping niches were present among agents released the number of agents responsible for success was lower than the number established.

Water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach in South Africa has more arthropod agents released against it than anywhere else in the world, yet control has been variable. If the biology and host utilisation of all the agents against water hyacinth is considered, a definite overlap of niches is apparent in at least one life stage of all the agents. Therefore the probability of these insects interacting is high, especially if they are established at the same site in the field. Three of the insects released in South Africa have been selected to investigate possible interactions. They are *Neochetina eichhorniae* Warner, *Neochetina bruchi* Hustache and *Eccritotarsus catarinensis* (Carvalho).

Y-tube olfactometer bioassays were used to measure responses of these insects to water hyacinth with prior feeding damage by either conspecifics or heterospecifics. This was done to determine whether olfactory cues played a role in host acceptability and avoidance of conspecifics or heterospecifics. The insects were given a choice between damaged and undamaged plants in various combinations. There was a significant preference for the undamaged plants when given a choice between undamaged and damaged plants. However when the insects were given a choice between two damaged plants there was no discrimination between heterospecific or conspecific damaged plants. This may indicate that there is little or no ecological cost for the insect to share a plant with other insects utilising a similar resource.

Insect – insect interactions were investigated in a common garden plot experiment to measure the impact that pairwise combinations of the insect may have on their performance. There was a significant interaction between the mirid *E. catarinensis* and the weevil *N. eichhorniae*, with the weevil not performing as well when in combination with the mirid than when alone. Interestingly there was a negative interaction between the two weevil species when in combination, however it was impossible to determine which species was being affected if not both. None of the insects performed significantly better when in combination with another insect.

A field study on Wriggleswade Dam in the Eastern Cape, South Africa was initiated to determine whether the relationship between the mirid *E. catarinensis* and the weevil *N. eichhorniae* could be determined in the field. The performance of the insects at the different sites in the field suggests that there was an interaction between the agents. This interaction did not limit the establishment of either insect at a site, but it did result in one insect dominating at a site over another.

Interactions between the three species of insect tested in this thesis suggest that there are both negative and neutral relationships between them. A basic comparison between the insect performances from 15 sites around the country was done to determine if the spatial segregation observed in the field could be extrapolated to the natural South African situation. The interaction observed between *N. eichhorniae* and *E. catarinensis* does seem to extrapolate to the general South African situation where there is definite spatial segregation on a landscape level. The co – occurrence of the two *Neochetina* weevils at these sites suggests that the negative relationship observed between them in the common garden experiment does not extrapolate to the field.

The results from this thesis suggest that the interactions between the agents tested would not limit establishment or have significant ramifications on performance. However, there may be spatial and temporal segregation of these species in the introduced range.

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Chapter 1: General Introduction

1.1 Introduction

Biological control of weeds is based on the release of host specific organisms in an attempt to reduce the plant's density in the introduced range (Harley 1985; McFadyen 1998). In most cases several agents are released in series, rarely altogether, against the same plant. In some cases all the agents establish and in others only a few or none establish in the introduced range (Julien & Griffiths 1998). Myers (1985) likened multi-species biological control programmes to "lottery models", where as many agents are released as possible until the best agent or proverbial silver bullet is found and control of the weed is achieved. In a review by McEvoy & Coombs (2000) several possible negative effects of releasing too many biological control agents were identified. These negative effects for which the term "revenge effects" was coined, result from multiple-species releases and can make the biological control programme potentially less effective and more risky (McEvoy & Coombs 2000). Where multiple-species releases are planned there must be an attempt to understand the interactions and these possible "revenge effects" before another agent is released.

Denoth *et al.* (2002) reviewed the biological control programmes against insect pests as well as weeds, placing emphasis on multi-species vs. single-species releases and the number of agents involved in success. The establishment rates of agents in insect biological control programmes were significantly lower in multi-species releases than in single-species releases. This was ascribed to competitive exclusion (Ehler & Hall 1982). Keller (1984) challenged this notion and put forward a convincing argument that pest species cannot be a limiting resource and therefore biological control agents should not be competing for resources. But competition between biological control agents must exist otherwise the populations of such agents on the pest or weed would be so low, or there would be so much available resource, that control could not possibly be achieved (Harris 1991). When the establishment of insects against weed biological control is considered there is no difference in the chance of establishment between multi-species and single-species introductions (Julien & Griffiths 1998, Denoth *et al.* 2002). It is possible that the level of competition between phytophagous insects required to shape the population structure and density is so tenuous (Hairston *et al.* 1960; Lawton & Strong 1981; Schoener 1983) that the probability of affecting the establishment rate of biological control agents is negligible. However, reviews of interspecific interactions between phytophagous insects have shown that competition is indeed an important factor in controlling the performance and overall fitness of phytophagous insects (Damman 1993; Denno *et al.* 1995; Kaplan & Denno 2007). Complete removal of the resource (i.e. complete defoliation of a plant) is rare (Hairston *et al.* 1960) and a plant may offer a range of niches for the hosts to occupy (Lawton 1982; Denoth *et al.* 2002). Competition therefore may not be responsible for differences in establishment rates but it does occur and could have implications for the performance and fitness of biological control agents.

1.2 Competition

Interactions between individuals of the same species or different species where the performance or fitness of a particular individual is reduced by the presence of the other is considered competition (Begon *et al.* 2005). The definition of competition presented by Keddy (1989), “the negative effects which one organism has upon another by consuming or controlling access to, a resource that is limited in availability” is sufficiently rigorous and general to satisfy most situations in nature. For the purposes of this thesis I have adopted this as a general definition for competition between biological control agents. It is possible to unpack the mechanisms of competition further, into “interference competition” and “exploitation competition” (Schoener 1983; Keddy 1989).

1.2.1 Interference competition

Interference competition occurs when an individual or organism engages with another directly through physical attack or fighting, production of toxins and chemicals or subtle indirect forms of threat behaviour and/or territoriality (Schoener 1983; Keddy 1989). The presence of interference competition in phytophagous insects is well documented (Gibson 1980; Faeth 1985; Faeth 1986; Hartley & Lawton 1987; Akimoto 1988; Finch & Jones 1989). There is evidence that interference competition is more common in mandibulate herbivores living in concealed niches, such as stem borers and seed feeders, and competitive superiority is strongly linked to body size and aggressive behaviour (see review by Denno *et al.* 1995). Interference competition was not completely absent in sap sucking herbivores, however, there was little evidence to suggest that competitive superiority was linked to body size or behaviour (Denno *et al.* 1995). It is widely accepted that interference competitors should be avoided in biological control programmes because they have the potential to reduce the exploitation of the host plant by the competing agents (Harris 1991). Interference competition is not common in biological control programmes and where it is, it seems to be at such a low level that it is relatively unimportant (Story *et al.* 1991).

1.2.2 Exploitative competition

Exploitative competition occurs when the activity of one individual reduces the available pool of resources, thus indirectly affecting another individual (Schoener 1983; Keddy 1989). This eventually results in a reduction in performance and fitness (Kaplan & Denno 2007). Exploitation can take several forms, for example a chewing insect removes a portion of the leaf, thus making it unavailable for another individual or insect species (Blakley & Dingle 1978). On the other hand the effects of a sap-feeding Homopteran would reduce the nutritional value of the plant or leaf, but would leave it still available (McClure 1980). Exploitative competition is of significant importance in shaping population dynamics and structure as well as individual performance and fitness of phytophagous insects (Damman 1993; Denno *et al.* 1995; Kaplan & Denno 2007) and therefore should be important in the performance and ultimate success of agents released for the biological control of weeds.

1.3 Competition in biological control programmes

It is well established that competition does occur in herbivorous insects. In 75% of all pairwise interactions studied, competition was found to be an important driver in fitness and performance of phytophagous insects (Damman 1993; Denno *et al.* 1995; Kaplan & Denno 2007). In biological control, host specific insect herbivores are imported into an area where their host (an invasive alien plant) is a problem (MacFadyen 1998), but in so doing another novel organism is introduced into the environment. The possible reduction in natural enemy pressure could result in an increase in the importance of competition. Interspecific competition was important in 91% of all pairwise interactions involving novel herbivorous insects which lacked specialised natural enemies (Denno *et al.* 1995). For example specialised natural enemies have been shown to maintain populations of scale insects and adelgids on hemlock and pine in the native range (Japan) below a competitive level (McClure 1980, 1986). In the introduced range (North America) or where specialised natural enemies are kept in check these insects reach such great population levels that competitive exclusion in not uncommon (McClure 1980, 1986). Huffaker & Kennett (1969) emphasised the importance of competition between biological control agents released for the control of weeds where natural enemies are often depauperate in the introduced range. If competition between biological control agents is important the possibility exists that the overall success of a particular biological control programme will be compromised. There is evidence worldwide that in several biological control programmes, including those involving *Centaurea diffusa* Lamarck (Storey *et al.* 1991; Crowe & Bourchier 2006; Seastedt *et al.* 2007), *Hypericum perforatum* Linnaeus (Briese 1997) and *Carduus nutans* Linnaeus (Woodburn 1996; Groenteman *et al.* 2007) that insect-insect interactions can reduce the overall efficacy and performance of the insects and ultimately the programme.

1.3.1 A South African perspective

To investigate the importance of insect-insect interactions that may compromise the efficacy of biological control programmes in South Africa, the latest catalogue of all biological control programmes initiated was consulted (Klein 2011). Insects with overlapping niches on the host plant are more likely to compete for resources and these interactions may be important in terms of performance and overall fitness (McClure 1980; Denno & Roderick 1992). It has been hypothesised that where there are overlapping niches between agents (i.e. competition) fewer agents than established would be responsible for the level of control achieved or fewer biological control programmes would reach any level of control.

The South African catalogue was chosen as a case study for several reasons. Success or levels of success in biological control are not consistent and the number of successful biological control programmes varying from author to author. In South Africa the biological control fraternity have agreed upon what is considered success in biological control and it has been clearly defined by Hoffmann (1995). Hoffmann's definitions for success are **1) Complete**: no other control measures are required to keep the weed under control due to the damage caused by the biological control agent(s), at least in areas where the agent(s) are established; **2) Substantial**: in spite of the damage caused by the biological control agent(s) other methods of control are required to decrease the impact of the weed but at a reduced rate (e.g. less herbicide or less frequent application); **3) Negligible**: despite the damage inflicted by the agent(s), control of the weed is still dependent on other control measures; and **4) Not**

determined: the programme may be too recent for a meaningful evaluation or it has not yet been evaluated. In addition to the level of control, the perceived level or degree of damage that each agent is inflicting on the weed has also been categorised. These categories include **a) Extensive**: very high levels of damage where few plants survive, growth is arrested and/or almost no seeds are produced; **b) Considerable**: relatively high levels of damage but some plants survive, growth rates are slowed and/or seed production is reduced by more than 50%; **c) Moderate**: noticeable damage by the agent, however most plants survive; growth may be slowed to some extent and/or seed production is reduced by less than 50%; **d) Trivial**: there is some damage by the agent but all plant parameters are relatively unaffected; and **e) Unknown**: the agent performance has not been evaluated or it is too soon after release for a full evaluation (Anon. 1999). In terms of comparing the levels of success for each programme, as well as the damage inflicted by the agents, there is thus a bench mark for all the South African data.

To summarise the data and extract the relevant information, plant species where no biological control agents have been released, or where the level of control of the weed has not been established, were discarded from the data set. From the remaining plants the data were summarised and are presented in Appendix 1. In analysing the data several assumptions have been made. These are as follows:

1. If the level of control in multi-species releases was either **substantial** or **complete** and the damage inflicted by any agent was **extensive**, that agent was assumed to be responsible for that level of control. If no agent produced **extensive** damage, the level of damage by the agents was assumed responsible for the level of control. For example, *Hypericum perforatum* is under complete control with two insects established on it in South Africa. These are *Chrysolina quadrigemina* (Suffrian) causing extensive damage and *Zeuxidiplosis giardia* (Kieffer) causing moderate

damage. It was assumed that only the insect that caused extensive damage, in this case *C. quadrigemina,* was responsible for the level of success achieved in this programme. When no agent in the programme produced an extensive level of damage, the next highest level of damage from an agent was considered responsible. For example, the biological control programme against *Eichhornia crassipes* (Martius) Solms-Laubach, has seen a substantial level of success with no agent performing better than a **considerable** degree of damage and therefore all the agents are assumed to be responsible for that level of control. It is accepted that this approach underestimates the subtle (interactive) effects that agents might be having.

2. Competition is relatively important only between species that have overlapping niches. For example, in the biological control programme against *Solanum elaeagnifolium* Cavanilles, competition was considered important because of the overlapping niches between the two leaf-feeding insects that have established - *Leptinotarsa defecta* (Stål) and *Leptinotarsa texana* Schaeffer. On the other hand competition between the three insects against *Sesbania punicea* (Cavanilles) was considered unimportant because there were no overlapping niches even though *Trichapion lativentre* (Béguin-Billecocq) feeds on the flower buds which would limit the potential amount of seed set for *Rhyssomatus marginatus* Fåhraeus. There is substantial evidence suggesting that competition is important between species utilising different niches of a plant through induced defences and reductions in plant nutrition, growth and output (Karban 1986, 1989; Inbar *et al.* 1999; Kessler & Baldwin 2004; Van Zandt & Agrawal 2004; Lynch *et al.* 2006; Kaplan *et al*. 2007). To consider all the possible interactions would be beyond the scope of this review.
3. Failure of an agent to establish in the field is not a function of competitive exclusion, but rather attributed to other factors. These might include limited release effort,

biotype mismatch or unsuitable climatic matching. All insects that were released in a biological control programme but did not establish in the field were excluded from the analysis.

From a total of 42 evaluated biological control programmes in South Africa, 18 have had a single agent established on them and 24 have had two or more agents established on them. In the single-agent biological control programmes, 28% of the weeds are under **complete** control, while only 21% of the multi-agent programmes are under some level of control. Multi-agent programmes have a higher number under **substantial** control, which accounts for 54% vs. 39% for single-agent programmes. For **negligible** levels of control single-agent programmes do slightly better with 33%. Only 25% of the multi-agent programmes are under **negligible** levels of control. Overall it seems that with multi-agent biological control programmes the chance of success is moderately higher.

Where only the multi-agent programmes are considered, 50% of the programmes where either **complete** or **substantial** control was achieved by fewer agents than were established, 75% of these had overlapping niches between agents. In 66% of these, control was achieved by a single agent. In the five programmes where all the agents that established were responsible for control, only two had overlapping niches between agents. In 73 % of the biological control programmes where competition was assumed to be important one agent was considered responsible for control. However overlapping niches in biological control programmes was not always the most important factor. This was shown by the biological control programmes on *Opuntia stricta* (Haworth), *Opuntia ficus – indica* (Linnaeus) and *Eichhornia crassipes*, where despite overlapping niches control was achieved by several agents acting apparently synergistically. The South African data suggest that where overlapping niches (assumed competition) between biological control agents exist, fewer agents than are present are responsible for control of the weed. The data show that interactions between agents released for biological control, and the potential effects on the efficacy of the programmes are important but inadequately understood in a biological control context.

New Zealand has taken into account the potential effects on the efficacy and interactions between biological control agents before the release of multiple agents into the field to maximise impact while minimising futile releases (Syrett *et al.* 1996). Potential interactions between agents have largely been ignored in South African biological control and agents are released without prior knowledge of potential "revenge effects". South Africa has released the highest number of agents against water hyacinth worldwide with several more potential agents being considered (Coetzee *et al.* 2011). An understanding of whether interactions can be recognised and their possible importance in terms of compromising the programme can be identified is important for the biological control against water hyacinth in South Africa.

1.4 The model: Water hyacinth

1.4.1 Background and distribution

Water hyacinth *Eichhornia crassipes* (Pontederiacae) was first described in 1823 by C.F.P. von Martius. It is indigenous to the New World tropics, with its centre of origin in Amazonia, Brazil (Barrett & Forno 1982). It has spread to other areas such as Venezuela, parts of central South America and the larger Caribbean islands (Edwards & Musil 1975). Since the first record of water hyacinth outside South America in New Orleans in 1884 (Penfound & Earle 1948), it has spread around the USA and by the end of the nineteenth century is was recorded in Egypt, India, Australia and Java (Gopal 1987). To date the distribution of water hyacinth is mostly pan-tropical, but it also occurs in warm temperate regions of the world, extending to latitudes 40^{o} N and S (Gopal 1987).

1.4.2 Description and biology

Water hyacinth has an extremely plastic morphology which is dependent on the conditions in which it grows. In mature dense stands the petioles are elongated and the leaves are commonly circular. The plants are usually short with bulbous petioles and kidney shaped leaves in less dense, actively growing stands or along the edges of mature stands (Center & Spencer 1981). Each plant typically has 6-10 functional leaves which originate from a central bud and are arranged in basal rosettes on the rhizome. The numerous roots are feathery and form on this rhizome (Center & Spencer 1981).

Reproduction of water hyacinth is both sexual and asexual, with asexual reproduction being the predominant mode of population growth in the introduced range (Center & Spencer 1981). Ramets or daughter plants are formed from auxiliary buds on stolons produced through elongation of internodes (Center & Spencer 1981). However, sexual reproduction does occur despite the paucity of suitable pollinators and lack of germination sites (Gopal 1987). Each flower can produce a large number of seeds that can remain viable for an extremely long time: in some cases up to 20 years (Gopal 1987). There are several sites in South Africa where seeds have been found in the substrate and several of these seeds are viable, with germination between 25 and 80 % (Albano Pérez *et al.* 2011).

Due to vegetative growth under suitable conditions water hyacinth populations can grow extremely quickly and in some cases double their biomass in as little as 11 to 18 days (Edwards & Musil 1975). As the nutrient concentrations (nitrogen and phosphorous) increase it has been shown that water hyacinth biomass also increases (Reddy *et al.* 1989, 1990). Gopal (1987) directly correlated water hyacinth growth with nutrient concentration. According to Gopal (1987) growth of water hyacinth ceases at 10 ^{o}C and has an optimal growth temperature range of between 28 and 30 ^{o}C. During unsuitable times carbohydrate reserves from the stems are used, however prolonged exposure to temperatures below 5 ^{o}C can result in death of the plant (Gopal 1987; Owens & Madsen 1995).

1.4.3 Biological control

In 1961 the United States Department of Agriculture launched the first research programme into the biological control of water hyacinth, with the first agents being released in Florida in 1972 (Harley 1990). Biological control of water hyacinth has largely been successful with agents having been released in at least 31 countries worldwide (Julien & Griffiths 1998). Of the seven agents released three are considered to be the most effective: the two weevils *Neochetina eichhorniae* Warner and *Neochetina bruchi* Hustache (Coleoptera: Curculionidae) and the moth *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae) (Cilliers 1991). Complete control of water hyacinth with the introduction of a suite of agents is not uncommon; the most notable African example is on Lake Victoria. The two weevils *N. eichhorniae* and *N. bruchi* were released into different parts of Lake Victoria from 1995. Within five years the weevils had reduced the stand from 20 000ha before release to 2000ha (Moorhouse *et al.* 2000). South Africa initiated a biological control program against water hyacinth in 1973 with the first agent, *N. eichhorniae* being released as early as 1974 (Cilliers 1991). South Africa has seen the largest number of agents released and established against this weed in the world (Hill & Cilliers 1999; Coetzee *et al.* 2011) and yet control is considered variable (Hill & Cilliers 1999; Hill & Olckers 2000).

1.4.4 Arthropod agent biology and host utilisation

All the agents released for biological control of water hyacinth in South Africa originate from South America and are host specific to water hyacinth (*Eichhornia crassipes*) (Hill *et al.* 1999; Julien *et al.* 1999; Cordo & DeLoach 1976). There is considerable niche overlap by the different agent species (Table 1.1). The two species of weevil have very similar biologies, with the adults feeding mainly on the leaf (feeding scars are indistinguishable) and the larvae mine the petioles and feed in the crown on auxiliary buds (DeLoach & Cordo 1976a). *Neochetina bruchi* has a shorter larval development and generation time than *N. eichhorniae*, however the adult longevity is similar at approximately 140 days (DeLoach & Cordo 1976a, Center 1994). *Niphograpta albiguttalis* adults do not feed on water hyacinth and are extremely short lived, surviving for only 4 to 9 days (Center 1994). Eggs are oviposited in the leaf and larvae begin feeding on the leaf for 1 to 2 days then mine the petiole (DeLoach & Cordo 1976a). The late instar larvae tend to feed on the rosette of the plant (Julien 2001). Larval development is short, ranging from 16 to 21 days with a total generation time of about 21 to 28 days (DeLoach & Cordo 1976a; Julien 2001). *Eccritotarsus catarinensis* (Carvalho), is fairly long lived with adults surviving about 50 days (Hill *et al.* 1999). The nymphs and the adults are both sap suckers having similar habits by feeding on the leaf. They can easily be distinguished: the nymphs are a pale white and the adults black (Hill *et al.* 1999). Eggs are oviposited into the leaf tissue and nymphal development is fairly short at approximately 15 days and a total generation time of only 22 days (Hill *et al.* 1999). The eggs of *Orthogalumna terebrantis* Wallwork, are laid under the lamina usually in wounds/damaged areas or protected locations of the leaf. After a 7 to 8 day incubation period the eggs hatch and one larval and three nymphal stages mine the leaf (Cordo & DeLoach 1975, 1976). During the 15 day development the larvae and nymphs produce galleries that are usually 2-5mm long and move towards the apex of the leaf. Adults produce emergence holes in the mines and feed on the surface of the leaf where they can survive for up to 85 days (Cordo & DeLoach 1975, 1976). The adults and nymphs of *Cornops aquaticum* (Bruner) feed on the leaf tissue of water hyacinth. The egg packets are deposited in the petiole, usually of the youngest leaf, just above the crown and take up to 30 days to hatch (Oberholzer & Hill 2000; Adis & Junk 2003). The nymphs go through between five and seven instars and development may take up to 64 days (Oberholzer & Hill 2000;

Adis & Junk 2003). Adult longevity is characteristically about 68 days (de Vieira & dos Santos 2003).

The potential for interactions between biological control agents on water hyacinth is relatively high, with overlapping niches not being uncommon. Some of these interactions have been identified as important. Del Fosse (1978) investigated the interactions between *O. terebrantis* and *N. eichhorniae* and Ajuonu *et al.* (2007) studied the impact of *Neochetina* weevils on the survival and fitness of *E. catarinensis.* An understanding of the potential effects of insect-insect interactions on water hyacinth will be crucial in the biological control programme so as not to disrupt the current efficacy of the programme by releasing a potentially incompatible agent.

1.5 Thesis outline and aims

The aim of this study was to determine whether insect – insect interactions (competition) are important in terms of the compatibility of biological control agents released against water hyacinth, and whether such competition has any significant effect on their performance. Invasive weeds afford an opportunity to study insect – insect interactions in a relatively simple and easily controlled system. Invasive plants in their introduced range often have an extremely small number of herbivorous insects associated with them which are usually the insects that are released by biological control practitioners. This results in a highly simplified plant herbivore system that is easy to control and manipulate. This study uses water hyacinth *E. crassipes,* and three insects *N. eichhorniae, N. bruchi* and *E. catarinensis* established in South Africa on the plant as a model to study insect – insect interactions in a biological control context. The water hyacinth system is one of the simplest as it never enters into the third trophic level. This is because none of the insects used has any specialised predators or parasitoids (Deloach & Cordo 1982; Hill pers. comm.). This affords us the ability to study insect – insect interactions, in this case competition for a common resource, with very few confounding factors.

It is well documented that olfactory cues are important in host determination by insects and there is substantial evidence that such cues apply to herbivorous insects as well (Visser 1986; Dicke & van Loon 2000). The aim of chapter 2 of this thesis is to determine the importance of olfactory cues released by water hyacinth after damage due to prior feeding by either conspecifics or heterospecifics. Some related work has been done by Perkins *et al.* (1976) and Del Fosse & Perkins (1977), looking at volatiles released by mechanically damaged water hyacinth. This is important work, however it gives little insight into the response of the plant to herbivore damage (known to be different) and how the insects respond to this difference in damage. There are potential ecological advantages and disadvantages for a herbivorous insect when selecting for a plant that has had prior feeding on it. Advantages might include a plant's defences having been overcome and/or the chance of coming into contact with a conspecific mate is greatly increased on an infested plant (Dicke & van Loon 2000). Disadvantages might include a plant has mounted its induced defences, and/or the danger of entering an enemy dense space or having to compete for a common shared resource (Dicke & van Loon 2000). Ultimately the response of an insect towards or away from a plant in a biological control context is important for the efficacy of the programme. If the plant or patch of plants becomes undesirable after one agent has been feeding, the likelihood of establishing additional agents in that area is reduced. This could result in a patchy distribution of insects on a local and landscape level.

Chapter 2 investigated the acceptability of a particular plant to the insect, while chapter 3 presents a "pre-release" type study on the interactions of these three insects in a pairwise combination experiment which was done as a measure of insect – insect interactions. The effect that combinations of insects have on insect performance can be related to a measure of coexistence or compatibility. There are several examples where the efficacy of a particular biological control programme has being compromised by insect – insect interactions (Story *et al.* 1991; Woodburn 1996; Briese 1997; Crowe & Bourchier 2006; Groenteman *et al.* 2007; Seastedt *et al.* 2007). Chapter 3 highlights the importance of pre-release studies to determine whether multi species releases in biological control programmes are likely to increase or decrease the efficacy of the programme.

A post-release study was initiated in April 2010 (Chapter 4) on Wriggleswade Dam in the Eastern Cape, South Africa, where both *N. eichhorniae* and *E. catarinensis* have been established for at least the last decade. The performance of the insects was measured at several sites to investigate whether results obtained in the laboratory-based experiment (Chapter 3) can be extrapolated to the field. The behaviour and interactions of insects in a laboratory are often different under natural conditions. The experimental setup in chapter 3 forced the coexistence of two insects. This may not be a true reflection of the distribution in the field, where the insects may have different preferences for particular plant phenotypes or environmental conditions. This would ultimately result in a spatial segregation of the insects and an apparent coexistence in the field.

The importance of both pre-release and post-release studies in multi species biological control programmes has been highlighted in this thesis. Ultimately the more agents that are released the more complex and difficult a system becomes to manage, with an increase in the possibility of “revenge effects”.

Table 1.1 Brief biology and host utilisation for the arthropod biological control agents released in South Africa against water hyacinth.

Species	**Feeding damage**		**Oviposition sites**	**Larval/ nymph development**	**Generation time**	**Adult longevity**
	Adults	**Larvae**				
Neochetina eichhorniae	Leaf[1]	Petiole & crown[1]	Leaf and petiole[1]	60-90 days[2]	96-120 days[1;2]	138 days[1;2]
Neochetina bruchi	Leaf[1]	Petiole & crown[1]	Leaf and petiole[1]	30-40 days[2]	72-96 days[1]	138 days[1]
Niphograpta albiguttalis	None[2]	Leaf & petiole[3]	Leaf[2]	16-21 days[3]	21-28 days[2;3]	4-9 days[2]
Eccritotarsus catarinensis	Leaf[4]	Leaf[4]	Leaf[4]	15 days[4]	22 days[4]	50 days[4]
Orthogalumna terebrantis	Leaf[5;6]	Leaf[5;6]	Leaf[5;6]	15 days[5;6]	22-23 days[5;6]	85 days[5;6]
Cornops aquaticum	Leaf[7;8;9]	Leaf[7;8;9]	Petiole[7;8;9]	64 days[7;8]	81 days[7;8]	68.7 days[9]

1 DeLoach & Cordo (1976a); 2 Center (1994); 3 DeLoach & Cordo (1978); 4 Hill *et al.* (1999); 5 Cordo & DeLoach (1975); 6 Cordo & DeLoach (1976); 7 Oberholzer & Hill (2000); 8 Adis & Junk (2003); 9 de Vieira & dos Santos (2003)

Chapter 2: The effect of previous feeding on water hyacinth leaf acceptability by three water hyacinth biological control agents measured with a simple Y – Tube olfactometer

2.1 Introduction

Herbivorous insects in general have restricted feeding habits, with most species feeding on a narrow range of plant species, and within that range only on specific plant parts (Visser 1986). It has been shown that phytophagous insects use olfactory cues for location and acceptance of their host plant (Visser 1986). This, coupled with visual cues, aids in host-finding and final acceptance (Miller & Strickler 1984; Prokopy 1986; Bernays & Chapman 1994; Quiroz *et al.* 1997). An example is the Colorado potato beetle *Leptinotarsa decemlineata* (Say), which adopts an upwind orientation and movement towards its host, the potato plant (Visser 1976). The physiological state as well as the age of the potato plant is important in this case, where adult Colorado potato beetles are most attracted to 4-8 week old healthy plants (Visser 1976). Moorhouse (1971) tested the orientation behavior of the locust *Schistocera gregaria* (Forskal), and found that grass odour (i.e. a food source) elicited a positive anemotactic response.

Healthy, undamaged plants release volatiles attractive to herbivorous insects, but they are released at low levels. Damaged plants or those infested with herbivorous insects dramatically increase the amounts of volatiles released (Dicke *et al.* 1990; Vet & Dicke 1992; Dicke & Baldwin 2009). In this response to herbivory the active over-production of new or already existing volatiles (Dicke *et al*. 1990; Turlings *et al*. 1990) may be a form of indirect defence (Karban & Meyers 1989; Takabayashi & Dicke 1996; Karban & Baldwin 1997; Dicke 2000; Dicke & Grostal 2001). Volatiles can be detected from a considerable distance (several meters) and an abundance of information is thus conveyed (de Moraes *et al.* 1998; Dicke 2000; Dicke & van Loon 2000; de Moraes *et al.* 2001). The cues can be so specific in ratio and/or blend that a parasitoid or predator can distinguish between infested plants and between species of herbivore feeding on a plant. The parasitoid *Cardiochiles nigriceps* Viereck can determine from olfactory cues alone whether its host *Heliothis virescens* (Fabricius), or a closely related species *Helicoverpa zea* (Boddie), is infesting a particular plant (de Moraes *et al.* 1998). De Moraes *et al.* (1998) showed that under laboratory and semi-field conditions *C. nigriceps* selected significantly more plants that were infested with its host *H. virescens.* This ability was extended over three different plant species. The predatory mite *Phytoseiulus persimilis* Athias Henriot, is able to differentiate between plants damaged by its favoured food source, the two-spotted spider mite *Tetranychus urticae* Koch, and the European red spider mite *Panonychus ulmi* (Koch) (Sabelis & van de Baan 1983). In this example the predatory mite *P. persimilis* selected significantly more for apple leaves infested with *T. urticae* than for apple leaves infested with *P. ulmi* (Sabelis & van de Baan 1983).

The odours released by a plant under herbivory pressure are also intercepted by herbivorous insects and behavioural changes can be elicited. The relevant information relayed to the herbivorous insect may include the nutritional quality of the plant, the presence, identity and abundance of potential competitors and the potential for entering an enemy-dense space (Dicke 2000; Dicke & van Loon 2000). It may be expected that with this information available, a herbivorous insect may be attracted to or repelled by a particular host plant, depending on the apparent ecological costs and benefits associated with that plant (Dicke & van Loon 2000).

The scarab beetle *Maladera matrida* Argaman is a polyphygous insect that exhibits aggregation behaviour (Gol'berg *et al.* 1989). In a study by Harari *et al.* (1994) it was observed that male *M. matrida* emerge first in the evenings and begin feeding on nearby plants, the females always emerging slightly later to join the males, resulting in roughly a 1:1 sex ratio. During a laboratory study the adult beetles were significantly attracted to damaged plant material irrespective of species of insect causing the damage (Harari *et al.* 1994). The aggregations are formed as more and more individuals join the initial feeding individuals, resulting in a positive feedback (i.e. the more individuals feeding the more volatiles released by the plant) (Harari *et al.* 1994). A similar response was elicited by the Colorado potato beetle *L. decemlineata* on young potato plants that had been mechanically damaged or fed upon by either conspecifics or the larvae of *Spodoptera exigua* (Hubner) (Bolter *et al.* 1997). Loughrin *et al.* (1995) tested the response of the Japanese beetle *Popillia japonica* Newman, and found a positive anemotactic response to apple plants that had been fed upon by either conspecifics or heterospecifics. There is no discrimination between plants under different treatments, so it is thought that these examples are a case of increased detectability of damaged plants that elicits the response in the insect.

Several examples highlight the importance and specificity of plant volatiles for herbivorous insects. A study by Pallini *et al.* (1997) showed a significant attraction of the two-spotted spider mite *T. urticae* when presented with cucumber leaves damaged by conspecifics, but the mites showed a strong repellence to plants infested with heterospecifics. In a similar study there was a significant repellence of the aphid *Rhopalosiphum maidis* (Fitch) to maize plants treated with caterpillar regurgitate (Bernasconi *et al.* 1998). The herbivore-induced volatiles released by tobacco plants that are infested by *H. virescens* significantly repelled female moths, which spent significantly more time in the area of the experimental chamber that contained undamaged plants (de Moraes *et al.* 2001). The responses of herbivorous insects to plant volatiles can be variable, but in general such responses may determine whether a patch or area is attractive. The implications for biological control could be the difference between establishment of one or several agents at a particular infestation of the weed.

Biological control of weeds uses host specific herbivorous insects to reduce a particular weed's density (McFadyen 1998). Several of these biological control programmes enlist the services of several species of insect (Julien & Griffiths 1998). There are two potential consequences for biological control programmes in terms of herbivore-induced odours and volatiles. A biological control agent could be attracted to the volatiles (produced in response to either conspecific or heterospecific feeding) which could result in an increase in the efficacy of the programme. Alternatively, the biological control agent could be repelled by the volatiles released by a damaged plant (produced in response to either conspecific or heterospecific feeding) and result in the reduction of the efficacy of the programme.

Several studies have shown that in many cases plants release specific blends of volatiles, depending on the species of herbivore (Sabelis & van de Baan 1983; Takabayashi *et al.* 1995; Pallini *et al.* 1997; de Moraes *et al.* 1998; Powell *et al.* 1998). The mirid *Eccritotarsus catarinensis* is a sap feeder, while the two *Neochetina* weevils are chewers and by virtue of different feeding guilds it is likely that the plants will respond differently (Vet & Dicke 1992; Dicke & Baldwin 2009). The aim of this chapter is to explore the question of whether feeding damage alters the response of the insects to water hyacinth plants and whether the type of damage from sap feeders and chewers (i.e. conspecific vs. heterospecific) is important in terms of host preference for these three insects.

2.2 Materials and methods

Y-tube olfactometer bioassays were used to measure the response of *N. eichhorniae, N. bruchi* and *E. catarinensis* to water hyacinth that had been fed upon by either conspecifics or heterospecifics.

2.2.1 Experimental plants

Twenty-four tubs (6 tubs per treatment) were set up in the poly eurathane tunnel on the Rhodes University campus to obtain plants for each of the treatments. These included undamaged control plants and plants fed upon by each insect species. The tubs were 65 cm by 44 cm and 37 cm deep and filled with 66 litres of borehole water. A slow release fertiliser, Multicoat 6 month formula, was added to obtain a total nitrogen of 50.5mg N L^{-1} (N:P:K ratio of 15:3:12) and approximately 6g of iron chelate was added to each tub. Fifteen water hyacinth plants of a similar size (between 30 and 40 cm tall with 6-8 healthy leaves) were selected from the stock ponds in the tunnel. The plants were cleaned, by removing all dead leaves and daughter plants. The plants were then covered with a mesh sleeve having a diameter of 0.5mm, and given two weeks to acclimate to the new environment before inoculation with insects. In six of the tubs approximately 200 adult *E. catarinensis* were inoculated per tub, while for both *N. eichhorniae* and *N. bruchi* 20 adults (10 males and 10 females) were inoculated per tub. For the undamaged plant treatment no insects were inoculated. This was a fairly high density of insects, to ensure that the resulting damage on the plants for the treatments was high. The insects were then allowed a period of 6 weeks to feed on the plants resulting in a moderate to high level of damage per plant. The level of damage recorded for the *E. catarinensis* treatment ranged from 80-100% surface area damage on leaf 2 with an intensity of 2-3 (Hill *et al.* (1999) characterised the feeding intensity of the mirid, where 1 is slight speckling and 5 is total chlorosis of the leaf). The numbers of feeding scars recorded varied for the weevil species, with *N. eichhorniae* averaging 30.7±6.4 feeding scars on leaf 2 and *N. bruchi* averaging 19.4±6.2 feeding scars on leaf 2.

2.2.2 Experimental insects

The insects used in the bioassays were all collected from cultures maintained in the Rhodes University tunnel. *Eccritotarsus catarinensis* cultures were grown in cages, where the water hyacinth plants were replaced at regular intervals from the stock ponds to ensure that food was never a limiting factor for the insects. The two species of weevil were initially obtained from the mass rearing facility at the South African Sugar Research Institute (SASRI), Durban, South Africa, but healthy populations were maintained in large ponds in the tunnel. The plants in the ponds were maintained using a high level of nitrogen and phosphorous (20-30 mg N L^{-1} and 1-2 mg P L^{-1}) in the water and fresh undamaged plants were also replaced at regular intervals. The insects were never deprived of food or ovipositon sites at any time before the initiation of the experiment. On the day of the trails (see below), the insects were collected and starved for 4 hours before the initiation of any of the experiments.

2.2.3 Y-tube olfactometer bioassay

All Y-tube bioassays were carried out in a controlled environment room on the Rhodes University campus in order to regulate ambient conditions. The temperature was maintained at a constant 25 ± 1°C and a constant relative humidity of 65-75%. Due to the diurnal nature of *E. catarinensis* all experiments involving this species were conducted in full white fluorescent light from an overhead source. Experiments involving both *Neochetina* weevils were conducted under a dull red light due to the nocturnal nature of these two species (Perkins 1972). This reduced the possibility of any phototactic responses of the insects confounding the results of the Y-tube bioassay.

The glass Y-tubes had a 1.0cm diameter. Each arm was 10.0cm long with a 10.0cm stem, 7.5cm up the arm was a marked decision line which the insects had to cross for a positive response to be recorded (Figure 2.1). At the end of each branch a clear 100ml plastic bottle was attached, into which the leaf of a treated plant was placed. The leaf used in the bioassay was always leaf 2 because Center & Wright (1991) showed that the youngest leaf of the plant had the lowest concentration of phenolics and was always significantly preferred by the weevils. The leaf was never mechanically damaged or removed from the plant as this may affect the results. Perkins *et al.* (1976) and Del Fosse & Perkins (1977) showed that *N. eichhorniae* was significantly attracted to mechanically damaged water hyacinth. If a leaf was damaged during the setup of the experiment that plant was discarded and all apparatus was washed. The test insect was inserted into the stem of the Y-tube, which was then stoppered with a cork. No air flow was maintained through the olfactometer apparatus as this can result in the desiccation of water hyacinth during the course of the experiment (Del Fosse & Perkins 1977). Only one novel insect was used for each replicate in each experiment (i.e. the same insect was never used more than once). The insects responded by walking up the stem of the Y-tube, into an arm and crossing a “decision line” marked 7.5cm up each branch of the Y-tube and remained there for at least 1 minute.

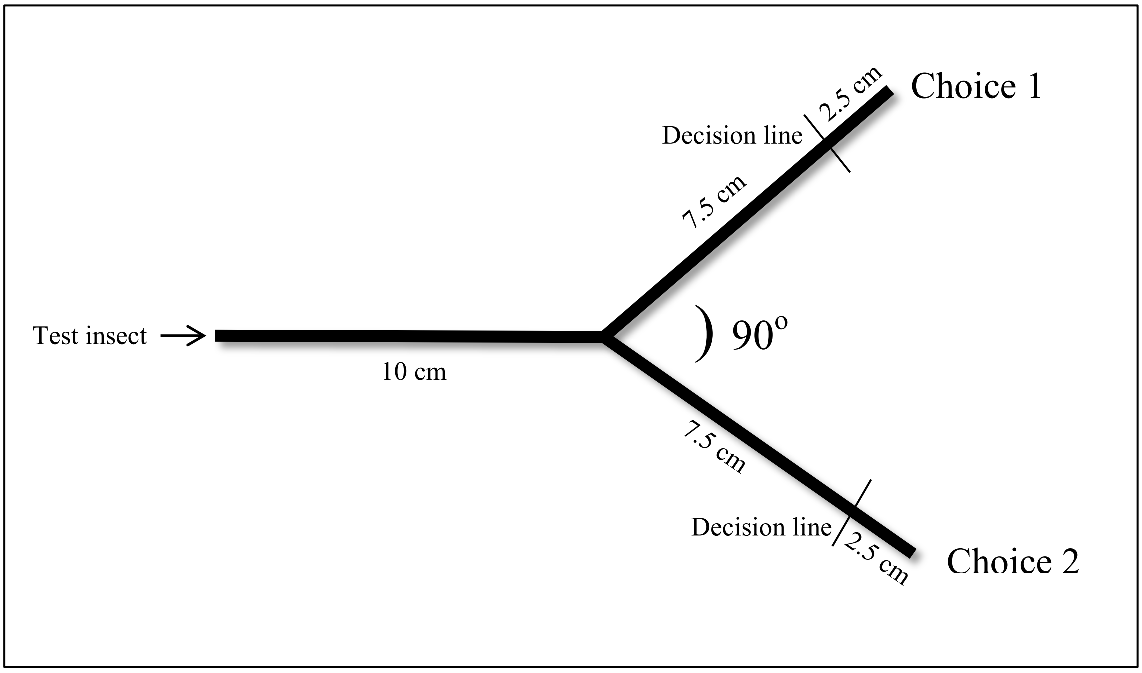

Figure 2.1 A schematic diagram of the experimental setup for the Y-tube olfactometer bioassay.

The insect was given five minutes to initiate a response, failing this the insect was discarded and a new insect placed in the apparatus. The bioassay was repeated until 30 responses had been recorded for each experiment or bioassay per species. The leaves used in the bioassay were randomly assigned branches of the Y-tube in order to control for any location effects. The Y-tube was rinsed with absolute ethanol and allowed to air dry between all replicates to control for any traces or alternative sources of chemical or plant odours. The experimental treatments conducted in this study are outlined in Table 2.1. Each treatment was conducted for all three species of insect.

Table 2.1 The experimental treatments for the Y-tube olfactometer bioassay. Hp = healthy undamaged plant; Np = No plant control; Ec = *Eccritotarsus catarinensis* damaged plant; Ne = *Neochetina eichhorniae* damaged plant; Nb = *Neochetina bruchi* damaged plant.

Treatment	Choice 1		Choice 2	Code
1	Healthy plant	vs.	No plant control	Hp vs. Np
2	*E. catarinensis* plant	vs.	No plant control	Ec vs. Np
3	*N. eichhorniae* plant	vs.	No plant control	Ne vs. Np
4	*N. bruchi* plant	vs.	No plant control	Nb vs. Np
5	*E. catarinensis* plant	vs.	Healthy plant	Ec vs Hp
6	*N. eichhorniae* plant	vs.	Healthy plant	Ne vs. Hp
7	*N. bruchi* plant	vs.	Healthy plant	Nb vs. Hp
8	*E. catarinensis* plant	vs.	*N. eichhorniae* plant	Ec vs. Ne
9	*E. catarinensis* plant	vs.	*N. bruchi* plant	Ec vs. Nb
10	*N. bruchi* plant	vs.	*N. eichhorniae* plant	Nb vs. Ne
11	Healthy plant	vs.	Healthy plant	Hp vs. Hp
12	*E. catarinensis* plant	vs.	*E. catarinensis* plant	Ec vs. Ec
13	*N. eichhorniae* plant	vs.	*N. eichhorniae* plant	Ne vs. Ne
14	*N. bruchi* plant	vs.	*N. bruchi* plant	Nb vs Nb

2.2.4 Statistical analysis

The differences between the choices made by the insects in each treatment were tested statistically using the χ^2_{Yates}-test. There was a relatively small data set with only 1 degree of freedom, which means there was a risk of over estimating the significance of the data. To reduce the amount of error and ultimately the risk the χ^2-test was performed with Yates correction for continuity (Fowler *et al.* 2005).

2.3 Results

The three insects tested responded in a similar manner (Figure 2.2). There was a significant positive response by the insects irrespective of species when presented a choice between a healthy water hyacinth plant and the no plant control (Hp vs. Np) (Figure 2.2, Table 2.2). Again there was a significant positive response towards the water hyacinth plant irrespective of prior damage when the test insects were offered a choice between water hyacinth plants that had previously been fed upon by both conspecifics and heterospecifics and the no plant control (i.e. treatments Ec vs. Np, Nb vs. Np and Ne vs. Np) (Figure 2.2, Table 2.2). There was a significance preference for undamaged healthy plants, again irrespective of insect species, when the insects were offered a choice between healthy water hyacinth plants and plants that had previously been fed upon by either conspecifics or heterospecifics (i.e. treatments Ec vs. Hp, Nb vs. Hp, Ne vs. Hp) (Figure 2.2, Table 2.2). No significant preference was shown by any insect species when offered a choice between differently damaged water hyacinth plants (i.e. treatments Ec vs. Nb, Ec vs. Ne, Nb vs Ne) (Figure 2.2, Table 2.2). There was also no significant preference shown by the insects when offered plants that were either both undamaged or had the same type of damage inflicted to them (i.e. treatments Hp vs. Hp, Ec vs. Ec, Nb vs. Nb, Ne vs. Ne) (Figure 2.2, Table 2.2).

There was always a positive response towards water hyacinth irrespective of damage or type of damage when the insects were given a choice between water hyacinth plants and the no plant control. If the insects were presented with two damaged plants, there was no preference for any type of damage by any species of insect. In summary, there was always a significant preference for undamaged water hyacinth plants.

Table 2.2 The χ^2_{Yates}-test results for each of the treatments as well as for each of the insect species. All the tests were performed under 1 degree of freedom ($F = 3.81$) and an * indicates a level of significance $P<0.05$.

Test insect	Treatment	χ^2 value	Significance
Eccritotarsus catarinensis	Hp vs. Np	30.03	*
Neochetina bruchi	Hp vs. Np	30.03	*
Neochetina eichhorniae	Hp vs. Np	22.57	*
Eccritotarsus catarinensis	Ec vs. Np	22.57	*
Neochetina bruchi	Ec vs. Np	13.37	*
Neochetina eichhorniae	Ec vs. Np	19.23	*
Eccritotarsus catarinensis	Ne vs. Np	26.17	*
Neochetina bruchi	Ne vs. Np	19.23	*
Neochetina eichhorniae	Ne vs. Np	19.23	*
Eccritotarsus catarinensis	Nb vs. Np	22.57	*
Neochetina bruchi	Nb vs. Np	10.83	*
Neochetina eichhorniae	Nb vs. Np	10.83	*
Eccritotarsus catarinensis	Ec vs. Hp	19.23	*
Neochetina bruchi	Ec vs. Hp	19.23	*
Neochetina eichhorniae	Ec vs. Hp	10.83	*
Eccritotarsus catarinensis	Ne vs. Hp	16.17	*
Neochetina bruchi	Ne vs. Hp	10.83	*
Neochetina eichhorniae	Ne vs. Hp	10.83	*
Eccritotarsus catarinensis	Nb vs. Hp	19.23	*
Neochetina bruchi	Nb vs. Hp	13.37	*
Neochetina eichhorniae	Nb vs. Hp	10.83	*
Eccritotarsus catarinensis	Ec vs. Ne	1.23	
Neochetina bruchi	Ec vs. Ne	0.57	
Neochetina eichhorniae	Ec vs. Ne	3.37	
Eccritotarsus catarinensis	Ec vs. Nb	0.17	
Neochetina bruchi	Ec vs. Nb	2.17	
Neochetina eichhorniae	Ec vs. Nb	0.03	
Eccritotarsus catarinensis	Nb vs. Ne	0.17	
Neochetina bruchi	Nb vs. Ne	0.03	
Neochetina eichhorniae	Nb vs. Ne	0.57	
Eccritotarsus catarinensis	Hp vs. Hp	0.03	
Neochetina bruchi	Hp vs. Hp	1.23	
Neochetina eichhorniae	Hp vs. Hp	0.17	
Eccritotarsus catarinensis	Ec vs. Ec	0.17	
Neochetina bruchi	Ec vs. Ec	0.17	
Neochetina eichhorniae	Ec vs. Ec	0.17	
Eccritotarsus catarinensis	Ne vs. Ne	0.57	
Neochetina bruchi	Ne vs. Ne	0.17	
Neochetina eichhorniae	Ne vs. Ne	0.57	
Eccritotarsus catarinensis	Nb vs. Nb	0.57	
Neochetina bruchi	Nb vs. Nb	1.23	
Neochetina eichhorniae	Nb vs. Nb	2.17	

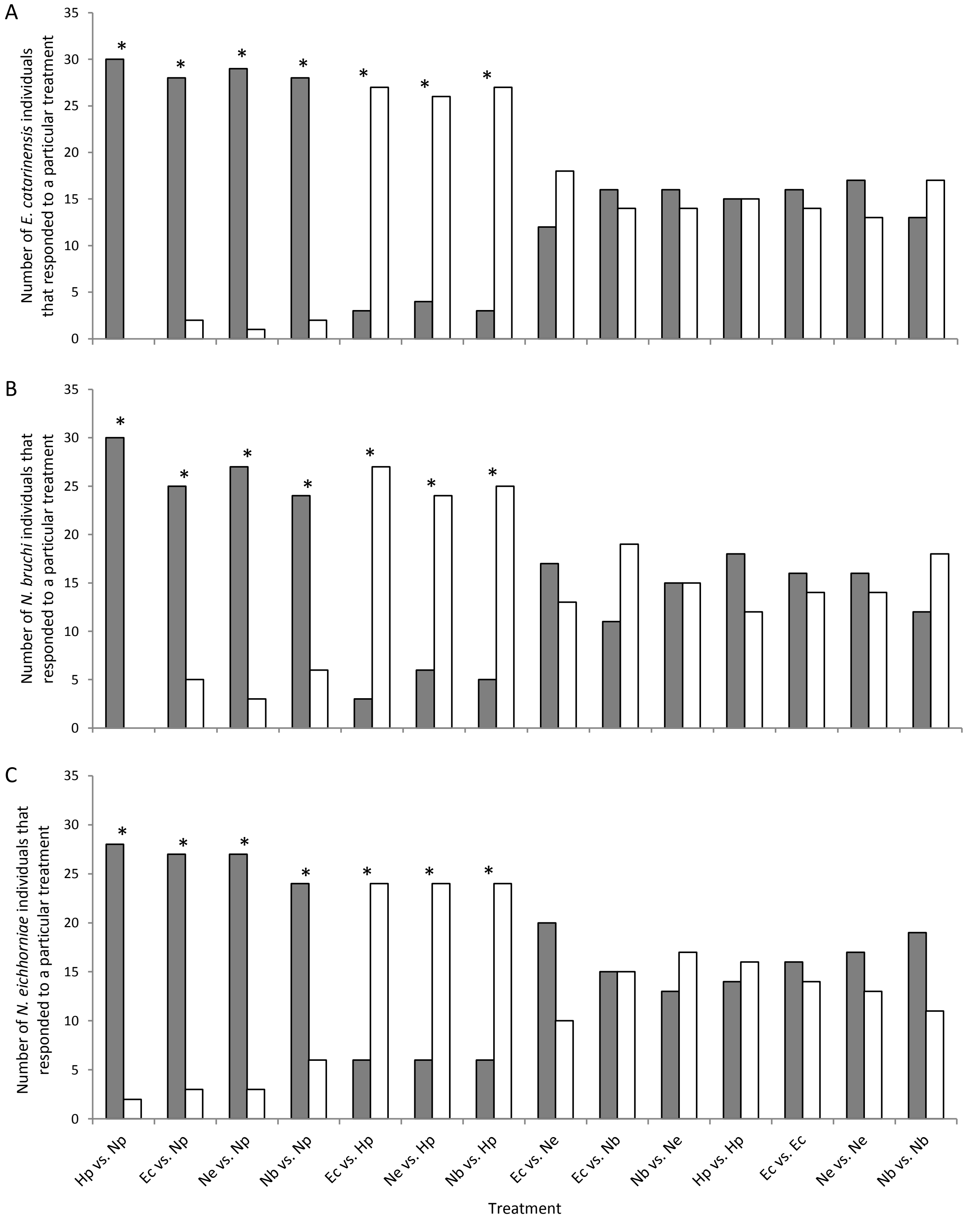

Figure 2.2 The frequency of individuals from each species tested, that responded to a particular treatment during the Y-tube olfactometer bioassay. A: *Eccritotarsus catarinensis,* B: *Neochetina bruchi,* C: *Neochetina eichhorniae*. For a detailed explanation of the treatments see Table 2.1. The * above the treatments indicates significant differences ($P<0.05$). The χ^2_{Yates}-test for each treatment was performed separately due to the independence of the experiments.

2.4 Discussion

Several studies have shown that herbivorous insects respond to plant volatiles (e.g. Visser 1986; Dicke & van Loon 2000). Del Fosse & Perkins (1977) identified a kairomone present in water hyacinth that is likely to serve as an attractant to the insects associated with it. During the current study the three insects, *E. catarinensis, N. bruchi* and *N. eichhorniae* were able to respond to undamaged water hyacinth plants in the control treatment as well as herbivore-damaged plants. This would suggest that the olfactory cues from both damaged and undamaged water hyacinth are sufficient for the insects to respond to them. This was not the case with the female Colorado potato beetle, *L. decemlineata*, which were not attracted to young undamaged potato plants (2-4 weeks old) (Visser 1976). However, after mechanical and herbivory damage the young plants were attractive to the beetles (Bolter *et al.* 1997), but this is probably related to the increased detectability of the plants and rather than a preference for damaged plants.

The attraction of the insects towards damaged plants in the control treatments, which involved a choice of herbivore-damaged plant and a no plant control, was not surprising. Behavioural responses of some of the water hyacinth insects to plant odours have been studied in the past. The weevil *N. eichhorniae* showed a significant attraction to mechanically crushed water hyacinth leaves (Del Fosse & Perkins 1977). In addition to this there are several unrelated cases where herbivorous insects are attracted to damaged plants (Landolt 1993; Harari *et al.* 1994; Loughrin *et al.* 1995; Bolter *et al.* 1997; Pallini *et al.* 1997; Anderson & Alborn 1999). When the insects were given a choice between a damaged plant (irrespective of type of damage) and a healthy plant there was a strong avoidance of the damaged plant. This does not concur with the results from Del Fosse & Perkins (1977), where there was a strong attraction of *N. eichhorniae* to plants that had been mechanically damaged. In addition to this Perkins *et al.* (1976) suggested that by manually damaging water hyacinth in the field there was a 10-fold increase in the number of *Neochetina* weevils on the plants a day later. Plants respond differently to mechanical damage and damage caused by herbivory (Vet & Dicke 1992). Volatiles released from mechanically damaged plants usually comprise of green leaf leafy volatiles (Turlings *et al.* 1998; Hoballah & Turlings 2005). The response of plants to insect herbivory, usually stimulated by regurgitate or saliva, most commonly comprise of other volatiles such as terpenoids and aromatics, including indole, which usually appear only several hours after the damage is caused (Turlings *et al.* 1998). In addition to this, Soti & Volin (2010) demonstrated that mechanical damage inflicted on water hyacinth in an attempt to mimic weevil damage did not elicit the same response by the plants that weevil damage would have. This suggests that plants respond differently to herbivore damage and mechanical damage in both the odours released and plant parameters. The response of the insects tested here was expected to be different from other studies testing mechanically damaged plants.

The test insects were given a choice between different types of damage to test whether there was a preference for conspecific or heterospecific insect damage. Plants can respond to herbivory by releasing specific blends or ratios of volatiles that can be specific either to the type of damage or to the species of herbivore involved (Sabelis & van de Baan 1983; Takabayashi *et al.* 1995; Pallini *et al.* 1997; de Moraes *et al.* 1998; Powell *et al.* 1998). Pallini *et al.* (1997) showed very clearly that the two-spotted spider mite *T. urticae* had to some degree a preference for cucumber plants damaged by conspecifics, but a strong avoidance of plants infested with the thrips *Frankliniella occidentalis* (Pergande). The thrips *F. occidentalis* represents not only a competitor, but also a potential predator of the spider mite *T. urticae* (Pallini *et al.* 1997). The plants infested with thrips could pose a high ecological cost to the spider mite, and therefore avoidance of the plant is expected. The two weevils *N. bruchi* and *N. eichhorniae* and the mirid *E. catarinensis* did not show any preference for any particular type of herbivory damage (either conspecific or heterospecific) to water hyacinth. This suggests that there may be no perceived ecological cost for the test insects in terms of entering a potentially enemy-dense space or competition between conspecifics or heterospecifics.

In the current study the insects showed a significant avoidance for herbivore damaged plants when given a choice between damaged and undamaged plants. In a study on tobacco budworm *H. virescens*, females were repelled by tobacco plants fed upon by conspecific larvae (de Moraes *et al.* 2001) because these plants could potentially represent competitors for the moth's offspring or an enemy-dense space/area (Thaler 1999). This is further supported by studies testing the attraction of damage caused by conspecifics on cotton plants. It was found that both the cabbage looper moth *Trichoplusia ni* (Hubner), and the African cotton leaf worm *Spodoptera littoralis* (Biosduval) were attracted to the plant, but oviposition occurred only on nearby undamaged plants (Landolt 1993; Anderson & Alborn 1999). An extensive study of the natural enemies of the two water hyacinth weevils *N. eichhorniae* and *N. bruchi* resulted in only generalist predators being found and not a single specialised parasitoid or predator (DeLoach & Cordo 1982). During the surveys in the native range of *E. catarinensis* no specialised parasitoids and predators were found (Hill, pers. comm.). Therefore the odours released by water hyacinth under the herbivore pressure in this study are unlikely to be related to potential predator or parasitoid attack but rather to an increase in potential competition, which explains the preference for undamaged plants by the insects tested.

In general herbivore-damaged plants could present a welter of information for a herbivorous insect to decipher. The volatile cues may represent a potentially enemy-dense space which may decrease the chances of survival (Thaler 1999), or the plant's induced defences such as the production of chemical toxins may have been initiated (Coley *et al.*

1985; Agrawal 1998). On the other hand the plant's induced defences may have been overcome. The cues may indicate that the plant has been weakened and is therefore more susceptible, but conversely could also indicate that the nutritional value of the plant is reduced (Coley *et al.* 1985; Agrawal 1998). Ultimately herbivore-induced plant odours indicate that the plant is being fed upon and those individuals may represent potential competitors (Dicke & van Loon 2000).

2.5 Conclusion

The insects tested here were attracted to both damaged and undamaged water hyacinth plants. However the damaged plant may not represent the "best option" and therefore the preference for undamaged plants was expressed in the response of the insects when given a choice. The insects did not show a preference for any particular type of damage, however, when presented with no choice, the insects were always attracted towards water hyacinth. In terms of biological control, this study may indicate that the insects will always be attracted to a water hyacinth infestation regardless of other agents present, but once at the site a particular insect may have a preference for a particular area over another. The next chapter explores the potential for these insects to compete, and whether the interactions are synergistic or antagonistic for insect performance.

Chapter 3: The effects of insect-insect interactions on the performance of three biological control agents released against water hyacinth in a common garden experimental design

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3.1 Introduction

Interactions between different species of herbivorous insect are common and fairly well documented in the literature, and these can be either direct or indirect interactions (for review see Damman 1993; Denno *et al.* 1995; Kaplan & Denno 2007). The direct insect – insect interaction has been shown to be important in mandibulate herbivores, such as beetle larvae that make use of concealed niches and can be strongly correlated to body size and aggression (Denno *et al.* 1995). The indirect interaction would be plant mediated and be either a consequence where the plant tissue is completely removed by the insect (Blakley & Dingle 1978) or a response such as induced defences and/or reduced nutritive value of the plant or plant part (Rhoades 1985; Denno & Kaplan 2007). This could be important for conspecifics, for example insect species ‘A’ feeding on the host plant could elicit a response in the plant resulting in an interaction on other individuals of the same species. This can be a significant driving force in the population dynamics of a particular species of insect, but for the purposes of this study this scenario will not be explored further. The importance of the interaction for heterospecifics would be insect ‘A’ feeding on the host plant, which elicits a response or has a consequence on the host plant which then affects insect species ‘B’.

Plant mediated interactions between species of herbivorous insect are common; after colonisation and feeding from one species on a host plant there is a reduction in the suitability of the host plant to other individuals (Kogan & Paxton 1983; Rhoades 1985; Denno & Kaplan 2007). Often this type of interaction is asymmetrical where one species will largely be unaffected, while the performance and fitness of the other species is greatly reduced (Lawton & Hassell 1985).

During an investigation into the potential interactions between two aphids *Melanocallis caryaefoliae* (Davis) and *Monellia caryella* (Fitch), the fitness of *M. caryaefoliae* was significantly reduced on plant material that had been previously damaged, irrespective of aphid species, while *M. caryella* was largely unaffected by prior damage from either species (Petersen & Sandström 2001). The competitive interaction between these two aphids was asymmetric. When both were released on greenhouse and field plants in equal numbers *M. caryella* was the superior competitor, performing better than *M. caryaefolia* (Petersen & Hunter 2001; Petersen & Sandström 2001). The reduction in performance and fitness of *M. caryaefoliae* can be directly attributed to changes in the host plant elicited by feeding damage of conspecifics and heterospecifics (Petersen & Sandström 2001).

The invasion of North America and Canada by the Musk thistle *Carduus nutans* has resulted in the initiation of a biological control programme in the 1960s and has seen the release of two species of weevil *Trichosirocalus horridus* (Panzer) and *Rhinocyllus conicus* Froelich (Kok 2001). The feeding, oviposition and development of *T. horridus* on musk thistles results in a modification of the flower heads that negatively affects the performance and fitness of *R. conicus* (Milbrath & Nechols 2004). The feeding of *T. horridus* larvae can reduce the quality and available quantity of floral resources (in some cases by up to 50%) that are available to *R. conicus* larvae, which has severe implications for recruitment (Milbrath & Nechols 2004). In addition to this, damage caused by *T. horridus* can delay flower head production by up to 2 weeks, ultimately resulting in a break in the oviposition synchrony. This reduced the overall fitness of *R. conicus* by 63% when in combination with *T. horridus* (Woodburn 1997; Milbrath & Nechols 2004). This is an asymmetrical relationship because if *R. conicus* gets in first it does not have a similar effect on *T. horridus*. However, the strong synchrony between *R. conicus* and its host *C. nutans* rarely allows it to take advantage of flower heads before *T. horridus* (Milbrath & Nechols 2004).

In a similar example the biological control programme on spotted knapweed, *Centaurea stoebe* Linnaeus, and diffuse knapweed, *C. diffusa* has had several agents released, some of which include two flower head feeding insects which are gall flies in the genus *Urophora* (Müller-Schärer & Schroeder 1993). The flower head weevils are in the genus *Larinus* (Seastedt *et al.* 2007). A negative relationship was observed between *Urophora* and *Larinus*, where the abundance of *Larinus* declined in the presence of *Urophora* due to the modification of a common resource - the flower heads (Crowe & Bourchier 2006). The results from this study suggest that the interaction between these agents is reducing the effectiveness of the biological control programme, with a less effective agent reducing the efficacy of a more effective agent (Crowe & Bourchier 2006).

Plant mediated insect – insect interactions may not necessarily result in a negative relationship and the presence of one insect species may enhance or increase the performance of either or both of the species (Williams and Myers 1984; Damman 1989; Gange and Brown 1989; Strauss 1991; Masters *et al.* 2001). The feeding or damage caused by one species can increase the resource availability to another, for example root feeding insects can increase nutrients for foliar herbivores, which increases the fecundity and performance of some leaf miners (Gange and Brown 1989; Masters 1995). This applies not only to foliar herbivores. The presence of root herbivores on *Cirisum palustre* (Linnaeus) resulted in higher population densities of the seed predators than on plants that were not subjected to root herbivory (Masters *et al.* 2001). This is possibly due to changes in plant physiology, potential resource allocation and nutrient balance when subjected to root herbivory (Masters & Brown 1992; Masters 1995). Root herbivory can in some cases mimic drought stress, which has been shown to increase nutrient allocation to reproductive organs - in this case the flower heads

(Chapin 1980). This potential increase in nutrient allocation to the flower heads would explain the increase in fitness and performance of the seed predator of the marsh thistle (Masters *et al.* 2001).

Foliar feeding by the western tent caterpillar, *Malacasoma californicum pluviale* (Dyar) on red alder, *Alnus rubra* Bongard, resulted in an increase in performance and fitness of fall webworm, *Hyphantria cunea* (Drury) (Williams & Myers 1984). The quality of available resources in red alder may have been increased by light feeding of the western tent caterpillar, resulting in a 12.5% increase in fecundity of individual fall webworms as compared to individuals that fed on undamaged red alder (Williams & Myers 1984). A similar interaction was found when the herbivores on smooth sumac, *Rhus glabra* Linnaeus were studied (Strauss 1991). The cerambycid beetle *Oberea ocellata* Linné showed a preference for and possible increased performance and fitness on, plants that had been feed upon previously by the chrysomelid beetle *Blepharida rhois* (Forster) (Strauss 1991).

Ajuonu *et al.* (2007) experimentally investigated the interactions between three agents on water hyacinth, the two *Neochetina* weevils and the mirid *Eccritotarsus catarinensis.* The results suggest that the weevils should not influence the establishment of *E. catarinensis* in the field. However when *E. catarinensis* was presented plants with a large number of old feeding scars from the weevils (200 scars per leaf) there was a significant decrease in the performance of both adults and nymphs. This was probably due to the decrease in plant quality due to the old feeding scars (Center & Van 1989), which can adversely affect the mirid (Coetzee 2004). In the presence of fresh feeding scars from the *Neochetina* weevils there was a significantly better performance of the mirid than in the presence of old feeding scars, and only a slightly better performance when compared with plants with no feeding scars (Ajuonu *et al.* 2007).

Del Fosse (1978) studied the interactions between two arthropod agents, *Orthogalumna terebrantis* and *N. eichhorniae* on water hyacinth. The results suggest that there was a positive relationship between these two agents, with significantly higher oviposition by *N. eichhorniae* in the presence of *O. terebrantis* (Del Fosse 1978). The concentrations and feeding intensity of both arthropods also increased when in combination, probably due to a kairomone released by injured water hyacinth acting as an attractant. The sympatric presence of these species can lead to higher population levels of both species (Del Fosse 1978).

The aim of the work reported in this chapter was to quantify the interactions between three biological control agents, *N. eichhorniae, N. bruchi* and *E. catarinensis* released for the control of water hyacinth in a common garden plot design.

3.2 Materials and methods

To investigate the interactions between three arthropod agents on water hyacinth a large scale experiment was set up to mimic as closely as possible, natural conditions in a common garden experiment. The insects were inoculated in pairwise combinations and various parameters of insect and plant performance were measured.

3.2.1 Experimental setup

In the poly eurathane tunnel at Rhodes University, Grahamstown, South Africa, 42 experimental cages were set up in late summer of 2010 running from March until June. The experimental cages consisted of tubs 65 cm by 44 cm and 37 cm deep, filled with 66 litres of borehole water and covered with a fine mesh sleeve with a mesh diameter of 0.5mm. The fertiliser Multicoat 6 month formula was used to introduce total nitrogen at a rate of 50.5 mg N L^{-1} (N:P:K ratio of 15:3:12) and approximately 6g of commercial iron chelate (13% Fe) was added to each tub. The nutrient levels for this experiment are classed by Holmes (1996) as hypertrophic and are the upper levels for water hyacinth growth (Reddy *et al.* 1989, 1990).

Ten water hyacinth plants of similar size (approximately 30 cm high) per tub were collected from insect free stock ponds in the tunnel which were grown under the same conditions. The common garden plot design was to simulate as closely as possible natural conditions, so 10 plants per tub were used to mimic the conditions presented in a mature water hyacinth mat. All dead material and daughter plants were removed and each plant was washed in fresh water and the tubs were immediately covered in the fine mesh sleeve. The mesh sleeve fitted snugly around the brim of the tub and extended 1 meter above the water surface. The plants were given a 14 day period for acclimation to the conditions of the experiment before any insects were released or plant parameters recorded. During the acclimation time the plants were sprayed with kelthane and pirimore to minimize the chance of red spider mite (Tentranychidae) and aphid contamination respectively. The plants were washed down with fresh water prior to the inoculation of insects.

The experimental treatments consisted of each insect singly, and then paired combinations of each insect (Table 3.1), resulting in 6 treatments and one control where no insects were released. The tubs were arranged in a randomised block design to minimize any confounding factors associated with position in the tunnel.

Both species of weevil *N. eichhorniae* and *N. bruchi* were sourced from the mass rearing facility at the South African Sugar Research Institute (SASRI) in Durban, South Africa. The weevils arrived two weeks before the experiment. The species were separated and kept in plastic containers, the containers were cleaned and leaves were replaced daily to ensure no food shortage. For each treatment containing the weevils (either *N. eichhorniae* or *N. bruchi*) ten adult weevils of the appropriate species were introduced at a 1:1 sex ratio (5 males and 5 females). This resulted in a stocking density of one pair per 2 plants which is a low density. This was lower than common field densities (Center *et al.* 1999) to ensure that the plants in the experimental tubs did not collapse before the end of the experiment due to weevil herbivory pressure.

Two cages were set up in the tunnel and inoculated with 500 adult mirids approximately two months before the experiment to culture *E. catarinensis*. Fresh plants were added to the cages on a regular basis. The mirids were collected from the cages as required for the experiment. The sex ratio of the population was tested where three batches of 100 mirids were collected and sexed. Statistically the population had a sex ratio of 1:1 (Mann-Whitney U – test, $U_{(1)} = 3.00$, $P = 0.513$) and therefore the assumed sex ratio for all inoculations was 1:1. For each *E. catarinensis* treatment 150 individuals were released per experimental cage, an initial stocking density of 15 adults per plant, which is the same as used by Coetzee *et al.* (2007) and Ajuonu *et al.* (2007).

Table 3.1 The combinations of species and total number of individual insects inoculated in the treatments of the experiment (Ne = *Neochetina eichhorniae,* Nb = *Neochetina bruchi* and Ec = *Eccritotarsus catarinensis*).

	N. eichhorniae	*N. bruchi*	*E. catarinensis*
N. eichhorniae	**Ne** (10 Ne)		
N. bruchi	**Ne + Nb** (10 Ne + 10 Nb)	**Nb** (10 Nb)	
E. catarinensis	**Ne + Ec** (10 Ne + 150 Ec)	**Nb + Ec** (10 Nb + 150 Ec)	**Ec** (150 Ec)

The daily maximum and minimum temperatures for tunnel air, water hyacinth canopy and water temperature inside an experimental cage were recorded using Thermochron iButtons (DS1921G; Maxim Dallas Semiconductor Corporation) which have an operational temperature range from -40°C to 85°C and an accuracy of ± 1°C (Hubbart *et al.* 2005). There was a general downward trend in the temperature data as the experiment ran from late summer into early winter (Figure 3.1).

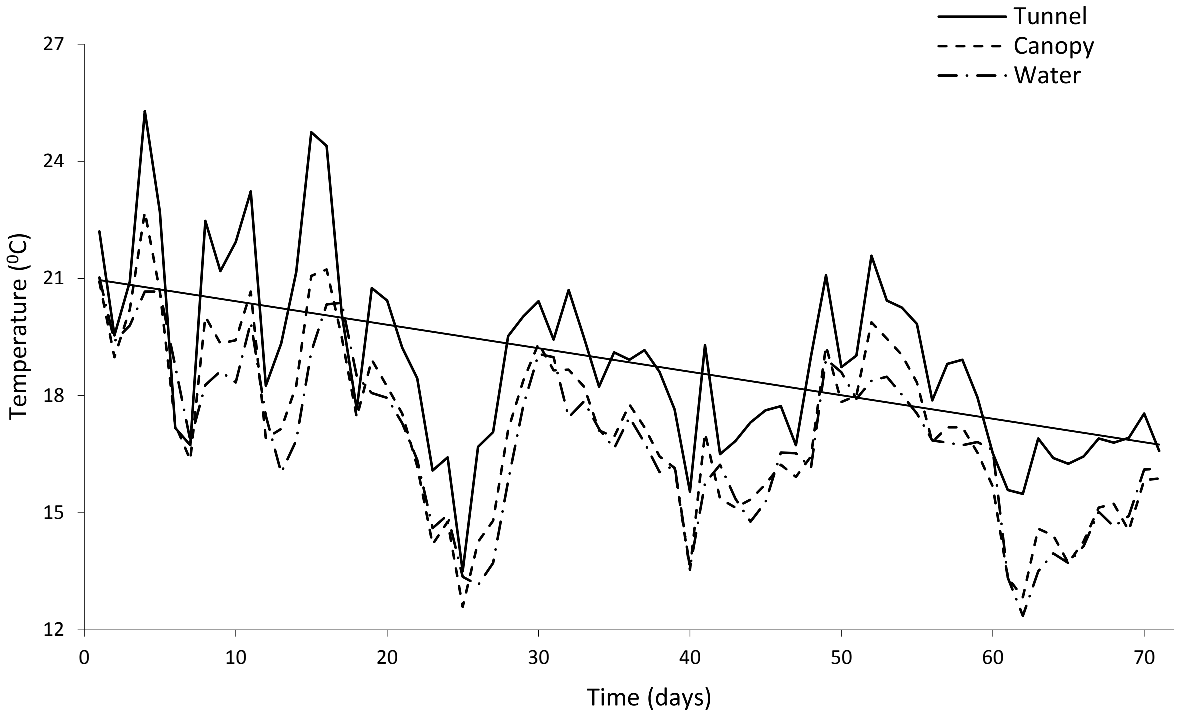

Figure 3.1 The daily average temperature (°C) recorded in the tunnel, water hyacinth canopy and the water for the duration of the experiment.

3.2.2 Data collection

Prior to the release of insects into the experimental treatments the plant parameters were taken from three randomly selected plants per cage. These three plants were labelled and utilised for all subsequent data collection. Thereafter all plant and insect parameters were taken every 14 days for a period of 10 weeks.

3.2.2.1 Plant parameters

The plant parameters collected included the number of leaves and ramets, leaf turnover, mat height at three independent points in the cage and the leaf surface area of leaves two, three and four. The leaf turnover was measured by labelling the youngest leaf and recording the position of that leaf on the next sampling event, then moving that label back to the youngest leaf. The biomass of the plants was separated into above water, below water and dead organic matter and weighed separately at the end of the experiment. The shoot to root ratio was investigated to determine if there was a difference in resource allocation.

3.2.2.2 Insect parameters

Neochetina weevils

The adult feeding scars were counted on both the upper and underside of leaf two, three and four. The total number of petioles mined by the larvae was recorded, however, the plants were not destructively sampled during the experiment so larval mining of the petioles could only be recorded once it was externally visible on the petiole.

Eccritotarsus catarinensis

Feeding damage was recorded as an estimate of percentage cover on leaf two, three and four. The intensity of feeding was also scored on a scale of 1 – 5, where one is slight speckling and five is almost total chlorosis of the leaf where it appears white to yellow (Hill *et al.* 1999). Feeding damage, as well as feeding intensity, is subject to observer bias so therefore all data collection was done by the same observer.

3.2.3 Statistical analysis

In each experimental tub or cage the same three plants were measured at each sampling event. These plants were pseudo-replicates but the measurements obtained were averaged for that replicate. The total number of replicates for each treatment was 6, but each measurement was an average for each experimental tub.

3.2.3.1 Plant parameters

According to the Levene test for homogeneity of variances, Kolmogorov-Smirnov test for normality and Mauchly sphericity test all the plant parameters fulfil the assumptions and requirements of a repeated-measures ANOVA. To test for significant differences and identify homogenous groups in the plant parameters, the Tukey HSD post-hoc test at a confidence interval of 0.05 was conducted (Fowler *et al.* 2005). The biomass parameters measured fulfil the Levene test for homogeneity of variances and the Kolmogorov-Smirnov test for normality requirements of a one-way ANOVA. Therefore a one-way ANOVA was performed on the biomass parameters to test for differences between them. A Tukey HSD post-hoc test was conducted at a confidence level of 0.05 to determine where the statistical differences were (Fowler *et al.* 2005).

3.2.3.2 Insect parameters

The level of feeding damage for each insect species was used to put a quantifiable value on performance; this assumes that the level of damage is an indication of insect performance. The difference between the performances of the insects in the treatments determined whether there was a synergistic, antagonistic or neutral relationship between them.

The weevil feeding scars were divided by the surface area of the leaf, and this relative number of feeding scars was then used for all subsequent analyses. This was especially important for comparisons between the percentage feeding area of the mirid because this is already relative to the surface area of the leaf. The numbers of feeding scars and percentage feeding area of the mirid were then multiplied by the leaf turnover at each sampling event to relate insect feeding to exposure time of the leaf to herbivory.

Treatments that had the paired combinations of the weevil had double the density of weevils than the control treatments. For a realistic comparison to the control treatments the number of feeding scars was divided by the number of individuals present for that treatment. This was done only for leaf 2 and only for the weevil control treatments and the paired combination of the weevils.

Before any of the statistical tests were performed on the percentage feeding area of the mirid the data were arcsin transformed. According to Levene test for homogeneity of variances, Kolmogorov-Smirnov test for normality and Mauchly sphericity test, all the insect parameters fulfil the assumptions and requirements of a repeated-measures ANOVA. The Tukey HSD post-hoc test was conducted to test for significant differences and identify homogenous groups at a 0.05 confidence interval (Fowler *et al.* 2005).

A Spearman's Rank order correlation was employed to test for correlations between the insect parameters.

3.3 Results

3.3.1 Plant parameters

The plant growth parameters of water hyacinth were largely unaffected by the different treatments or combinations of insects. The mat height, which is a reflection of the amount of resource available to the insects, had an overall downward trend (Figure 3.2). At the start of the experiment the plants were between 400 and 500 mm, and by the end the plants ranged between 150 and 250 mm. There were no significant differences between any of the treatments at any sampling event, but there were significant differences between sampling events (Table 3.2).

The number of leaves increased from approximately 6 leaves to between 7 and 8 leaves per plant during the course of the experiment, with an increase in the number of leaves between the second last and the final sampling event (Figure 3.3). There were no significant differences between insect combinations at any sampling event, however there were significant differences between the sampling events (Table 3.2).

The surface area for leaf 2 was initially between 160 and 189 cm^2, with no significant differences between treatments (Figure 3.4, Table 3.2). The surface area steadily decreased and by the end of the experiment the area was between 33 and 44 cm^2, with no significant differences between treatments at any sampling event (Figure 3.4, Table 3.2). The surface area of leaf 3 and leaf 4 showed a similar trend, where the surface area started at between 160 and 190 cm^2 and 130 and 170 cm^2 respectively, with no significant differences between treatments (Figures 3.5 and 3.6, Table 3.2). During the course of the experiment this declined steadily to 40 – 65 cm^2 for leaf 3 and 70 – 100 cm^2 for leaf 4 with no significant differences between treatments at any sampling event (Figures 3.5 and 3.6, Table 3.2).

Initially the leaf turnover was high, at about 2 leaves every 14 days, but declined between sampling event T1 and T2, where the turnover rate was less that 1 every 14 days, this then had a steady but only slight increase in the leaf turnover rate to approximately 1 leaf per 14 days (Figure 3.7). There were no significant differences between insect combinations at any sampling event, but there were significant differences between the sampling events (Table 3.2).

The number of ramets the plants were producing was variable between sampling events but remained at approximately 2 ramets per plant for the duration of the study (Figure 3.8). There were no significant differences between insect combinations at any sampling event, but there were significant differences between the sampling events (Table 3.2).

There were no differences in the above water biomass ($F_{(6,35)}$= 1.70, P= 0.15), with the average weight being 2.93 ± 0.1 kg and the below water biomass ($F_{(6,35)}$= 2.21, P= 0.07) with an average of 2.21 ± 0.09 kg. There was a significant difference in the dead biomass with the treatment where *N. bruchi* and *E. catarinensis* were in combination having less dead biomass than the other treatments ($F_{(6,35)}$= 3.35, P= 0.01). The average dead organic matter was 1.6 ± 0.07 kg. There was no difference between the treatments for the shoot to root ratio, which ranged from 1.2 to 1.3 ($F_{(6,35)}$= 0.36, P= 0.89).

Table 3.2 The *F*-statistic and *P*-values for the repeated-measures ANOVA performed on the plant parameters, illustrating significant differences between insect combination, sampling event and the interaction between the two. The numbers in brackets are the sample size and degrees of freedom for each source of variation. Values in **bold** identify significant differences between means.

Parameter	Source of variation					
	Treatment		Time		Treatment X Time	
	F (6, 35)	*P*	*F* (5,175)	*P*	*F* (30,175)	*P*
Mat height	1.20	0.31	356.9	**0.00**	1.00	0.51
Number of leaves	1.60	0.17	98.06	**0.00**	1.50	0.06
Area of leaf 2	1.22	0.32	417.23	**0.00**	0.89	0.62
Area of leaf 3	2.10	0.08	306.27	**0.00**	0.89	0.68
Area of leaf 4	2.66	**0.03**	175.78	**0.00**	1.74	**0.01**
Leaf Turnover	0.50	0.80	197.40	**0.00**	0.80	0.68
Number of ramets	0.47	0.82	27.55	**0.00**	1.50	0.05

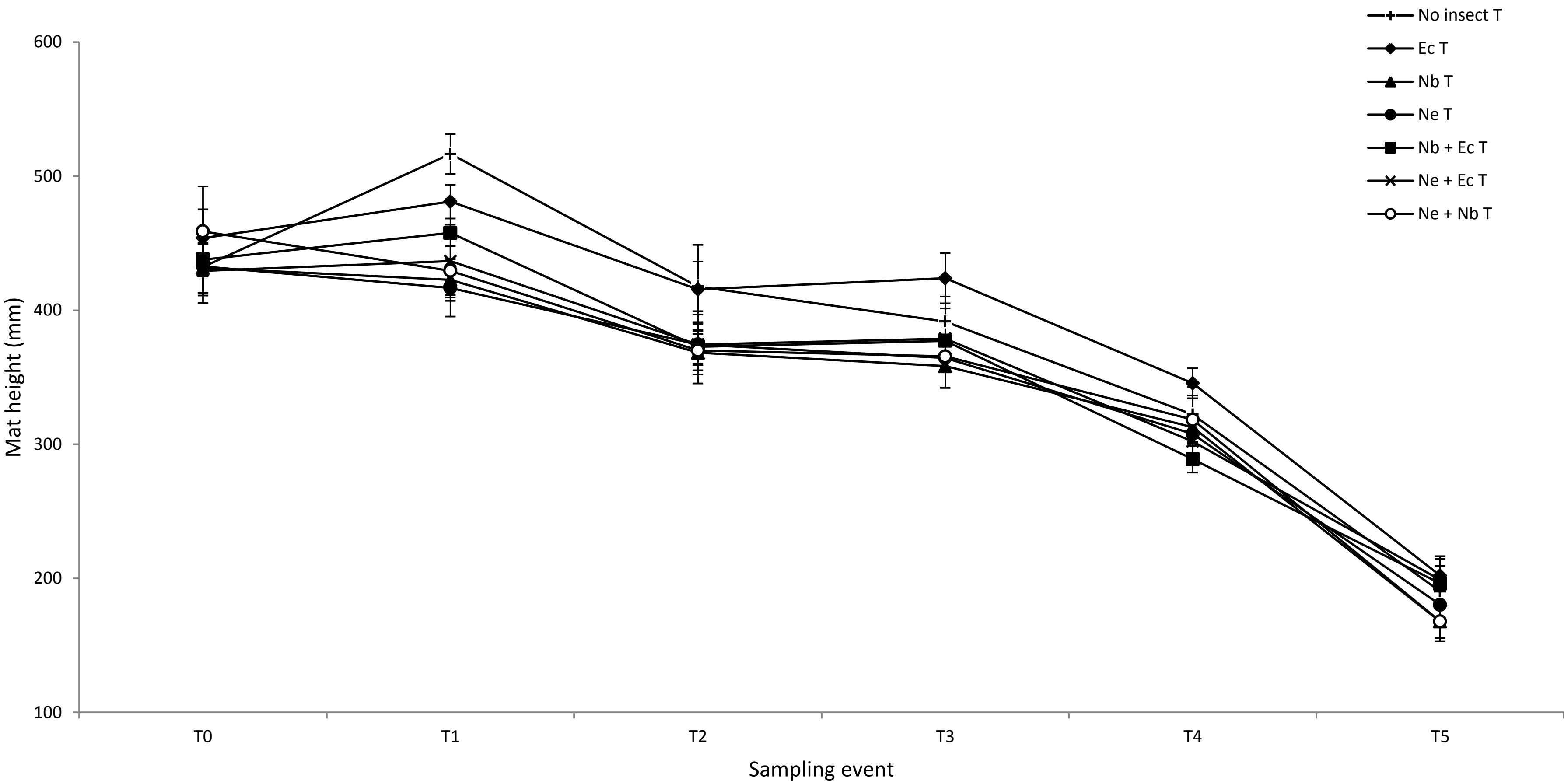

Figure 3.2 The water hyacinth **mat height** (mm) at each sampling event for each of the treatments. The treatments included are No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

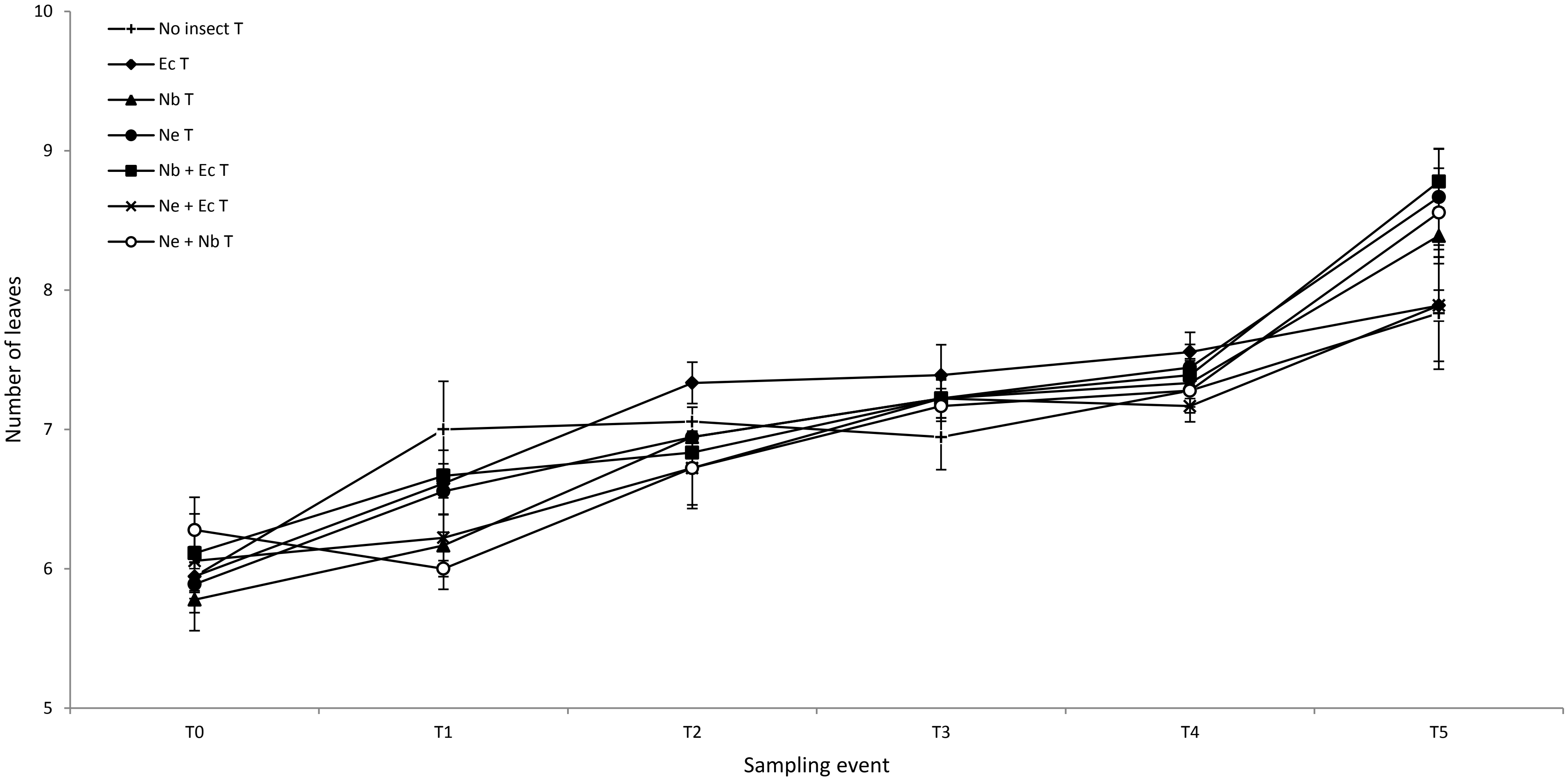

Figure 3.3 The **number of functional leaves** per water hyacinth plant at each sampling event for each of the treatments. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

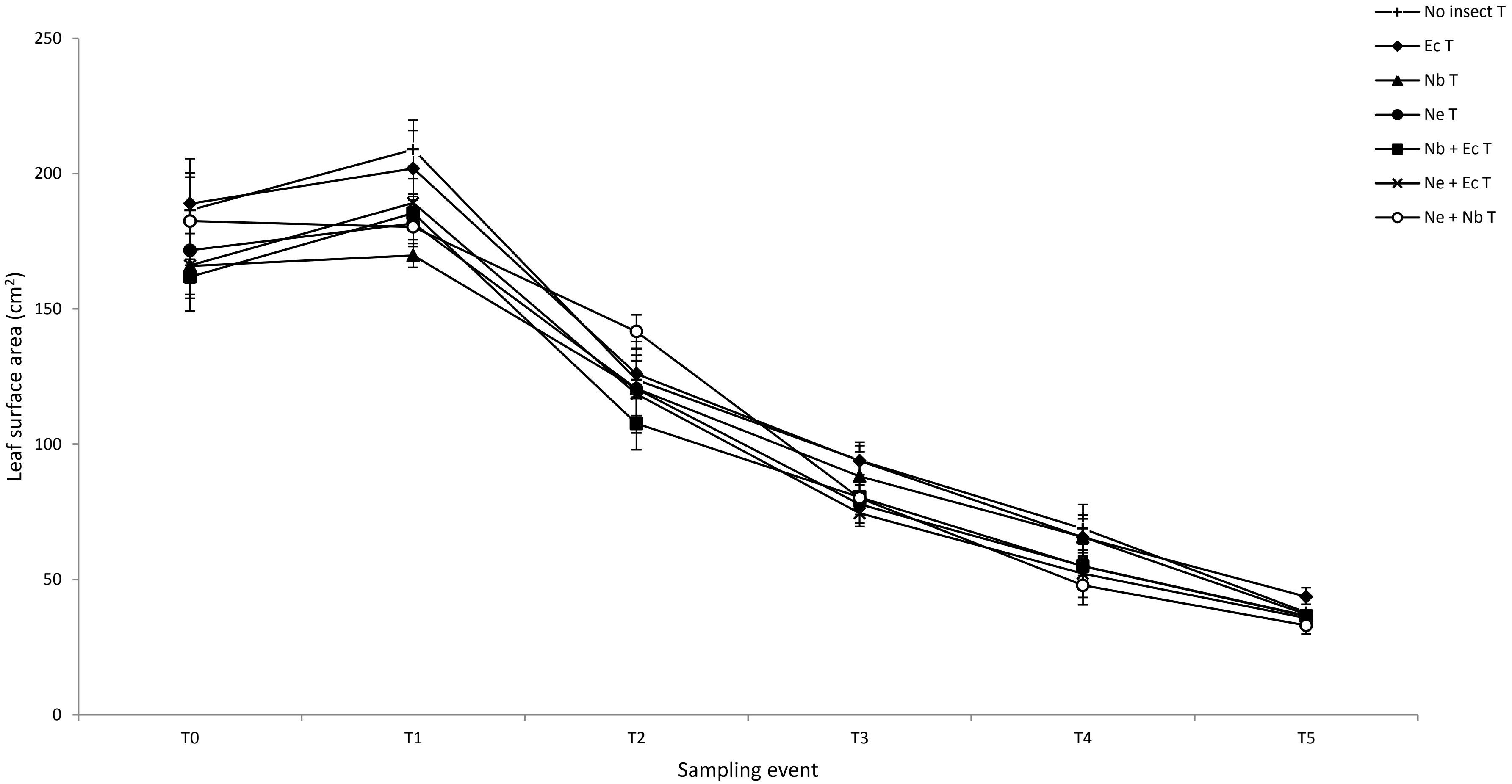

Figure 3.4 The **surface area for leaf 2** for the water hyacinth plants at each sampling event for each of the treatments. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

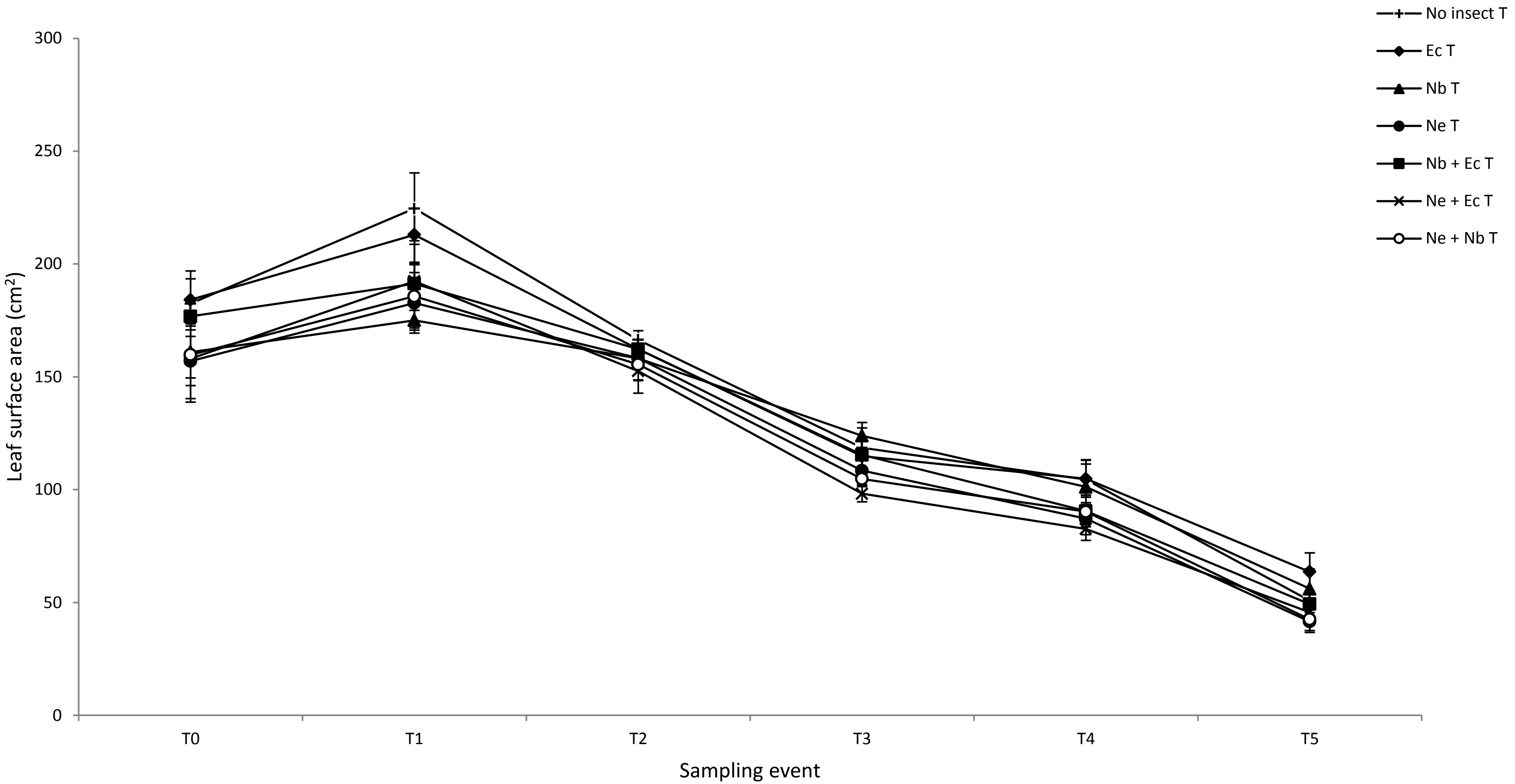

Figure 3.5 The **surface area for leaf 3** for the water hyacinth plants at each sampling event for each of the treatments. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

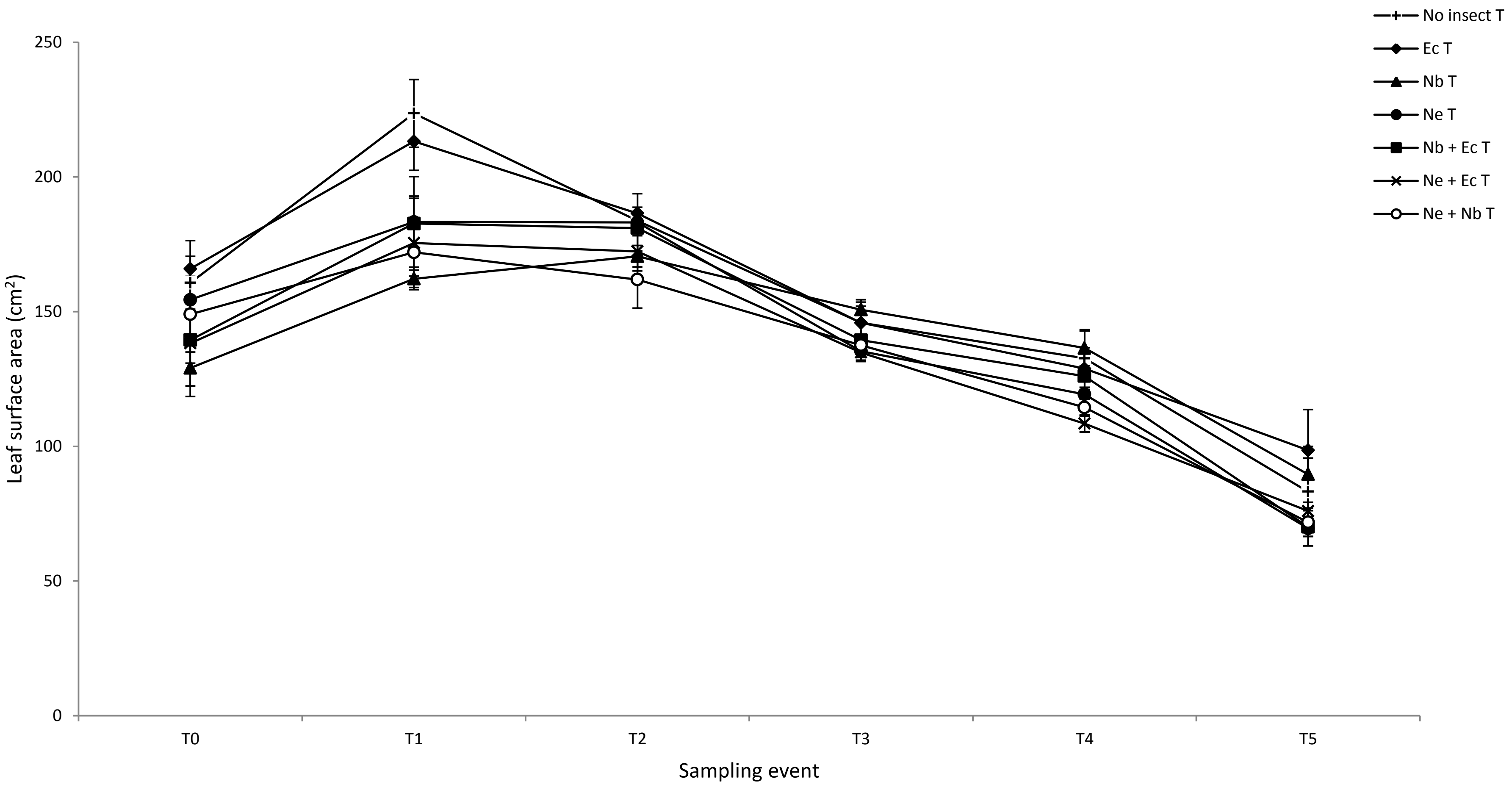

Figure 3.6 The **surface area for leaf 4** for the water hyacinth plants at each sampling event for each of the treatments. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

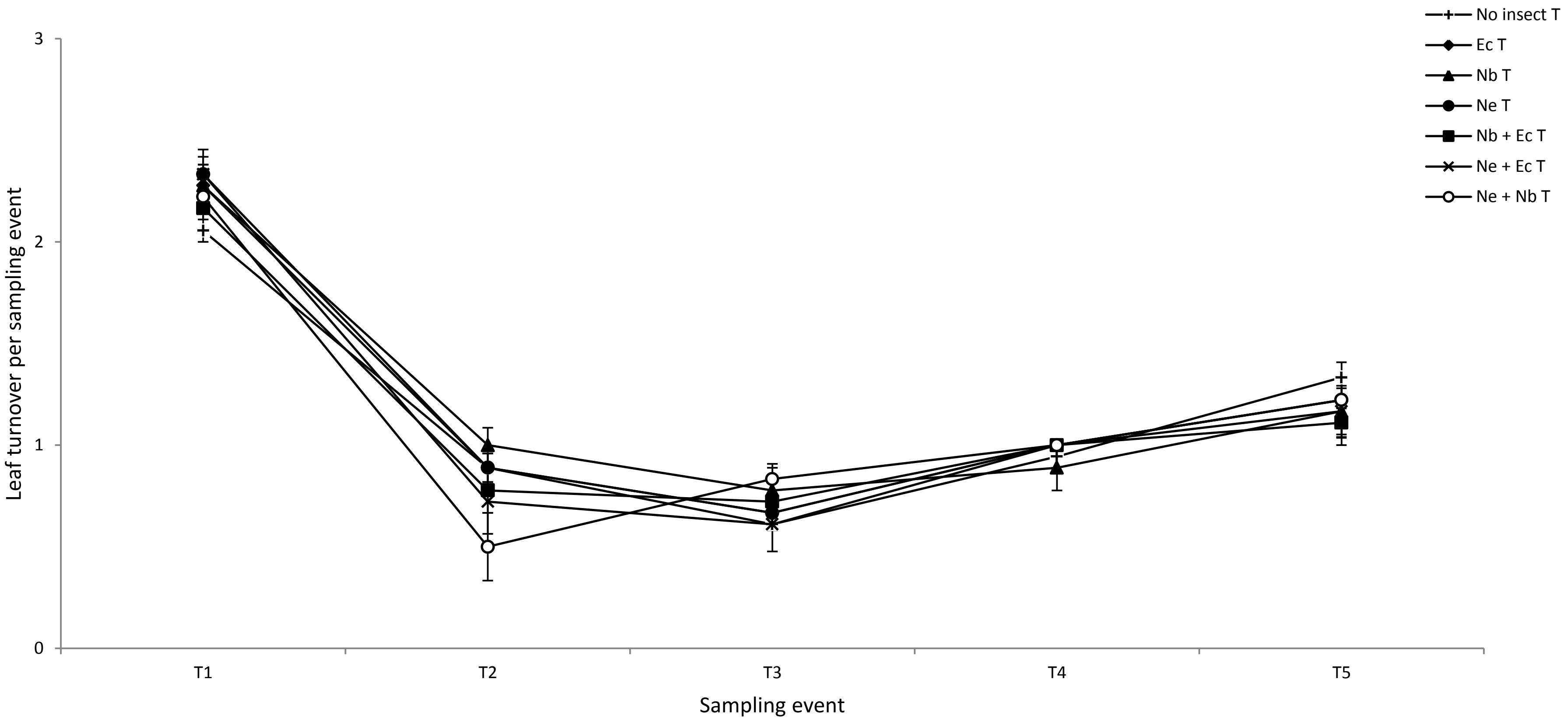

Figure 3.7 The number of water hyacinth **leaves turned over** at each sampling event for each treatment. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

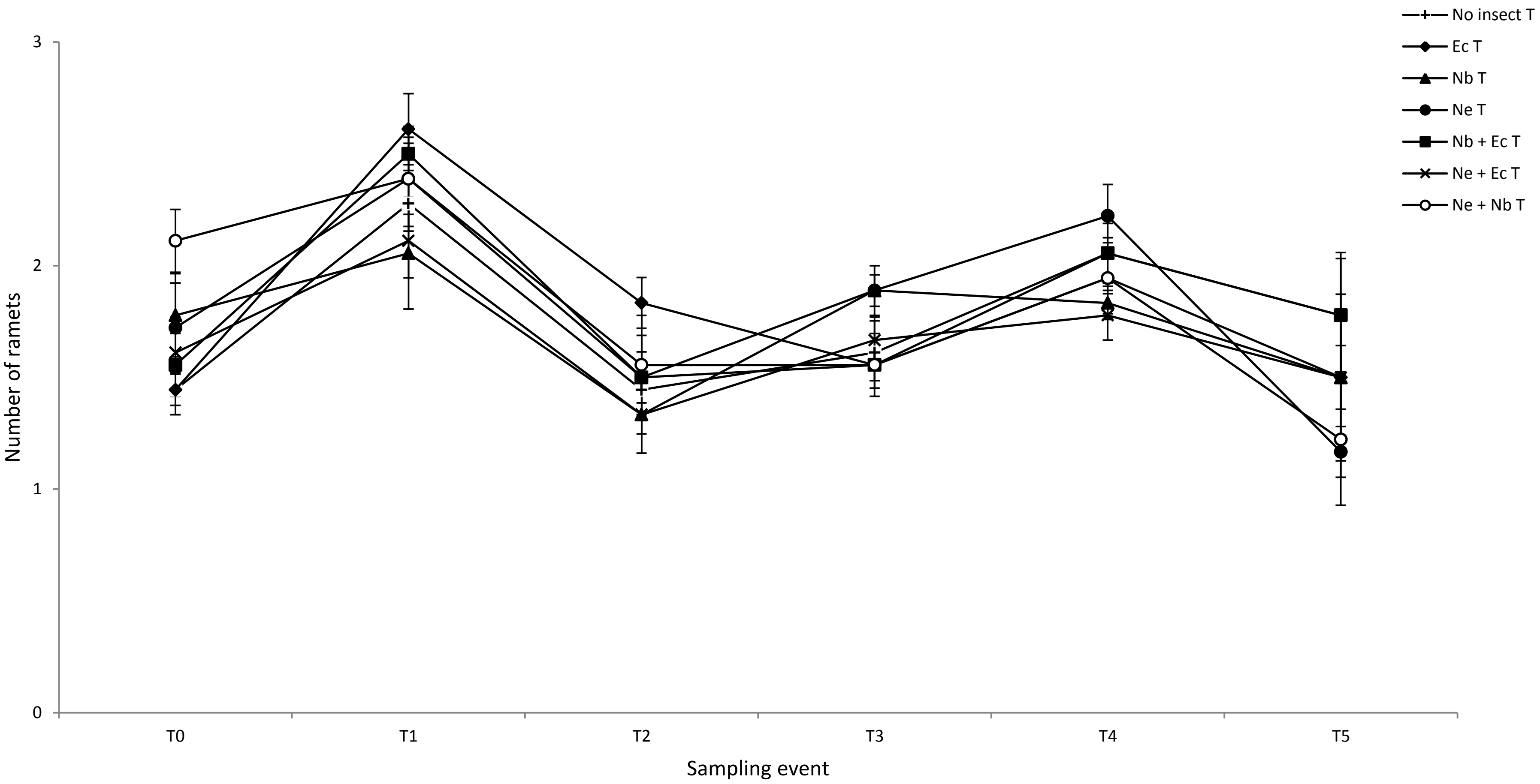

Figure 3.8 The **number of ramets** per water hyacinth plant at each sampling event for each treatment. The treatments included are: No insect T = no insect control, Ec T = *Eccritotarsus catarinensis* alone treatment, Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

3.3.2 Insect parameters

In all treatments the number of feeding scars per cm^2 remained relatively low and at no point did the level of feeding reach more than 0.8 scars per cm^2 (Figures 3.9, 3.10 and 3.11). The numbers of adult weevil feeding scars per cm^2 on leaf 2 were always significantly more abundant in the *N. eichhorniae* and *N. bruchi* treatment (Ne + Nb) than any other treatment, with the exception of the *N. eichhorniae* alone treatment at sampling event T2 and T4 (Figure 3.9). The number of feeding scars per cm^2 on leaf 2 for *N. eichhorniae* was always significantly higher when alone than when in combination with *E. catarinensis*, with the exception of sampling event T1 (Table 3.3, Figure 3.9). There was no difference in the number of feeding scars per cm^2 on leaf 2 when *N. bruchi* was in combination with *E. catarinensis* compared to the *N. bruchi* alone treatment (Figure 3.9, Table 3.3). There was a sharp decline in the number of feeding scars on leaf 2 for all treatments during the last sampling event T5 (Figure 3.9).

The number of feeding scars per cm^2 on leaf 3 reveal a similar result, where the two weevil combination (Ne + Nb T) has significantly more feeding scars than any other treatment with the exception of sampling event T2 and T5 (Figure 3.10, Table 3.3). The numbers of feeding scars per cm^2 were always significantly fewer when *N. eichhorniae* was in combination with *E. catarinensis* compared to *N. eichhorniae* alone except for sampling event T1 (Figure 3.10, Table 3.3). There were no significant differences measured between the number of feeding scars per cm^2 on leaf 3 from the *N. bruchi* alone treatment compared to the *N. bruchi* and *E. catarinensis* combination (Figure 3.10, Table 3.3). There is a slight decline in the number of feeding scars per cm^2 between sampling event T4 and T5 for all the treatments (Figure 3.10).

The number of feeding scars per cm^2 on leaf 4 for the two weevils in combination is again significantly greater than those in all other treatments at all sampling events (Figure

3.11, Table 3.3). There were significantly more feeding scars per cm^2 on leaf 4 for *N. eichhorniae* alone treatment at sampling event T3, T4 and T5 than in *N. eichhorniae* alone treatment and *N. eichhorniae* and *E. catarinensis* combination (Figure 3.11, Table 3.3). No significant differences were measured between the number of feeding scars per cm^2 on leaf 4 for the *N. bruchi* alone treatment and the *N. bruchi* and *E. catarinensis* combination with the exception of sampling event T4 (Figure 3.11, Table 3.3). Between sampling event T4 and T5 there was a slight decrease in the number of feeding scars per cm^2 for most of the treatments (Figure 3.11).

The number of feeding scars per individual weevil for *N. eichhorniae* was significantly higher and almost double that of the weevils in combination for leaf 2, with the exception of sampling event T1 and T5 (Figure 3.12, Table 3.3). There are no significant differences between the feeding scars from the *N. bruchi* treatment and the *N. eichhorniae* and *N. bruchi* combination treatment (Figure 3.12, Table 3.3).

The number of petioles mined per water hyacinth plant was always significantly greater for all the treatments that involved *N. bruchi* at the T3, T4 and T5 sampling events (Figure 3.13, Table 3.3). The number of petioles mined ranged from 4 to 5 petioles per plant for *N. bruchi* and between 2 to 3 petioles per plant for *N. eichhorniae.*

The percentage feeding area of the mirid for leaves 2, 3 and 4 increased significantly between sampling events for all the treatments (Figures 3.14, 3.15 and 3.16, Table 3.3) but a difference between the treatments was observed only for leaf 4 at sampling events T4 and T5 (Figure 3.16, Table 3.3). There is a slight decrease in the percent feeding area between sampling events T4 and T5 for leaves 3 and 4 (Figures 3.15 and 3.16).

The correlation between the percentage feeding area of the mirid and the number of feeding scars was investigated for the treatments *N. bruchi* and *E. catarinensis* combination and *N. eichhorniae* and *E. catarinensis* combination. There is a weak but significant negative correlation between the percent feeding of *E. catarinensis* and the feeding of *N. bruchi* (Spearman rank correlation r_s = -0.161, *P* <0.05) (Figure 3.17). The correlation between *E. catarinensis* and *N. eichhorniae* revealed slightly stronger and also significant negative correlation (Spearman rank correlation r_s = -0.569, *P* <0.05) (Figure 3.17).

There is a strong positive correlation between the percentage feeding area and the intensity of feeding for leaf 2 (Spearman rank correlation r_s = 0.848, *P* <0.05), leaf 3 (Spearman rank correlation r_s = 0.792, *P* <0.05) and leaf 4 (Spearman rank correlation r_s = 0.759, *P* <0.05).

Table 3.3 The *F*-statistic and *P*-values for the repeated measures ANOVA performed on the insect parameters, illustrating significant differences between insect combination, sampling event and the interaction between the two. Numbers in brackets are the sample size and the degrees of freedom for each source of variation. Values in **bold** indicate significant differences between the means.

Parameter	Source of variation					
	Treatment		Time		Treatment X Time	
Weevils	*F* (4,25)	*P*	*F* (4,100)	*P*	*F* (16,100)	*P*
Feeding scars on leaf 2	13.72	**0.00**	18.64	**0.00**	2.33	**0.01**
Feeding scars on leaf 3	14.96	**0.00**	25.15	**0.00**	1.49	0.12
Feeding scars on leaf 4	11.21	**0.00**	67.89	**0.00**	1.75	0.05
Number of mined petioles	35.40	**0.00**	255.2	**0.00**	7.50	**0.00**
	F (2,15)	*P*	*F* (4,60)	*P*	*F* (8,60)	*P*
Feeding scars on leaf 2 per individual weevil	6.38	**0.01**	35.83	**0.00**	2.77	**0.01**
Mirids	*F* (2,14)	*P*	*F* (4,56)	*P*	*F* (8,56)	*P*
% damage leaf 2	2.87	0.09	37.83	**0.00**	1.24	0.29
% damage leaf 3	2.58	0.11	82.84	**0.00**	1.86	0.09
% damage leaf 4	5.13	**0.02**	66.99	**0.00**	1.06	0.40

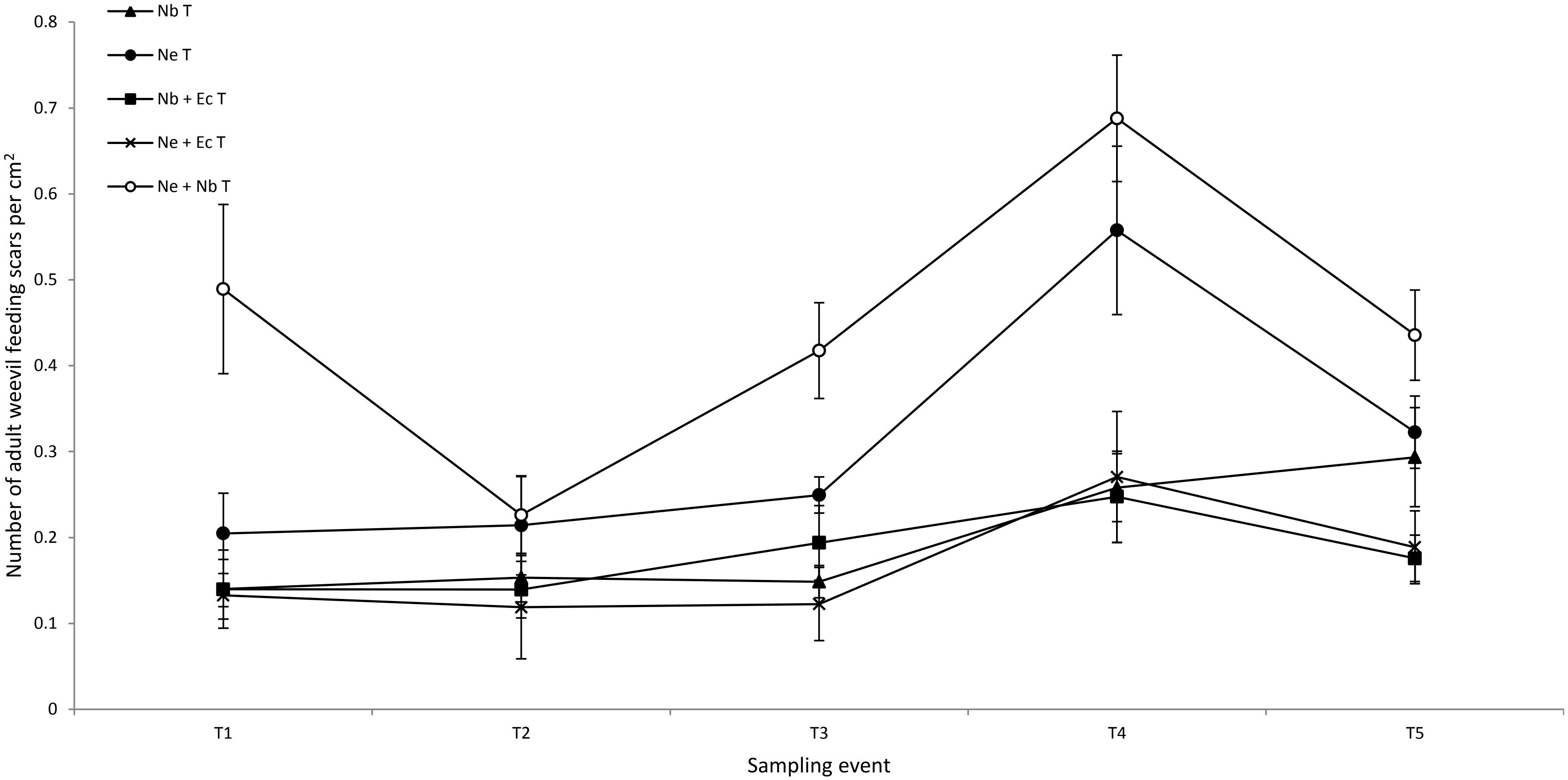

Figure 3.9 The number of adult weevil **feeding scars per cm^2** corrected for exposure time on **leaf 2** at each sampling event for the treatments that included weevils. The treatments included are: Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

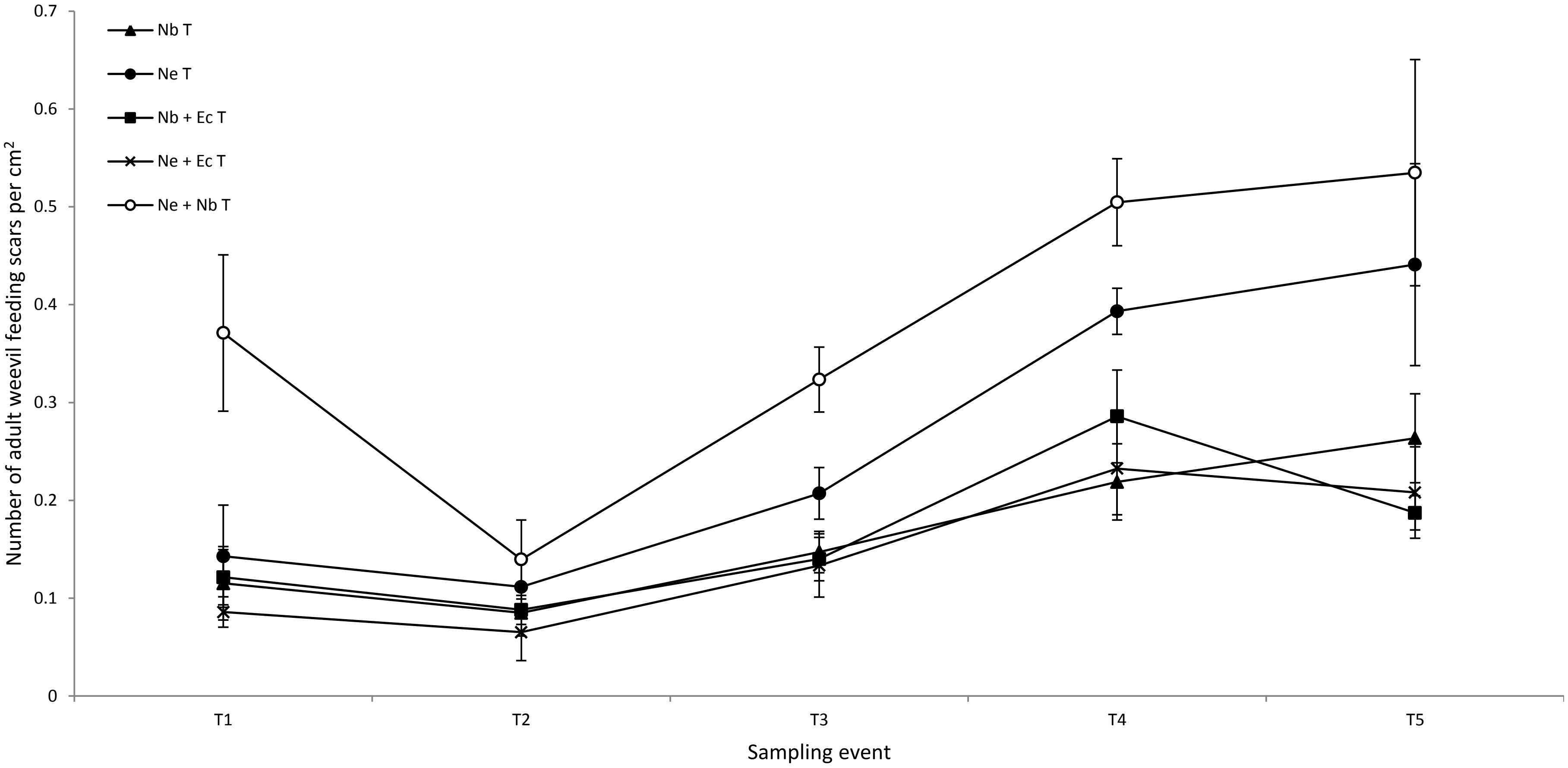

Figure 3.10 The number of adult weevil **feeding scars per cm^2** corrected for exposure time on **leaf 3** at each sampling event for the treatments that included weevils. The treatments included are: Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

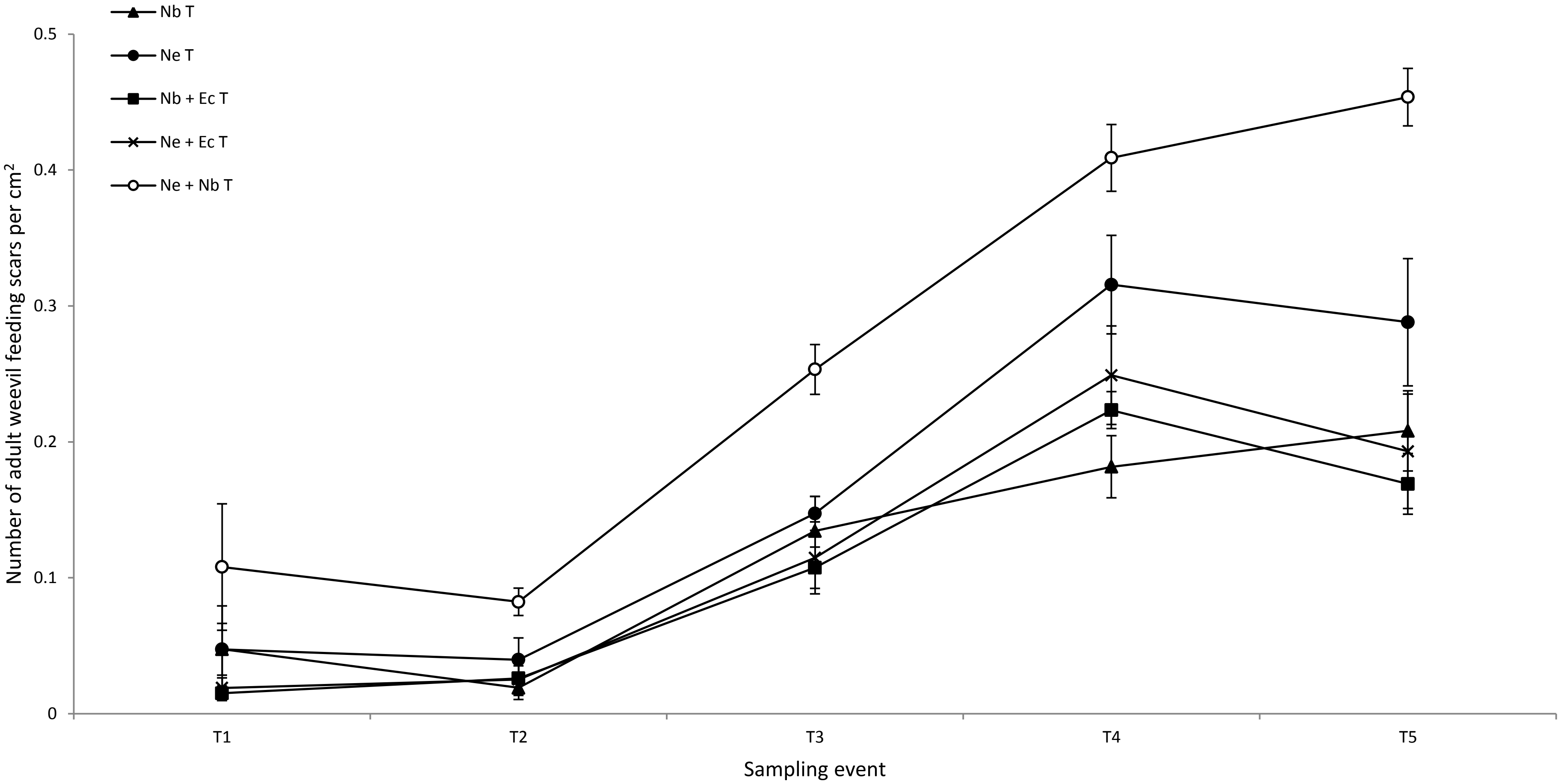

Figure 3.11 The number of adult weevil **feeding scars per cm^2** corrected for exposure time on **leaf 4** at each sampling event for the treatments that included weevils. The treatments included are: Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

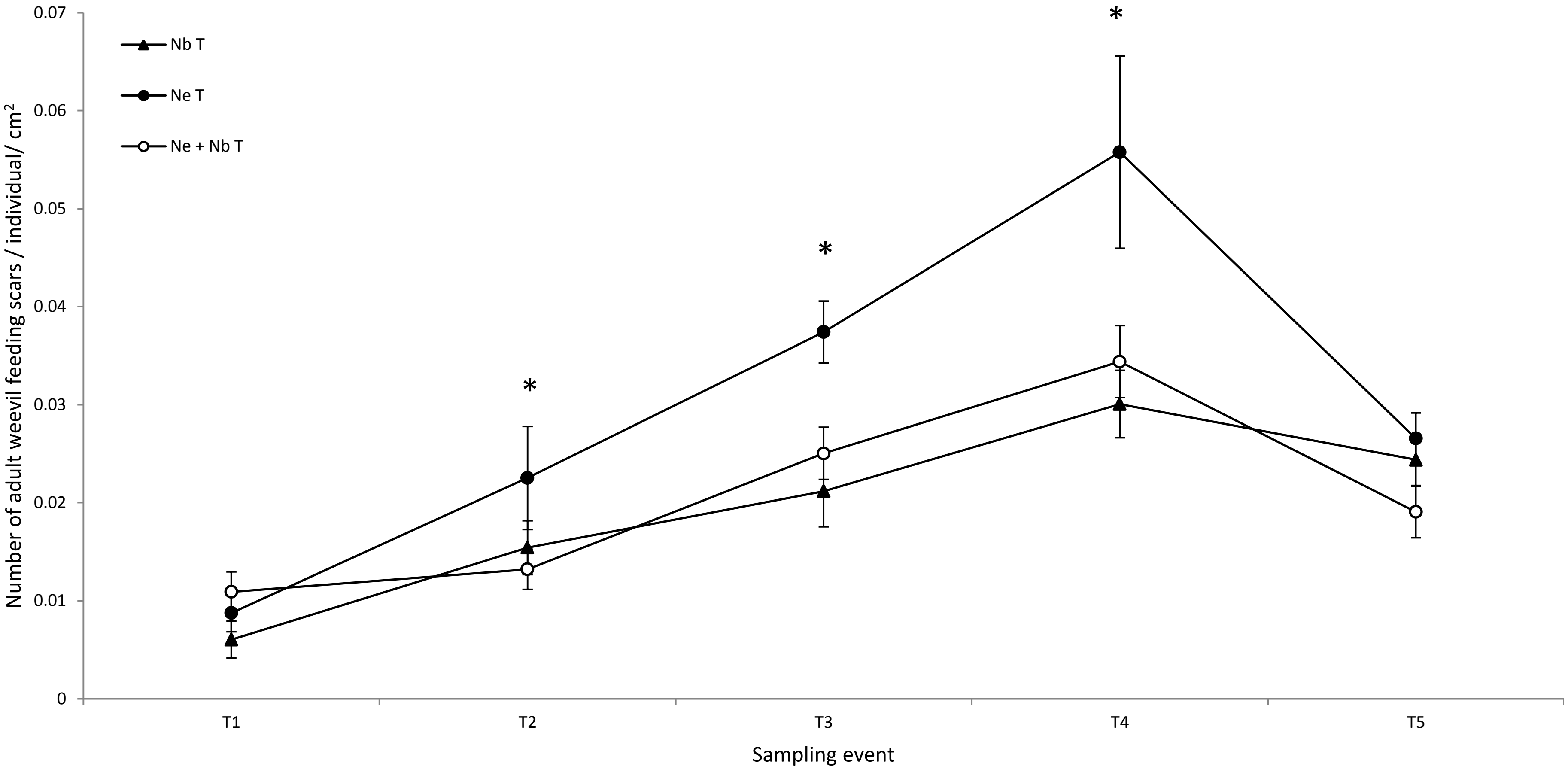

Figure 3.12 The number of adult weevil **feeding scars per individual weevil per cm^2** on **leaf 2** at each sampling event for the treatments that included weevils. The treatments included are: Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean and the * represents significant differences of the mean.

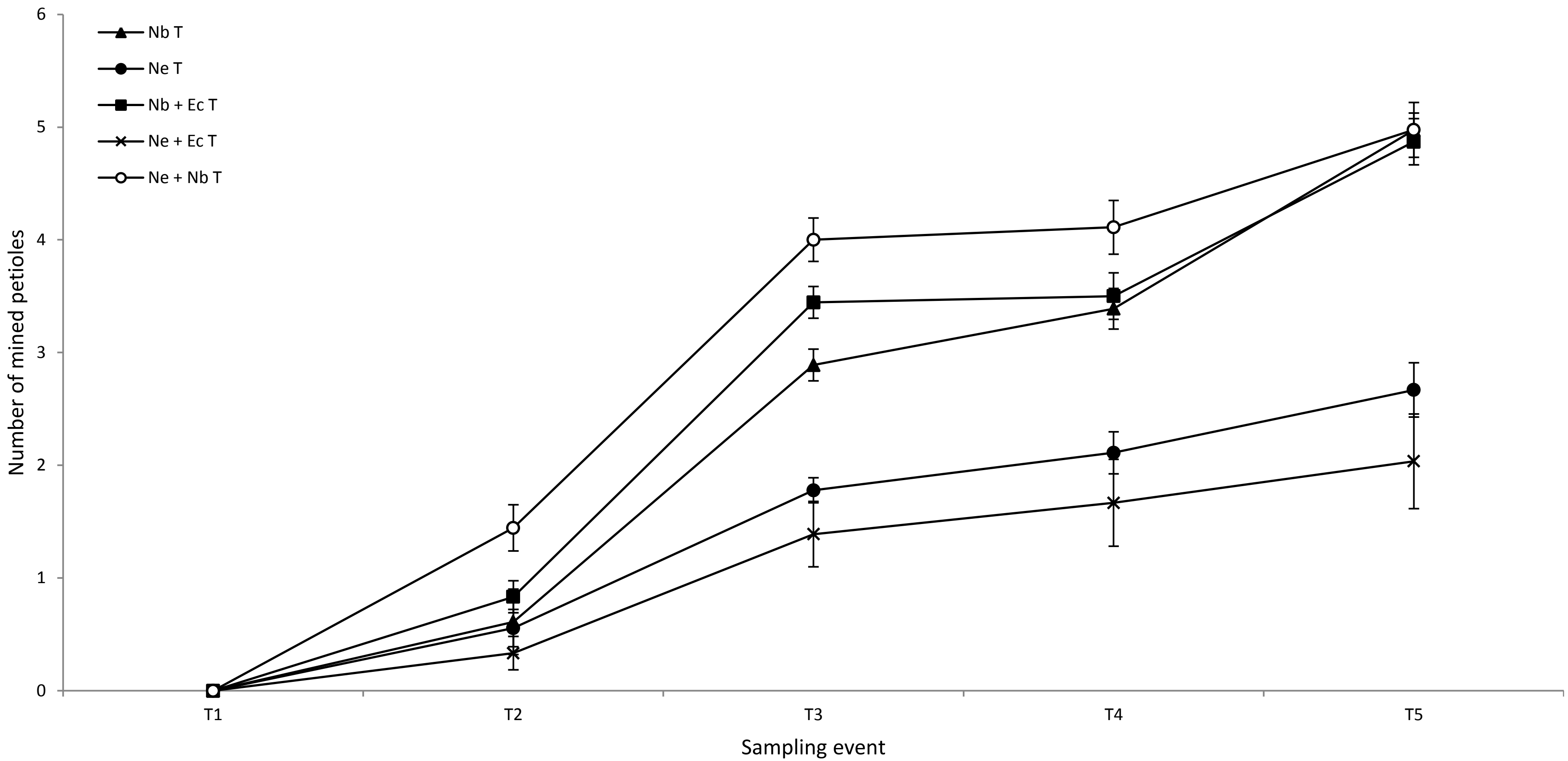

Figure 3.13 The number of water hyacinth **petioles mined** by weevil larvae at each sampling event for the treatments that included weevils. The treatments included are: Nb T = *Neochetina bruchi* alone treatment, Ne T = *N. eichhorniae* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination, Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination and Ne + Nb T = *N. eichhorniae* and *N. bruchi* combination. The error bars indicate the standard error around each mean.

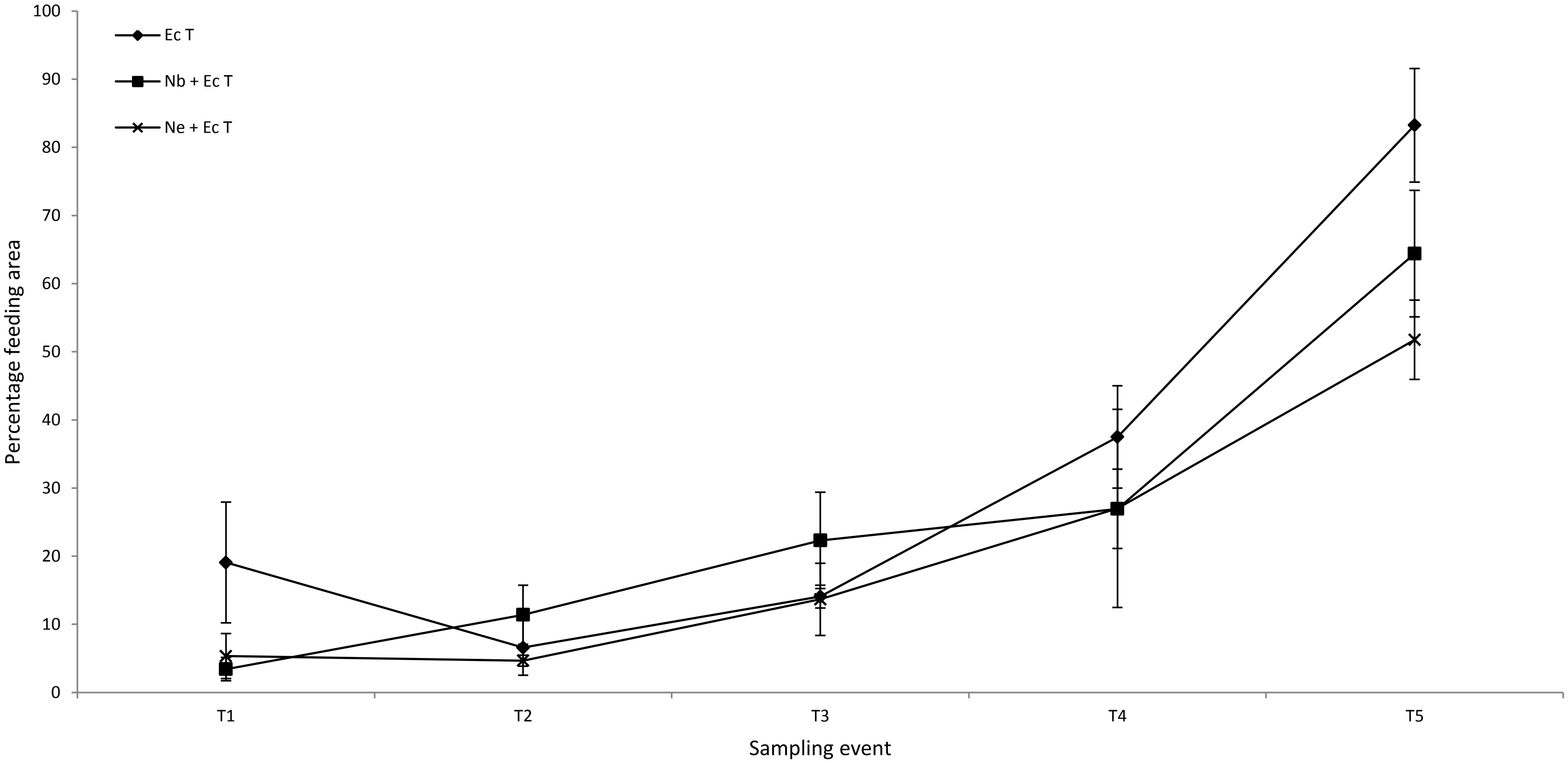

Figure 3.14 The **percentage feeding area** of the mirid corrected for exposure time on **leaf 2** at each sampling event for the treatments that included the mirid. The treatments included are: Ec T = *Eccritotarsus catarinensis* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination and Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination. The error bars indicate the standard error around each mean.

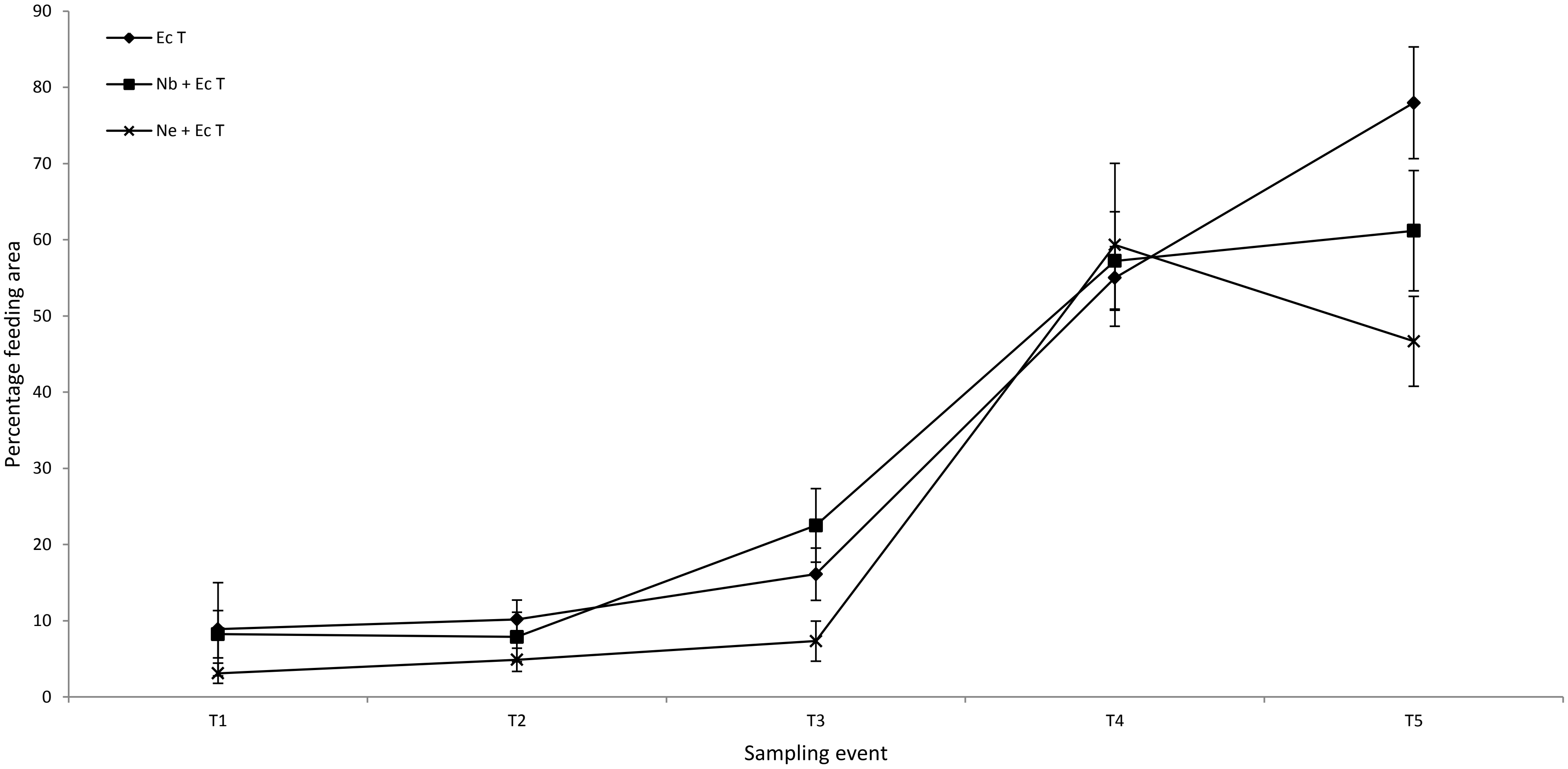

Figure 3.15 The **percentage feeding area** of the mirid corrected for exposure time on **leaf 3** at each sampling event for the treatments that included the mirid. The treatments included are: Ec T = *Eccritotarsus catarinensis* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination and Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination. The error bars indicate the standard error around each mean.

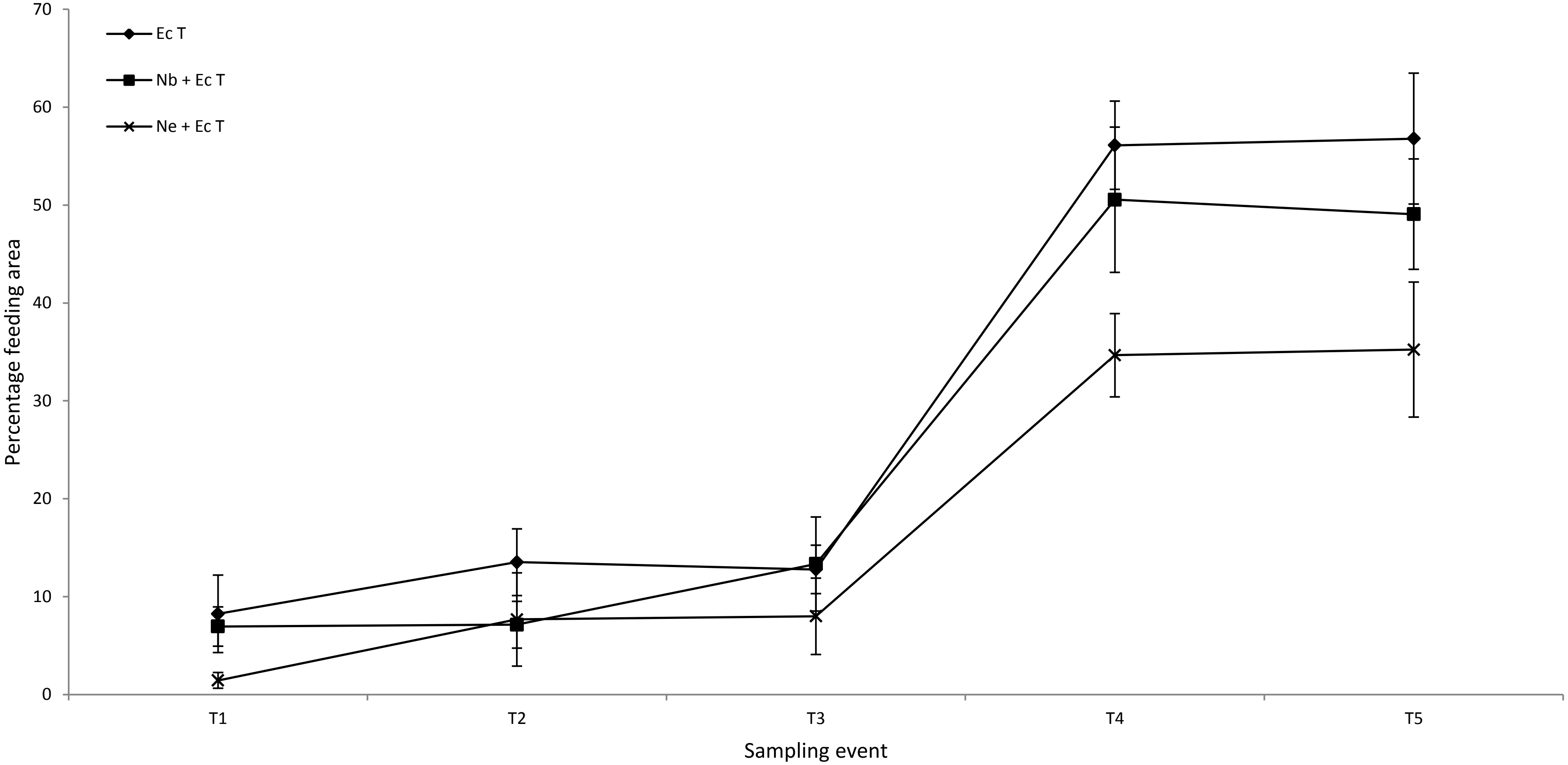

Figure 3.16 The **percentage feeding area** of the mirid corrected for exposure time on **leaf 4** at each sampling event for the treatments that included the mirid. The treatments included are: Ec T = *Eccritotarsus catarinensis* alone treatment, Nb + Ec T = *N. bruchi* and *E. catarinensis* combination and Ne + Ec T = *N. eichhorniae* and *E. catarinensis* combination. The error bars indicate the standard error around each mean.

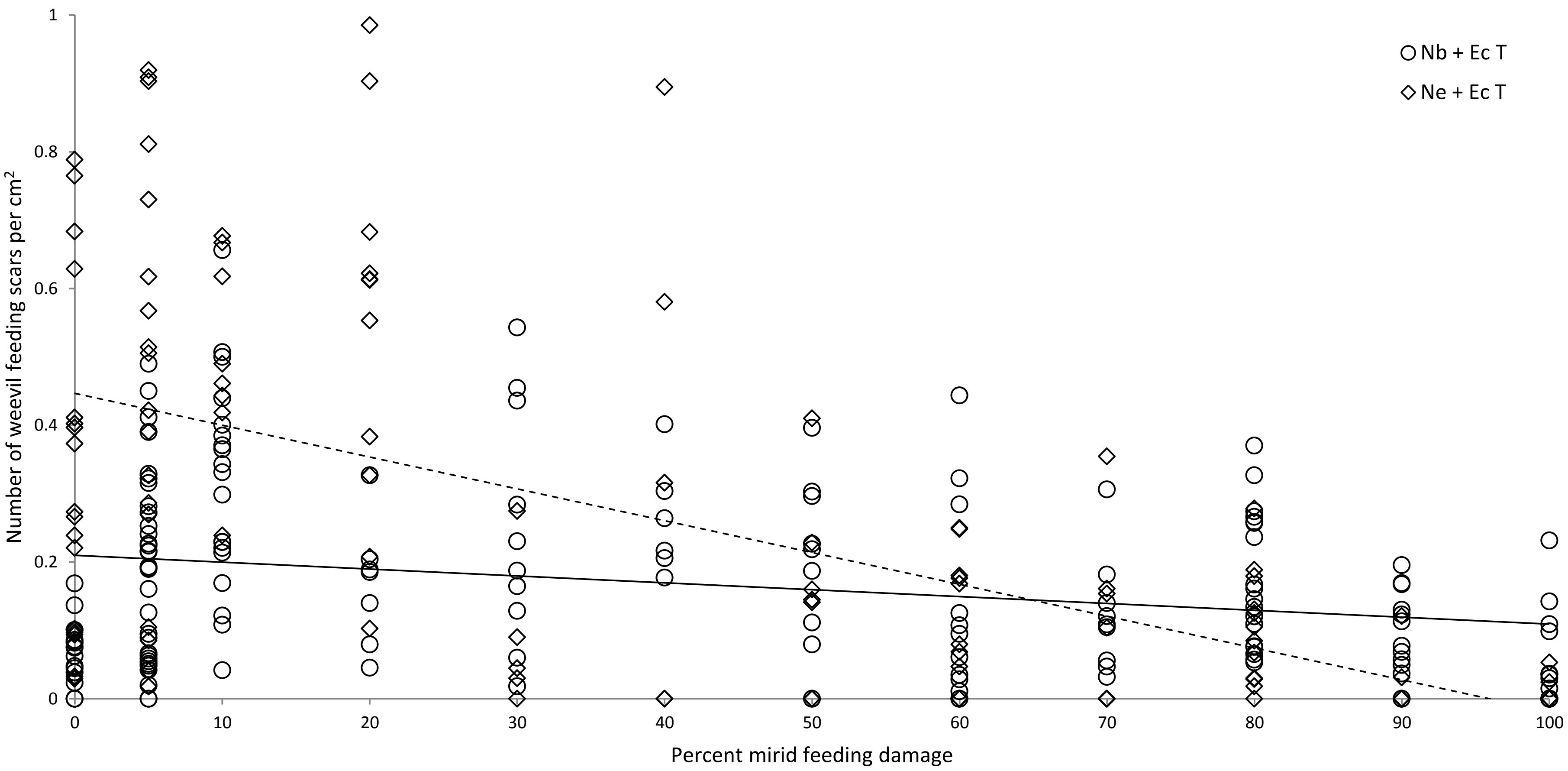

Figure 3.17 The correlation between the percent mirid feeding area and the number of weevil feeding scars. The solid line represents the trend between *Eccritotarsus catarinensis* and *Neochetina bruchi* (Spearman rank correlation $r_s = -0.161$, $P<0.05$) while the dashed line represents the trend between *E. catarinensis* and *N. eichhorniae* (Spearman rank correlation $r_s = -0.569$, $P<0.05$).

3.4 Discussion

The plant parameters for water hyacinth did not differ significantly between treatments at any of the sampling events during the course of the experiment. This is not entirely surprising because nutrients are an important driver of water hyacinth plant parameters, in some cases more than insect herbivory (Heard & Winterton 2000; Coetzee *et al.* 2007; Center & Dray 2010; Coetzee & Hill 2011). When the mirid *E. catarinensis* was inoculated on plants grown under high concentrations of nitrogen and phosphorous (50.5 mg N L^{-1} and 2.36 mg P L^{-1}), there was little suppression of water hyacinth vigour (Coetzee *et al.* 2007). The damage inflicted on the plants by the two *Neochetina* weevils did have an effect on plant parameters under high nutrients (1.6 mg NO_3-N L^{-1} and 1.0 mg PO_4-P L^{-1}), but not as pronounced as on the plants grown under medium nutrients (0.4 mg NO_3-N L^{-1} and 0.025 mg PO_4-P L^{-1}) (Heard & Winterton 2000). The nutrient conditions used for the current experiment were chosen to best mimic the conditions from Coetzee *et al.* (2007), where the total nitrogen levels were extremely high at 50.5 mg N L^{-1}, and represent the level at which maximum nitrogen storage occurs in water hyacinth (Reddy *et al.* 1989). These high nutrient levels are indicative of natural South African conditions, where nutrient pollution is one of the worst in the world (Van Ginkel *et al.* 2000). The treatments that involved insect damage never differed from each other or the control, so it is likely that the nutrient levels were allowing the plants to overcome the herbivory pressure.

When the start and end plant parameters are compared, there was a decrease in plant performance in all cases except the number of leaves per plant and number of ramets per plant. Over time the plants were getting smaller. Even in the control treatment where there was no herbivory, the mat height reduced from between 400 and 500mm to between 150 and 250mm, the leaf surface area also reduced and the leaf turnover slowed. There was a consistent decline in the daily average temperature from between 22 and 25°C in the tunnel at the start to 16 and 17°C by the end of the experiment. In addition to this, all the experimental tubs were covered by a fine mesh sleeve which in some cases can reduce the photosynthetically active radiation (PAR) by up to 36% (Weyl, unpub.). The mesh sleeve reduces the chance of contamination of the treatments by other insects and keeps the densities of insects in the treatments constant (no immigration or emigration of individuals) in order to compare within and between treatments. This reduction in PAR due to the sleeves could have had an impact on the vigour of the plants, however this would have been common to all treatments. The fertiliser Multicoat 6 month formula used in this experiment is designed for a terrestrial environment. In an aquatic environment there seems to be an initial rapid increase or burst in the release of nitrogen and phosphorous, which is not maintained for the time period advised by the manufacturer: in this case 6 months (Pretorius, Hill & Weyl, unpub.). It is possible that the level of nutrients was not kept constant throughout the study period and the plants were getting smaller due to a reduction in the amount of available resources. The plant parameters did change between sampling events, but there were no differences between treatments that may have had an implication for the performance of the insects.

Both the water hyacinth weevil species have been shown to perform better in terms of overall growth rates under high levels of nutrients (Heard & Winterton 2000; Wilson *et al.* 2006), so the high nutrients levels were unlikely to negatively affect the weevils' performance. During the study by Coetzee *et al.* (2007) high nutrient concentrations actually yielded low numbers of mirids. When grown under high levels of N and P the concentrations of phenolics have been shown to increase in water hyacinth (Center & Wright 1991), which could have influenced the performance of the mirid (Coetzee *et al.* 2007). The high nutrients in this study did not affect the mirid in the same way and populations in all treatments increased over time.

A decline, which was often significant, was seen in most of the insect parameters measured. This is not likely to be due to a decline in insect performance, but rather to a leaf turnover event occurring just prior to the sampling event. There was also an increase in the number of leaves between the last and second-last sampling event, which supports this suggestion. This would have resulted in a reduction in the exposure time of the leaf to herbivory, as was reflected in the insect performance, especially the weevil parameters.

There was a negative interaction between the mirid *E. catarinensis* and the weevil *N. eichhorniae*, with the weevil performing less well when in combination with the mirid. There were significantly fewer feeding scars of *N. eichhorniae* in the presence of *E. catarinensis* as compared to the *N. eichhorniae* control, and there was a negative correlation between the percentage feeding area of the mirid and the number of feeding scars. Wright & Center (1984) developed a formula to indirectly determine the number of *N. eichhorniae* weevils on a population of water hyacinth though the feeding scars. The formula is

$$I = 0.0366S^{0.775},$$

where I is the number of weevils and S the number of feeding scars. The average number of feeding scars on leaf 2 from the *N. eichhorniae* control treatment (28 scars) gives a result of 1.02 weevils per plant. The inoculation density of weevil was one weevil per plant and the formula is in accordance with this. However, if we take the average number of feeding scars on leaf 2 from the combination of *N. eichhorniae* and *E. catarinensis* treatment (12.44 scars) the result reduces to 0.54 weevils per plant, even though the inoculation rate was one weevil per plant. This possibly indicates that the adults of *N. eichhorniae* are only feeding approximately half as much as they potentially could be. There are several examples worldwide of one biological control agent reducing the potential efficacy of another (Story *et al.* 1991; Woodburn 1996; Briese 1997; Crowe & Bourchier 2006; Groenteman *et al.* 2007; Seastedt *et al.* 2007). However, in these cases it is normally the complete removal or reduction of a resource. In the case of *Hypericum perforatum* the leaf-feeding beetle *Chrysolina quadrigemina* causes a boom – bust scenario in the plant populations which has inhibited the establishment of the root borer *Agrilus hyperici* (Creutzer) (Briese 1997). The impact of the receptacle weevil, *Rhinocyllus conicus*, on populations of nodding thistles, *Cardus nutans*, has not been satisfactory in both New Zealand and Australia, resulting in the release of a second agent for its control (Woodburn 1996; Groenteman *et al.* 2007). There is evidence that the weevil in this case is reducing the potential efficacy of the woody gall forming fly *Uphora solstitialis* Linnaeus (Woodburn 1996; Groenteman *et al.* 2007). However in this case it is not so much a reduction in food quality as a complete domination of the resource and possible predation on the larvae of the gall fly by *R. conicus* (Groenteman *et al*. 2007). There are few examples in the biological control literature where the potential reductions in food quality or induced plant defences have resulted in the reduction in a biological control agent's potential efficacy.

The release of a benign Willamette mite *Eotetranychus willametti* (McGregor), on grape vines has resulted in an induced defence to be initiated in the plant, which "protects" it from the much more aggressive Pacific mite *Tetranychus pacificus* McGregor (Karban *et al.* 1991; Karban *et al.* 1997). In addition to this it is well documented in unrelated studies that the feeding of one insect especially a sap feeder, can reduce the nutritional value of a plant or plant part and thus have implications for the performance of another insect (Kaplan & Denno 2007).The mirid *E. catarinensis* is a sap sucking bug and both the adults and nymphs feed on the leaf tissue (Hill *et al.* 1999). The consequence of this sap sucking is the yellowing, chlorosis and premature death of the leaf (Hill *et al.* 1999). This could result in a reduction in the nutritional quality of the plant or the potential initiation of an induced defence by the water hyacinth, which would have resulted in a reduction in the adult performance of *N. eichhorniae.* From personal observations it seems that *N. eichhorniae* avoids feeding in areas where *E. catarinensis* has previously fed. This explains the negative correlation between percent feeding damage and number of feeding scars. There were no significant differences in the numbers of petioles mined by the weevil larvae but in combination with *E. catarinensis* there was always slightly fewer petioles mined. The negative effect of *E. catarinensis* on the performance of adult *N. eichhorniae* does not seem to extend significantly to fitness of the females. The current experiment was only run over 10 weeks, so this slight reduction in the fitness of the females may be amplified over several generations.

The weevil *N. bruchi* was compatible with the mirid *E. catarinensis*, which was unexpected. The number of feeding scars was never significantly different from the control treatment and in some cases the number of feeding scars was slightly higher when in combination with the mirid. Oviposition was also unaffected by the presence of the mirid. *Neochetina bruchi* performs best on high quality plants grown under high nutrients (Heard & Winterton 2000). If the mirid was reducing plant quality it may be expected that *N. bruchi* would perform worse between the two weevils tested. However the reduction in plant quality due to mirid herbivory may not be the same as the reduction in plant quality due to low nutrient levels. This study investigated the interactions between the insects and did not consider or determine the mechanisms or drivers of the interactions. In conclusion adult performance and fitness of *N. bruchi* was completely unaffected by the presence of *E. catarinensis.*

The performance of *E. catarinensis* in combination with either of the weevil species was largely unaffected and there were no differences in the performance between pairwise combinations or the control. The only significant differences were on leaf 4 at the second last and last sampling event, however, it is unlikely for this to be of any biological significance. This is in agreement with the findings of Ajuonu *et al.* (2007), who studied the possible effect of adult weevil feeding on the survival and performance of the mirid. When the mirids were presented with plants that had a large number of old feeding scars, their performance was significantly reduced, but when presented with a large number of old scars mixed with fresh feeding scars there was no difference in their performance (Ajuonu *et al.* 2007). In the present study weevil feeding scars remained low at about 22.5±12.9 for *N. bruchi* and 19.2±15.7 for *N. eichhorniae* for leaves two, three and four in the pairwise combination treatments. There were always live weevils during the course of the experiment which would mean there were always fresh scars available.

Despite the significantly higher number of feeding scars per cm^2 for the treatment with the two weevils in combination, there is still a potential antagonistic relationship. The life histories of both the *Neochetina* weevils are extremely similar with considerable niche overlap for both adults and larvae (Deloach & Cordo 1976a; Center 1994). Adults of both species are nocturnal and feed primarily on the mesophyll tissue of the upper and lower surface of the leaf, creating nicks and scars ranging from a surface area of 0.5 to $25mm^2$ (Deloach & Cordo 1976a). These scars are unfortunately indistinguishable between the species. Eggs are laid in the petiole and leaf tissue, the larvae develop and mine the petiole down to the crown (Deloach & Cordo 1976a), where the third instar larvae feed on the axillary buds (Center 1994). When the interaction between these two species was considered it was not surprising to find an antagonistic relationship between them, but it is unfortunately impossible to determine which species was performing better. The available leaf tissue is not likely to have played a major role in determining the number of feeding scars because even with the higher inoculation rate the number of feeding scars remained relatively low, with averages for leaf 2 = 30.22 scars, for leaf 3 = 45.39 scars and for leaf 4 = 46.22 scars. In terms of adult performance, the combination of weevils did not perform as well as the *N. eichhorniae* control, on the other hand when the number of petiole mines is considered *N. bruchi* always had significantly more petioles mined per female. In the native range these two weevils do coexist, but this is not without a continually shifting balance in favour of one or other species (Deloach & Cordo 1976b). There is definite temporal and spatial segregation of the two species under natural conditions, and the coexistence of *N. bruchi* and *N. eichhorniae* is thought to be linked to the seasonal shifts in relative abundances (Deloach & Cordo 1976b). These shifts are dependent on seasonal changes in the growth form of water hyacinth with the associated ovipositional behaviour, rates of increase and temperature tolerances of the weevils (Deloach & Cordo 1976b). In addition to this the weevils seem to dominate in particular areas on a geographical scale and a particular area may be dominated by either *N. bruchi* or *N. eichhorniae* (Deloach & Cordo 1976b). This may also be explained by the differences in performance of the two weevil species under different nutrient regimes, where *N. bruchi* has a higher level of performance under high nutrient conditions (Heard & Winterton 2000) and may under such conditions outcompete *N. eichhorniae.* They also prefer different positions on the plant (*N. bruchi* prefer the outer leaves while *N. eichhorniae* prefers the central wrapper leaf), which may also explain the apparent coexistence in the native range (Deloach & Cordo 1976b). The negative relationship observed in this study may be explained by the forced coexistence of these two species in a relatively small area (in terms of geographic scale the experimental cages were small). In future it may be worth running experiments over several generations, however the weevils are so damaging that the plants rarely survive more than one generation of insect. The interactions and coexistence of the two weevils in the native range may also be related to the coevolution of the weevils with their specialised natural enemies and other population regulating factors.

Some interactions identified in this study are contradictory to the literature. Other studies have found no interference or interactions between insects. The study by Center & Dray (2010) showed little evidence of interference or competition between the two *Neochetina* weevil species, and they were compatible in the experimental setup. The interactions presented in this study indicated a competitive interaction between the weevils. Marlin (2010) investigated the interactions between the mirid *E. catarinensis* and the weevil *N. eichhorniae* and found no negative interactions between them, while the interactions observed in this study showed that the weevil was negatively affected by the presence of the mirid. The differences observed between the studies may be attributed to experimental design in terms of insect and plant stocking densities, as these would play an important role in insect behaviour and ultimately interactions between the species. The study by Marlin (2010) used individual plants in small tubs. Center & Dray (2010) used 15 plants in large mesocosm tanks and the current study used 10 plants placed in each cage. In the studies done by these workers the plants would have been sparse, while in the current study the plants were quite tightly packed by the end of the experiment. The stocking densities of the weevils were also higher. Marlin used 4 weevils per plant and Center & Dray used 2 weevils per plant, while the current study used only 1 weevil per plant.

3.5 Conclusion

The insect-insect interactions observed between the pairwise combinations of the two weevil species *N. bruchi* and *N. eichhorniae* and the mirid *E. catarinensis* on water hyacinth revealed both negative and neutral relationships. There was a neutral or potentially synergistic relationship between the mirid and the weevil *N. bruchi*, with neither species performing significantly better or worse when in combination than when alone. The negative relationship between the weevil *N. eichhorniae* and the mirid *E. catarinensis* was interesting with the weevil performing significantly less well when in combination with the mirid. *Eccritotarsus catarinensis* was relatively unaffected by the presence of the weevil. The only significant difference in feeding damage was at leaf 4, and for this to be of any biological significance is unlikely. Considering the extreme overlap of niches between the two weevils it was not surprising to find a reduction in adult performance and fitness of the weevils when in combination.

The next chapter deals with the relationship between *E. catarinensis* and *N. eichhorniae* and determines whether it can be measured in the field.

Chapter 4: The insect – insect interactions and performance of two field established natural enemy species against water hyacinth in the Eastern Cape, South Africa

4.1 Introduction

Biological control of weeds has a long history: the first agent released over 200 years ago in India (Johnston & Tyrone 1914). In relatively recent years, biological control has gained popularity and more and more agents are being released against a growing number of weeds. For a worldwide perspective see Julien & Griffiths (1998) and for a more recent South African perspective see Klein (2011). According to McEvoy & Coombs (1999) biological control in North America is practiced primarily in one dimension, where natural enemy function is enhanced by the introduction of new natural enemies. This concept could be extrapolated to a global scale where the number of agents released far exceeds the number of target weeds (Julien & Griffiths 1998). With the exception of Syrett *et al.* (1996), most additional biological control agents are released into already existing programmes without consideration of the possible interactions between agents and possible effects on the efficacy of the programme (McEvoy & Coombs 2000). In most cases an understanding of the interactions and population dynamics of both the weed and the insects allows informative decisions to be made for the biological control programme while reducing any potential "revenge effects".

The biological control programme against *Sesbania punicea* is an example. In this case the release of three biological control agents, a flower bud-feeding apionid, *Trichapion lativentre* (Béguin-Billecocq), the seed-feeding curculionid, *Rhyssomatus marginatus* Fåhareus, and the stem-boring curculionid, *Neodiplogrammus quadrivittatus* (Olivier), was essential to exert a cumulative stress on the plant to reduce its density (Hoffmann 1998). A modelling study revealed that, for the weevils to be most effective, *T. lativentre* and *R. marginatus* had to be released first, followed approximately five years later by *N. quadrivittatus* (Hoffmann 1990). The post-release evaluation in the field showed that all three weevil species were necessary for sufficient reduction in plant density to be considered under long term sustainable control (Hoffmann & Moran 1998). By investigating and understanding the interactions and population dynamics of both the tree and the insects, a successful biological control programme was achieved.

Interactions between the three agents *Neochetina eichhorniae*, *N. bruchi* and *Eccritotarsus catarinensis* released against water hyacinth have been investigated in a common garden experimental study (Chapter 3). The results suggest that there is a negative relationship between the weevil *N. eichhorniae* and the mirid *E. catarinensis*, with the weevil not performing as well when in the presence of the mirid.

A post-release evaluation of the two insects was initiated to assess the influence that this negative relationship may have on the population dynamics and performance of these two insects in a natural field environment. The field site chosen for the post-release study was Wriggleswade Dam in the Eastern Cape, South Africa. The first release of 3600 *N. eichhorniae* and 2000 *E. catarinensis* was done in 1999, with an additional large release of 3200 *N. eichhorniae* in 2003 (Hill, pers. comm.). All the releases were on infestations of water hyacinth on the Kubusie River upstream of the current post-release evaluation study sites (Hill, pers. comm.). Since the first release in 1999 both insects have been established at several sites on the Kubusie River as well as the infestations on Wriggleswade Dam (Hill, pers. comm.).

The aims of this study were to quantify the performance of the insects and determine if there is an interaction between the two species in a natural environment that may limit insect establishment, fitness and performance. In addition to this the population dynamics of the two species of insect were assessed to determine whether there was any spatial segregation of the two insects on a geographic scale.

4.2 Materials and Methods

4.2.1 Study area

The study area (Figure 4.1) was situated in the upper reaches of Wriggleswade Dam on the Kubusie River system (32°33'S 27°29'E). The surrounding landscape is characterised by low mountain ranges developed on sedimentary rocks from the Beaufort Group and overlaid by deep, freely drained, highly weathered soils (Mucina & Rutherford 2006). The area is approximately 720 meters above sea level and the vegetation is predominantly Amatole montane grassland (Mucina & Rutherford 2006). The climate typically has bimodal rainfall with spring and late summer peaks with an average rainfall of 570-700mm per annum. Isolated areas receiving 1000mm are relatively common (Mucina & Rutherford 2006). Rainfall in the year prior to the study was limited and variable. During the study period (April 2010 to September 2010), very little rain was recorded at the Dohne weather station. This was followed by very good rains, January and February 2011 receiving over 100mm (Figure 4.2.) (El Tiempo 2011). The temperatures for the region are typically mild with a temperate type climate (Figure 4.3) (El Tiempo 2011). In the Stutterheim area, which is close to the study site, winter frost is frequent with up to 80 days per annum in the higher elevation areas (Mucina & Rutherford 2006).

4.2.2 Study sites

The upper reaches of Wriggleswade Dam had five permanent water hyacinth mats at the beginning of the study period (Figure 4.1). Study sites were located at each of these mats (sites 1 – 4) and three points on the large plug (top = site 7, within = site 6 and at the bottom = site 5) which is approx. 2km long. Each site was sampled on a monthly basis from April 2010 to April 2011 (13 months). However, due to the low rainfall in mid-2010, water began to be diverted from Wriggleswade Dam to Nahoon Dam to serve East London. This resulted in a steady decline in the water levels from 85% to less than 60% from May to November (Figure 4.4) (Department of Water Affairs 2011). Some of the sites therefore dried up and were lost during the course of the study (Table 4.1). After the increased precipitation in October and November there was a slight increase in the water levels, resulting in site 7 being washed out and lost (Table 4.1). Between December and January there was a huge influx of water, which increased the levels from 60% to 98%, and by February 2011 the dam level was 100%. This was maintained until the termination of the study after the April 2011 sampling period. During the flood the plug was washed out and into the dam, however, with the exception of site 7, the sites were not clear of water hyacinth for very long when more water hyacinth was washed down from the Kubusie River, all this had an important impact on the result (Table 4.1).

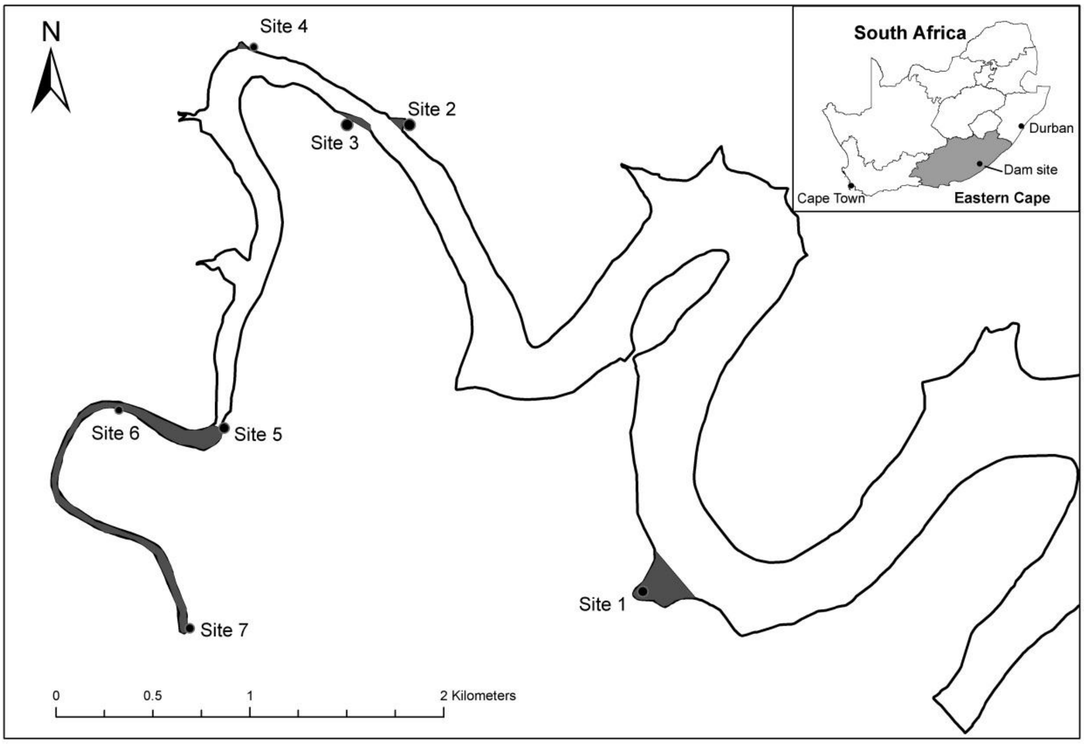

Figure 4.1 The upper reaches of Wriggleswade Dam on the Kubusie River ($32^{o}33'$S $27^{o}29'$E). The grey areas indicate the permanent water hyacinth mats on the dam at the initiation of the study in April 2010.

Table 4.1 Study sites that were sampled monthly. The √ indicates a positive sample, while an x, indicates that the site had dried up and was subsequently lost. The o symbol indicates that the water hyacinth at the site had been washed away due to floods.

Sampling date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
April-10	√	√	√	√	√	√	√
May-10	√	√	√	√	√	√	√
June-10	x	√	√	√	√	√	√
July-10	x	√	√	x	√	√	√
August-10	x	x	√	x	√	√	√
September-10	x	x	√	x	√	√	√
October-10	x	x	x	x	√	√	√
November-10	x	x	x	x	√	√	√
December-10	x	x	x	x	√	√	√
January-11	x	x	x	x	√	√	o
February-11	√	x	√	√	√	o	o
March-11	√	x	√	√	√	√	o
April-11	√	x	√	√	√	√	o

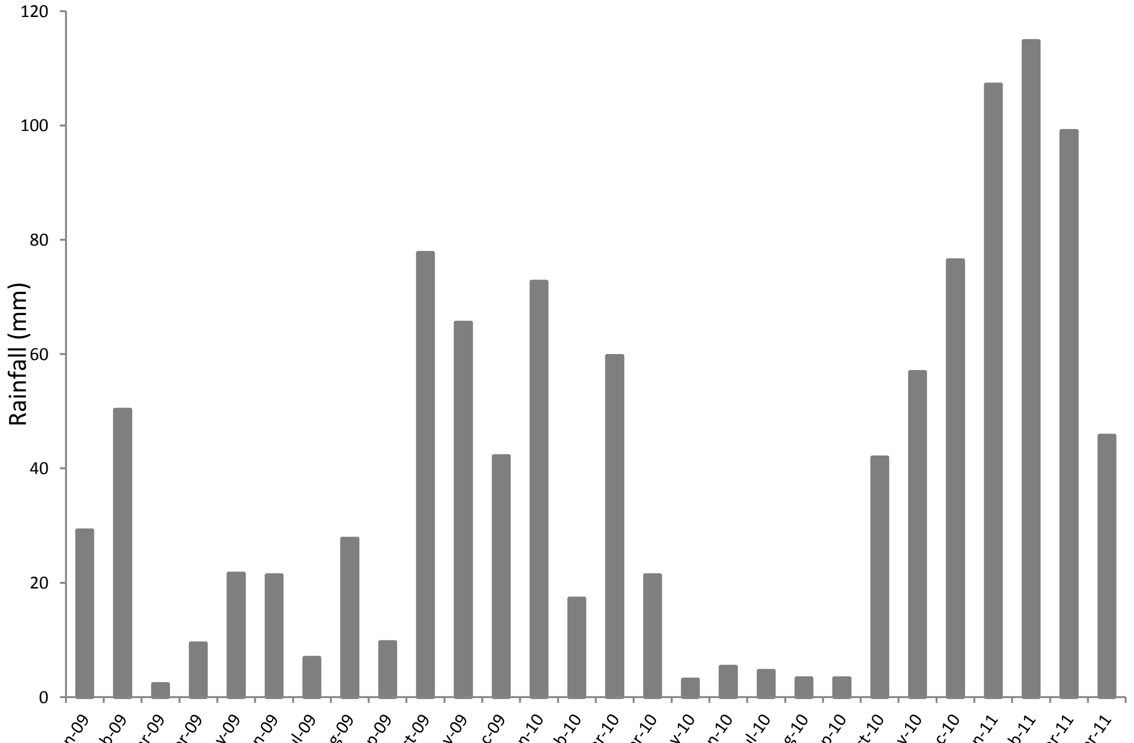

Figure 4.2 Total monthly rainfall for the Dohne weather station from January 2009 to April 2011.

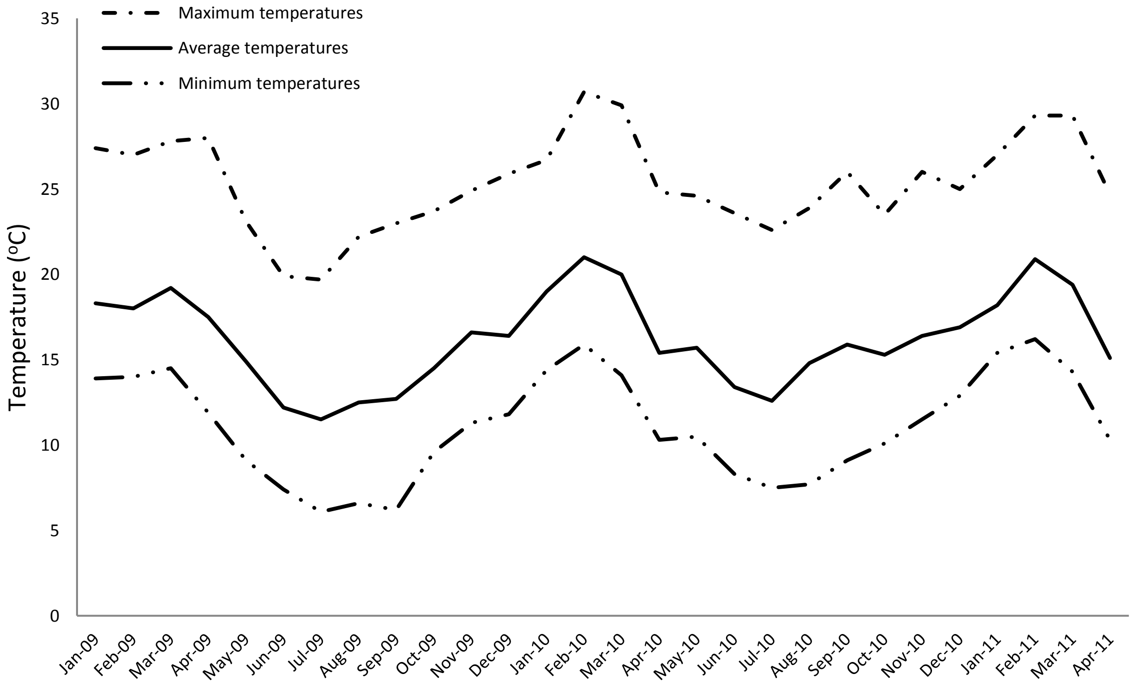

Figure 4.3 Average monthly, maximum and minimum temperatures for the Dohne weather station from January 2009 to April 2011.

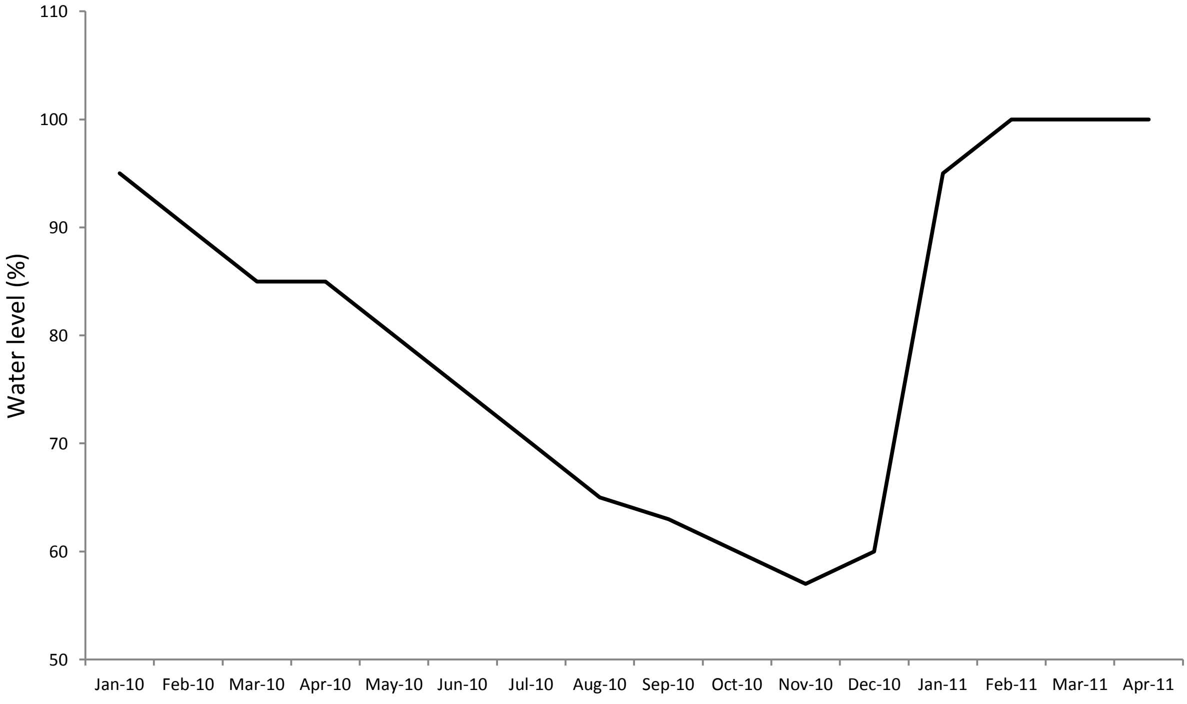

Figure 4.4 Wriggleswade Dam water levels from January 2010 until the termination of the study in April 2011.

4.2.3 Plant parameters

The water hyacinth plant condition at each site was determined by measuring various plant parameters. All the water hyacinth plants from three 0.25m^2 quadrats were collected at each site. The plants were then counted and above water material, below water material and dead material were separated and weighed. At each site an additional 10 plants were randomly collected and various parameters measured. Length of longest petiole gives an indication of the height of the water hyacinth mat and ultimately the maturity of the water hyacinth population at the site. Length of the second petiole gives an indication of how old the leaf is (the closer the length to the longest petiole the older the leaf) and therefore, how long it could have been potentially exposed to herbivory. The number of ramets on each adult plant gives an indication of the reproductive output of the population. The total number of leaves and the total number of petioles were counted. This was important during the winter months where after frost the leaves of the plant were burnt, however the petioles were still potentially available for weevil oviposition. The maximum root length was measured to give an indication of the nutrient status of the surrounding environment.

4.2.4 Insect parameters

The performance and population dynamics of the mirid *Eccritotarsus catarinensis* and the weevil *Neochetina eichhorniae,* two biological control agents that have been established in the area for at least a decade (Hill, pers. comm.) were investigated at each site. Using the developmental data from King (2011) and the time elapsed since the first release it can be estimated that *N. eichhorniae* and *E. catarinensis* would have gone through about 22 and 59 generations respectively. The high number of generations for both insects suggests that they are well established at this field site and it is likely that populations on a landscape level are consistent. The same 10 plants that were randomly collected were used for the insect parameters. On each plant the total numbers of feeding scars were counted on the second leaf.

On the same leaf the percentage feeding area as well as the feeding intensity of the mirid was estimated. Due to this estimation being subjected to observer bias, the same observer was utilised at each sampling event. The petioles were dissected and the numbers of petioles mined by the weevil were recorded. During this process the plant was also searched and any adult weevils were counted. The mirid *E. catarinensis* is extremely mobile and it was difficult to count the exact number of adults per plant and so an overall estimation of the site was made at each sampling event.

4.2.5 Physico-Chemical parameters

Various physical and chemical parameters of the surface waters (± 30cm depth) underneath the water hyacinth mats were measured at each site. This was done once in April 2010 at the initiation of the study to test for differences in abiotic conditions between sites. The HANNA model Hl 9828 multi-parameter probe was used to measure pH, conductivity, total dissolved solids (TDS) and salinity.

Thermochron iButtons (DS1921G; Maxim Dallas Semiconductor Corporation) which have an operational temperature range from -40°C to 85°C and an accuracy of ± 1°C (Hubbart *et al.* 2005) were placed at each site. However, due to the receding waters and floods the data logger was unable to collect any continuous temperature data. The temperature data from the weather station in Dohne were reported in this study (Figure 4.3) (El Tiempo 2011) because there is a strong correlation between the Kubusie River at 32°35’07”S 27°28’09”E and the closest weather station, the Dohne weather station (Coetzee, unpub.).

4.2.6 Statistical analysis

According to the Levene test for homogeneity of variances and Kolmogorov-Smirnov test for normality both the insect and plant parameters measured in this field study did not fulfil the requirements of parametric statistics. Non-parametric statistical analyses were therefore employed. The percentage feeding area of the mirid was arcsin transformed before any analyses were run. During the course of the study some of the sites were lost resulting in a disjointed data set, so a Kruskal-Wallis ANOVA was employed to test for differences between insect and plant parameters measured between sites at each sampling event. The trends over time were described. This test increases the chance of a type II error being committed (Fowler *et al.* 2005). A type II error is when the test is over conservative and differences may not be found significant when they are, however, with the amount of missing data this increased risk is justified (Fowler *et al.* 2005).

To test whether the performance of one insect had an effect on the performance of another, the Spearman rank order correlation was employed to test for any correlations between the insect parameters.

4.3 Results

4.3.1 Plant parameters

The plants sampled at each site were from the original mats for the months April 2010 through to January 2011. The initial small flood from November 2010 to December 2010 (Figure 4.4) washed away the plants from site 7 but had little or no impact on the other sites. Between December 2010 and January 2011 there were major floods and the original water hyacinth mats from all the sites were washed out. From most of the plant parameters it was clear that the plants from site 6 were washed down to site 3, and sites 1, 4 and 5 received new plants, possibly from an infestation higher up the river. However, by March 2011 all plant parameters had equalised which was possibly due to all the plants coming from the same infestation on the Kubusie River (Figure 4.5 to 4.14).

The sites were made up of relatively permanent mature water hyacinth mats. The plants at sites 1, 2, 3, 5 and 6 were characterised by being medium to tall with well attenuated petioles, while the plants at sites 4 and 7 were small with bulbous petioles (Figure 4.5 and 4.6, Table 4.2). The height of the tall plants (sites 1, 2, 3, 5 and 6) ranged from approx. 350 to

700mm, while the shorter bulbous plants ranged from 150 to 300mm (sites 4 and 7) (Figure 4.5 and 4.6, Table 4.2). The number of leaves was relatively consistent between sites with the majority of the plants having between 6 and 8 leaves, but up to 10 leaves was not uncommon. There was a sharp decline in the number of leaves between June 2010 and July 2010, which is due to frosting events over winter and the leaves being burnt, however the number of petioles per plant were variable but remained fairly consistent (Figure 4.7 and 4.8, Table 4.2). The ramet production was fairly low at sites 1, 2, 3, 5 and 6 and characteristic of large tall plants in relatively dense mats, while the ramet production for sites 4 and 7 was high and characteristic of sites with small plants in sparse mats (Figure 4.9, Table 4.2). There was a dramatic increase in the production of ramets between October 2010 and November 2010 (Figure 4.9, Table 4.2) when the plants were probably responding to the warmer summer conditions (Figure 4.3). All the sites, with the exception of site 7, had long roots, with over 1000mm being common (Figure 4.10, Table 4.2).

The number of plants per $0.25m^2$ was relatively low (between 8 and 15 individuals) at sites with tall plants (sites 1, 2, 3, 5 and 6) and relatively high (between 18 and 25 individuals) at the sites with small plants (sites 4 and 7) (Figure 4.11, Table 4.3). The biomass, whether above water, below water or dead material was extremely variable between sites at a sampling event and within sites over time, however there is a general trend towards lighter plants through the duration of the study (Figure 4.12 – 4.14, Table 4.3).

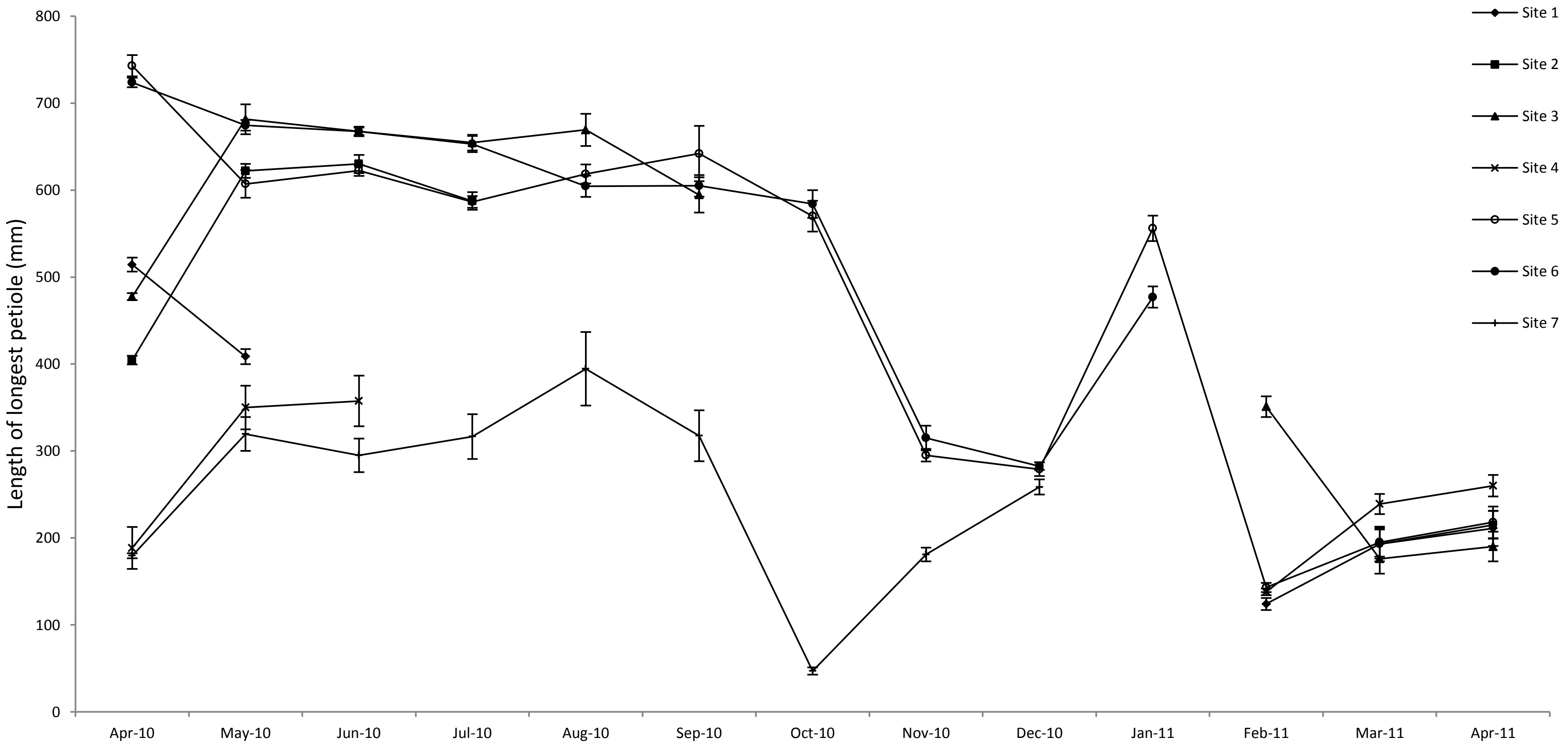

Figure 4.5 The average length (mm) of the **longest petiole** of water hyacinth at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

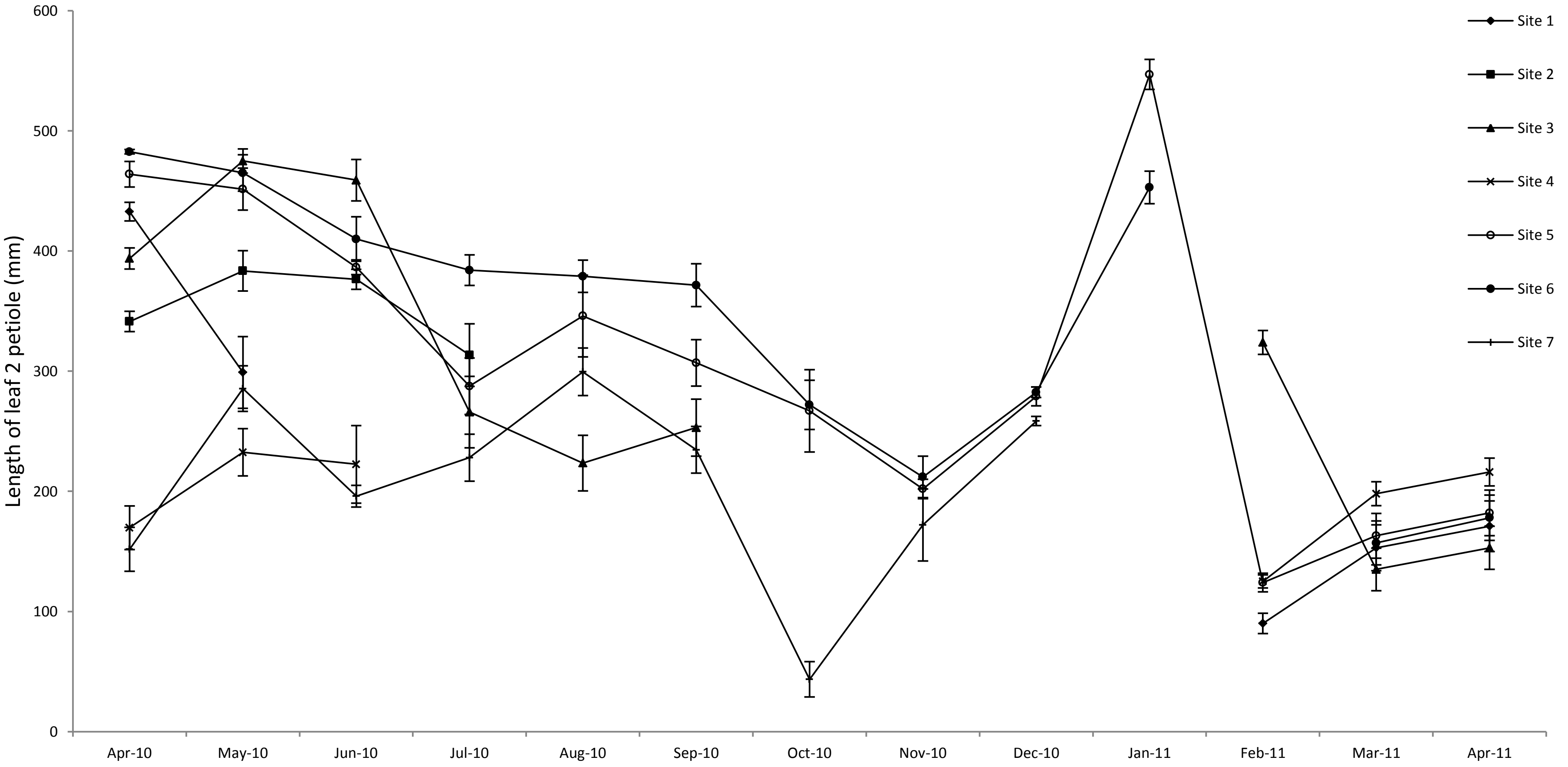

Figure 4.6 The average length (mm) of the **leaf 2 petiole** of water hyacinth at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

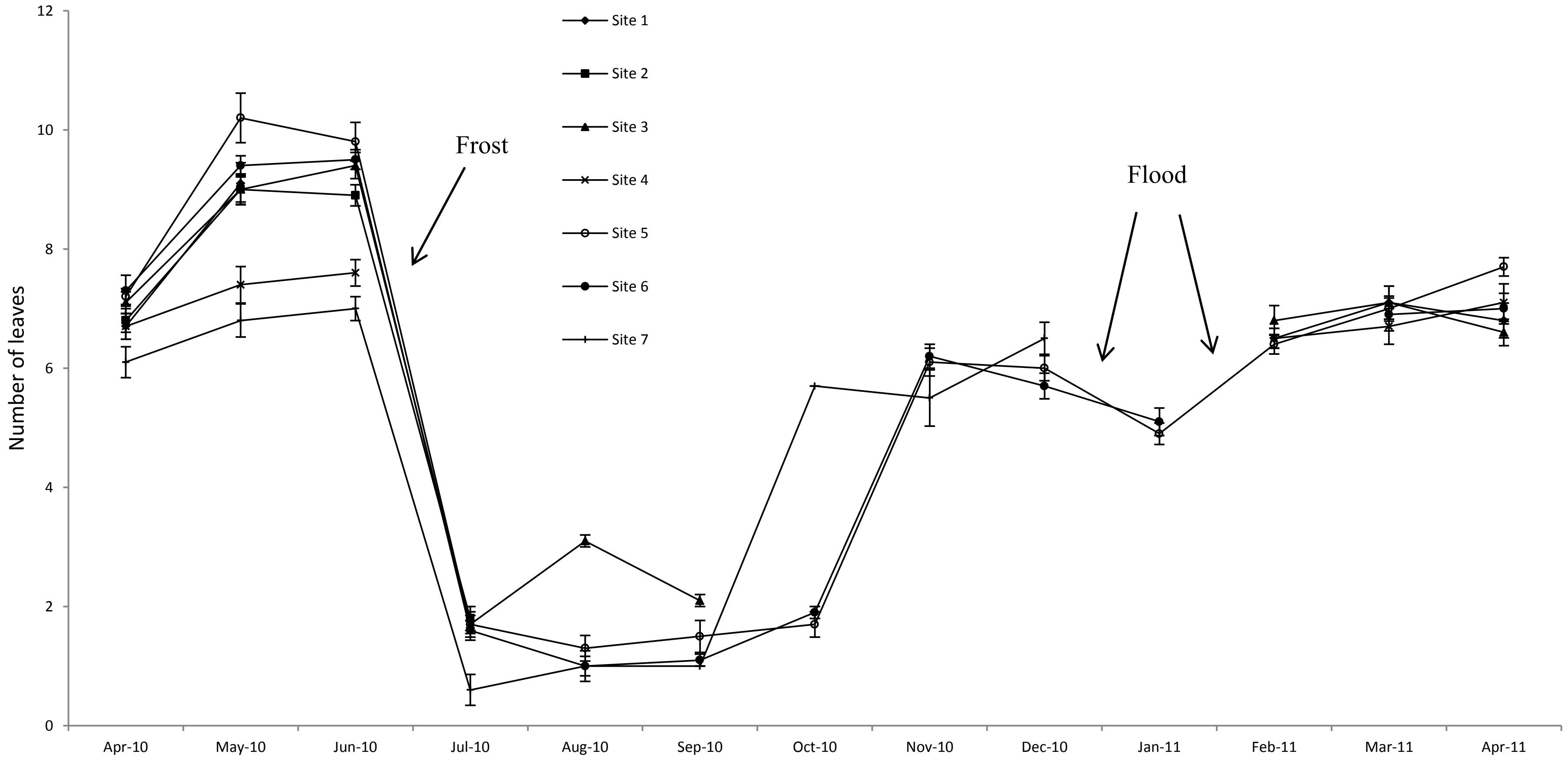

Figure 4.7 The average **number of leaves** for each water hyacinth plant at the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

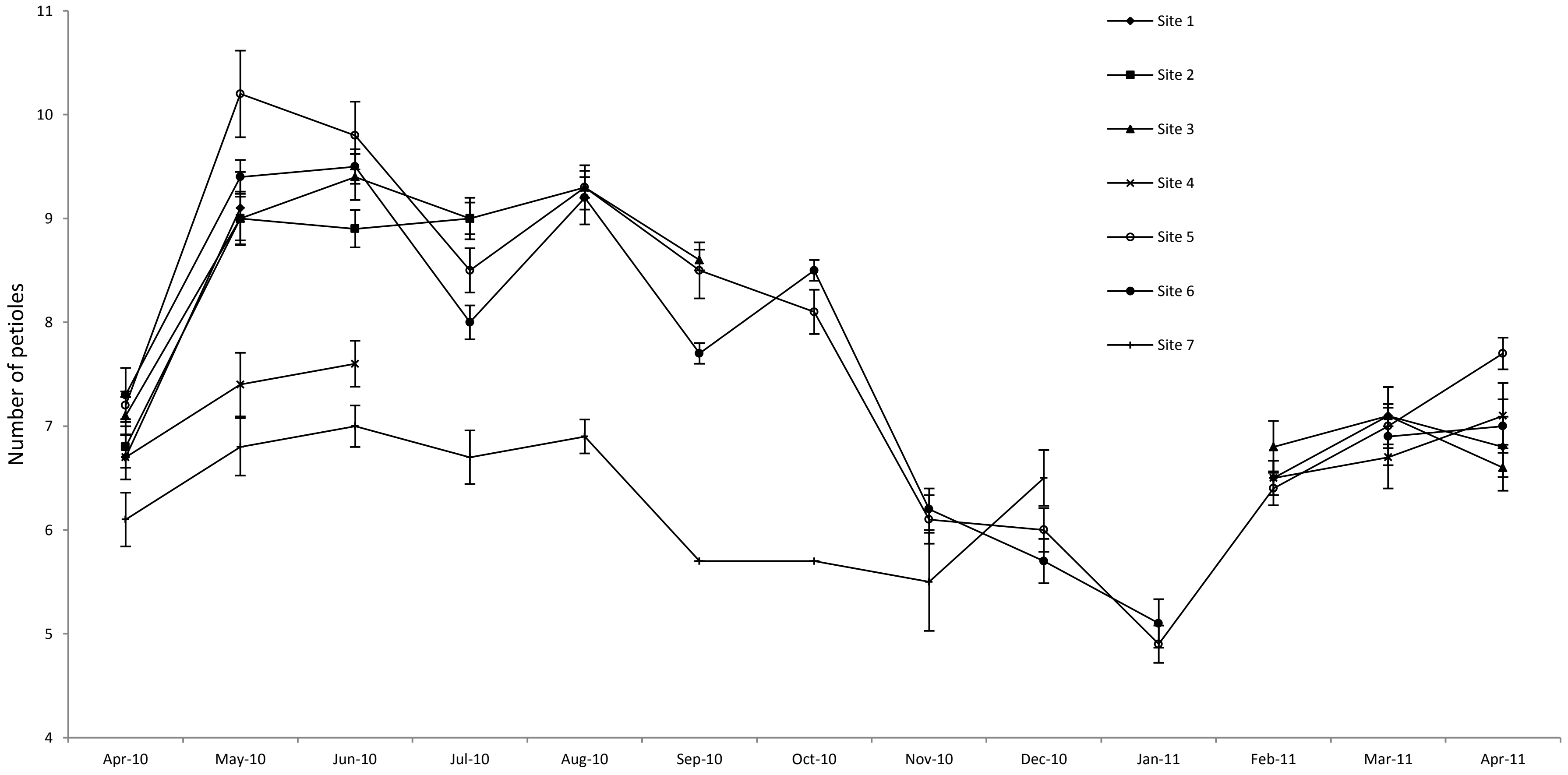

Figure 4.8 The average **number of petioles** for the water hyacinth plants at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

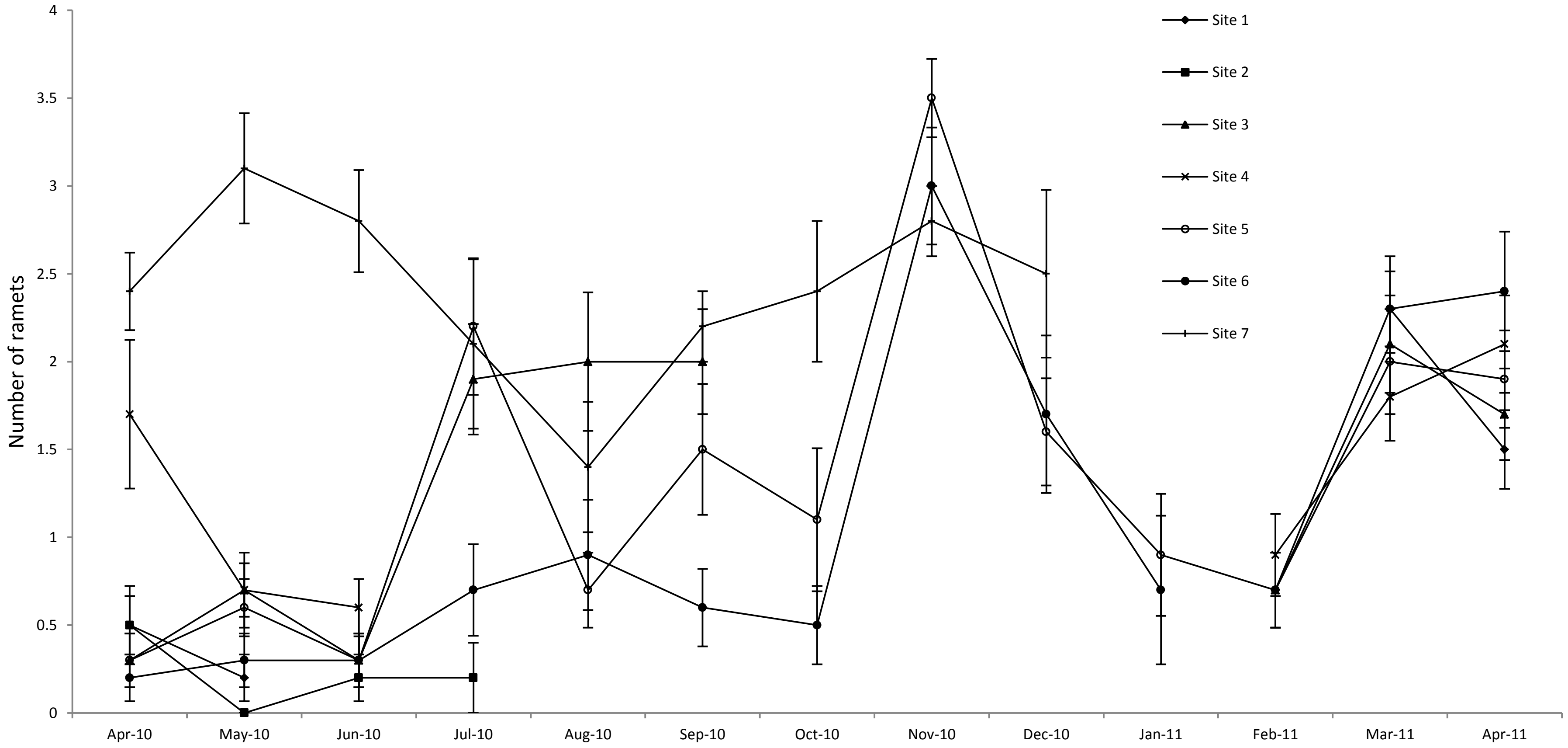

Figure 4.9 The average **number of ramets** for the water hyacinth plants at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

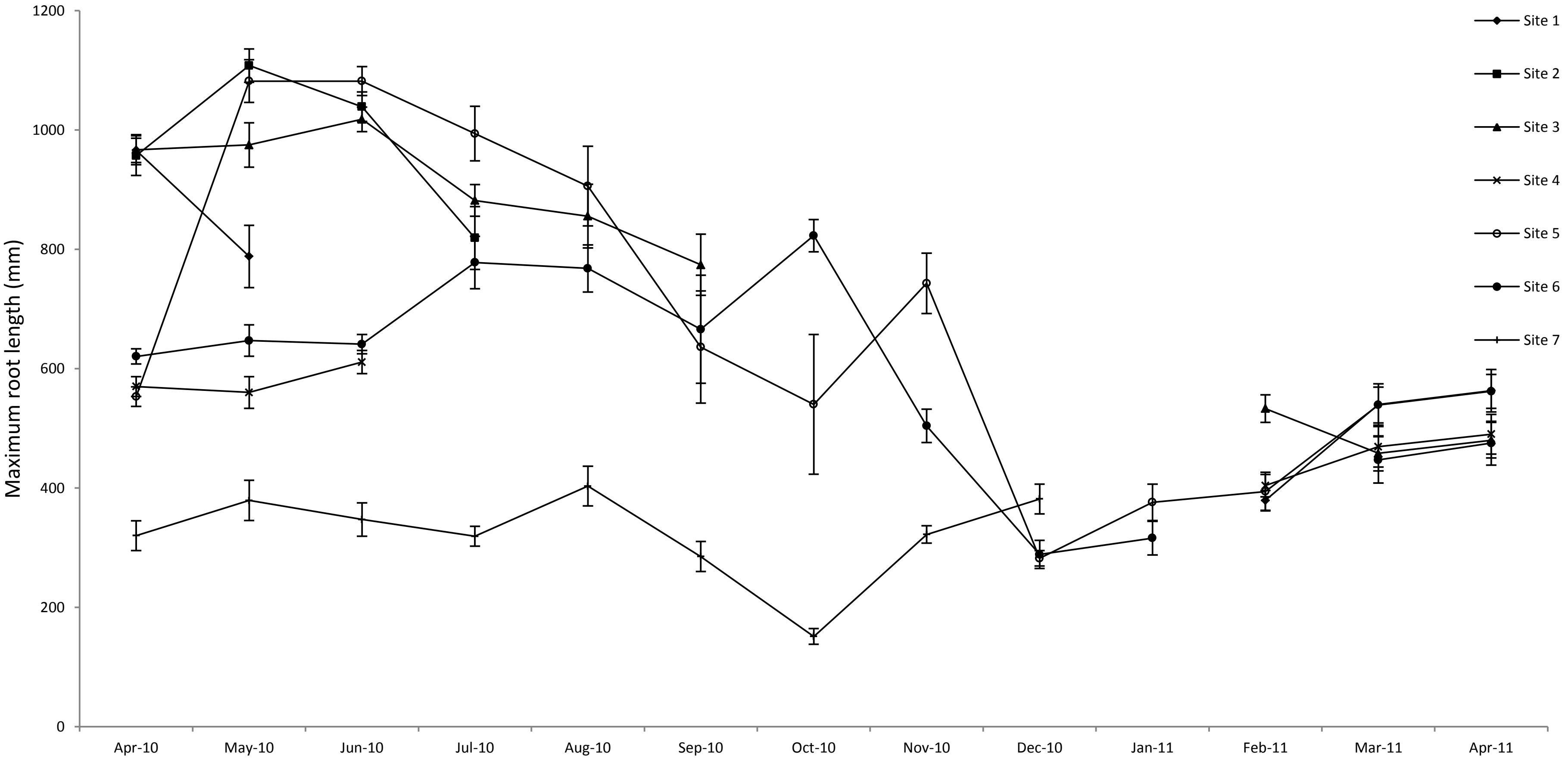

Figure 4.10 The average **maximum root length** (mm) of the water hyacinth plants at the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

Table 4.2 The H-test statistic and *P*-values for the monthly Kruskal-Wallis test performed on the plant parameters for each available site for each sampling event. The numbers in brackets indicate the degrees of freedom and the total sample size. The values in **bold** indicate significant differences.

April-10			May-10			June-10			July-10		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (6,70)	*P*	Parameter	H (6,70)	*P*	Parameter	H (5,60)	*P*	Parameter	H (4,50)	*P*
Longest petiole	65.04	**0.00**	Longest petiole	59.69	**0.00**	Longest petiole	49.27	**0.00**	Longest petiole	39.48	**0.00**
Leaf 2 petiole	62.53	**0.00**	Leaf 2 petiole	50.62	**0.00**	Leaf 2 petiole	40.17	**0.00**	Leaf 2 petiole	17.46	**0.00**
Leaf 2 surface area	32.82	**0.00**	Leaf 2 surface area	41.91	**0.00**	Leaf 2 surface area	24.66	**0.00**	Leaf 2 surface area	15.55	**0.00**
Max root length	59.69	**0.00**	Max root length	58.31	**0.00**	Max root length	50.30	**0.00**	Max root length	30.23	**0.00**
Number of ramets	32.23	**0.00**	Number of ramets	38.70	**0.00**	Number of ramets	30.90	**0.00**	Number of ramets	20.86	**0.00**
Number of leaves	15.61	0.16	Number of leaves	41.05	**0.00**	Number of leaves	39.86	**0.00**	Number of leaves	18.17	**0.00**
									Number of petioles	30.27	**0.00**
August-10			September-10			October-10			November-10		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (3,40)	*P*	Parameter	H (3,40)	*P*	Parameter	H (2,30)	*P*	Parameter	H (2,30)	*P*
Longest petiole	25.86	**0.00**	Longest petiole	24.01	**0.00**	Longest petiole	19.74	**0.00**	Longest petiole	19.84	**0.00**
Leaf 2 petiole	14.40	**0.00**	Leaf 2 petiole	16.00	**0.00**	Leaf 2 petiole	19.40	**0.00**	Leaf 2 petiole	6.47	**0.04**
Leaf 2 surface area	7.64	**0.05**	Leaf 2 surface area	16.80	**0.00**	Leaf 2 surface area	20.06	**0.00**	Leaf 2 surface area	1.79	0.41
Max root length	22.98	**0.00**	Max root length	16.17	**0.00**	Max root length	15.25	**0.00**	Max root length	22.74	**0.00**
Number of ramets	6.84	0.08	Number of ramets	13.59	**0.00**	Number of ramets	9.80	**0.01**	Number of ramets	4.23	0.12
Number of leaves	25.89	**0.00**	Number of leaves	23.30	**0.00**	Number of leaves	22.01	**0.00**	Number of leaves	4.30	0.12
Number of petioles	19.28	**0.00**	Number of petioles	22.18	**0.00**	Number of petioles	14.28	**0.00**			
December-10			January-11			February-11			March-11		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (2,30)	*P*	Parameter	H (1,20)	*P*	Parameter	H (3,40)	*P*	Parameter	H (4,50)	*P*
Longest petiole	5.02	0.08	Longest petiole	8.76	**0.00**	Longest petiole	4.57	0.21	Longest petiole	7.52	0.11
Leaf 2 petiole	4.99	0.08	Leaf 2 petiole	11.90	**0.00**	Leaf 2 petiole	10.58	**0.01**	Leaf 2 petiole	6.46	0.17
Leaf 2 surface area	0.17	0.92	Leaf 2 surface area	1.65	0.20	Leaf 2 surface area	22.28	**0.00**	Leaf 2 surface area	0.95	0.92
Max root length	9.25	**0.01**	Max root length	1.97	0.16	Max root length	14.66	**0.00**	Max root length	7.05	0.13
Number of ramets	2.07	0.36	Number of ramets	0.53	0.47	Number of ramets	0.60	0.90	Number of ramets	2.59	0.63
Number of leaves	5.73	0.06	Number of leaves	0.48	0.49	Number of leaves	1.66	0.65	Number of leaves	1.67	0.80
April-11											
	Kruskal-Wallis test										
Parameter	H (4,50)	*P*									
Longest petiole	8.64	0.07									
Leaf 2 petiole	6.15	0.19									
Leaf 2 surface area	0.56	0.97									
Max root length	7.02	0.13									
Number of ramets	5.42	0.25									
Number of leaves	9.46	0.05									

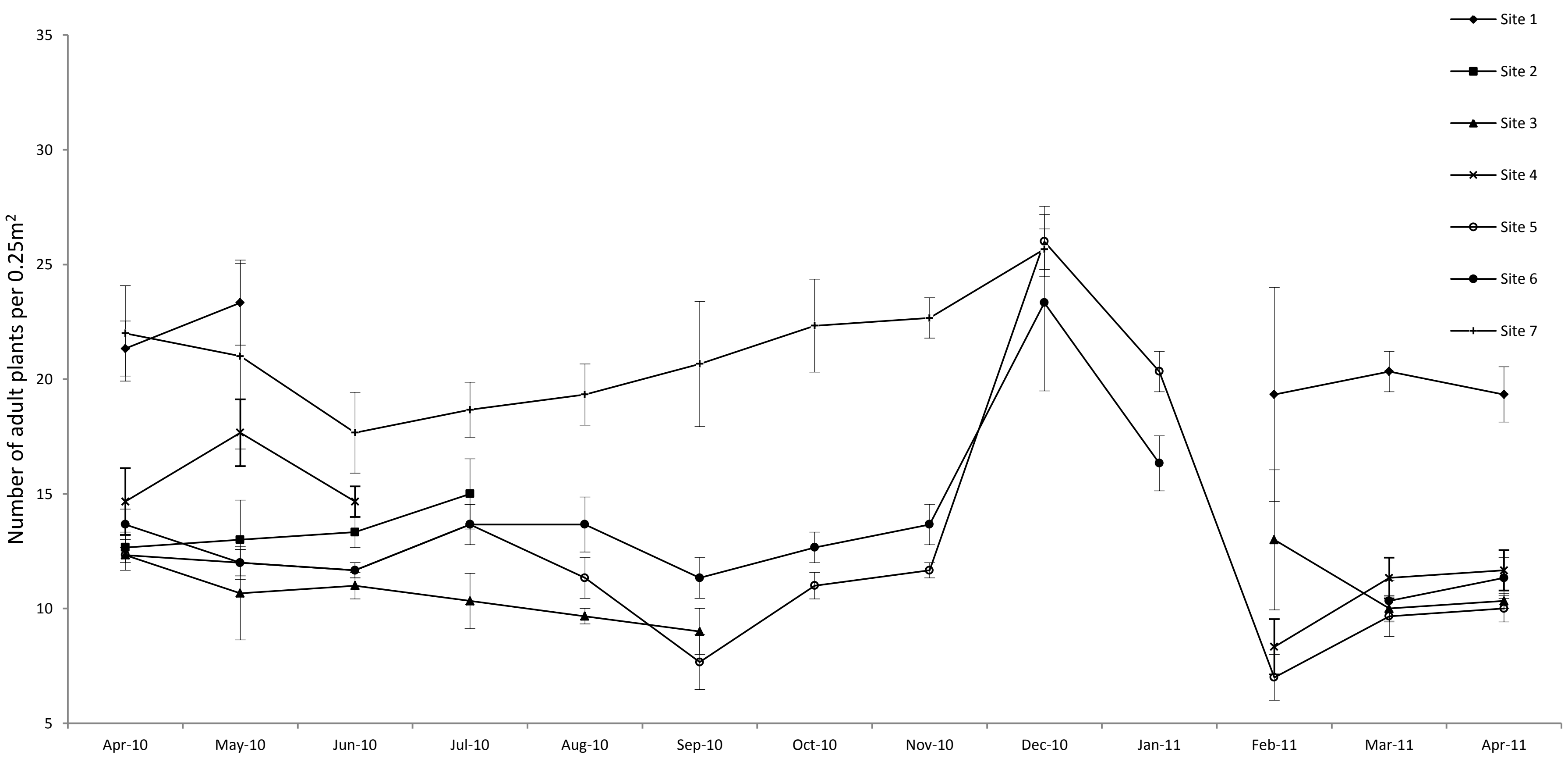

Figure 4.11 The average **number of water hyacinth plants** per 0.25 m^2 at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

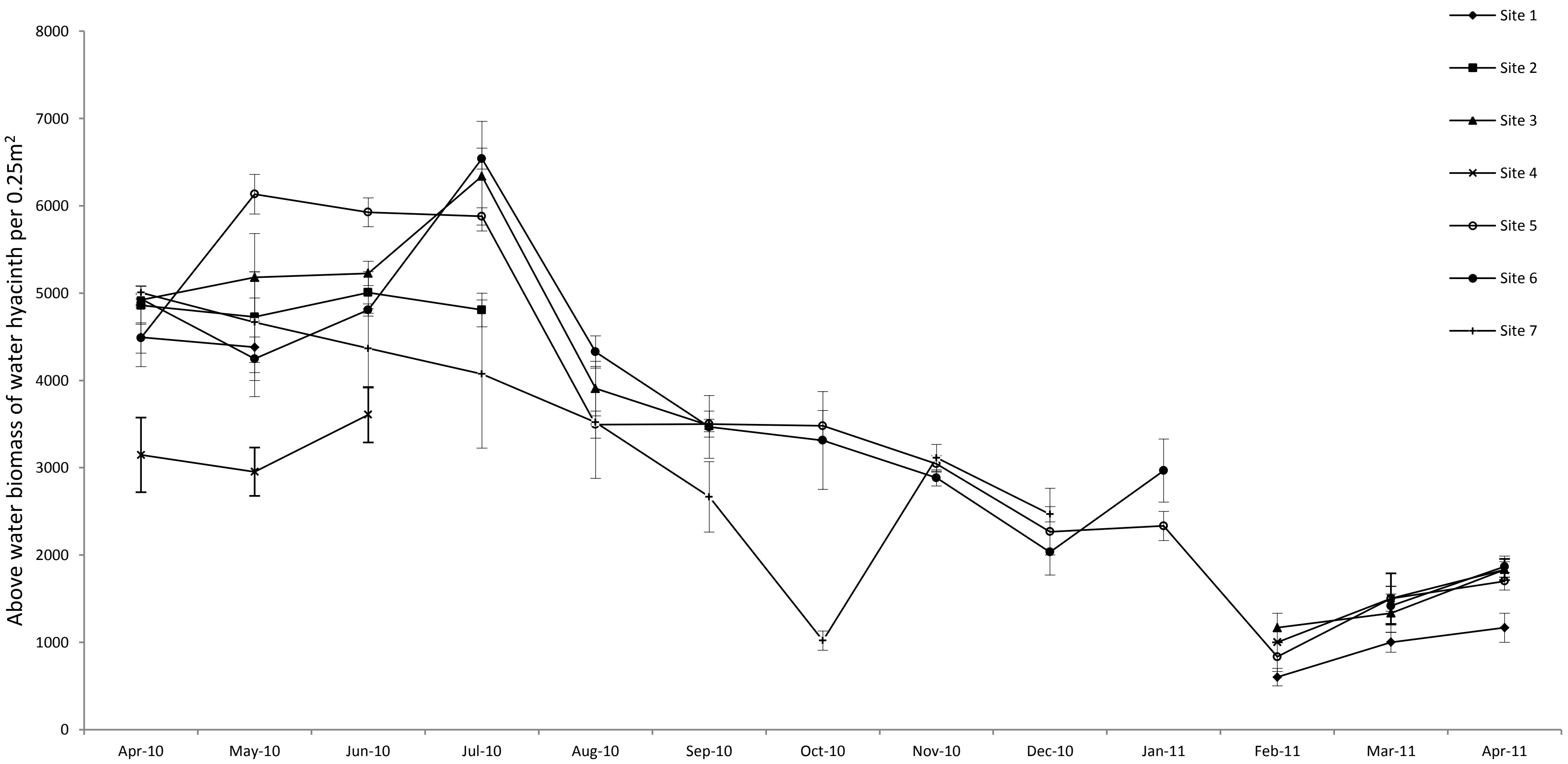

Figure 4.12 The average **above water biomass** of water hyacinth plants per 0.25 m^2 at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

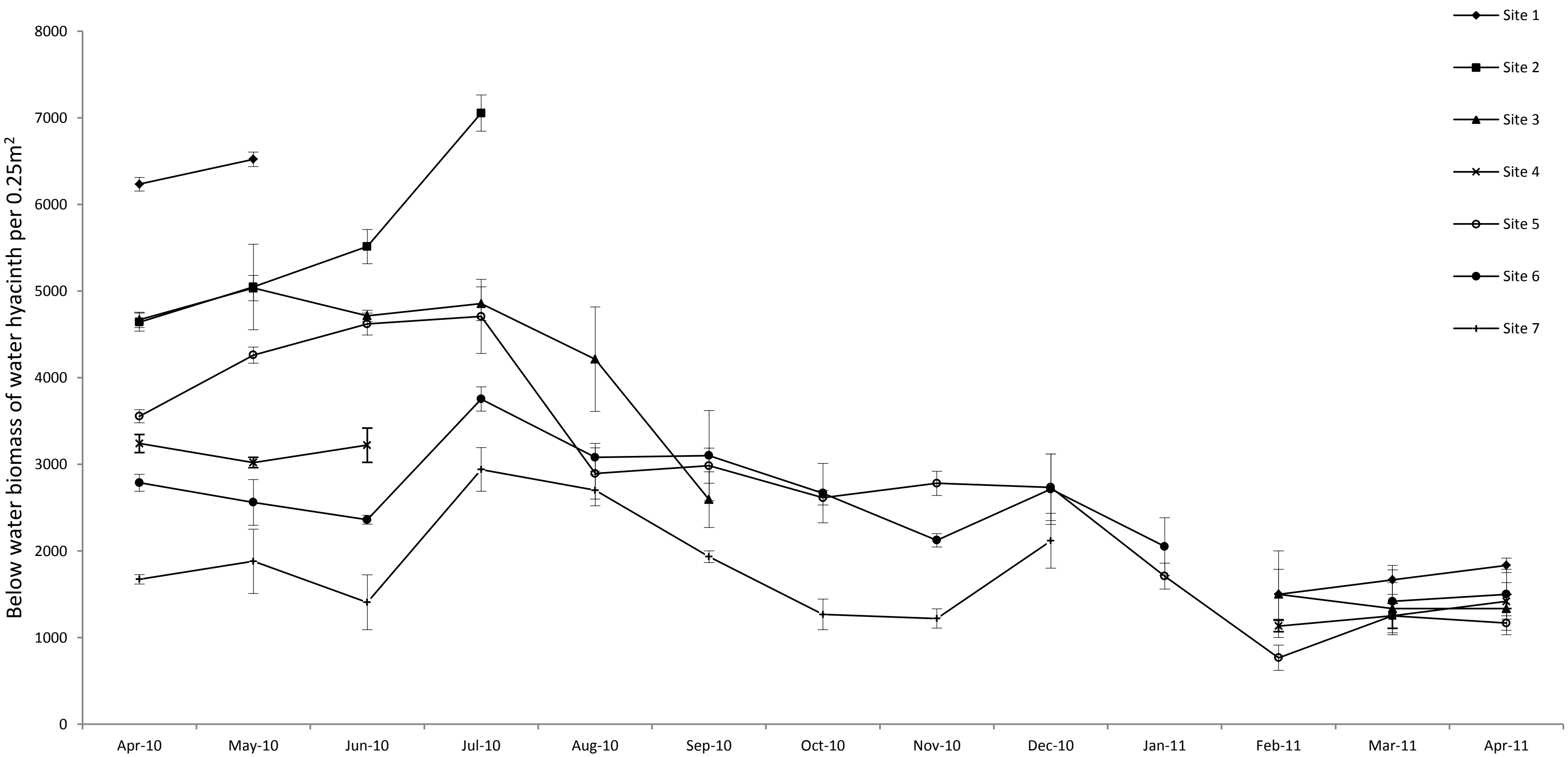

Figure 4.13 The average **below water biomass** of water hyacinth plants per 0.25 m^2 at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

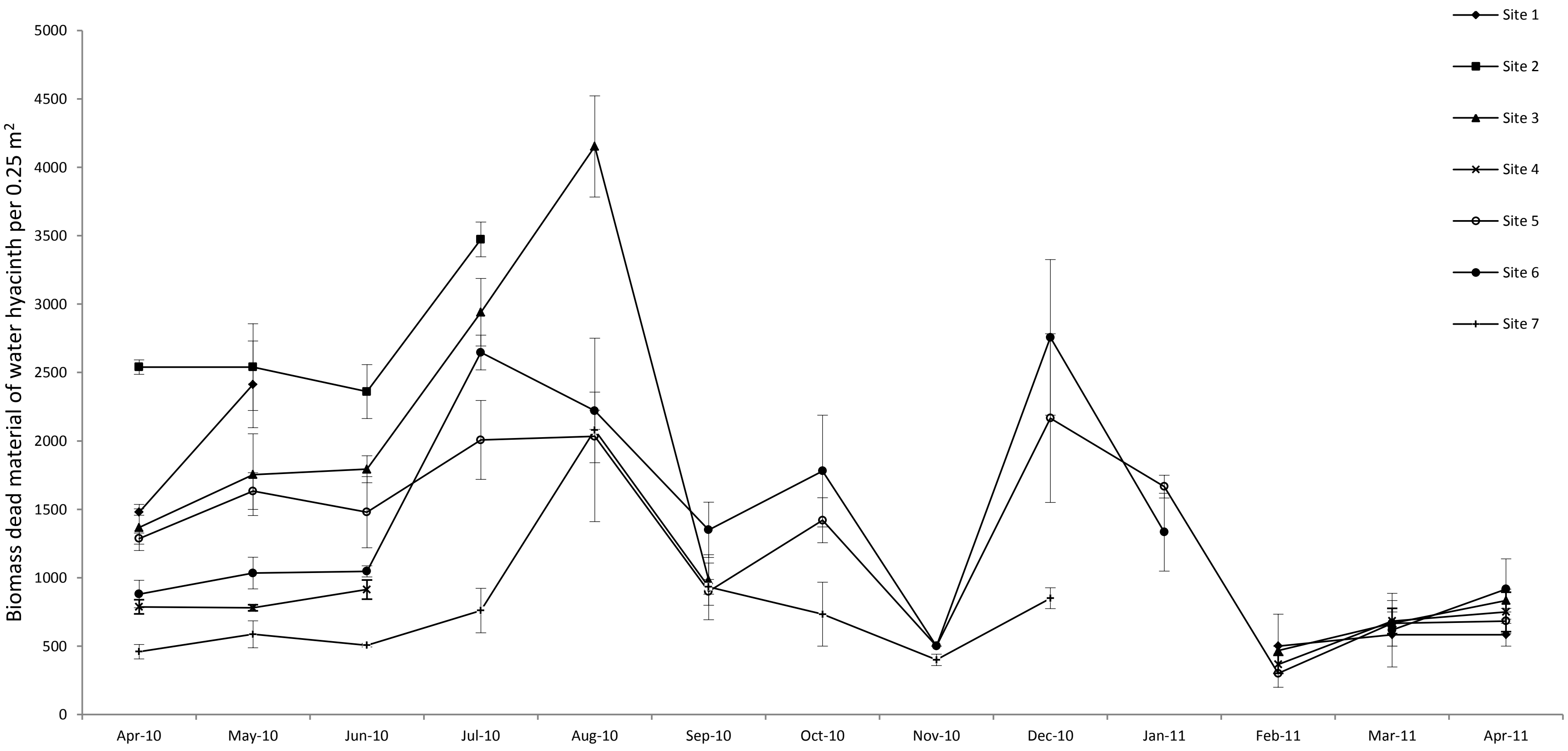

Figure 4.14 The average **dead biomass** of water hyacinth plants per 0.25 m^2 at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

Table 4.3 The H-test statistic and *P*-values for the monthly Kruskal-Wallis test for number of plants and biomass of water hyacinth for each available site for each month. The numbers in brackets indicate the degrees of freedom and the sample size. The values in **bold** indicate significant differences.

April-10			May-10			June-10			July-10		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (6,21)	*P*	Parameter	H (6,21)	*P*	Parameter	H (5,18)	*P*	Parameter	H (4,15)	*P*
# of plants	14.56	**0.02**	# of plants	13.47	**0.04**	# of plants	14.09	**0.02**	# of plants	9.81	**0.04**
Above water biomass	12.36	**0.05**	Above water biomass	13.52	**0.04**	Above water biomass	13.45	**0.02**	Above water biomass	9.59	**0.05**
Below water biomass	19.23	**0.00**	Below water biomass	18.30	**0.01**	Below water biomass	16.19	**0.01**	Below water biomass	12.65	**0.01**
Dead material	18.10	**0.01**	Dead material	17.96	**0.01**	Dead material	15.73	**0.01**	Dead material	12.17	**0.02**
August-10			September-10			October-10			November-10		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (3,12)	*P*	Parameter	H (3,12)	*P*	Parameter	H (2,9)	*P*	Parameter	H (2,9)	*P*
# of plants	9.52	**0.02**	# of plants	8.67	**0.03**	# of plants	6.71	**0.03**	# of plants	6.71	**0.03**
Above water biomass	3.21	0.36	Above water biomass	3.48	0.32	Above water biomass	5.42	0.07	Above water biomass	5.60	0.61
Below water biomass	7.43	0.06	Below water biomass	5.87	0.12	Below water biomass	5.60	0.06	Below water biomass	7.20	**0.03**
Dead material	6.69	0.08	Dead material	3.44	0.33	Dead material	3.50	0.17	Dead material	7.62	**0.02**
December-10			January-11			February-11			March-11		
	Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test			Kruskal-Wallis test	
Parameter	H (2,9)	*P*	Parameter	H (1,6)	*P*	Parameter	H (3,12)	*P*	Parameter	H (4,15)	*P*
# of plants	0.16	0.92	# of plants	3.86	0.05	# of plants	7.35	0.06	# of plants	8.16	0.09
Above water biomass	3.01	0.22	Above water biomass	1.23	0.27	Above water biomass	7.37	0.06	Above water biomass	4.33	0.36
Below water biomass	3.32	0.19	Below water biomass	0.43	0.51	Below water biomass	5.18	0.16	Below water biomass	2.74	0.62
Dead material	5.96	0.05	Dead material	0.44	0.51	Dead material	3.49	0.32	Dead material	0.74	0.95
April-11											
	Kruskal-Wallis test										
Parameter	H (4,15)	*P*									
# of plants	8.92	0.06									
Above water biomass	8.00	0.09									
Below water biomass	6.14	0.19									
Dead material	3.22	0.52									

4.3.2 Insect parameters

The number of adult weevils per plant was extremely variable between the sites and between months (Figure 4.15, Table 4.4). The number of weevils at site 7 never exceeded one adult per plant, while all the other sites were variable between months, but generally from April 2010 to November 2010 range between 2 and 7 weevils per plant. This is with the exception of site 4 which, in April 2010, had an average of 4 weevils per plant. This steadily decreased to 3 weevils per plant in May 2010, and by June 2010 there was an average of less than 1 weevil per plant. By November 2010 the remaining three sites had an average of 6.5 weevils per plant for site 6, 2.5 weevils per plant for site 5 and characteristically for site 7, approximately 0.1 weevils per plant (Figure 4.15, Table 4.4). In December 2010 there was a decrease in the number of weevils per plant and by January 2011 at site 5 there was approximately 0.1 weevils per plant. There was a slight increase in the number of weevils per plant for all the available sites between February 2011 and April 2011 but it never exceeded 1.5 weevils per plant (Figure 4.15), and there were no significant differences between the sites (Table 4.4).

When the numbers of feeding scars on leaf 2 per plant at each site were considered, there was a similar amount of variation between sites and within sites over time (Figure 4.16). For majority of the sites the number of feeding scars was relatively high, generally ranging between 20 – 60 scars on leaf 2. This was with the exception of site 7, which always remained extremely low and never exceeded 10 scars on leaf 2. There was a general decreasing trend for site 4, where by June 2010 the number of feeding scars had reduced to approximately 10 on leaf 2. At site 7 in June, there were no recordings of feeding damage because all the leaves, including leaf 2, were frosted over. In August 2010 there was a sharp increase in the number of feeding scars at all the available sites, but this decreased again by September 2010 to follow the usual trend (Figure 4.16, Table 4.4). The low number of feeding scars at site 7 remained consistent thoughout the study until the site was washed away by the small November – December 2010 flood (Figure 4.4). After the floods in December 2010 – January 2011, there was a transfer of plants from site 6 to site 3, shown in the number of weevil feeding scars, where site 3 in February 2011 mirror those of site 6 in January 2011 (Figure 4.16). The number of feeding scars settled down by March 2011 when all the plants at all the available sites were homogenised (Figure 4.16, Table 4.4) possibly from an infestation further up the Kubusie River.

The number of petioles mined by weevil larvae was consistent through the study period with sites 1, 2, 3, 5 and 6 having a high number of mined petioles, ranging from approximately 2.5 to 3.5 per plant (Figure 4.17, Table 4.4). Site 4 had a decrease in the number of petioles mined over time, with a decrease from 2 petioles in April 2010 to less than 1.5 petioles by June 2010. Site 7 had a similar result with a steady decline in the number of mined petioles. In April 2010 there was approximately 1 mined petiole per plant dropping to zero petioles mined in August 2010. After this there was a slight increase in the number of petioles mined but it never exceeded 0.5 mined petioles per plant (Figure 4.17, Table 4.4). From November 2010 there was a sharp and steady decline in the number of mined petioles for sites 5 and 6, and by January 2011 there were only about 1 in 10 plants that had a petiole mined at both of the sites. There was a small increase in the number of petioles mined but by April 2011 all the sites had less than 1 petiole mined per plant (Figure 4.17).

The numbers of mirids were estimated at each site and sites 1, 2, 3, 5 and 6 always remained low with between 5 and 10 mirids per plant. Site 7 consistently had a very high number of mirids, which varied from 30 to 40 per plant at any sampling event. In April site 4 had approximately 5 mirids per plant, by May 2010 there were approximately 10 mirids per plant and by June 2010 there were approximately 20 mirids per plant.

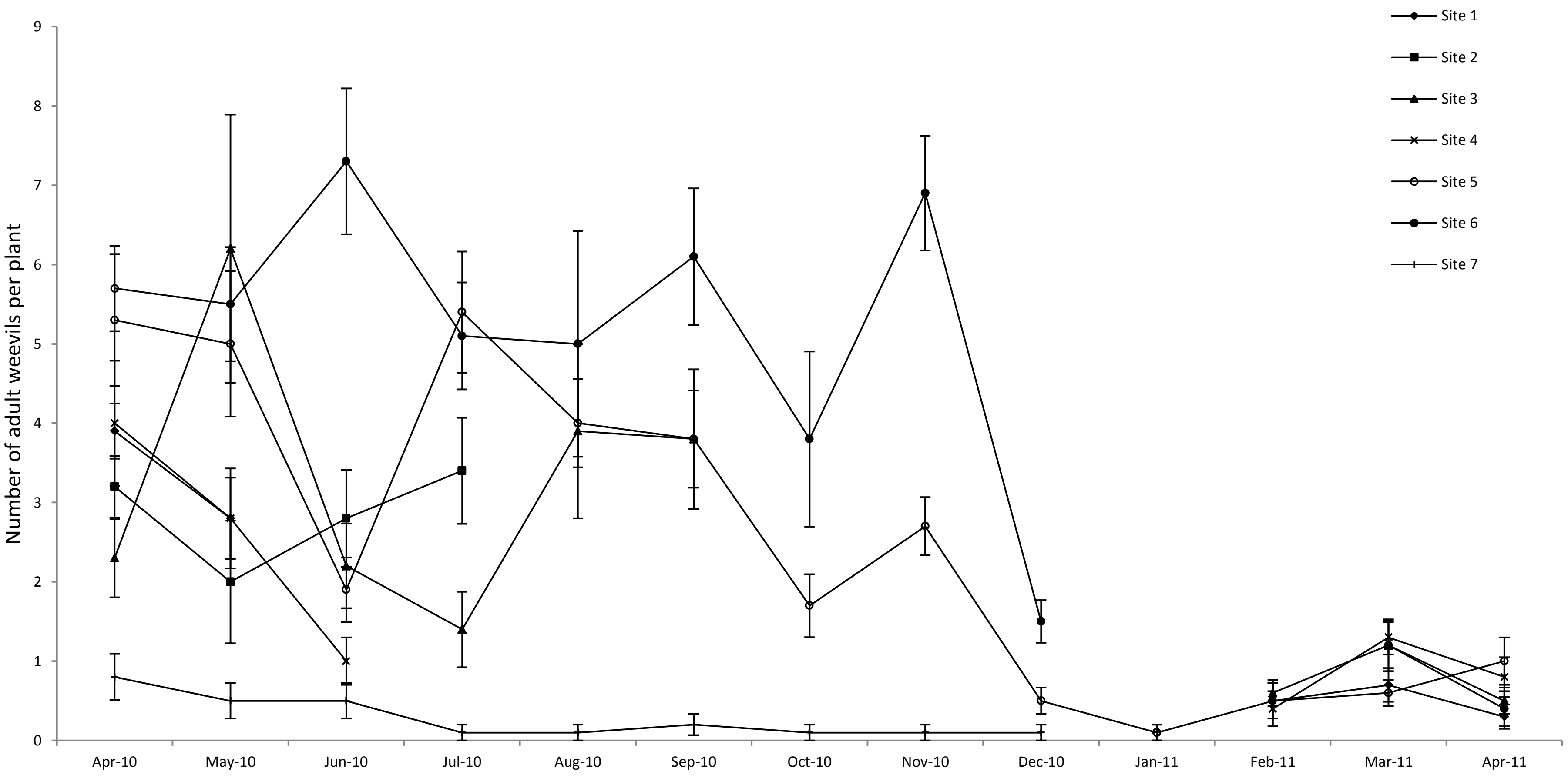

Figure 4.15 The average **number of adult weevils** per water hyacinth plant at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

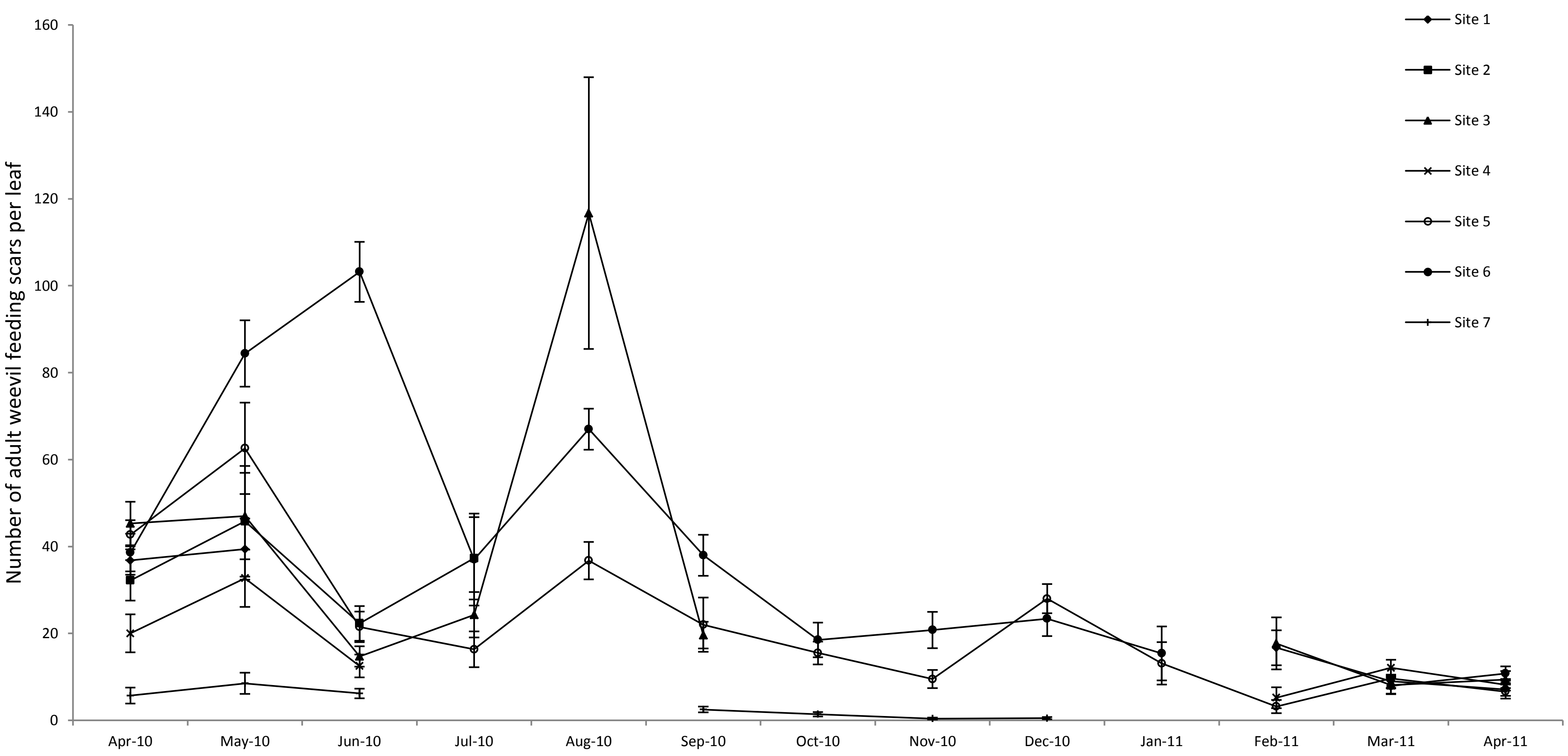

Figure 4.16 The average number of adult weevils **feeding scars on leaf 2** per water hyacinth plant at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

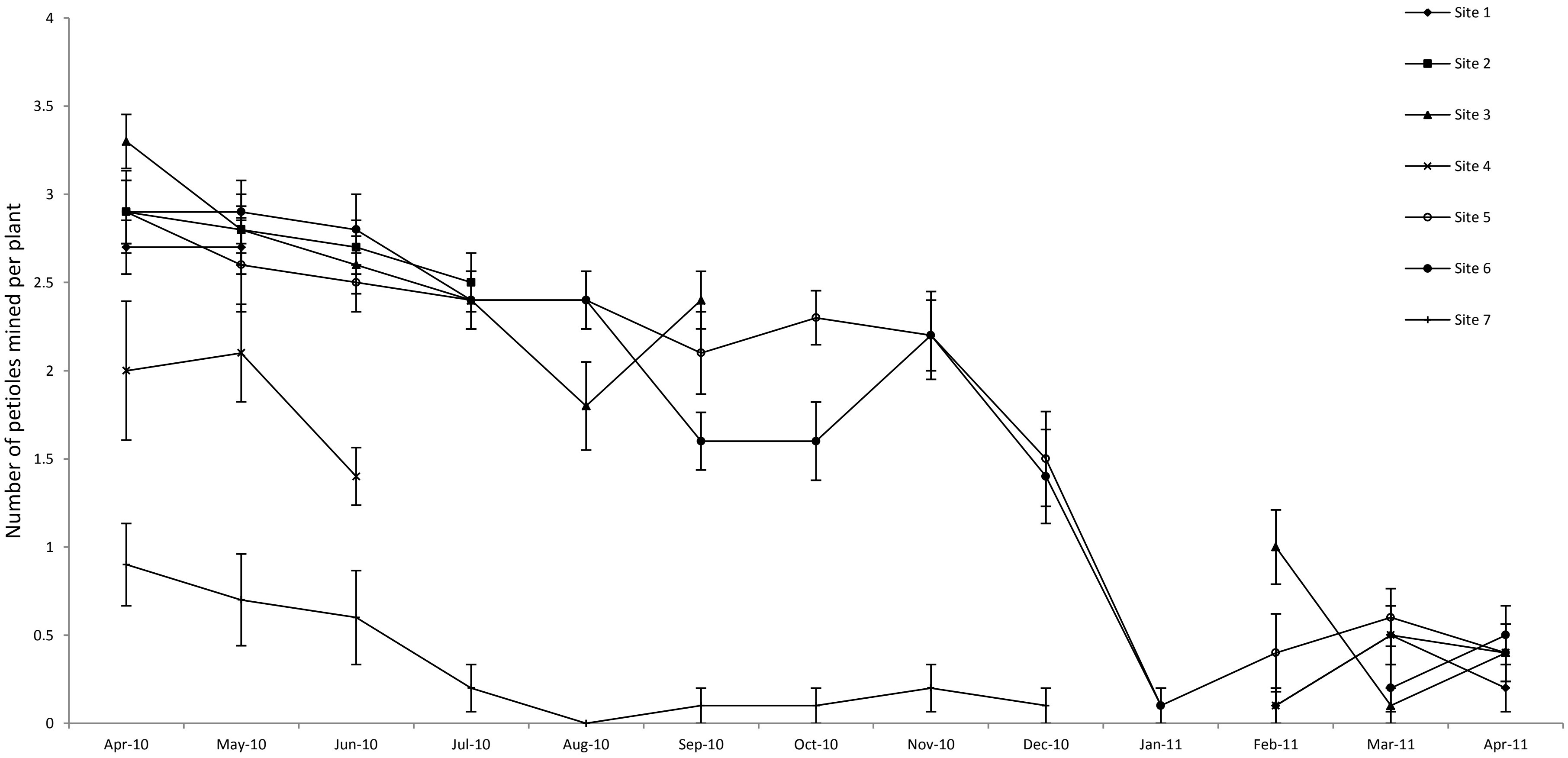

Figure 4.17 The average number of **mined petioles** per water hyacinth plant at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

In April 2010 site 6 had a relatively high percentage feeding area of 30%, however there was a sharp decrease to 10% feeding area and this remained fairly consistent for the duration of the study (Figure 4.18). Initially the feeding area at site 4 was low, where in April 2010 it was approximately 5%, by May 2010 it had risen to approximately 10%, then there was a sharp increase in the feeding area to approximately 50% by June 2010 (Figure 4.18). There were no significant differences in percentage feeding area between sites 1, 2, 3 and 5 at any of the sampling events and it remained fairly low at approximately 10% or below (Figure 4.18, Table 4.4). The percentage feeding area for site 7 was always significantly higher than for all other sites, with the feeding area ranging between approximately 70 and 90% on leaf 2 (Figure 4.18, Table 4.4).

There were some strong correlations identified between the insect parameters using Spearman rank correlations (r_s). There was a significantly positive correlation between number of adult weevils and number of adult weevil feeding scars ($r_s = 0.595$, $P < 0.05$), the number of weevil adults and the number of mined petioles ($r_s = 0.609$, $P < 0.05$), the number of mirids per plant and the percent feeding damage ($r_s = 0.697$, $P < 0.05$), the number of mirids and the intensity of feeding ($r_s = 0.749$, $P < 0.05$) and the percent feeding damage and the intensity of feeding ($r_s = 0.818$, $P < 0.05$).

There was no significant difference between the number of weevil adults and percent feeding damage of the mirid ($r_s = -0.014$, $P > 0.05$). There was a significant negative correlation between the number of adult weevil feeding scars and percent feeding damage of the mirid ($r_s = -0.207$, $P < 0.05$). As the percentage feeding damage of the mirid increased there were fewer weevil feeding scars recorded (Figure 4.19). If the mirid feeding damage got to over 20% there were fewer weevil feeding scars than at a lower mirid percentage feeding damage, and when the mirid damage exceeded 30% the number of weevil feeding scars rarely exceeded 20 per leaf (Figure 4.19).

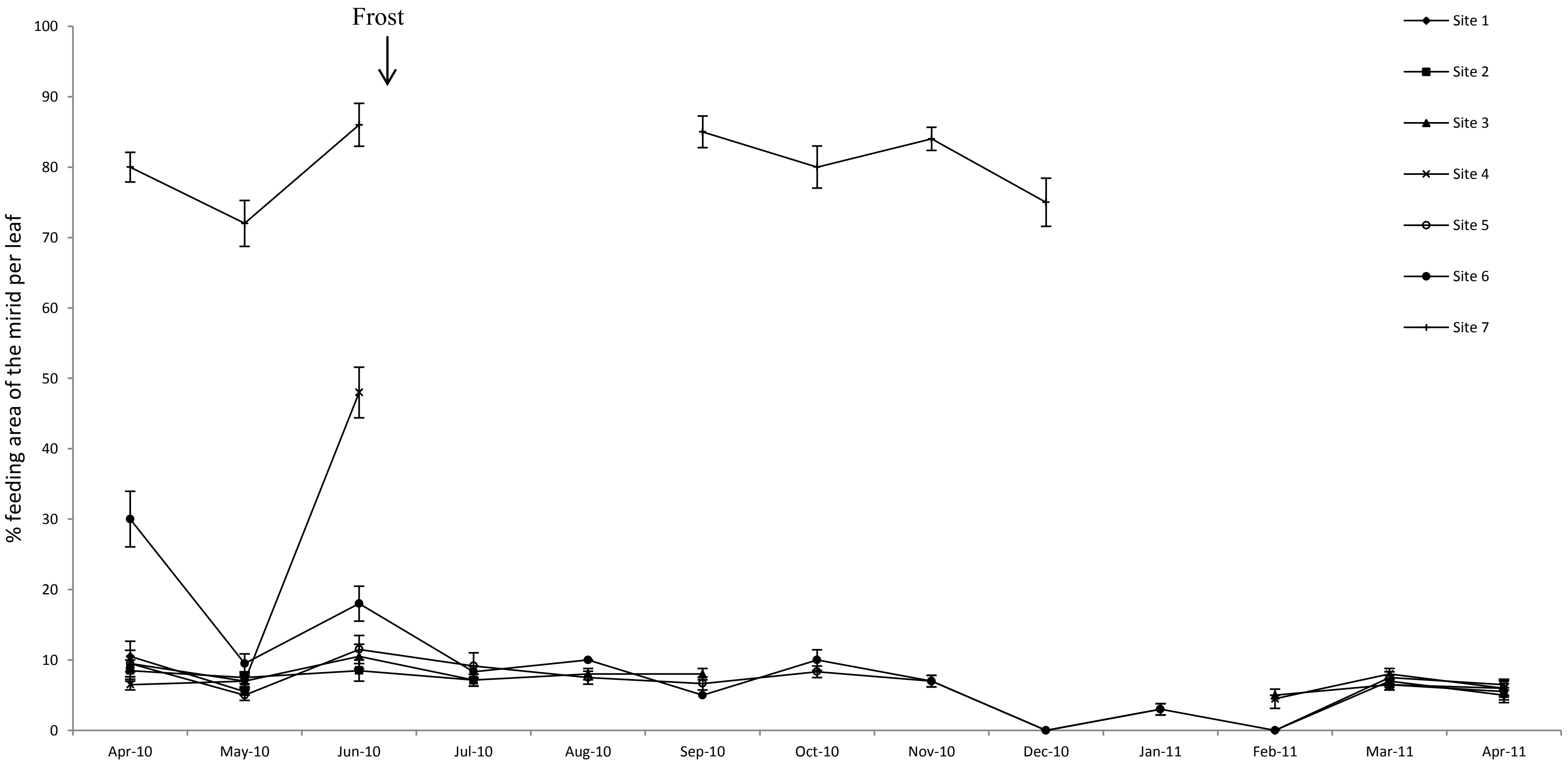

Figure 4.18 The average **percentage feeding damage** by the mirid on leaf 2 per water hyacinth plant at each of the seven sites from April 2010 to April 2011. Error bars indicate the standard error around each mean.

Table 4.4 The H-test statistic and *P*-values for the monthly Kruskal-Wallis test on the insect parameters for each available site for each month. The numbers in brackets indicate the degrees of freedom and the total sample size. The values in **bold** indicate significant differences.

April-10		
	Kruskal-Wallis test	
Parameter	H (6,70)	*P*
# of weevils	33.35	**0.00**
# of feeding scars	35.36	**0.00**
# mined petioles	33.46	**0.00**
% mirid damage	44.61	**0.00**

May-10		
	Kruskal-Wallis test	
Parameter	H (6,70)	*P*
# of weevils	33.40	**0.00**
# of feeding scars	32.73	**0.00**
# mined petioles	29.80	**0.00**
% mirid damage	39.07	**0.00**

June-10		
	Kruskal-Wallis test	
Parameter	H (5,60)	*P*
# of weevils	31.67	**0.00**
# of feeding scars	36.09	**0.00**
# mined petioles	36.46	**0.00**
% mirid damage	46.29	**0.00**

July-10		
	Kruskal-Wallis test	
Parameter	H (4,50)	*P*
# of weevils	32.47	**0.00**
# of feeding scars	10.37	**0.03**
# mined petioles	27.54	**0.00**
% mirid damage	12.51	**0.01**

August-10		
	Kruskal-Wallis test	
Parameter	H (3,40)	*P*
# of weevils	19.35	**0.00**
# of feeding scars	2.86	0.41
# mined petioles	26.62	**0.00**
% mirid damage	20.98	**0.00**

September-10		
	Kruskal-Wallis test	
Parameter	H (3,40)	*P*
# of weevils	24.51	**0.00**
# of feeding scars	18.39	**0.00**
# mined petioles	27.20	**0.00**
% mirid damage	20.15	**0.00**

October-10		
	Kruskal-Wallis test	
Parameter	H (2,30)	*P*
# of weevils	14.69	**0.00**
# of feeding scars	18.37	**0.00**
# mined petioles	22.46	**0.00**
% mirid damage	3.92	0.14

November-10		
	Kruskal-Wallis test	
Parameter	H (2,30)	*P*
# of weevils	25.71	**0.00**
# of feeding scars	21.68	**0.00**
# mined petioles	19.63	**0.00**
% mirid damage	21.32	**0.00**

December-10		
	Kruskal-Wallis test	
Parameter	H (2,30)	*P*
# of weevils	14.93	**0.00**
# of feeding scars	20.05	**0.00**
# mined petioles	15.38	**0.00**
% mirid damage	27.58	**0.00**

January-11		
	Kruskal-Wallis test	
Parameter	H (1,20)	*P*
# of weevils	5.43	**0.02**
# of feeding scars	9.89	**0.00**
# mined petioles	5.61	**0.02**
% mirid damage	3.17	0.08

February-11		
	Kruskal-Wallis test	
Parameter	H (3,40)	*P*
# of weevils	1.08	0.78
# of feeding scars	13.02	**0.00**
# mined petioles	14.65	**0.00**
% mirid damage	26.25	**0.00**

March-11		
	Kruskal-Wallis test	
Parameter	H (4,50)	*P*
# of weevils	6.43	0.17
# of feeding scars	3.68	0.45
# mined petioles	7.82	0.10
% mirid damage	2.74	0.60

April-11		
	Kruskal-Wallis test	
Parameter	H (4,50)	*P*
# of weevils	5.30	0.26
# of feeding scars	4.46	0.35
# mined petioles	2.00	0.74
% mirid damage	1.13	0.89

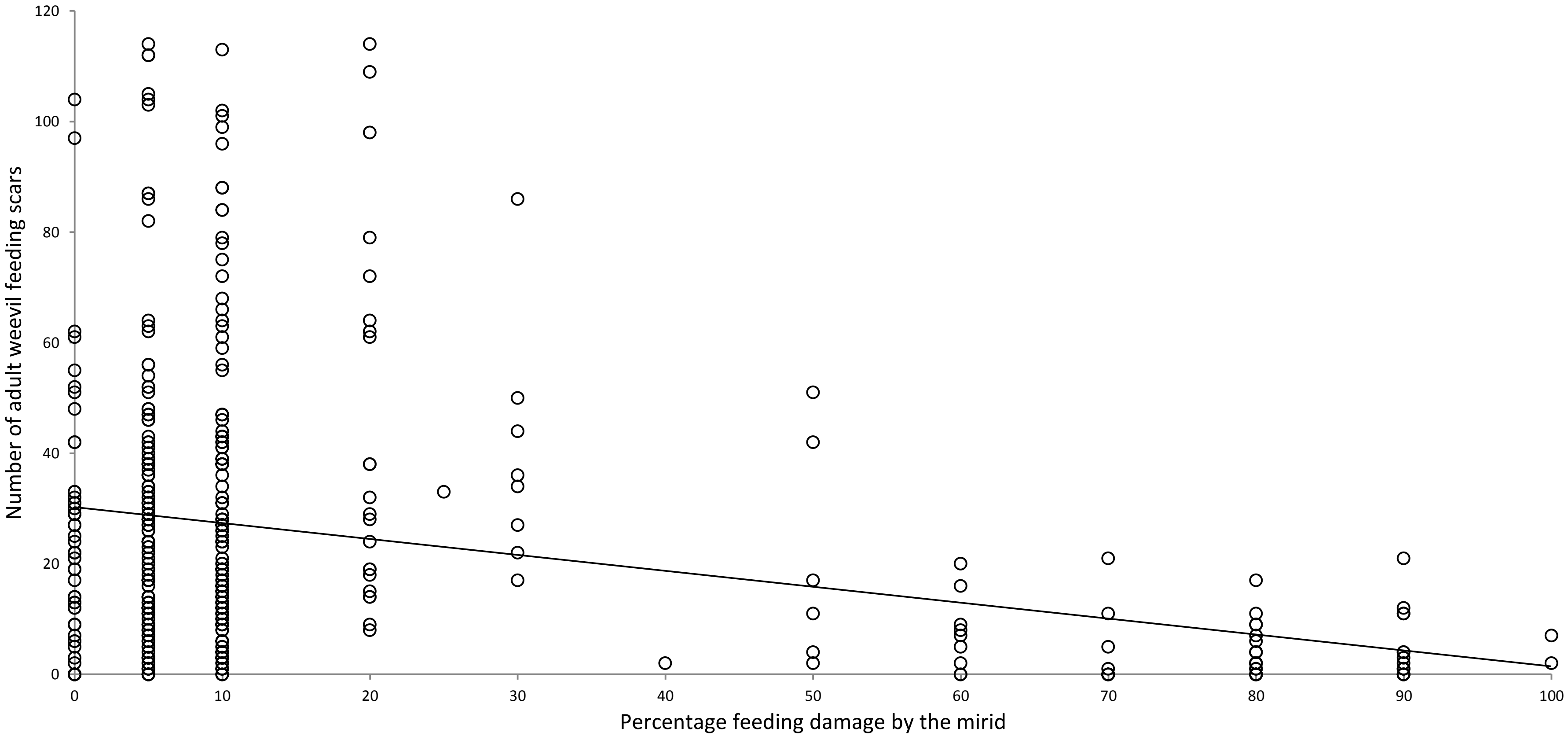

Figure 4.19 The correlation between the percent feeding area and the number of weevil feeding scars at all sites for the duration of the experiment (Spearman rank correlation $r_s = -0.207$, $P<0.05$)

4.3.3 Physico-Chemical parameters

The physico-chemical parameters were similar at each site, the pH ranged from 6.8 to 7.4, the conductivity from 195.6 to 230.7µS/cm, while the TDS ranged from 99.3 to 119.6 ppm, and salinity never varied from 0.1 (Table 4.5).

Table 4.5 Water quality parameters measured at each study site on Wriggleswade Dam in April 2010 at the initiation of the study.

Location	pH	Conductivity (µS/cm)	TDS (ppm)	Salinity
Site 1	7.1	198.8	99.3	0.1
Site 2	7.3	199.8	101.5	0.1
Site 3	7.4	230.7	119.6	0.1
Site 4	7.2	203.0	105.7	0.1
Site 5	6.9	215.1	115.2	0.1
Site 6	6.8	215.6	111.8	0.1
Site 7	7.4	195.6	100.5	0.1

4.4 Discussion

The management and ultimate success of a biological control programme can in many cases only be achieved by understanding the population dynamics, spatial and temporal distribution and interactions of the insects in their introduced range. The collecting of data and describing field sites are variable which can be amplified when working on dynamic systems such as floating aquatic plants, in this case water hyacinth. The plant and insect performance at the water hyacinth infestations in the upper reaches of Wriggleswade Dam highlight the dynamic nature of water hyacinth and associated insect populations in the field. At the initiation of the study the water hyacinth mats were in relatively permanent stands with both insect species *N. eichhorniae* and *E. catarinensis* present and established at all the sites. The water levels of the dam consistently dropped from January 2010 until November 2010. This resulted in several of the sites drying up and being lost during the course of the study.

Then there was a major influx of water into Wriggleswade Dam between November 2010 and February 2011, which pushed all the original water hyacinth from the sites out and replaced it with water hyacinth from a stand further up the Kubusie River system. This essentially reset the system and homogenised the sites which is clearly highlighted in the plant and insect parameters measured from February 2011 until April 2011.

The distribution and performance of *E. catarinensis* and *N. eichhorniae* at the field sites suggest that there was spatial segregation, with one species dominating or performing better at certain sites. At each of the field sites both species of insect were present throughout the study period, which suggests that no environmental conditions excluded a particular insect species from a site. The field site situated at the top of the plug of water hyacinth (site 7) was dominated by the mirid throughout the study period. The numbers of the mirid *E. catarinensis* adults were always high, which resulted in an equally high percentage feeding damage and feeding intensity on leaf 2. The weevil *N. eichhorniae* did not perform well at this site and the numbers of adults were well below 1 per plant and the number of feeding scars was low. Interestingly site 4 at the beginning of the study period was a weevil-dominated site at the back of a small bay, with the mirid not performing well. By the third sampling event weevil performance had dropped and mirid performance significantly increased. This suggests that the insect populations were shifting from being weevil-dominated to mirid-dominated at this site. The insect performance at site 6, which is in the middle of the plug, suggests a shift from mirid-dominated to weevil-dominated. In April 2010 the number of mirid adults was high and there was a correspondingly high percentage feeding area and intensity, however by May 2010, just one month later, the weevils had dominated the site.

In a similar study involving the geographic distribution of two agents, *Oxyops vitiosa* Pascoe and *Boreioglycaspis melaleucae* Moore, released against *Melaleucae quinquenervia*

(Cavanilles), in Florida, Balentine *et al.* (2009) found that there was no difference in their distributions. In addition to this there was a positive correlation between the densities of these insects, suggesting that the presence of one species does not limit the performance of the other (Balentine *et al.* 2009). This would suggest that any potential antagonistic relationship between these species through interspecific competition does not limit performance and potential fitness of the insects involved. There are inherent differences between this biological control programme and the water hyacinth programme in South Africa. The programme against water hyacinth, at least on the Kubusie River system, has been classical, with no re-releases of agents after the initial release over a decade ago (Hill, pers. comm.) whereas the agents on *M. quinquenervia* have been actively distributed and re-released (Center *et al.* 2006). The classical biological control approach on water hyacinth on the Kubusie system may allow a particular insect species to dominate over another, which may have more recently dispersed into an area with relatively low numbers.

The interaction between *E. catarinensis* and *N. eichhorniae* is clear, with the correlation between percentage feeding area of the mirid and the number of weevil feeding scars showing a significant negative relationship. Once the percentage feeding area of the mirid reaches 30% the number of feeding scars on that leaf rarely exceed a total of 20. This is a similar trend to that seen in the experiment described in chapter 3, where after about 20-30% feeding damage the number of feeding scars tailed off at about 0.4 scars per cm^2. Ajuonu *et al.* (2007) showed that there was a potential negative relationship between the number of “old” weevil feeding scars on the performance and fitness of *E. catarinensis.* The data from chapter 3 suggest that the mirid was not significantly affected by the presence of *N. eichhorniae*, but the weevil performed significantly less well in the presence of *E. catarinensis.* Either way, there is a potential negative relationship between *E. catarinensis* and *N. eichhorniae.* The field data from the current study suggest that as the population of one species increases in an area it is likely that there will be a decrease in the population of the other. The differences were in the abundance and performance of the species and not in presence or absence, suggesting that neither species would limit the establishment of the other. The interaction between *Megamelus scutellaris* Berg and *Taosa longula* Remes Lenicov suggests that these insects can coexist on a shared host plant (water hyacinth) through spatial segregation on a local scale (Hernandez *et al.* 2011). Both of these insects are salivary-sheath feeders (Wang *et al.* 2008), with most of the sheaths ending in the phloem tissue, resulting in a very similar utilisation of the host resources (Hernandez *et al.* 2011). *Taosa longula* prefer the abaxial lamina of the leaf and down to the mid-petiole, while *M. scutellaris* prefer the basal petiole, with some individuals being found up the petiole to the lamina of the leaf (Hernandez *et al.* 2011). This spatial distribution or pattern was consistent for each species irrespective of the presence of heterospecifics. This example suggests that the possible negative effects of interspecific competition for a common resource may be cushioned by local segregation and a subtle use of different niches or niche partitioning on the shared host plant.

The coexistence of *E. catarinensis* and *N. eichhorniae* at the sites irrespective of dominance may be explained by this spatial segregation of the species on a local scale. This was not specifically tested in the current study but certain inherent differences between these species would suggest local segregation. The mirid is diurnal and both adults and nymphs spend the day on the upper and lower lamina of the leaf respectively (Hill *et al.* 1999). The weevil, *N. eichhorniae*, is nocturnal and spends the day preferentially in the area around the base of the central petiole near the wrapper leaf (DeLoach & Cordo 1976b). There is overlap between the mirids and the adult weevils in the adult feeding areas and potentially oviposition. Both feed on the lamina of the leaf, but the mirid is a sap sucker (Hill *et al.* 1999) while the weevil is a mandibulate beetle which removes leaf tissue (DeLoach & Cordo

1976a; Center 1994). The feeding areas are the same, however, there is a potential for segregation in resource utilisation and on a local scale, which suggests that they are able to coexist. The data from this study demonstrate, however, that one insect may dominate over another at a particular site.

Plant phenology may play an important role in the spatial distribution of species on a landscape or geographic scale. Center & Dray (2010) suggest that bottom up forces are one of the most important factors driving insect populations and densities. In an unrelated study the interactions between two root-feeding weevils, *Sphenoptera jugoslavica* Obenberger, and *Cyphocleonus achates* (Fahraeus), released for the biological control of both *Centaurea stoebe* and *C. diffusa*, may be mitigated by spatial or temporal separation (LeJeune *et al*. 2005). Each of these insects has a specific set of conditions that favour performance and fitness. *Sphenoptera jugoslavica* performed better when the plants were grown under nutrient rich conditions and were relatively large, while *C. achates* performed better when the plants were grown under nutrient poor conditions and were small (LeJeune *et al*. 2005). In reality, a pasture or rangeland will not be homogenous in terms of growing conditions for plants, and these two insects have the potential to coexist in particular areas through spatial or temporal separation. The water hyacinth phenology at sites sampled in the current study was variable, but a general trend did emerge. The plants at the weevil-dominated sites tended to be taller and in mature stands that were not actively expanding, shown by the low number of ramets, while the plants at the mirid-dominated sites were small in actively growing stands. This may reflect the mirid’s ability and propensity to disperse. *Eccritotarsus catarinensis* has established at some sites around South Africa that are 100’s of kilometres from the nearest release site (Coetzee, pers. comm.), while the weevils on this system or at least at the sites surveyed were persisting on the available plants.

In another study two agents were released for the control of *Mimosa pigra* Linnaeus. The moth, *Neurostrota gunniella* (Busck) and the fungal pathogen, *Phloeospora mimosae-pigrae* H.C. Evans & Carrión, had a relatively benign interaction in the laboratory (Paynter & Hennecke 2001). The moth was released first into Australia and is well established, while the fungal pathogen was released later (Heard *et al.* 1997). Populations of the pathogen have declined and it seems to be established only at the original release sites (Paynter & Hennecke 2001). Despite the weak competition between these species there does seem to be a geographic segregation between them where the moth prefers to attack plants that are either on the edges of infestations or isolated (Smith & Wilson 1995). What is unclear from this is whether the segregation is directly related to this potential competition or whether it is the plants that are limiting the establishment of the fungal pathogen. In the introduced range *M. pigra* grows much taller than in the native range, which changes the dynamics and microclimates (i.e. reduction in humidity around the stem of the plant) necessary for the fungal pathogen to establish (Lonsdale & Segura 1987). The spatial segregation between these two agents may not be related to their interactions but rather to the differences in plant preference and potential requirements for establishment.

4.5 Conclusions

The water hyacinth parameters and insect populations and densities were not manipulated or controlled during this study. This was a descriptive study to determine whether the negative interactions that were observed in the controlled common garden experiment would be expressed in the field or natural conditions. To a certain degree these insects are interacting, but such interactions were expressed in the spatial distribution of insect populations and their respective performances in different areas. This is probably because the insects have the opportunity to disperse to more favourable areas and are not confined to relatively few plants in a cage as in the experimental setup of chapter 3. Spatial segregation in herbivorous insects sharing a common resource or host plant is not uncommon (De Loach & Cordo 1976b; Tedders 1978; Paynter & Hennecke 2001;Viswanathan *et al.* 2008; Hernandez *et al.* 2011), but is not necessarily a prerequisite for establishment and population growth (Ferrenburg & Denno 2003; Balentine *et al.* 2009).

Chapter 5: General discussion

Globally, biological control of weeds has been successful with several biological control programmes reaching a level of control which is considered complete. In many of these successful biological control programmes more than one biological control agent has been released against a particular weed (Julien & Griffiths 1998). This success with multiple agents is thought to have been achieved for one of two possible reasons. Harris (1981) suggested that a cumulative stress from several different agents working synergistically was necessary for control to be achieved. On the other hand Myers (1985) suggested that most biological control programmes represent a lottery model, where numerous agents are released until one effective agent is found and control is achieved. Despite the increased success with an increase in the number of agents released most of these agents are released without consideration of potential interactions between already established agents that could have implications for the efficacy of the programme (McEvoy & Coombs 2000). Additional biological control agents are often released before the full potential of the already existing agents has been achieved or, possibly more importantly, measured (MacFadyen 1998; McEvoy & Coombs 2000). These additional agents result in an increase in the complexity of the system and management of these systems can become difficult (McEvoy & Coombs 1999). Despite this some biological control programmes benefit from the introduction of additional agents into the programme and the overall efficacy can be greatly increased (Hoffmann & Moran 1998), while the efficacy of other programmes could be compromised.

Several factors regulate herbivorous insect populations, but one of the most important biotic factors is competition for a limited resource (Damman 1993; Denno *et al.* 1995; Kaplan & Denno 2007). McEvoy & Coombs (2000) suggest that competition between biological control agents is important and can have consequences on the performance of the agents. An instance of competition between biological control agents potentially having an effect on the efficacy was the biological control programme against *Acacia cyclops* Cunningham in South Africa. The flower galling cecidomyiid *Dasineura dielsi* Rübsaamen may have out-competed the weevil *Melanterius servulus* Pascoe which could have had serious implications for the efficacy of the programme. The seed-feeding weevil, *M. servulus,* was first released in 1991, for the control of *A. cyclops*, with more extensive releases being made in 1993 (Impson *et al.*2004). Despite the 95% seed mortality at sites where the weevil was established, dispersal of the weevil was limited. In 2001 *D. dielsi* was released as a supplement to the biological control programme. The cecidomyiid dispersed extremely rapidly and the level of galling was high; initially it was considered a very effective agent (Impson *et al.* 2008). However, over time the midge populations went through boom and bust population dynamics due to the lack of natural enemies from the native range, which could have resulted in a variable supply of pods for the *M. servulus* weevils to persist on (Impson *et al.* 2008). This could have had implications for the weevil populations and the unpredictable populations of *D. dielsi* and may have resulted in an unstable biological control programme against *A. cyclops*. However, the ability to predict these interactions between the agents at the time of the release was limited (Impson *et al.* 2008). In an on-going investigation it is apparent that the expected negative implications for the biological control programme have not been observed and *M. servulus* are able to persist at sites with a variable supply of pods between years (Impson, pers. comm.). This is considered exploitative competition, where the interactions between insects are usually manifested in the responses of the plant to herbivory, in this case a reduction in the number of seed pods. Some other plant-mediated interactions may include a feeding-induced change in plant odours and volatiles, changes in nutritional value and induced chemical and morphological defences (Heshula & Hill 2011). The latter two responses elicited in a plant would have a direct effect on the performance and compatibility of different species of insect, while the changes in plant odours and volatiles would affect the acceptability of a host plant. In a biological control context it is important to investigate how the different plant-mediated interactions between the insects affect the performance and compatibility of the agents and ultimately the efficacy of the programme.

In a biological control context this thesis is a retrospective study aimed at identifying whether interactions between three insects (*Neochetina eichhorniae*, *N. bruchi* and *Eccritotarsus catarinensis*) released for the control of water hyacinth *Eichhornia crassipes* could be identified and measured in a series of laboratory and field studies. In addition to this the impact of the interactions on the performance and ultimately compatibility of the insects was assessed to give an indication of the potential effect on the efficacy of the programme. In a South African context for the biological control of water hyacinth this is important because several additional agents are under consideration for release into the biological control programme (Coetzee *et al.* 2011).

The importance, in terms of host acceptability, of the olfactory cues released from water hyacinth that had been previously fed upon by the insects was investigated in chapter 2. The olfactory cues and volatiles from plants change when they have been fed upon by insects. The changes may include the active production of new volatiles and odours or an increase in the amounts and ratios of already existing volatiles (Dicke *et al.* 1990; Vet & Dicke 1992; Dicke & Baldwin 2009). The response of herbivorous insects to these volatiles could simply be a case of increased detectability (Visser 1976; Bolter *et al.* 1997), or the plants pose a potential ecological cost (through competition for a common resource) or gain (when plant defences have been overcome) to the insect (Coley *et al.* 1985; Agrawal 1998; de Moraes *et al.* 1998; Thaler 1999; Dicke 2000; Dicke & van Loon 2000; de Moraes *et al.* 2001).

Results from chapter 2 confirm that all three insects are able to detect and respond to volatiles released by both damaged and undamaged water hyacinth. Interestingly the insects tested did not distinguish between types of herbivore damage. This suggests that there was no perceived ecological cost or gain for the insects in the olfactory cues released by water hyacinth due to damage caused by conspecifics or heterospecifics. In a biological control context this is a positive result as it would mean that the insects would not necessarily avoid plants that were previously damaged and therefore it is unlikely that one insect would limit the establishment of another. When the insects were offered a choice between damaged plants and undamaged plants there was a preference for the undamaged plants irrespective of type of herbivore damage. This means that the undamaged plant potentially represents the "best option" and the insects tested would have an inclination to disperse to sites that have a lower level of insect damage. This would ultimately result in homogenous density of insects on a landscape level if olfactory cues and odours released by water hyacinth was the only factor playing a role in their distribution.

If this type of investigation where the acceptability of a host plant through olfactory cues was used as a "pre-release" study, the results would indicate that these insects would not limit establishment of heterospecifics and would to a certain degree be compatible. However this study gives little insight into the effect that one insect could have on the performance of another, albeit through plant-mediated interactions.

Plant-mediated interactions between insects would be reflected in the reduction in the suitability of the plant due to prior colonisation (including feeding and oviposition) of an insect (Kogan & Paxton 1983; Rhoades 1985; Denno & Kaplan 2007). Chapter 3 reported the impact that the combination of different species of insect had on their performance (the amount of feeding damage was an index of performance). The results indicated that there was a negative relationship between two sets of combinations, *E. catarinensis* and *N. eichhorniae* treatment, and the two *Neochetina* weevils in combination, where the feeding and possibly oviposition of one species of insect on water hyacinth resulted in that plant becoming undesirable for the other.

In the case of the weevils in combination there was always a significantly lower number of feeding scars per individual when the weevils were in combination as compared to the *N. eichhorniae* treatment, while no differences were observed between the *N. bruchi* treatments. The similar feeding habits and ovipostional preferences made it impossible to determine which weevil species was being affected and extended studies would be necessary to determine through population growth indicies which species was the better competitor. There is substantial evidence that these two species can coexist in a laboratory or experimental environment (Center & Dray 2010) and under natural conditions in the native range (De Loach & Cordo 1976b). In the native range the coexistence is largely due to spatial segregation both on a local (per plant) scale and on a geographic scale (De Loach & Cordo 1976b). The experimental cages in chapter 3 were to best mimic a natural mature water hyacinth mat in a small confined space. This may not have been adequate for the spatial segregation of these species and this forced coexistence may have highlighted this negative relationship.

In the case of the *E. catarinensis* and *N. eichhorniae* combination, the weevil was affected by the presence of the mirid. The number of adult feeding scars was always significantly lower than in to the *N. eichhorniae* control treatment. According to the formula by Wright & Center (1984) for predicting the populations of *N. eichhorniae,* the feeding performance of *N. eichhorniae* was approximately half of what it potentially could be (Chapter 3). The interaction between these two insects suggests they were competing for a common resource and in the confines of the experimental cages the mirid *E. catarinensis* was the superior competitor with no implications on their performance in the presence of the weevil. The oviposition of the weevil was also always slightly less in the combination treatment, but this was never statistically significant. This may be biologically significant over several generations, but it was not tested in this experiment. It is a consideration for future studies of this type.

It is possible that interference competition could play a role in the interactions observed in chapter 3. Denno *et al.* (1995) showed that interference competition was important for mandibulate herbivores utilising concealed niches and is strongly linked to body size. The larvae of both species of *Neochetina* weevils mine the petioles (De Loach & Cordo 1976a; Center 1994) so there is a possibility that if a larva were to come into contact with another in the petiole there would be a conflict and the larger larvae would probably consume the smaller one. Cannibalistic and predaceous behaviour among herbivorous insects especially stem borers such as the Cerambycidae, is not uncommon and has been shown to be an important population regulator in some cases (Dodds *et al.* 2001; Ware & Stephen 2006). A manipulative experiment would have to be set up to determine the importance of this type of competition, and to establish whether or not a female weevil would oviposit in a petiole that had already received an egg.

Del Fosse (1978) showed that *N. eichhorniae* did not actively search for *Orthogalumna terebrantis* eggs, however it was possible that by chance some eggs may have been eaten by the weevil. It is possible that the weevil feeding scars could destroy mirid eggs, but this is likely to be a chance occurrence. The performance of the mirid was slightly less when in combination with the weevil, but not significantly so, suggesting that if this does occur it is unlikely to have a significant effect on the population performance of the mirid. A manipulative experiment would be needed to determine the importance of weevil predation on mirid eggs.

In a biological control context this type of “pre-release” study (Chapter 3) is extremely valuable in identifying species or combinations of agents that are likely to have “revenge effects” in the field. The results presented in chapter 3 have been able to identify potential interactions between biological control agents in a simple garden plot experimental design. The negative interactions between the agents suggest that there are implications for their performance but it is unlikely that there would be effects on the establishment of the insects in the field.

In the field study (Chapter 4) both *N. eichhorniae* and *E. catarinensis* were established at all the sites investigated during the field study so neither species is limiting the establishment of the other despite the negative interaction in the laboratory. The distribution of the insects in the field shows that there is spatial segregation between them with one insect dominating at a site over the other, however the driving forces of this segregation were unclear. It is possible that the plants are driving the insect population dynamics in the field. Several studies highlight the importance of bottom-up forces in insect performance on water hyacinth (Heard & Winterton 2000; Coetzee *et al.* 2007; Center & Dray 2010). The plant phenologies were different between field sites, with the weevils dominating at sites with tall mature water hyacinth and the mirid dominating at sites with short actively growing water hyacinth. This may not necessarily indicate the insect’s preference for a particular plant phenology, but the mirid’s ability to disperse, coupled with a shorter development time would allow them to dominate at newer sites more quickly than the weevil. This would suggest that although there does not seem to be an effect on the establishment of the insects in the field there is an effect on their distribution and performance at particular sites. In this case it would seem that the insects are complimenting each other by not occupying the same areas and covering a wide range of plant phenologies. However, it was not possible to determine the distribution of the insects in the event that one had been absent from the field site (i.e.

conclusions on whether the distribution of the weevil would be different if the mirid was completely absent, and vice versa, were not possible).

A recent study by Byrne *et al.* (2010) looked at 15 water hyacinth systems (Table 5.1) around South Africa and measured insect performance over two years. *Eccritotarsus catarinensis* was not present at every site for the duration of the study period, and the year of release varied between insects and between sites (Table 5.1). This does not necessarily mean that the insects are not established in the system as a whole, for example *E. catarinensis* is established in Warrenton Weir (Hill, pers. comm.), however, it was not recorded in the data collected at the site studied by Byrne *et al.* (2010). Large scale landscape type studies as in Byrne *et al.* (2010) miss the subtle interactions and effects on a local scale, especially when only a small section of the infestation is measured and the same site is revisited on successive dates. For example the Kubusie system was studied by Byrne *et al.* (2010) and the results showed that the mirid dominates in this system, but the intensive field study reported in chapter 4 of the present work suggested that both *N. eichhorniae* and *E. catarinensis* dominated at different sites in the system. During the field study described in chapter 4 there were two sites not more than 1km apart, yet the mirid dominated at the one and the weevil dominated at the other.

The insect performance from Byrne *et al.* (2010) was used to determine whether there was any spatial segregation on a landscape level in South Africa. A Pearson's correlation test was conducted between the performance of *N. eichhorniae, N. bruchi* and *E. catarinensis* at the sites where all three insects were present (N=8). The number of feeding scars of the weevil was not a good index for the performance of *N. eichhorniae* because *N. bruchi* was established and present at every site as well. So the number of individuals recorded at each site where the mirid was present (Table 5.1) was compared to the percentage feeding damage of the mirid at that site using a Pearson correlation test. The feeding performance of the mirid was averaged over the entire study period and compared to the average number of *N. eichhorniae* and *N. bruchi* weevils at those sites.

Table 5.1 The water hyacinth systems studied in Byrne *et al.* (2010), the year of release where available (Hill, unpub.) and the presence of *Eccritotarsus catarinensis*, *Neochetina eichhorniae* and *N. bruchi* feeding damage at each site.

Water hyacinth system	*Eccritotarsus catarinensis*		*Neochetina eichhorniae* and *N. bruchi*	
	Year of release	Present at study site	Year of release	Present at study site
Breede River	2003	yes	N/A	yes
Crocodile River	N/A	yes	1993	yes
Delta Park	1999	no	N/A	yes
Enseleni River	1996	yes	1996	yes
Farm Dam	Never released	no	N/A	yes
Feesgronde	N/A	no	1989	yes
Hammarsdale Dam	1996	yes	1989	yes
Kubusi River	1999	yes	1999	yes
Mbozambo Swamp	N/A	yes	1990	yes
Mkadhzi Spruit	N/A	no	N/A	yes
New Years Dam	1999	no	1999	yes
Princess Vlei	N/A	yes	N/A	yes
Warrenton Weir	1999	no	1999	yes
Wolseley	2000	no	2000	yes
Yamorna Weir	2001	yes	2001	yes

No relationship was observed between the percentage feeding damage of *E. catarinensis* and the presence of adult *N. bruchi* (Pearson correlation test $r^2 = 0.002$, $P > 0.05$) (Figure 5.1). This accords with the results obtained from the common garden experiment reported in chapter 3, which found a neutral interaction between these two insects, with no effect on their performance when in combination. This would suggest that these two species are compatible under field conditions in South Africa. Again, however, the subtle effects would not have been identified in this large scale study.

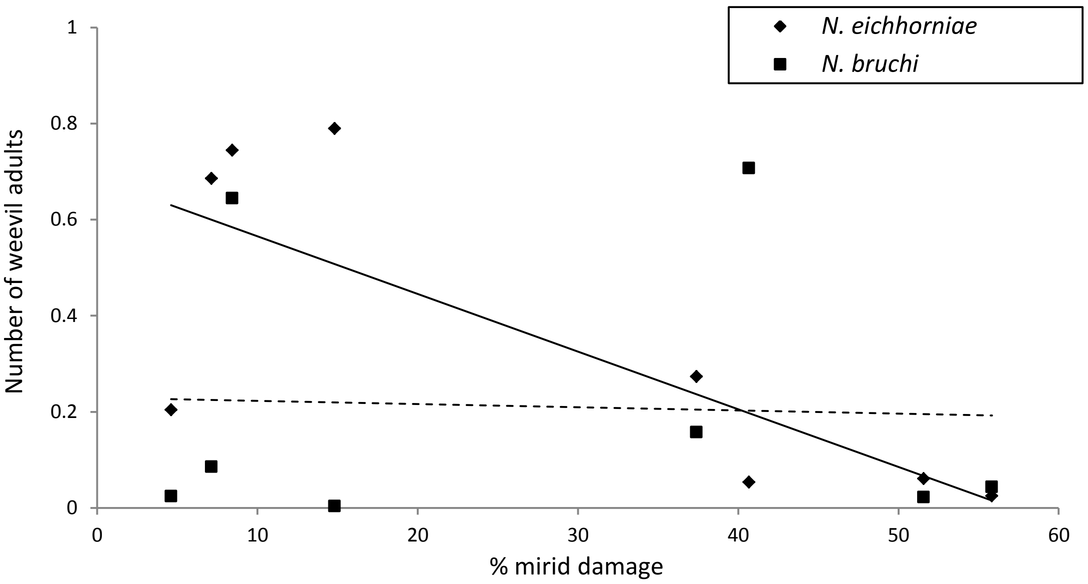

Figure 5.1 The correlation between the percentage feeding damage of *Eccritotarsus catarinensis* and the average number of weevil adults. The solid line represents the trend between *E. catarinensis* and the average number of adult *Neochetina eichhorniae* (Pearson correlation test $r^2 = 0.59$, $P < 0.05$) and the dashed line represents the trend between *E. catarinensis* and *N. bruchi* (Pearson correlation test $r^2 = 0.002$, $P > 0.05$).

On the other hand there was a strong and significant negative relationship between the percentage feeding damage of *E. catarinensis* and the presence of *N. eichhorniae* adults (Pearson correlation test $r^2 = 0.59$, $P < 0.05$) (Figure 5.1). This means that as the percentage feeding of the mirid increases there is a decrease in the number of *N. eichhorniae* adults at eight independent sites around the country. Although this is correlative, it corroborates the results from the field study in chapter 4, which suggested spatial segregation of these two species but not necessarily on a landscape level as the data would suggest, because subtle interactions are not observed in these large scale studies. It is possible that the sites used in each system were dominated by either the mirid or the weevil. For example the Breede River site had good *E. catarinensis* damage but very few *N. eichhorniae* adults present, while the Mbozambo Swamp site had poor *E. catarinensis* damage but high numbers of *N. eichhorniae* adults present.

The correlation between the number of *N. eichhorniae* and *N. bruchi* adults was also investigated to determine whether there was an interaction between these species in a field situation. The average number of adults of each species at all the sites was compared and a Pearson correlation test was conducted. The results from chapter 3 suggest that there was a negative relationship between these species, however this was not observed in the field sites with no relationship between the number of individuals (Pearson correlation test $r^2 = 0.00$, $P > 0.05$) (Figure 5.2). The field data from Byrne *et al.* (2010) suggest that these two species are compatible. However, intensive field studies at these sites would be necessary to reveal any subtle interactions that these insects may be having under natural conditions, as have been highlighted in studies done in the native range (De Loach & Cordo 1976b).

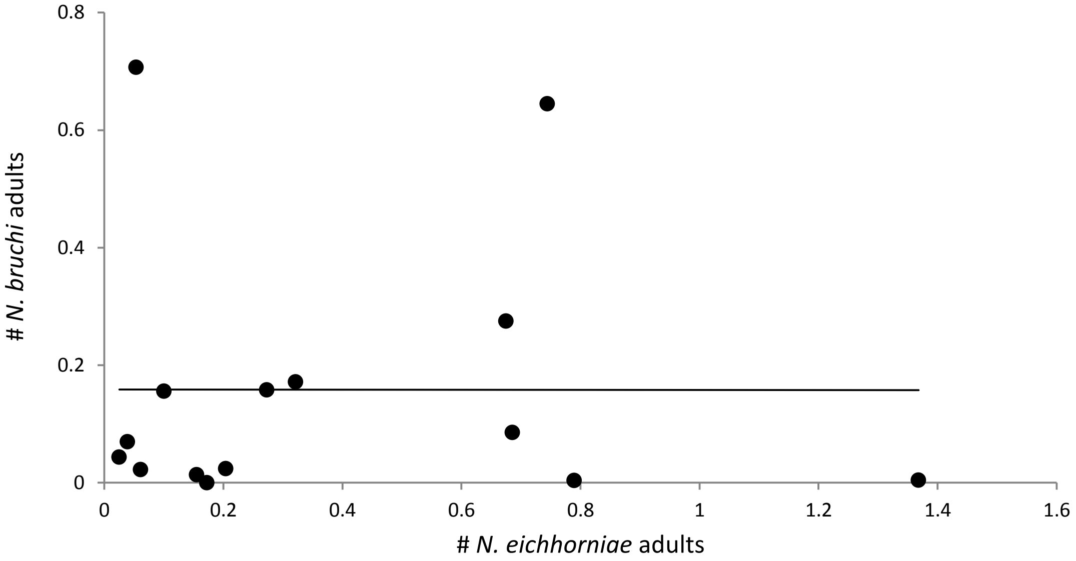

Figure 5.2 The correlation between the number of adult *N. eichhorniae* and *N. bruchi* (Pearson correlation test $r^2 = 0.00$, $P > 0.05$).

Biological control agents that are introduced are usually free of all specialised predators and parasites which can amplify the importance of competitive interactions on their population dynamics (Huffaker & Kennet 1969). Because two insects coexist in the native range the assumption cannot be made that the same two insects will be compatible in the introduced range. Several studies highlight the competitive interactions of introduced insects which in some cases can result in complete exclusion from an area in a wide range of systems (McClure 1980; 1989; Denno *et al.* 1995). The results from this thesis suggest that the most appropriate insect be released first into an area or infestation, and that this can be done based on the preferences of the insect, which may include plant phenology, nutrient status preferences and climate matching. If additional agents are justified after a full evaluation has been made of the efficacy of the already released agent, the next appropriate agent should be released after a "pre-release" study has been done to determine the possible interactions between the agents.

The prediction of the interactions between biological control agents is an extremely difficult task, with several uncontrollable factors playing a role. These may include attributes of the insect, the target weed and the environment (Cullen 1995; Zalucki & van Klinken 2006; Impson *et al.* 2008). Despite the difficulties of predicting the interactions, this thesis shows that pre-release studies investigating interactions between agents are able to identify combinations of insects that have the potential to compete in the field. The more agents that are released into a system the more complex the system becomes and ultimately the more difficult it becomes to investigate and predict the results of interactions between agents.

To a certain degree the agents released for the biological control of water hyacinth, tested in this study, are likely to be complimentary in the field and it is unlikely that there are any implications for the efficacy of the programme. However any additional agents released into an already established biological control programme should be well justified, done with extreme caution, and only where the best predictions can be made.

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Appendix 1

An adapted catalogue from Klein (2011) highlighting the biological control programmes that have been initiated in South Africa and where at least one agent has established. R, the number of species released; E, the number of species established; I, the number of species involved in success; O, the presence of overlapping niches.

Target weed	**Level of control**	**R**	**E**	**I**	**O**	**Species that have established**	**Feeding guild**	**Damage inflicted**
Acacia baileyana F. Muell.	Negligible	1	1	1	n/a	*Melanterius maculatus* Lea	Seed feeder	Moderate
Acacia decurrens (Wendl.) Willd.	Negligible	1	1	1	n/a	*Melanterius maculatus* Lea	Seed feeder	Moderate
Acacia melanoxylon R.Br.	Substantial	1	1	1	n/a	*Melanterius acaciae* Lea	Seed feeder	Extensive
Ageratina riparia (Regel) R.M.King & H.Rob.	Complete	1	1	1	n/a	*Entyloma ageratinae* Barreto & Evans	Leaf pathogen	Considerable
Azolla filiculoides Lam.	Complete	1	1	1	n/a	*Stenopelmus rufinasus* Gyllenhal	Frond feeder	Extensive
Caesalpinia decapetala (Roth) Alston	Negligible	1	1	0	n/a	*Sulcobruchus subsuturalis* (Pic)	Seed feeder	Trivial
Cirsium vulgare (Savi) Ten.	Negligible	2	1	0	n/a	*Rhinocyllus conicus* (Froelich)	Seed feeder	Moderate
Cylindropuntia imbricata (Haw.) Knuth.	Substantial	2	1	1	n/a	*Dactylopius tomentosus* (Lamark)	Cladode sucker	Considerable
Cylindropuntia leptocaulis (DC.) Knuth.	Complete	1	1	1	n/a	*Dactylopius tomentosus* (Lamark)	Cladode sucker	Extensive
Harrisia bonplandii (Pfeiff.) Britton & Rose	Substantial	1	1	1	n/a	*Hypogeococcus pungens* Granara de Willink	Stem sucker	Extensive
Leucaena leucocephala (Lam.) de Wit	Negligible	1	1	0	n/a	*Acanthoscelides macrophthalmus* (Schaeffer)	Seed feeder	Trivial

Target weed	Level of control	R	E	I	O	Species that have established	Feeding guild	Damage inflicted
Myriophyllum aquaticum (Vell.) Verdc.	Substantial	1	1	1	n/a	*Lysathia* sp.	Leaf feeder	Extensive
Opuntia salmiana J.Parm. Ex Pfeiff.	Substantial	1	1	1	n/a	*Cactoblastis cactorum* (Berg)	Cladode borer	Considerable
Paraserianthes lophantha (Willd.) Nielsen	Substantial	1	1	1	n/a	*Melanterius servulus* Pascoe	Seed feeder	Considerable
Pereskia aculeata Mill.	Negligible	1	1	0	n/a	*Phenrica guerini* Bechyné	Leaf feeder	Trivial
Pistia stratiotes L.	Complete	1	1	1	n/a	*Neohydronomus affinis* Hustache	Leaf and stem borer	Extensive
Salvinia molesta D.S.Mitch.	Complete	1	1	1	n/a	*Cyrtobagous salviniae* Calder & Sands	Stem borer	Extensive
Solanum sysimbriifolium Lam.	Substantial	1	1	1	n/a	*Gratiana spadicea* (Klug)	Leaf feeder	Extensive
Acacia cyclops A. Cunn. ex G. Don.	Substantial	2	2	2	no	*Dasineura dielsi* Rübsaamen	Flower galler	Extensive
						Melanterius servulus Pascoe	Seed feeder	Considerable
Acacia longifolia (Andr.) Willd.	Substantial	2	2	2	no	*Melanterius ventralis* Lea	Seed feeder	Extensive
						Trichilogaster acaciaelongifoliae (Froggatt)	Bud galler	Extensive
Acacia pycnantha Benth.	Substantial	2	2	1	no	*Melanterius maculatus* Lea	Seed feeder	Moderate
						Trichilogaster signiventris (Girault)	Bud galler	Considerable/ Extensive
Acacia saligna (Labill.) H.L.Wendl.	Substantial	2	2	1	no	*Melanterius compactus* Lea	Seed feeder	Considerable
						Uromycladium tepperianum (Sacc.) McAlpine	Gall former	Extensive

Target weed	**Level of control**	**R**	**E**	**I**	**O**	**Species that have established**	**Feeding guild**	**Damage inflicted**
Ageratina adenophora (Spreng.) R.M.King & H.Rob.	Negligible	2	2	0	no	*Passalora ageratinae* Crous & A.R. Wood	Leaf spot pathogen	Moderate
						Procecidochares utilis Stone	Stem galler	Moderate
Cereus jamacaru DC.	Complete	2	2	1	yes	*Hypogeococcus pungens* Granara de Willink	Stem sucker	Extensive
						Nealcidion cereicola (Fisher)	Stem borer	Considerable
Cylindropuntia fulgida (Engelm.) Knuth.	Complete	2	2	1	yes	*Dactylopius tomentosus* (Lamark)	Cladode sucker	Extensive
						Dactylopius tomentosus (Lamark)	Cladode sucker	Trivial
Hakea gibbosa (Sm.) Cav.	Negligible	2	2	0	no	*Aphanasium australe* (Boisduval)	Stem borer	Unknown
						Erytenna consputa Pascoe	Green-seed feeder	Trivial
Harrisia martinii (Labour.) Britton & Rose	Complete	2	2	1	yes	*Hypogeococcus pungens* Granara de Willink.	Stem sucker	Extensive
						Nealcidion cereicola (Fisher)	Stem borer	Considerable
Hypericum perforatum L.	Complete	6	2	1	no	*Chrysolina quadrigemina* Suffrian	Leaf feeder	Extensive
						Zeuxidiplosis giardi (Kieffer)	Shoot-tip galler	Moderate
Leptospermum laevigatum (Gaertn.) F.Muell.	Negligible	2	2	0	no	*Aristaea thalassias* (Meyrick)	Leaf feeder	Considerable

Target weed	**Level of control**	**R**	**E**	**I**	**O**	**Species that have established**	**Feeding guild**	**Damage inflicted**
						Dasineura strobila Dorchin	Bud galler	Considerable
Opuntia aurantiaca Lindl.	Substantial	5	2	1	yes	*Cactoblastis cactorum* (Berg)	Cladode borer	Moderate
						Dactylopius austrinus De Lotto	Cladode sucker	Extensive
Opuntia engelmannii Salm-Dyck ex Engelm.	Substantial	2	2	1	yes	*Cactoblastis cactorum* (Berg)	Cladode borer	Extensive
						Dactylopius opuntiae (Cockerell)	Cladode sucker	Considerable
Opuntia monacantha Haw.	Substantial	2	2	1	yes	*Cactoblastis cactorum* (Berg)	Cladode borer	Considerable
						Dactylopius ceylonicus (Green)	Cladode sucker	Extensive
Opuntia stricta (Haw.) Haw.	Substantial	2	2	2	yes	*Cactoblastis cactorum* (Berg)	Cladode borer	Extensive
						Dactylopius opuntiae (Cockerell)	Cladode sucker	Extensive
Prosopis L. sp.	Negligible	3	2	0	yes	*Algarobius prosopis* (LeConte)	Seed feeder	Considerable
						Neltumius arizonensis (Schaeffer)	Seed feeder	Unknown
Solanum elaeagnifolium Cav.	Substantial	4	2	1	yes	*Leptinotarsa defecta* (Stål)	Leaf feeder	Moderate

Target weed	Level of control	R	E	I	O	Species that have established	Feeding guild	Damage inflicted
						Leptinotarsa texana (Schaeffer)	Leaf feeder	Extensive
Solanum mauritianum Scop.	Negligible	2	2	0	no	*Anthonomus santacruzi* Hustache	Flowerbud feeder	Unknown
						Gargaphia decoris Drake	Leaf sucker	Trivial
Sesbania punicea (Cav.) Benth.	Complete	3	3	3	no	*Neodiplogrammus quadrivittatus* (Olivier)	Stem borer	Extensive
						Rhyssomatus marginatus Fåhraeus	Seed feeder	Extensive
						Trichapion lativentre (Béguin-Billecocq)	Flowerbud feeder	Extensive
Macfadyena unguis-cati (L.) A.H.Gentry	Negligible	5	4	0	yes	*Carvalhotingis hollandi* Drake	Leaf sucker	Unknown
						Carvalhotingis visenda Drake & Hambleton	Leaf sucker	Moderate
						Charidotis auroguttata Boheman	Leaf feeder	Trivial
						Hylaeogena (*Hedwigiella*) *jureceki* Obenberger	Leaf miner	Unknown
Opuntia ficus-indica (L.) Mill.	Substantial	4	4	3	yes	*Cactoblastis cactorum* (Berg)	Cladode borer	Extensive
						Dactylopius opuntiae (Cockerell)	Cladode sucker	Extensive
						Lagocheirus funestus (Thompson)	Stem borer	Trivial

Target weed	Level of control	R	E	I	O	Species that have established	Feeding guild	Damage inflicted
						Metamasius spinolae (Gyllenhal)	Stem borer	Extensive
Hakea sericea Schrad. & J.C.Wendl.	Substantial	5	5	1	yes	*Aphanasium australe* (Boisduval)	Stem borer	Unknown
						Carposina autologa Meyrick	Seed feeder	Considerable
						Cydmaea binotata Lea	Leaf & shoot borer	Trivial
						Dicomada rufa Blackburn	Flowerbud feeder	Unknown
						Erytenna consputa Pascoe	Green-seed feeder	Extensive
Eichhornia crassipes (C.Mart.) Solms.	Substantial	7	6	6	yes	*Cercospora rodmanii* (Conway)	Leaf pathogen	Considerable
						Eccritotarsus catarinensis (Carvalho)	Leaf sucker	Considerable
						Neochetina bruchi Hustache	Stem borer	Considerable
						Neochetina eichhorniae Warner	Stem borer	Considerable
						Niphograpta albiguttalis Warren.	Petiole borer	Considerable
						Orthogalumna terebrantis Wallwork	Leaf miner	Considerable
Lantana camara L.	Negligible/ Substantial	24	16	0	yes	*Aceria lantanae* (Cook)	Flower galler	Extensive
						Calycomyza lantanae (Frick)	Leaf miner	Moderate
						Coelocephalapion camarae Kissinger	Petiole galler	Unknown

Target weed	Level of control	R	E	I	O	Species that have established	Feeding guild	Damage inflicted
						Crocidosema lantana (Busck)	Flower- & receptacle miner	Trivial
						Falconia intermedia (Distant)	Leaf sucker	Moderate
						Hypena laceratalis Walker	Leaf feeder	Moderate
						Lantanophaga pusillidactyla (Walker)	Flower miner	Trivial
						Longitarsus bethae Savini & Escalona	Root feeder	Unknown
						Octotoma scabripennis Guèrin-Mèneville	Leaf miner	Considerable
						Ophiomyia camarae Spencer	Leaf miner	Considerable
						Ophiomyia lantanae (Froggatt)	Seed miner	Moderate
						Salbia haemorrhoidalis (Guenée)	Leaf feeder	Trivial
						Teleonemia scrupulosa Stål	Leaf & flower sucker	Considerable
						Uroplata girardi Pic	Leaf miner	Considerable