A study on the interaction between two weevils *Neochetina eichhorniae* and *N. bruchi*, and the mirid *Eccritotarsus catarinensis*, as biological control agents of water hyacinth *Eichhornia crassipes*.

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Abstract

Water hyacinth *Eichhornia crassipes* (Martius) Solms-Laubach is an invasive floating aquatic weed pest of South American origin that was distributed around the world primarily because of its attractive purple flowers. Due to its rapid growth, it is an important aquatic weed in tropical and subtropical regions worldwide. In Benin, it affects boat traffic, fishing and domestic use of water by about 200,000 inhabitants in several communities. When it became a serious problem in the late 1980s, the only control option approved by the government of Benin was biological control because it is sustainable and environmentally friendly.

This dissertation examined the biological control agents released against the weed. Two studies were conducted on the interaction between arthropod biological control agents, using the mirid *Eccritotarsus catarinensis* (Carvalho), and two weevils, *Neochetina eichhorniae* (Warner) and *N. bruchi* Hustache. The third study, investigated the displacement of *E. catarinensis* in the laboratory culture by an indigenous mirid *Nycticapsus* sp.

The objective of the first study was to determine the influence of adult weevil feeding scars on the mirid released to complement biological control by the weevil. Result show that adults and nymphs of *E. catarinensis* had high mortality (3-5 folds) on plants with high levels of old feeding scars by adult *N. eichhorniae* and *N. bruchi*. In contrast, mirids survived well on plants with recent adult weevil feeding scars or on undamaged plants. These results indicate that the mirid is compatible with the two weevil species under certain conditions and will therefore contribute to enhanced biological control of the weed.

The second study investigated the impact of *N. eichhorniae* and *E*. *catarinensis* separately and in combination on water hyacinth grown in culture with another aquatic plant, water lettuce (*Pistia stratiotes*), at varying planting densities. Without herbivory by either agent, water hyacinth was 18 times more competitive than water lettuce, as estimated from the fresh weight. Reduction in the production of flowers by herbivory was highest (100%) in the treatment with both species. On free water, where both herbivores can reproduce well, mirids might have a short-term initial effect, but in the longer term, when all water hyacinth plants become infested, the weevil out-competes the mirids completely. In treatment with both species, the combined use of the weevils and the mirids is, however, highly justified essentially in situations where the weevil is not efficient, as on stranded water hyacinth where the weevil pupae cannot survive.

Nycticapsus sp was regarded as one of the reasons for the failure of *E. catarinensis* to establish in Benin. Therefore in the third study, *E. catarinensis* was reproductively more competitive, producing 816.8 adults compared to 451.1 adults by *Nycticapsus* in single species treatments and (372.2) compared to (268.0) in combination experiment did not show that *Nycticapsus* sp. could displace *E. catarinensis* in a laboratory culture. In conclusion, where the weevils have already established, it will not prevent the establishment of the mirid. Combining the mirid with the weevil will have either an additive effects on water hyacinth populations, or, at worst no effect. The mirid could provide also alternative control at sites where the weevils do not establish because of unfavourable/unsuitable environmental conditions.

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Masters of Science in the University of Witwatersrand, Johannesburg. It has not been submitted for any degree or examination in any other University.

_____ _____6th__Day of February 2009

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CHAPTER ONE

INTRODUCTION

1. 1 Aquatic weeds, as pests

Some of the most troublesome floating aquatic plant species were deliberately or accidentally introduced by man, as ornamental plants or for use in the aquarium trade, without their natural enemies into new areas where they have became invasive. The most notorious of these, are water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae); water lettuce, *Pistia stratiotes,* L. (Araceae); giant Salvinia or Kariba weed, *Salvinia molesta* D. S. Mitchell, (Salviniaceae); and the red water fern, *Azolla filiculoides* Lamarck (Azollaceae) (Cilliers *et al*., 2003). Other, possibly less notorious species include alligator weed, *Alternanthera philoxeroides* and hydrilla, *Hydrilla verticillata* (L.F.) Royle) (Center *et al*., 1989).

Water hyacinth is considered the most damaging aquatic weed worldwide and was spread around the world as an ornamental plant because of its attractive flowers. Water hyacinth is now established throughout tropical, subtropical and warm temperate regions of the world (Julien *et al*., 1999). As an invader of aquatic communities, water hyacinth out-competes other plant species, both native and introduced (Wright and Purcell, 1995). Some inherent characters that contribute to water hyacinth's success as an invader are: (1) it invades fresh waters in nutrient-rich eutrophic environments; (2) it has a high rate of growth and multiplication; (3) it produces seeds that remain viable for a long period; (4) it has a wide ecological impact and exhibits great phenotypic plasticity (Gopal, 1987; Wright and Purcell, 1995).

1.2 Origin and the distribution of water hyacinth in Africa

Water hyacinth originates in the Amazon basin of Brazil, (Harley, 1990; Henderson, 2001) from where it has spread to many parts of Central and Southern America (Julien *et al.,* 1999), and to the USA in 1884 (Center et al., 1989) as an ornamental. It was introduced to Africa, in Egypt in the late 1800s, Tanzania in 1960 (Navarro and Phiri, 2000). Its presence was reported in Zimbabwe in 1937 (Chikwenhere and Phiri, 1998) and in the Congo Basin, Central Africa in the 1950s (Mbati and Neuenschwander, 2004). In West Africa, it was observed in Senegal by 1964 (Bennett and Zwölfer, 1968), and Benin by 1977 (van Thielen *et al*., 1994), Côte d'Ivoire, and Ghana in 1984 (ECOWAS, 1995; De Graft Johnson, 1988) and at the same time in Nigeria (Akinyemiju, 1987, Onyia *et al*., 1988). Water hyacinth was first reported in the province of Natal, South Africa in 1910 (Navarro and Phiri, 2000). The warm tropical and subtropical climates in these regions have particularly favoured the growth and proliferation of water hyacinth (Center *et al*., 1989).

1.3 Habitat and the biology of water hyacinth

The habit of the species is floating, erect with aerial leaves and submerged roots and rhizome (Center and Spencer, 1981). The petiole contains air that causes it to float, a character that distinguishes it from other members of the Pontederiaceae family (Julien *et al*., 1999). There are two leaf forms of water hyacinth plants; those with bulbous petiole up to 25cm long but usually less, and plants with slender petioles, 60cm or longer (Wright and Purcell, 1995). It grows best in tropical and subtropical fresh waters, rivers and lakes (Center and Spencer, 1981). Biomass doubling times of 6-18 (Schmitz *et al*., 1993) and 12-15 days (Wright and Purcell, 1995) have been reported. The growth form of water hyacinth is very plastic according to environmental changes and in responding to such changes, it will vary growth characters such as biomass, leaf and petiole lengths, petiole forms, sexual and vegetative reproduction.

Water hyacinth can survive for several months on a substrate of low moisture (Wright and Purcell 1995). Water bodies enriched with nutrients such as nitrogen, phosphorus and potassium favour its growth (Imaoka and Teranishi, 1988; Wilson *et al*., 2006). It can tolerate variations in pH levels but dies in waters with salinity higher than 0.06% (Wright and Purcell, 1995).

Reproduction in water hyacinth is by vegetative propagation as well as through seeds. Vegetative reproduction is through vertical branching of a ramet or stolon that is identical to the parent plants (Center, 1981). Low nutrient conditions are known to induce flowering (Watson and Brochier 1988). Seed germination requires warm, shallow water and high light intensity (Center and Spencer, 1981) and in the field, germination is usually observed on shorelines of lagoons (Wright and Purcell, 1995; Ajuonu personal observations).

1.4 Importance of water hyacinth

Water bodies are important for the socio-economic activities of human populations living around them. They play important roles in the equilibrium of climatic factors and also provide homes for a rich diversity of plant species and animals.

The presence of water hyacinth on water bodies is detrimental because its rapid growth and accumulation of large biomass enables it to block waterways, thereby impeding communication, trading, fishing and transport by boat or canoe, leading to increased transport costs and loss of revenue. In Benin, the most important impact was on fishing activities for men and lost revenues in trade for women (de Groote *et al*., 2003). On Lake Victoria, along lake side communities, landing of boats weighing less than 700 tonnes were hampered by the presence of water hyacinth (Mailu, 2001).

Water hyacinth negatively affected fish production in the Niger Republic on the Niger River, where about 40% loss in fish yield due to interference with fishing nets was recorded (ECOWAS, 1995). On Lake Victoria, due to the inability of fishermen along the lake sides to access fishing grounds, the quantity of some fish species caught declined by 14-59% (Mailu, 2001). Similarly, effects on human health, by providing refuge for disease causing organisms such as mosquitoes and the vectors of schistosomiasis (bilharzia), have been reported (Harley, 1990; de Groote *et al*., 2003).

Water hyacinth has negative effects on habitat qualities. Because of its rapid growth, it quickly forms thick mats over water bodies (Akinyemiju, 1987), and can out-compete native aquatic species (Wright and Purcell, 1995). The ecological impact of water hyacinth has been reviewed (Schmitz *et al*., 1993) where the authors noted that changes in the physiochemical environment beneath water hyacinth mats leads to increased dissolved carbon dioxide concentration, lower dissolved oxygen and phosphorous concentration and decreased phytoplankton populations. Water bodies maintain a host of natural ecosystems and by altering the habitat quality, the population of waterfowl and other wild life are reduced (Harley, 1990). In a study at two impoundments, Midgley *et al*. (2006) reported a reduction of the benthic invertebrate communities and algal biomass due to cover by water hyacinth.

Water hyacinth is expected to increase evatranpiration by 3.7 times compared to open water (Timer and Weldon, 1967). Water that could be useful for human activities such as drinking and irrigation is lost through increased evapo-transpiration.

1.5 Control of water hyacinth

Complete removal of water hyacinth is not possible in most areas. Where an infestation has been eradicated, this is short-term because re-infestation readily occurs either by seed, floating mats or transport by man (Julien *et al*., 1999).

The techniques for the control of water hyacinth include: (1) the use of herbicides, (2) mechanical control and manual clearing, (3) biological control using host-specific natural enemies and (4) applying different techniques in an integrated approach. Utilisation is often proposed as a means of control, however, factors such as difficulties in harvesting, due to its high water content, proper disposal and market acceptance of its products must be considered (Harley, 1990). Among these methods, biological control has gained importance following an increasing awareness to reduce herbicides in the environment, health and for sustainability (Greathead, 2003; Neuenschwander, 2004). Although arthropod biological control agents are widely used and have been effective in many locations, there is the need to develop pathogens into bio-herbicides, to further improve control through an integrated approach (Charudattan, 2001).

1.6 Review of global biological control of water hyacinth

Exploration in South America, the native home of water hyacinth, for biological control agents started in 1960 by the United States Department of Agriculture (USDA) scientists supported by funds from the U.S. Army Corps of Engineers, with the first release of insect agents in 1972 (Center *et al*., 1989). The search for new agents has been continuing while laboratory testing is being conducted on some potential agents (Julien, 2001).

Around the world, seven arthropod agents have been released for the classical biological control of water hyacinth (Julien *et al*., 1999; Harley, 1990). The agents

are; two Coleoptera (Curculionidae), *Neochetina eichhorniae* Hustache and *Neochetina bruchi* (Warner), three Lepidoptera, *Bellura densa* Walker, *Niphograpta albiguttalis* (Warren) (formally *Sameodes albiguttalis*) and *Xubida infussella* (Walker), a mirid (Miridae), *Eccritotarsus catarinensis* (Carvalho), and a mite Galumnidae, *Orthogalumna terebrantis* (Wallwork). Six arthropod agents have been released across 40 countries of the world (Table 1). Among the six agents (Harley, 1990; Julien *et al*., 1999), the two weevils, *N. eichhorniae* and *N. bruchi*, are considered the most efficient (DeLoach and Cordo, 1976; Center and Van, 1989; Center *et al*., 1999). The species *Bellura densa* is native to the USA and where augmentative releases are carried out (Harley, 1990). It was considered for release in South Africa but was rejected because plants in the Araceae family were at risk of being attacked by this species (Center and Hill, 2002).

The biology of the two weevils is similar (DeLoach and Cordo, 1976; Stark and Goyer 1983; Julien *et al*., 1999). The generation time is 120 days for *E. eichhorniae* and 96 days for *N. bruchi* (Harley, 1990). Adult weevils feed nocturnally on water hyacinth leaves, by removing the epidermis together with some of the lower layers of underlying cells, causing feeding scars (Deloach and Cordo, 1976). They prefer young leaves in the first and second leaf positions. Females insert their eggs into the leaf blade, petiole and ligules. Larvae develop inside the petiole and rhizomes, and then bore out to pupate under water in a cocoon that is attached to the water hyacinth roots.

The biology of the mirid *E. catarinensis* was described by Hill *et al*., (1999b) and Stanley and Julien (1999). It is a sap-sucking bug with a life cycle of approximately around three weeks, active mostly during the day when females insert their eggs into water hyacinth leaf tissue. Both adults and immatures feed on water hyacinth leaves causing yellowing or chlorosis.

Table1.1 Countries where arthropod control agents have been released on water hyacinth and the dates of initial releases (data modified from Harley, 1990; Julien 2001).

	Neochetina bruchi	*Neochetina eichhorniae*	*Niphograpta albiguttalis*	*Eccritotarsus catarinensis*	*Orthogalumna terebrantis*	*Xubida infusellus*
Australia	1990	1995	1977			1981;1996
Benin	1992	1991	1993	1999[b]		
Burkina Faso	1997[a]	1997[a]				
China	1996	1996		2000		
Congo Brazaville	1999[b]	1999[b]				
Cotê d'Ivoire	1997[a]	1997[a]	1997[a]			
Cuba	1995					
Egypt	2000	2000				
Fiji		1977	1996			
Ghana	1994	1994				
Honduras	1989	1990				
India	1984	1983			1986	
Indonasia	1996	1977				
Jamaica						
Kenya	1995	1993	2004		2004[e]	
Malawi	1995	1995	1996	1996		
Malaysia	1992	1983	1996			
Mali	2000[c]	2000[c]				
Mexico	1995	1972				
Mozambique	1972	1972				
Myanmar		1980				
Niger Republic		1999[d]				
Nigeria	1995	1993				
Panama	1997		1977			
Philippines	1992	1992				
PNG	1993	1986	1994			1996
Rwanda	2000	2000				
Solomon Islands		1988				
South Africa	1989	1974	1990	1996	1989[f]	
Sri Lanka		1988				
Sudan	1979	1978	1980			
Taiwan	1993	1992				
Tanzania	1995	1995				
Thailand	1991	1979	1995			1999
Togo	2000[c]	2000[c]				
Uganda	1993	1993				
USA	1974	1972	1977			
Vietnam	1996	1984				
Zambia	1997	1971;1996	1971;1997	1997	1971	
Zimbabwe	1996	1971	1994	1999		
Totals	33	36	14	6	5	3

a. IITA (1997); b. IITA (1999); c. de Graft Johnson (pers. comm.); d. Ajuonu et al., (2003) (spread from Nigeria to Niger Republic); e. Gitonga Waweru (pers. comm.); f. Martin Hill (pers com.)

The biological control of water hyacinth has been successful in many countries; Papua New Guinea (Julien and Orapa, 1999), Sudan (Beshir and Bennett, 1985), South Eastern USA (Center *et al*., 1989), Lake Victoria (Ochiel *et al*., 2001; Wilson *et al*., 2007), in Benin (Ajuonu *et al*., 2003) and other countries in West Africa (Cilliers et al., 2003). How long it takes to achieve effective control of water hyacinth ranges from several months to many years with the level of control varying among environments, between sites and countries. A control period of eight years has been reported at some sites in Benin (Ajuonu *et al*., 2003), four to six years in Argentina (Harley, 1990) and five years in Uganda, on Lake Victoria (Ogwang James, Namulonge Agricultural Research Institute, Kampala, Uganda. pers com).

1.7 Factors constraining biological control of water hyacinth

Success in biological control of water hyacinth has been limited by several factors and this varies among countries and location. In South Africa, major constraints are cold winters, highly eutrophic waters, periodic removal of weeds and natural enemies through flooding, drought and interference from other control methods (Hill and Cilliers, 1999a; Hill and Olckers, 2001).

In Benin, complicated interactions between water flows, wind, rain/dry seasons, activities of fishermen, and salinity influence the survival and dispersal of water hyacinth (van Thielen *et al*., 1994; Neuenschwander *et al*., 1996) and therefore biological control. In Southern Benin, development of water hyacinth is influenced mainly by the annual rainfall, usually between May–October in the North (from about 9^0N) and partly by those in the South, from March to November. During this period, water flows South, drifting water hyacinth in small mats and combined with the inflow of fresh water, plant growth becomes favourable. When water flow is weak, prevailing winds from the South drift water hyacinth upstream.

During the dry season, which lasts from November to April, water levels decline in many locations particularly in lakes with shallow banks and water hyacinth plants eventually root in the mud (Ajuonu *et al*., 2003). Under these circumstances, plants suitable for weevils are not readily available and therefore the population build-up of weevils is slow (Ganga and Jayanth, 1996).

Fishing activities have also influenced biological control activities. Fishermen remove water hyacinth to free space for the installation of a fish trap call “acadja”. In the process, some plants which are either carrying adult beetles or with pupae attached to the roots are destroyed. However, this action did not prevent establishment at this particular site (van Thielen *et al*., 1994).

Nutrient status is a limiting factor for growth of water hyacinth (Sastroutomo *et al*., 1978; Wright and Purcell, 1995) and therefore, biological control agents (Heard and Winterton, 2000; Center *et al*., 2005; Wilson *et al*., 2006). At high nutrient levels, water hyacinth will produce 0.15 leaves per day compared to 0.07 in conditions of low nutrients (Center, 1981). Besides nutrients, salt concentrations of 2.5% were found to be toxic to water hyacinth (Haller *et al*., 1974) and in the Badagry creeks (Lagos, Nigeria), water hyacinth was less abundant in February, April and May when the water had the highest levels of salinity (Eborge, 1988).

Temperature is also an important factor. Growth of water hyacinth is rapid in the temperature range 22-35^{o}C (Wright and Purcell, 1995). During the dry season, when temperatures are very high (e.g. 40^{o}C), reduction in water hyacinth growth was observed which indirectly affected biological control (Femi Daddy, National Institute for Freshwater Fisheries Research, New Bussa, Nigeria, pers. comm).

Some of the limitations of the biological control of water hyacinth are linked to the biology of the agents themselves. For example, the developing larvae of *N. eichhorniae* and *N. bruchi* tunnel downward inside the petioles towards the base of the plant (Delfosse, 1978) and pupation occurs under water within the water hyacinth roots (Deloach and Cordo, 1976). Water hyacinth can survive for several months on a substrate of low moisture (Wright and Purcell, 1995) but the pupae are subjected to high mortality when the plants are silted (Ganga and Jayanth, 1996) or stranded in shallow mud banks when the water recedes (Ajuonu *et al*., 2003). Under such conditions, biological control is ineffective or slow. Also, the first and second instar larvae are subject to leaf mortality, because they can not easily bore from one petiole to another when compared to third and fourth larval stages, (Center, 1987; Wilson, *et al*. 2006).

The preference of agents for specific forms of water hyacinth or plant parts can be a limiting factor. The moth *N. albiguttalis* prefers the water hyacinth form with young bulbous petioles (DeLoach and Cordo, 1978) and therefore may not be effective in conditions of dense mats with tall petioles. The mite *O. terebrantis* spends its life on the water hyacinth leaf lamina and rarely kills the shoots of water hyacinth (Center, 1984). Similar to the mite, the mirid *E. catarinensis* lives on leaf lamina and causes subtle damage (Coetzee *et al*., 2003) in which case water hyacinth plants could recover (Ajuonu *et al*. submitted and in review).

In response to these constrains some countries have introduced several control agent species, yet control has remained slow or ineffective, even many years after their release and establishment, e.g. in South Africa (Hill and Cilliers, 1999a) and Benin (Ajuonu *et al*., 2003). This is in contrast to the other three aquatic weed species, *P. stratiotes* (Mbati and Neuenschwander, 2004; Harley *et al*., 1990), *S. molesta*, (Room, 1986), *A. filiculoide*s (Hill, 1997; Hill, 1999) where one, host-specific agent has successfully controlled each of them in many countries, particularly in Africa (Hill and Cilliers, 1999b; Cilliers *et al*., 2003).

1.8 Water hyacinth in Benin

Benin (Fig. 1.1) is situated between latitude 06^{o}30$^{'}$ and 12^{o}30$^{'}$N and longitude 01^{o} and 03^{o}40$^{'}$E. About sixty percent of the population is concentrated in the three southern regions namely Atlantique, Mono and Ouémé (Adam and Boko, 1993).

Water hyacinth was first recorded in 1977 and was recognised as a serious problem 10 years later (van Thielen *et al*., 1994), with infestations mostly in the southern regions, on the Ouémé, Zou, and the Mono River systems, below latitude 07°.30N (Fig 1.1). The Mono River was until mid 1993, free of water hyacinth (Neuenschwander *et al*., 1996). The highest infestation is found on the Ouémé followed by the Zou River system and stretches about 100km into the upper reaches of both rivers.

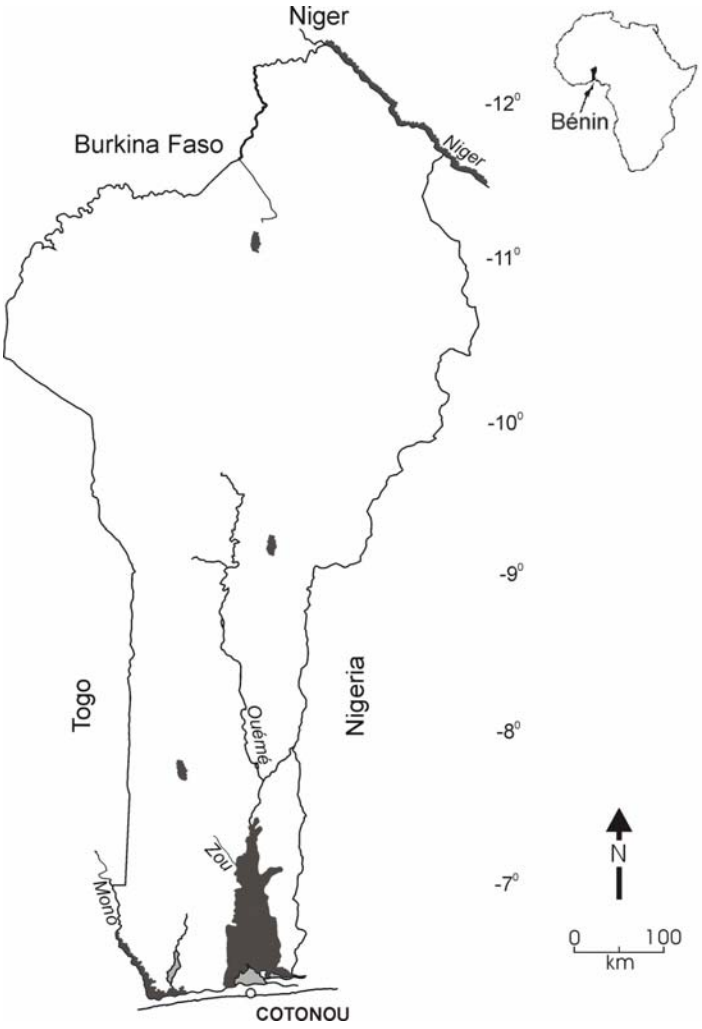

Figure 1.1. Map of Benin showing distribution of water hyacinth in 1995. Dark shaded areas are infested by water hyacinth.

Water hyacinth populations in Benin follow a seasonal (rainy and dry) pattern, and are influenced by the interaction between salinity, water flow, wind and fishermen (van Thielen *et al*., 1994). In Benin, seeds set in shallow banks and germinate when conditions are favourable, ensuring large seasonal populations of water hyacinth. According to the 1989 census of Benin, villages situated in the flood plain of the Ouémé and the Zou River systems had 194,000 people belonging to 39,000 households (De Groote *et al*., 2003). In these villages, water hyacinth obstructs transport which is mostly by paddled canoe or motorised boats, carrying tons of goods such as wood and agricultural produce e.g. maize

and vegetables. Water hyacinth causes a reduction in fishing activities and commerce in the lagoon/river systems, shared with neighbouring Nigeria.

1.9 Biological control of water hyacinth in Benin

Biological control of water hyacinth in Benin was initiated in 1991. The two weevils, *N. eichhorniae* and *N. bruchi*, imported from the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Brisbane, Australia, were released in 1991 and 1992 respectively (van Thielen *et al*., 1994). The moth *N. albiguttalis*, also imported from CSIRO, was released in 1993 (Neuenschwander *et al*., 1996) while the mirid, *E. catarinensis*, was imported from the Plant Protection Research Institute in Pretoria, South Africa (PPRI) and released in 1999 (IITA, 1999). However, only the two weevils have so far established in Benin and *N. eichhorniae* appears better suited than *N. bruchi* (Ajuonu *et al*., 2003).

In Benin, the weevils have brought water hyacinth under control at some sites, such as Tévèdji, Lihu, Lake Azili, Kafedji. At Lake Tévèdji, reduction in surface area of the weed from 100% to 5% was recorded about eight years after the release of the weevil (Ajuonu *et al*., 2003). In Lake Azili, control was achieved within five years probably because *N. eichhorniae*, released in Tévèdji in 1991 about 15km downstream, had spread to Lake Azili just before water hyacinth covered most of the lake (Ajuonu *et al*., 2003).

In other sites such as Savalou and Kpokissa, the impact of the weevils has been slow or ineffective (Ajuonu *et al*., 2003). This is in contrast to the control of water lettuce where one host specific agent successfully controlled water lettuce infestations in Benin (Ajuonu and Neuenschwander, 2003), similar to many countries such as Zimbabwe (Chikwenhere and Forno, 1991), South Africa, and Papua New Guinea (Harley, 1990), and recently other West African countries

(Cilliers *et al*., 2003).

In order to improve the level of control of water hyacinth in Benin, additional agents were considered. Although the mirid, *E. catarinensis* was released and did not establish, it was reconsidered. Because the laboratory culture of *E*. *catarinensis* (maintained at IITA since 1998) was apparently displaced after two years by an indigenous mirid *Nycticapsus* sp., a new shipment of *E*. *catarinensis* was requested from PPRI in South Africa. The new shipment of the exotic mirid *E. catarinensis* arrived in May 2002 and is now being maintained in the laboratory.

There are risks associated with introducing additional agents. One is that they might interfere with those agents already established, thus reducing the effectiveness of biological control (Denoth *et al*., 2002). This could occur through competitive displacement (Reitz and Trumble, 2002). Antagonistic interaction of control agents could be a potential threat to successful control of water hyacinth in Africa. Therefore, in this study, I attempted to answer three broad questions; first, will *E. catarinensis* improve the level of water hyacinth biological control in Benin. Second, is the mirid compatible with the two weevil species? Third, does the indigenous mirid, *Nycticapsus* sp.,which displaces *E*. *catarinensis* in the laboratory, prevent establishment of *E*. *catarinensis* in the field?

1.10 Specific Aims

Eccritotarsus catarinensis and the *Neochetina spp.* were used to test the hypotheses that the use of multiple agents in weed control is more effective than the use of a single agent, and if one agent can reduce the quality of the host plant, thereby affecting the performance of a second agent.

1.11 Thesis outline

The objective of this study was to investigate the interaction between biological control agents, namely the two weevils *N. eichhorniae*, *N. bruchi*, and the mirid *E. catarinensis*, and their impacts as single agents and in combination, on the competitive ability of water hyacinth. In addition, the displacement of the mirid *E. catarinensis* in the laboratory culture by a non host-specific indigenous mirid *Nycticapsus* sp. was investigated.

Chapter two reports an experiment investigating the survival of *E. catarinensis* using treatments of water hyacinth plants with feeding scars by adult *N. eichhorniae* and *N. bruchi*. Chapter three reports on an experiment investigating the interactions and impact of the weevil, *N. eichhorniae* and the mirid *E. catarinensis* as single species and species combinations on the competitiveness of water hyacinth against water lettuce at three planting densities of water hyacinth and water lettuce. Chapter four reports on the displacement of the biological control agent, *E. catarinensis*, in the laboratory by an indigenous mirid *Nycticapsus* sp. and chapter 5 discusses the relevance of these findings.

CHAPTER TWO

SURVIVAL OF THE MIRID *ECCRITOTARSUS CATARINENSIS* ON WATER HYACINTH (*EICHHORNIA CRASSIPES*) PLANTS WITH AND WITHOUT FEEDING SCARS OF *NEOCHETINA EICHHORNIAE* AND *NEOCHETINA BRUCHI* [1]

2.1 Introduction

In many countries across the world, several arthropod biological control agents have been successfully used to reduce water hyacinth infestations (Hill *et al*., 1999a; Navarro and Phiri, 2000; Cilliers *et al*., 2003). For example, the species *N. eichhorniae, N. bruchi*, *E. catarinensis*, *N. albiguttalis* and *O. terebrantis* are established in South Africa and Malawi (Cilliers *et al*., 2003). In West, East and Central Africa, four bio-control agents have been released, but only the two weevil species, *N. eichhorniae* and *N. bruchi* have established (Ajuonu *et al*., 2003; Mbati and Neuenschwander, 2004).

In locations where more than one agent has established, they co-exist and exploit water hyacinth for feeding, reproduction or both. The mirid *E*. *catarinensis* was first released in Africa in 1996, several years after the two weevil species *N. eichhorniae* and *N. bruchi* had established across many countries. Although the biology of the mirid and the two weevil species differ greatly, there is potential for interference between the two taxa because of adult feeding and selection of oviposition sites on the water hyacinth leaves.

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[1] The results of this study have been published as "Ajuonu, O., M. Byrne, M. Hill, P. Neuenschwander and S. Korie, 2007. Survival of the mirid *Eccritotarsus catarinensis* as influenced by *Neochetina eichhorniae* and *Neochetina bruchi* feeding scars on leaves of water hyacinth *Eichhornia crassipes*. BioControl: 52: 193-205.

Previous studies have shown the importance of adult weevil feeding scars for other agents. Such scars are used as oviposition sites by *N. albiguttalis* (DeLoach and Cordo, 1978) and also provide entry for pathogens (Del Fosse, 1978: Moran, 2005). In addition, the mite *O. terebrantis* concentrates around fresh weevil feeding scars (Del Fosse and Perkins, 1977). The sizes of feeding scars vary, from small nicks of ca 0.5 mm^2 to a maximum of 25 mm^2 (DeLoach and Cordo, 1976) and the density per leaf will depend on the rate of leaf production and density of the weevil population (Wright and Center, 1984). For the mirid, *E. catarinensis,* no studies on the effects of adult weevil feeding scars have been carried out.

In Africa, the mirid is established only in South Africa and Malawi (Cilliers *et al*., 2003) and there are plans to release the mirid in the USA (Coetzee *et al*., 2003) as well as in other countries where water hyacinth remains problematic. The effectiveness of multiple agents in biological control has been queried due to the risks of negative interactions among agents (Denoth *et al*., 2002; Reitz and Trumble, 2002). However, very little information is available on how the long established weevil species might co-exist with the recently released mirid. Therefore, the effects of adult weevil feeding damage on survival of the mirid *E. catarinensis* were studied by simulating different naturally occurring intensities of old feeding scars on water hyacinth plants and by adding adult weevils to obtain fresh feeding scars. This study was done to determine if there was a possible interaction between weevil feeding and mirid performance.

2.2 Materials and Methods

2.2-1 Water hyacinth production

All experiments were conducted at the International Institute of Tropical Agriculture (IITA) station near Cotonou, Benin. Water hyacinth plants were grown outdoors in round plastic lattice wall pools (265 x 67.5 cm) under shade to protect

them from direct intensive sunlight, which was observed to reduce plant growth, and away from insect rearing facilities to prevent accidental infestation. When the rearing pools were set up, liquid fertilizer (FERTIGOFOL ® 737) was applied at the rate of 0.5 ml/ liter of water.

2.2-2 Rearing agents, *Neochetina* spp. and *Eccritotarsus catarinensis*

Cultures of both weevil species were established in December 2003 using plants maintained outdoors in similar plastic pools and under similar conditions as those for growing water hyacinth. Periodically, new plants were added to the weevil rearing pools, in order to maintain overlapping weevil generations and ensure that plant material was not limiting weevil production. The weevil rearing pools also served as a source of plants with adult weevil feeding scars.

Eccritotarsus catarinensis was reared in the laboratory, where temperatures varied between 26 and 28 °C, with relative humidities of 61-77% and a photoperiod of 12/12hrs archived by 36 fluorescent light bulbs of 36W each. Two or three water hyacinth plants were placed in a plastic pot (16cm high, 15cm diameter) filled with water, and then installed in wooden cages measuring 44 x 45 x 58 cm, with fine mesh screen sides, and a glass top. Seven cages were each inoculated with adult *E. catarinensis* on separate dates so that freshly emerged adults were available at all times and adults used in the experiment were of the same age. Every week, one or two fresh plants were added into each cage, while old and dead water hyacinth plants without mirids were removed.

2.2-3 Experimental design

The experiments were conducted in the same laboratory used for rearing *E. catarinensis*. Trials with *N. eichhorniae* were set up from March to mid May while those with *N. bruchi* lasted from mid May to July 2004. Four conditions were represented by four treatments, each consisting of two potted water hyacinth

plants A and B enclosed in a cage. The treatments were: (1) leaves of both plants, A and B had weevil feeding scars. This represented conditions of a high weevil population and where adult weevils had moved to new water hyacinth shoots; (2) leaves of plant A had adult weevil feeding scars, and those on plant B had none. This offered the mirids an option of moving to plants with no adult weevils and no feeding scars; (3) was similar to treatment (2) but, in addition, ten adult weevils were introduced in order to ensure the presence of fresh feeding scars. Here, both adult weevils and the mirids could potentially interact; (4) both plants, A and B, were without feeding scars or weevils and this treatment served as the control, representing conditions of water hyacinth before the release of *Neochetina* spp. (Table 2.1) The experiment had a randomized design with five blocks for the different treatment dates, separately for each weevil species (*N. eichhorniae* and *N. bruchi*). The experiment thus comprised 40 cages (10 per treatment) with a total of 80 potted water hyacinth plants, half with and half without feeding scars.

Table 2.1 The distribution of feeding scars and adult weevils per cage used in the experimental set-up to test the effect of water hyacinth weevil feeding on the mirid *Eccritotarsus catarinensis*.

Treatment	**Status of plants A or B in each treatment (per cage)**	
	A	**B**
1	Feeding scars	Feeding scars
2	Feeding scars	No feeding scars
3	Feeding scars + adult weevils	No feeding scars + adult weevils
4	No feeding scars (control)	No feeding scars (control)

2.2-4 Selection of plants

Water hyacinth plants of 35 to 40 cm height, with and without weevil feeding scars were selected from weevil or water hyacinth rearing pools. As the age of a water hyacinth leaf is directly related to its position on the plant (with newly emerging leaves in the apex having furled lamina, while the oldest leaves are situated at the base: Center, 1981), selecting plants with unfurled first leaves and removing one or two lower leaves, led to uniform experimental plants. They had 4-5 healthy mature leaves with leaf blades measuring between 7 and 11 cm across.

In order to minimize leaf mortality, the petiole bases of the leaves with old feeding scars were carefully examined to exclude plants with the late instar weevil larvae which cause greatest damage (Center, 1984). Plants from the two weevil rearing cultures bearing live adult weevils were rejected. Therefore, on selected plants, fresh weevil feeding scars were rare and the feeding scars on plants from weevil rearing were from a few days to several weeks old, depending on leaf age. These were classified as ‘old feeding scars’. (In the experiment, ‘young feeding scars were created by the addition of adult weevils to one experimental set-up.).

The experimental plants were washed with tap water to prevent red spider mite and aphids, commonly observed feeding on water hyacinth, from being carried into the laboratory.

2.2-5 Setting up experiment in the laboratory

A replicate consisting of four labelled cages for *N. eichhorniae* and four cages for *N. bruchi*, requiring a total of 2x8 pots was set up on each of five dates. On each date, experimental plants were sorted into those with and those without old feeding scars. Plants were randomly picked from each group and placed into labelled plastic pots. Each pot was filled with 1.5 litres of tap water and the

number of leaves per plant was noted. On each plant, old feeding scars per leaf were counted, according to the age of the leaf as described by Center (1981). The two potted plants (A and B, Table 2.1) of each treatment were then transferred into a labelled cage and kept close to each other.

Adults of *E. catarinensis* were collected from the laboratory culture with an aspirator into plastic vials (size, 9 dram) covered with a ventilated cap. Forty adults were released into each cage by positioning the vial vertically on the floor of the cage and opening its cap. Adult mirids were allowed 72 hours for oviposition. At the same time, for treatment 3, 10 adult weevils (five females and five males) collected from the weevil rearing culture, were added through the sleeve of the cage.

After 72 hours, all surviving mirids and weevils were removed. This period was chosen because in an initial trial of five days oviposition, plants with old feeding scars were found not to survive long enough to allow immature mirids to develop due to the damage from weevil larvae already in the plants. Thereafter, the two potted water hyacinth plants per treatment were separated and each was transferred to an individual cage for subsequent development of the immature mirid stages.

2.2-6 Data collection

Surviving adult mirids removed from each cage after 72 hours were counted to provide a measure of mortality. On day 15, the numbers of immature mirids per leaf on individually caged plants were counted, according to leaf position to provide a measure of population size then combined into a count per plant. (In an earlier trial, counting per leaf had been found to be easier than counting per plant). Dead and new leaves found on each plant during the experimental period were also recorded.

2.2-7 Data analysis

To determine the possible effect of weevil feeding scars on the mortality of adult *E. catarinensis,* numbers of surviving adult mirids in each cage, recovered after 72hrs of oviposition, were log 10(x+1) transformed. Treatments were compared separately for each weevil species that had produced the feeding scars, in an analysis of variance (ANOVA). For separation of means, pair-wise comparisons as well as orthogonal contrasts of the treatment means were employed (SAS, 2002). The effect of old feeding scars from *Neochetina* spp. on survival of immature mirids was examined by comparing the number of immatures recovered from the different treatments of "A" plants bearing old scars, and those without scars (control) using ANOVA. Similarly, the effect of fresh feeding scars, made by the introduction of adult weevils, was examined on "B" plants of treatment 3, compared to treatment 2. Immature mirids counted on newly produced leaves were included in the analysis, and the number of surviving leaves, which ranged between three and five leaves per plant, was used as a covariate to adjust the treatment means in the ANOVA.

2.3 Results

2.3-1 Counts of old feeding scars by adult weevils

Old weevil feeding scars observed on water hyacinth plants ranged from 0-212 per leaf. The distribution (with intervals of 50) is given in Table 2.2. The total number of old feeding scars on forty plants made by *N. eichhorniae* was substantially lower (6,374) than those of *N. bruchi* (15,395) on the same number of plants.

Table 2.2. Number of feeding scars (intervals of 50) by adult weevils and the number of leaves distributed in six groups A-F for both weevil species *Neochetina eichhorniae* (Ne) and *N. bruchi* (Nb).

Groups of adult weevil feeding scars (range)	**Number of leaves in each category**		**% of leaves* (Ne and Nb)**
	Ne	**Nb**	
A (0)	2	0	1.05
B (1-50)	40	23	33.16
C (51-100)	20	37	30.00
D (101-150)	20	30	26.32
E (151-200)	6	8	7.37
F (>200)	2	2	2.10

*= Based on a total of 190 leaves for both weevil species (90 for *N. eichhorniae* and 100 for *N. bruchi*).

2.3-2 Mortality of the mirid *Eccritotarsus catarinensis*

The survival of adult mirids after three days of oviposition was similar for the two weevil species and therefore the results of mortality were combined (Table 2.3). Mortality in treatment 1 (25.17%), with old feeding scars on both plants, was significantly higher than the control. This was followed by treatment 2 (12.64%), where only one “A” plants had scars, and then the control, treatment 4 (4.83%). The lowest mortality of 4.33% was found in treatment 3, with old feeding scars on plant A and none on B, but where adult weevils had provided fresh feeding holes. Mirid mortality on treatments 2 and 3 did not differ significantly from that of the control.

Table 2.3. Mortality per cage, of adult *Eccritotarsus catarinensis* after three days of oviposition on leaves of water hyacinth. Each treatment from 1 to 4 has two plants (A, B), with (+) or without (-) old adult weevil feeding scars of *Neochetina* spp. Data are means ± SE.

Treatment	**Plants status** **A B**	**Mean % adult mirid mortality**
1	+ +	25.17 ± 1.43 (a)
2	+ -	12.64 ± 1.39 (ab)
3 + adult weevils*	+ -	4.33 ± 1.56 (b)
4 (control)	- -	4.83 ± 1.12 (b)

*= Adult weevils were added to provide fresh feeding scars. Means followed by the same letter(s) do not differ significantly at *P*= 0.05 (Pair-wise comparison of means in ANOVA).

2.3-3 Effect of old weevil feeding scars and the presence of adult weevils on the survival of immature mirids

Survival of immature mirids (Fig 2.1) is presented on a per plant basis because counts taken per leaf did not show any trend according to leaf position. Overall, immature mirids survived on plants with old scars from adult weevils (even on leaves with more than 200 old scars), with fresh scars as they did on plants without scars. Counts of immature mirids taken 15 days after introducing adult mirids, differed between the two weevil species treatments, where the mean number of mirid immatures found was lower for *N. bruchi* compared to *N. eichhorniae*. But the general trends for each species were similar. Overall, the control plants offered the best reproductive environment, which was not

significantly different to treatment 3, where adult weevils were present.

Details are as follows: On the control plants without scars (treatment 4), the number of immature mirids were significantly greater for both weevil species ($F_{1, 11}$ = 9.10 for *N. eichhorniae* and $F_{1, 11}$ = 35.99 for *N. bruchi*; P <0.01) compared to plants with old weevil scars ("A" plants, treatments 1-3). Treatment 2 was not significantly different from treatment 1 ("A" plants) for *N. eichhorniae* ($F_{1, 11}$ = 0.10, P=0.75), but was different for *N. bruchi* ($F_{1, 11}$ = 9.36, P< 0.01) (Figure 2.1b). In treatment 3 where only the "A" plant was scarred and adult *N. eichhorniae* were introduced to provide fresh feeding scars, the number of immatures on "B" plants with fresh scars was significantly higher ($F_{1, 10}$ = 8.96, P<0.01) than on the corresponding "B" plants in treatment 2 without adult weevils. For *N. bruchi*, although the mean number of immatures recovered on "B" plants in treatment 3 was nearly twice that of "B" plants in treatment 2, the difference was not significant ($F_{1, 11}$ = 1.39, P=0.26) (Fig 2.1b).

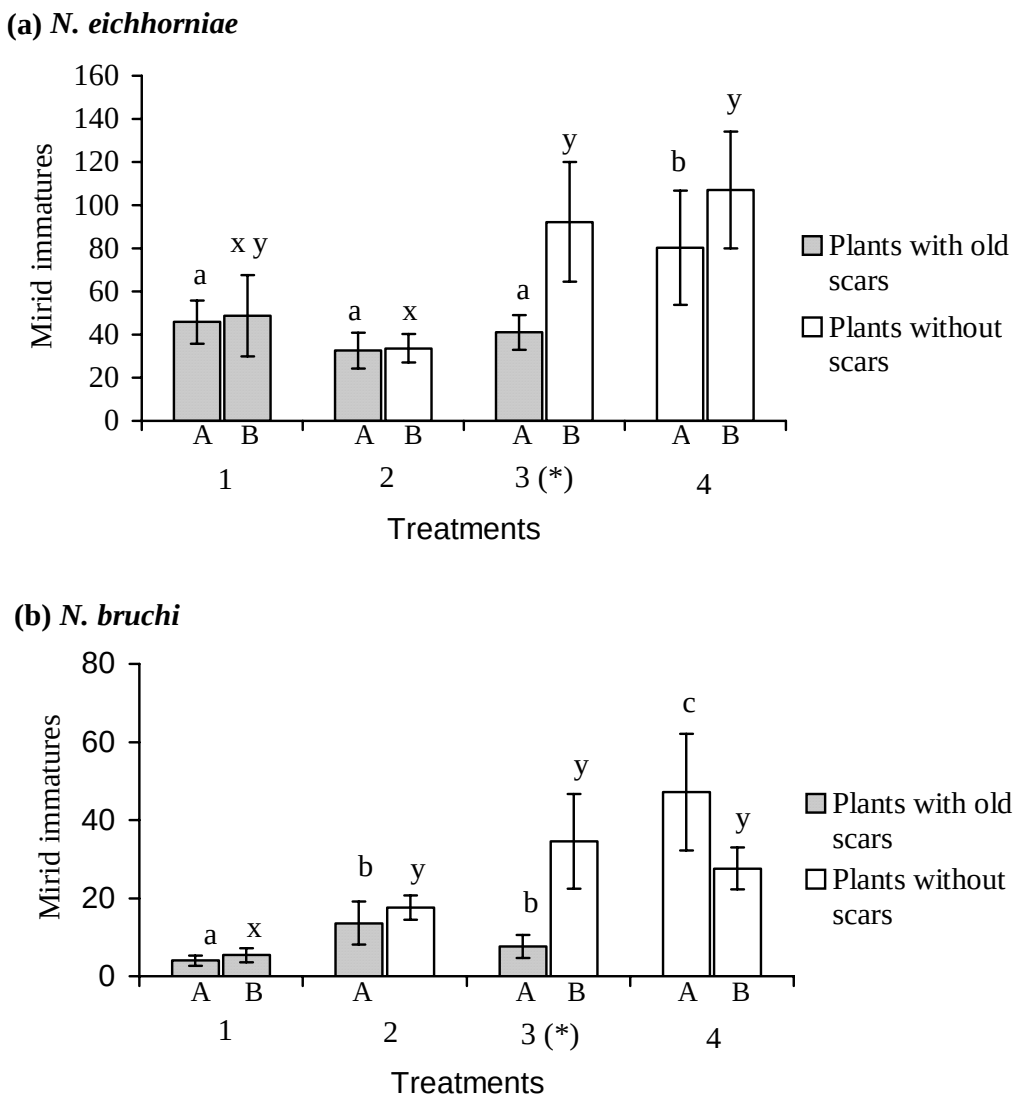

Figure 2.1. Mean numbers of *Eccritotarsus catarinensis* immatures recovered from water hyacinth plants with and without old feeding scars inflicted by either adult *Neochetina eichhorniae* (a) or *N. bruchi* (b). Means across treatments followed by the same letter(s) above the bar are not different at *P*= 0.05. Each treatment (1 to 4) had two sets of plants (A, B) and vertical bars represent ± standard error of the mean. (*)= Adult weevils were added to provide fresh feeding scars.

2.4 Discussion

Displacement of arthropods by other species is a well established phenomenon across a broad range of taxa (Reitz and Trumble, 2002) and is known from several examples of biological control agents (Louda *et al*., 2003). However, it would appear from the data presented here, in conjunction with field observations from South Africa, that the weevils *N. eichhorniae* and *N. bruchi* are unlikely to prevent establishment of *E. catarinensis* on water hyacinth.

Numbers of old feeding scars on the water hyacinth plants differed among leaves and between the two weevil species treatments. Old scars from the culture of *N. bruchi* were two fold higher than those from *N. eichhorniae*. This reflects the difference in population pressure in the open weevil colonies from where the experimental plants were taken (Wright and Center, 1984). Feeding-scar densities seen in the four experimental treatments corresponded to naturally occurring patterns of feeding scars where, 2-3 years after the release of *Neochetina* spp in Benin, the highest average number of feeding scars reached 250 per leaf, but mostly stayed between 25 and 150 (Ajuonu *et al*., 2003).

Feeding scars of *Neochetina* spp. affected the survival of the adult mirids. There was high mortality of adult mirids (5 fold in treatment 1, about 3 fold in treatment 2) on water hyacinth plants with old weevil feeding scars compared to the control, which we attribute to a reduction in tissue nutrients due to adult weevil feeding (Center and Van, 1989). When adult *Neochetina* spp. were present and caused fresh feeding scars on water hyacinth leaves, mortality of adult mirids was low and similar to the control. Fresh adult weevil scars release moisture and exudates (Del Fosse, 1978). In fact, we occasionally observed *E. catarinensis* crowding on leaves with fresh feeding scars and this suggests that adult mirids may profit from the easily available fluids around fresh weevil feeding scars.

Development of immature mirids occurred on leaves without adult weevil feeding scars, leaves with few scars and also on leaves with more than 200 old scars from *Neochetina* spp. In the control treatment without scars, counts of immature mirids were high (80.2/plant), but low (45.8/plant) in the treatment 1, where both plants had old feeding scars, for the species *N. eichhorniae*, plants A (Figure. 2.1a). Since plants attacked by *N. bruchi* had double the number of old feeding scars (15,395) than found with *N. eichhorniae* (6,374), the overall number of mirid immatures found was correspondingly lower in treatments with this weevil species, but does not explain the low numbers of mirids on the control plants. Low quality plants have been shown by Coetzee (2004) to adversely influence mirid development. However, due to the high rate of increase and short life cycle of the mirid (Hill *et al*., 1999b; Stanley and Julien, 1999), even on leaves with more than 200 old feeding scars, some immatures developed, thus ensuring a new generation.

The weevil and the mirid generally have separate diel activity patterns. Fresh weevil feeding scars are made mainly in the night because adults are primarily nocturnal (DeLoach and Cordo, 1976). On the contrary, the mirid is mainly active in the day (Hill *et al*., 1999b), but despite this temporal separation, both taxa potentially compete for the same resources on the second and third leaf of their shared host. Both weevil species and the mirid have been established in South Africa and Malawi, (Cilliers *et al*., 2003) and the mirid may well be released in other countries where the weevils are already established (Coetzee *et al*., 2003). The mirid co-occurs in high numbers with both weevil species at several sites in South Africa (Byrne, School of Animal, Plan and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa, unpublished data), and failure to establish at other sites has been attributed to severe winters, rather than interference from the weevils (Coetzee, 2004). Movement of the three species between their respective feeding resources on a water hyacinth plant as it turns over its leaf production should be measured to determine if they interfere, or assist each other on a long term, seasonal basis.

Because feeding by adult *Neochetina* spp. reduces the nutritive quality of water hyacinth leaves with old feeding scars (Center and Van, 1989), this may well have had a negative effect on the survival of adults and immatures of *E. catarinensis* as seen in treatments 1, 2 and 3. In contrast, where adult weevils create fresh feeding scars, survival of both stages of *E. caterinensis* was more favourable. The mirids are highly mobile within the water hyacinth canopy and would be expected to seek out plants in the field that have minimal weevil feeding damage.

CHAPTER THREE

THE IMPACT OF TWO BIOLOGICAL CONTROL AGENTS, THE WEEVIL *N. EICHHORNIAE* AND THE MIRID *E. CATARINENSIS* ON THE COMPETITIVENESS OF WATER HYACINTH *EICHHORNIA CRASSIPES*, AGAINST WATER LETTUCE, *PISTIA STRATIOTES* [2].

3.1 Introduction

Water hyacinth *E.* crassipes and water lettuce *P. stratiotes* are found in similar habitats such as slow moving or still water bodies (Harley, 1990). However, due to its rapid growth (Chadwick and Obeid 1966; Wright and Purcell, 1995) and its ability to shade other aquatic plants such as water lettuce, water hyacinth in the absence of its natural enemies is the dominant species (Center and Spencer, 1981).

Biological control is the preferred method of controlling water hyacinth because it is environmentally friendly (Greathead, 2003) and has successfully reduced infestations in many countries (Hill *et al*., 1999a; Navarro and Phiri, 2000; Cilliers *et al*., 2003; Mbati and Neuenschwander, 2004). The most recent agent to be released against *E. crassipes* is the mirid *E. catarinensis* (Chapter 1, Table 1). It was released in Benin to complement biological control by the weevils, *N.bruchi* and *N. eichhorniae in* ecological conditions where the weevils were not fully efficient, and have not established.

[2] A version of this chapter has been submitted to the Journal, BioControl as: Ajuonu, O., M. Byrne, M. Hill, P. Neuenschwander and S. Korie, 2007. The impact of two biological control agents, the weevil *Neochetina eichhorniae* and the mirid *Eccritotarsus catarinensis* on water hyacinth *Eichhornia crassipes* grown in culture with water lettuce, *Pistia stratiotes*.

Water lettuce sometimes re-colonizes water bodies after water hyacinth cover has been reduced by weevils (Harley, 1990; Ajuonu and Neuenschwander, 2003). This response of competing vegetation has therefore been used as an indicator for evaluating the impact of biological control agents against water hyacinth, particularly when measuring their direct effect on the host plant (Center *et al*., 2001). This was based on the hypotheses that both competition and herbivory would reduce plant performance. Center *et al*. (2005) compared the impact of the two weevils and Coetzee *et al*. (2005) evaluated the effect of the mirid *E. catarinensis* alone. Using the same methodology, the present study compared the subtle impact of the mirid with the well established efficiency of the weevil to test whether the two agents are more effective than one. This will aid in the decision making process of whether to invest further resources in and efforts to establish the mirid in Benin.

3.2 Materials and methods

3.2-1 Location of experimental site

This experiment was conducted outdoors in a shade screen house (40%) in the centre of a fallow field at the International Institute of Tropical Agriculture (IITA) near Cotonou, Benin. The first replicate lasted from 24 July to 6 October 2004, the second from 11 December 2004 to 26 February 2005 and the third from 9 March to 1 June 2005. The daily minimum and maximum temperature ranges were 19-22.8 °C and 25.7-30.9 °C, respectively for the first replicate; 14-25.5 °C and 29.5-34.7 °C for the second and 20-25 °C and 30-33.8 °C for the third replicate.

3.2-2 Rearing plants and insects

Plants were grown in plastic tubs (30 cm deep; 54 cm diameter) buried 22 cm in soil and covered by a 95 cm high white mosquito net canopy (Figure 3.1). Heat accumulation in small water containers adversely affects water hyacinth growth;

therefore, burying the rearing tubs lowered water temperatures by 0.5° C at 8 hrs and by 2° C from 12-17 hrs compared to surface placement. On the first day, the plastic tubs were filled with 20 L of water. Liquid fertiliser (FERTIGOFOL ® 737) was used at the application rate of 0.5 mL per litre of water. Water levels and nutrient status in the tubs were maintained constant throughout the experiment.

Figure 3.1. Experimental site, plastic tubs buried 22 cm in the soil and mosquito net canopy hanging over each plastic tub.

Water hyacinth plants were collected from Sazoué (08° 18.32N, 001°50.13E) on the Mono River where the weed has remained free of weevil establishment. The initial fresh weight of individual plants ranged from 170-258 g. Water lettuce plants were taken from a stock maintained at IITA in plastic pools (265 x 67.5cm). Before placement in the tubs, all plants were washed by spraying with tap water to remove all arthropods, and then covered with the net-canopy. Adults of *N. eichhorniae* were collected from a field population on the Sô River (06°40.07N, 002°24.59E). The mirid *E. catarinensis* was reared in the laboratory (Ajuonu *et al*., 2007).

3.2-3 Experimental design and set up

The trial followed an additive series competition experimental design (Spitters, 1983), consisting of factorial combinations of the two competing species in a randomised complete block design, with the plant density ratios nested in species treatment levels.

A mixture of water hyacinth and water lettuce was planted with the following numbers of plants: 3:0, 3:3, 3:9, 9:0, 9:3, 9:9, 0:3 and 0:9 per tub, respectively. Four sets of each combination were set up, of which three were each infested with a single species or a combination of agents; the fourth served as a control (C). The single species infestations were: *E*. *catarinensis* (E) at the rate of 40 adults per plant, or *N. eichhorniae* (N) at a rate of two pairs of adults per plant. In the combined species treatment (E+N), the infestation rate was 20 adult *E*. *catarinensis* and one pair of *N*. *eichhorniae* per plant, which is ½ the inoculum used in the single treatments. This was necessary in order to facilitate collection, counting the large number of mirids required for the experiment and to enable inoculation on same day. The experimental setup was replicated three times on the dates given above.

In contrast to previous studies using the same methodology, which either dealt with two very similar weevils (Center *et al*., 2005) or with the impact of a single agent (Coetzee *et al*., 2005), we faced the problem of having to compare the impact of two highly different organisms. The two species were combined in numbers approximating observed field population levels, and their weights were determined in order to reach a common standard. Ten adult weevils were frozen for 24 hours and air dried on a filter paper for seven hours. The weevils were individually weighed (Denver Instrument M-220D, measuring a minimum of 0.0001mg), yielding an average weight of 0.0077mg (range 0.0058 – 0.0094). Forty *E*. *catarinensis* adults weighed using the same procedure were found to equal one quarter the weight of adult *N. eichhorniae*. This implies that it would

have taken 160 mirids to match the mass per mg, the weight of the 2 pairs of *N. eichhorniae* or *N. bruchi* per plant used in a similar study by Center *et al*. (2005). Coetzee *et al*. (2005) used 15 adult *E. catarinensis* (50:50 sex ratio) per water hyacinth plants; however this number was considered too small to cause severe damage, therefore the decision to use 40 adults per plant. In this experiment, the mirid inoculation rate was therefore 25% of the weight of the weevil.

3.2-4 Data collection

Comparing the impact of mirids and weevils was difficult because their impacts are so different. Therefore plant growth parameters were considered as standard measurable units (Center *et al*., 1999) and indirect indicators of impact by both agents (Del Fosse, 1978). Data were recorded at week zero on the same date the agents were introduced, and at weeks three, six and eight. Measurements taken were: (1) length of the second leaf and (2) petiole, (3) number of daughter plants (only those with three unfurled leaves each were considered), (4) number of inflorescences produced per plant by water hyacinth, (5) estimates of surface cover of basins (in 5% increments) by water lettuce (Ajuonu *et al*., 2003), and (6) fresh weights of water hyacinth and water lettuce taken at weeks zero and eight, only.

The three week sampling period corresponded to the generation time of the mirid (Hill *et al*., 1999b). However, destructive sampling was carried out at week eight instead of week nine, because in an initial trial with the mirid, production of new water hyacinth leaves occurred after eight weeks. At this time, most of the leaves were chlorotic and drying and due to a lack of suitable food, the mirid population collapsed.

The numbers of adult and immature mirids were counted at week three and eight, on 10 leaves in the tubs initially containing three plants and on 20 leaves for those initially containing nine water hyacinth plants. On the same date, the number of adult weevil feeding scars on the second leaf was counted, as an indicator of the presence of adults (Wright and Center, 1984).

3.2-5 Data analysis

Multivariate principal component analysis (PCA) was used to explore the relationship between all plant growth parameters and the treatment units (i.e. insect species and plant species density combinations). PCA is a practical technique for examining the interrelationships among a set of correlated variables by transforming the original variables into new, uncorrelated variables called principal components (PC's). The PCA allows the identification of the growth parameters that are most important in explaining a multivariate system and, hence, allow the data to be interpreted meaningfully (Afifi and Clark, 1990; Cody and Smith, 1997).

Further, analysis of variance by GLM ANOVA procedure (SAS, 2002) was used to assess differences in species treatments (single agent, species combination and the control), followed by Student-Newman-Keuls tests at P=0.05. To achieve normality, count data were log10(x + 1) transformed before analysis. The means of fresh weight (water hyacinth and water lettuce), number of daughter plants and inflorescences of water hyacinth were calculated on the basis of the initial planting densities. Means of leaf and petiole lengths of water hyacinth (on the second leaf position) were based on the number of mature plants (plants with 5 unfurled leaves) per plastic tub on each sampling date.

Multiple regression analysis was carried out, using an inverse linear model, $1/w_h = a_{h0} + a_{hh}d_h + a_{hi}d_I$ (Spitters, 1983). Here, $1/w_h$ is the inverse biomass yield of

individual water hyacinth plants, while d_h and d_l represent water hyacinth and water lettuce planting densities, respectively. The coefficient a_{hh} estimates intraspecific competition, while the coefficient a_{hi} estimates interspecific competition. The ratio a_{hh}/a_{hi} measures the effect of intraspecific competition by water hyacinth on its own yield, relative to the effect of interspecific competition by water lettuce.

3.3 Results

3.3-1 Numbers of mirids and adult weevil feeding scars

Three weeks after introducing the agents, there were 15.12 ± 1.29 adult and immature mirids per leaf in the treatment (E) with the mirid alone, where 40 adults (20 female: 20 males) per plant had been used. In the treatment (E+N) with both species (with ½ the inoculation rate of single species), the mean was 9.19 ± 0.67 adult and immature mirids. At week eight the mean counts (adults and immatures) per leaf declined to 7.13 ± 0.61 (52.84%) in treatment E and to 1.97 ± 0.21 (78.56%) in treatment E+N, when plants became less suitable due to declining vigour resulting from the continuous effect of the weevil.

Some plants continued to produce new leaves. Although they decreased in size towards the end of the experiment, the new leaves provided suitable food which ensured the survival of some of the initial adult weevils used for inoculation throughout the period of the experiment. In treatment (N) adult weevil feeding scars on the second young leaf numbered 61.61 ± 6.30 per leaf at week three and 56.41 ±5.51 at week eight. In the treatment with both species (E+N), at half the inoculation rate in treatment with the weevil alone, feeding scars numbered 41.5 ± 3.50 on week three and 36.0 ± 3.74 at week eight. During the destructive sampling (week 8) several weevil pupae were observed in treatments with the weevil alone and in the combination of weevil and mirid, indicating that full larval development had occurred.

3.3-2 Plant growth parameters

Fresh weight at the end of the experiment from the first replicate (24 July to 6 October 2004) ranged from 121-390 g/plant and for the third replicate (9 March to 1 June, 2005) from 49.7-415.0 g/plant and differed greatly from the second replicate (11 December, 2004 to 26 February, 2005) which ranged between 0-19 gm/plant. This shows that the periods of the first and third replicates, which corresponded to the short and long rainy periods of the region, supported plant and insect population growth better than the second replicate conducted during the dry season and the 'harmattern' a dry dusty wind originating in the Sahara desert. The block effect created by repeating the experiment three times but on different dates, was therefore removed and replication was introduced as a factor in the ANOVA model.

The results of the principal component analysis (Table 3.1) indicate that the total variance, contributed to by the growth parameters in the data, was accounted for mainly by the first principal component (PC) (41.7%), the second PC (23.4%) and to a lesser extent by the third PC (17.8%). Together, the first three PC's accounted for 82.9% of the total variance of the six plant growth variables analysed.

Examination of the component coefficients indicates that the first PC was a weighted average of the following, in order of importance: (1) water hyacinth fresh weight (2) petiole length (3) leaf length and (4) the number of flowers produced. In the second PC, the number of new shoots produced was the most important variable and this was followed by the number of flowers produced. Percentage surface cover by water lettuce was the most important variable in the third factor. Graphical representation of the two principal factors 1 and 2 by treatments (Figure 3.2) indicated a negative displacement of the centroids on Axis 1 in treatments with agents (E, N, and E+N) compared to the control (C), without agents. On Axis 2, the centroids aggregated according to the initial

planting density in all treatments-with and without agents (Figure 3.2).

Table 3.1. Principal component analysis (PCA) of water hyacinth plant growth parameters and water lettuce surface cover measured on three dates, week 3, 6 and 8 after treatments with biological control agents.

Growth parameters (variables)	**Week**	**Factor 1**	**Factor 2**	**Factor 3**
Fresh weight	8	0.94752	-0.05230	-0.13973
Leaf length	3	0.77946	-0.22586	0.03085
	6	0.92783	0.06701	-0.06622
	8	0.88199	0.36854	0.02496
Flowers	3	0.10077	0.53890	0.17869
	6	0.67206	0.41491	0.06631
	8	0.80086	0.24892	0.12066
Petiole length	3	0.73138	-0.60459	0.00073
	6	0.93170	-0.22000	-0.06786
	8	0.92138	0.21057	-0.02318
Number of new shoots	3	-0.14649	0.92435	0.09373
	6	0.03499	0.91086	0.14782
	8	0.29943	0.87300	0.25600
Cover by WL	3	0.09405	0.14631	0.94357
	6	0.01935	0.27112	0.95041
	8	-0.20827	0.15730	0.93678
Percentage variance contribution		41.7	23.4	17.8
Cumulative percentage		41.7	65.1	82.9

The low planting density (points 3:0, 3:3, 3:9) allied on the positive side and the high density points (points 9:0, 9:3, 9:9) on the negative side of axis 2, thus indicating intraspecific competition in accordance with the initial planting densities (Figure 3.2). In addition, the results of ANOVA (Table 3.2) show that fresh weight ($F_{1, 62}$ =34.60, P<0.01) and number of shoots produced ($F_{1, 39}$ = 94.86, P<0.01) were significantly greater at low initial planting density (3H:0L, 3H:3L, 3H:9L) than at high density (9H:0L, 9H:3L, 9H:9L) of water hyacinth. Other parameters were not significant but followed a similar pattern.

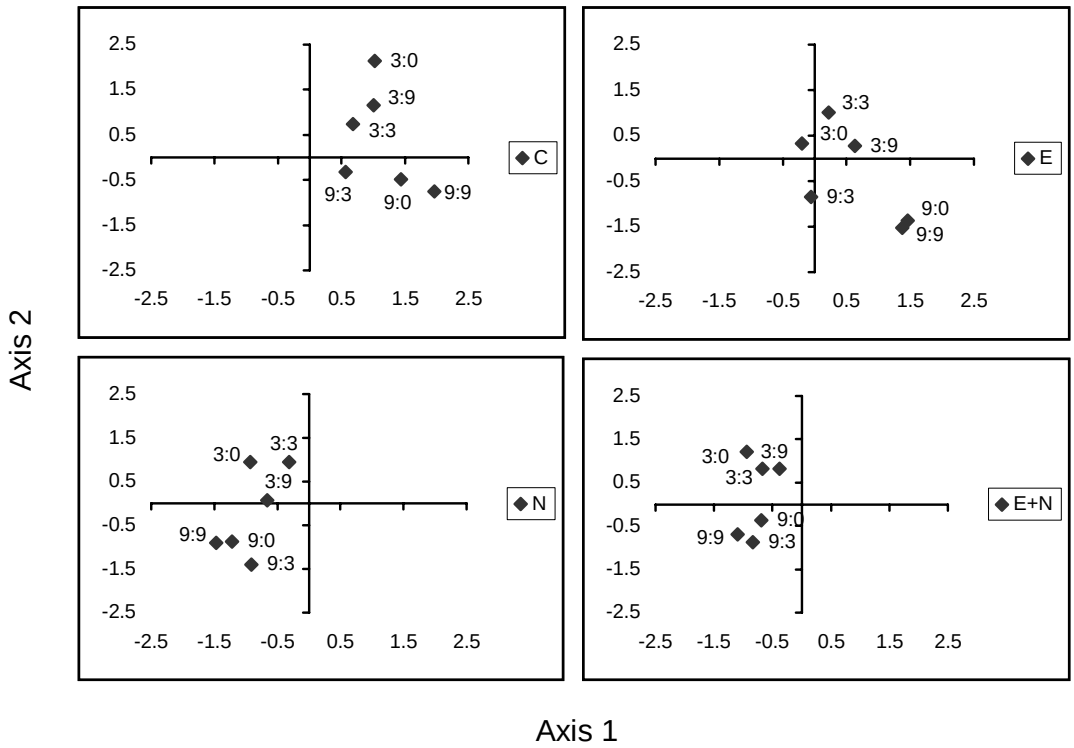

Figure 3.2. The principal component analysis (PCA), showing factor 1 (Axis 1) and factor 2 (Axis 2) of plant growth parameters (fresh weight, number of new shoots, leaf/petiole lengths etc.) at different planting densities (0, 3 and 9) of water hyacinth:water lettuce ratios according to the different insect treatments (C=control, E= *Ecrritotarsus catarinensis*, N= *Neochetina eichhorniae* and E+N= the combination of *E. catarinensis* and *N. eichhorniae* using ½ the inoculum in single treatments).

Table 3.2. Water hyacinth fresh weight, leaf/petiole lengths, number of new shoots and flowers produced, according to the initial planting densities (3=low and 9=high) of water hyacinth (H) and water lettuce (L) taken 8 weeks after introducing biological control agents. Means (per plant) with the same letter are not significantly different at (P<0.05).

Planting densities	**Mean fresh weight in grams (n=12)**	**Length in cm**		**Number produced**	
		Leaf (n=12)	**Petiole (n=8)**	**New shoots (n=24)**	**Flowers (n=8)**
3H:0L	210.413 a	6.58 a	19.64 ba	1.54 a	0.20 a
3H:3L	221.941 a	7.39 a (n=11)	21.94 a (n=7)	1.39 a	0.34 a (n=7)
3H:9L	235.691 a	7.30 a	20.59 ba	2.0 a	0.43 a
9H:0L	151.765 b	6.95 a (n=11)	21.55 a (n=7)	0.09 b	0.19 a (n=7)
9H:3L	128.422 b	5.78 a	15.10 b	0.06 b	0.17 a
9H:9L	148.885 b	5.92 a	18.12 ba	0.05 b	0.37 a

In summary, water hyacinth plant fresh weight and production of new shoots were the most important variables for measuring impact of agents and were strongly influenced by the initial planting density and treatment. The subsequent discussion, therefore, focuses on plant weight and new shoots, with less emphasis on the other variables.

3.3-3 Plant competition

Regression results (Table 3.3) shows that yield in fresh weight was influenced by intraspecific competition (a_{hh} >0) in all treatments, and in some cases (where a_{hi} >0) by interspecific competition. Without herbivores, the competition ratio a_{hh}/a_{hi}

was significantly different (p= 0.04) and water hyacinth was 18 times more competitive than water lettuce. The ratio declined to 2.64 in the treatment with the weevil *N. eichhorniae*, (still significant p= 0.05) and to -0.32 in the treatment with *E. catarinensis,* which was not significant (p=0.28). It further declined significantly to -23.87 (p=0.01) for both species together.

Table 3.3. Multiple regression analysis on water hyacinth fresh weight showing effects of the initial water hyacinth and water lettuce planting densities in treatments without and with agents (C= control, E= *E. catarinensis*, N= *N. eichhorniae* and E+N= combination of *E. catarinensis* and *N. eichhorniae* (using ½ the inoculum in single treatments), using the inverse linear model.

Treatment	**Regression coefficients**		**Intercept**		$\mathbf{R^2}$	***F* (2, 15) (*p*)**
	a_{hh}	a_{hi}	a_{hh}/a_{hi}	a_{ho}		
Ec	0.00010859	-0.00033080	-0.32	0.00725	0.1538	1.36 (0.2859)
Ne	0.01603	0.00607	2.64	-0.00801	0.4731	6.74 (0.0529)
Ec+Ne	0.00067129	-0.00002812	-23.87	0.00445	0.4767	5.92 (0.0149)
C	0.00017358	0.00000936	18.54	0.00274	0.35	4.07 (0.0388)

3.3-4 Effect of treatments

The effect of treatments varied according to species treatment and species combination, and on the growth parameter being measured. In figure 3.3, except for new shoots in the treatment with the mirid, plant growth parameters taken at the end of the experiment decreased compared to those at the beginning of the experiment. In the control (C), fresh weight increased significantly and differed from the treatments with agents ($F_{1,\ 48}$ =62.85, P<0.01) (i.e. E, N, and E+N combined), which showed fresh weight reductions.

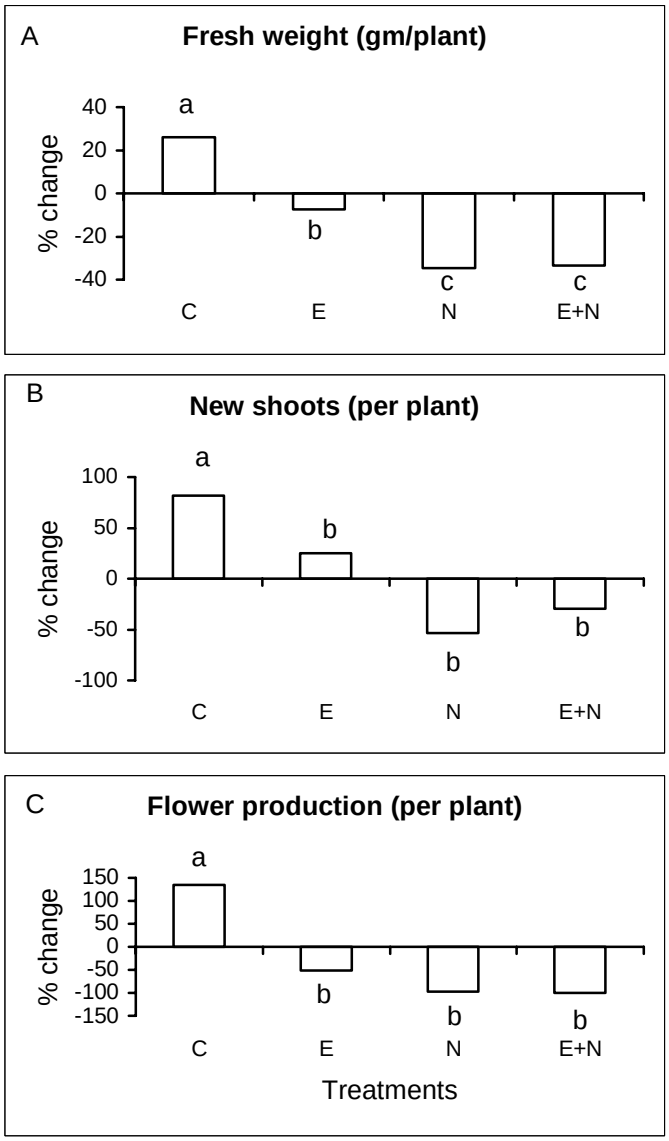

Figure 3.3. Percentage change (+)=increase; (–)=decrease) in plant growth parameters of water hyacinth at 8 weeks after introducing agents (E=*E. catarinensis*, N=*N. eichhorniae*, a combination both species =E+N at ½ inoculum in species treatments and the C=control without agents. Labels on the bars in letters are based on the value of the mean (ANOVA) and bars with the same letters did not differ statistically (P<0.05) (formula used for to calculate the percentage change= Initial measurement (taken on week 0) – Final measurement (taken on week 8) ÷ Initial measurement ×100).

Compared to the control, the treatment with the mirid alone reduced water hyacinth fresh weight by 35.2%, the weevil alone by 52.7%, and both species together (E+N) by 51.1 % after eight weeks, following the introduction of agents. Effects on the numbers of new shoots and flower production followed a similar pattern, indicating a decreased reproductive capacity of water hyacinth (Figure 3.3, B and C).

Surface cover by water lettuce, taken when the experiment was terminated at week eight, increased in all treatments with the highest increase in treatment (N) with the weevil alone (44.7%). This was followed by treatment (E+N) with the combination of the mirid and weevil (37%), and treatment (E) with the mirid alone (14.3%). In the control (C), surface cover by water lettuce increased by 2.4% only.

Leaf and petiole lengths, not shown here, varied according to treatments. Compared to the control, by the sixth and eight week, leaf and petiole length declined significantly in all treatments with agents, except in the treatment with the mirid alone, where no significant difference was found in petiole lengths.

3.3-5 Planting density and herbivory

Initial water hyacinth planting densities and herbivory influenced one another as indicated by fresh weights and the numbers of new shoots measured after eight weeks (Figure 3.4). At low densities of water hyacinth (3H), there was no statistical difference among treatments with agents, although each differed significantly from the control. At high water hyacinth planting densities (9H), fresh weights decreased and differed depending on treatment.

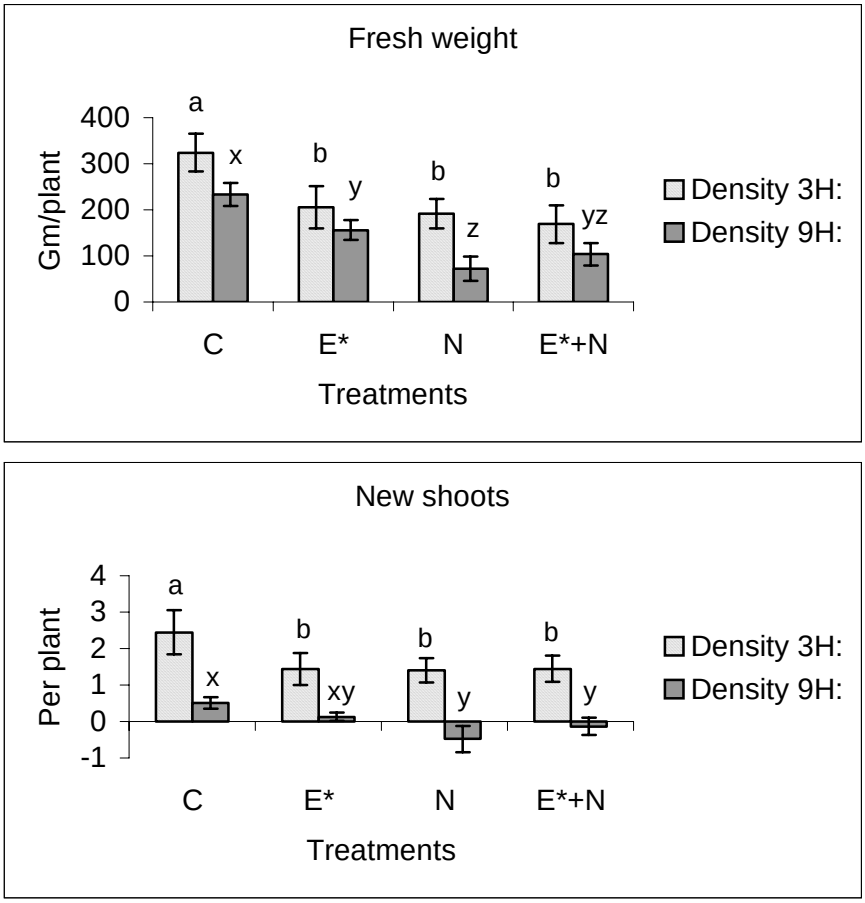

Figure 3.4. Influence of initial planting density and herbivory on water hyacinth fresh weight and production of new shoots 8 weeks after introducing agents (C=the control without agent, E=*E. catarinensis*, N=*N. eichhorniae* and E+N=combination both species). Means with the same letter are not significantly different (P<0.05); bars represent means ± standard error.

3.5 Discussion

This experiment assessed, for the first time, the impact of two biological control agents from different insect orders (a mirid and a weevil) both with very different modes of feeding, by measuring the reduction in growth of the host plant, water hyacinth, which was experimentally subjected to competition by another floating water weed. The principal component analysis (PCA) was used to identify fresh weight, new shoots and water lettuce cover (as the first, second, and third factors

respectively) (Table 3.1) as the most important growth parameters among the six parameters measured (Cody and Smith, 1997). PCA has been used to identify variables most important in a set of data (Badu-Apraku *et al*., 2006). Plotting the components of the first and second factors shows that plants at low density with less intraspecific competition had higher turnover (weight, new shoots, petiole and leaf lengths and number of flowers produced) and therefore, the centroids were on the positive side of axis 2, higher than those of higher planting densities (Figure 3.2). Further analysis (ANOVA) was therefore carried out on the most important growth parameters.

Water hyacinth is generally assumed to be a superior competitor over water lettuce (Wright and Purcell, 1995). This study quantifies this competitive advantage which is, however, only shown when the plant is not attacked by biological control agents which, as measured by the competition ratio a_{hh}/a_{hi}, were 18.5 times greater than water lettuce (Table 3.3). Where water lettuce already exists, this confirms its displacement following the introduction of water hyacinth (Center *et al*., 2001; Ajuonu and Neuenschwander, 2003). With agents, the ratio declined to -0.32 in the presence of the mirid (Table 3.3). In a similar study, the ratio was 23.6 times greater without agent, but declined to 10 in the presence of the mirid (Coetzee *et al*., 2005). In this study, the weevil alone reduced the ratio to 2.64. Center *et al*., (2005) have shown that without agents, water hyacinth was 41 times more competitive than water lettuce, but the weevil *N. eichhorniae* reduced the ratio to 1.4. In this study, the mirid combined with the weevil for the first time, reduced the ratio to -23.87, which was better than the mirid alone (-0.32).

The differences in damage severity of the two biological control agents in this study are reflected in their impact on plant growth parameters in single species, treatments with the weevil having greater impact. The mirid lives entirely on the leaf of water hyacinth (Hill *et al*., 1999b) and damage is usually mild (Coetzee *et al*., 2003). For the weevil, the primary damage is due to larval stages that bore

into the crown of water hyacinth (DeLoach and Cordo, 1976) leading to shoot mortality (Center and Van, 1989). In the present experiment, with the host plant simultaneously subjected to competition by water lettuce, the mirid reduced water hyacinth growth significantly similar to the weevil, when compared to the control (Figure 3.3).

In treatments with both species, the impact of agents is expected to occur in an overlapping sequence according to their respective life cycles, i.e. three weeks for the mirid (Hill *et al*., 1999b) and 96 days for the weevil (Julien *et al*., 1999). In treatment (E), the number of adults and immature mirids per leaf after one generation, was 15.12 ± 1.29, compared to 9.19 ± 0.67 in the mixed treatment with half the inoculum of mirids. This relatively better performance may be due to access to fresh feeding holes made by the weevil, as reported in a laboratory study (Ajuonu *et al*., 2007, Chapter 2). At this time, the increased mirid population caused severe leaf damage and added to damage on young leaves and petioles caused by adult weevils (DeLoach and Cordo, 1976). By the sixth and eighth week (when the mirid population had dropped by 78.6% due to poor leaf quality) weevil larvae had developed into second/third instars and were old enough to cause severe damage (DeLoach and Cordo, 1976) that overlapped again with increased damage by mirids. This explains why despite the 78.6% decrease in the mirid population at week eight, treatment with both agents (E+N) produced similar effects as those with the weevils alone (N) (Figure 3.3).

Initial planting density influenced water hyacinth growth and impact by agents. At the end of the experiment, fresh weights at low water hyacinth densities (3H) were 1.5 times higher, while the numbers of new shoots were 27.3 fold greater compared to those at high densities (9H) (Table 3.2). Center and Van (1989) have shown that at low plant densities, leaf production is accelerated. This explains the low impact of agents on water hyacinth fresh weight and production of new shoots recorded at low planting densities in this study (Figure 3.4). In contrast, at high initial planting densities, the effect of herbivory on fresh weight

and production of new shoots was more severe in all treatments with insect agents. This can be attributed to the combined effects of intraspecific interaction among plants at high densities and interspecific interactions with water lettuce under the influence of biological control agents, similar to the results of Center *et al*. (2005).

Competition design, addition series (Spitters, 1983), were used in studies by Center *et al*. (2005), Coetzee *et al*. (2005), both similar to this study. In all previous cases, studies have shown that herbivory can reduce the competitive ability of water hyacinth grown with another aquatic plant. This study combined the mirid and the weevil for the first time. Although there are studies that advocate that one biological control agent is better than multiple agents, in weed control, the rate of success has also been shown to increase with number of agents (Denoth *et al*., 2002). In conclusion, this experiment has demonstrated that although *E. catarinensis* is less damaging than the weevil, it is able to contribute to the biological control of water hyacinth. When combined with the weevil, this may be particularly important under conditions where the weevils are not as effective, e.g. lakes with shallow banks where water hyacinth has rooted in mud (Ajuonu *et al*. 2003).

CHAPTER FOUR

DISPLACEMENT OF THE BIOLOGICAL CONTROL AGENT *E. CATARINENSIS* IN THE LABORATORY BY AN INDIGENOUS MIRID *NYCTICAPSUS* SP.

4.1 Introduction

Eccritotarsus catarinensis imported into Benin in 1998 was maintained in the laboratory for eventual supply to other countries within the West African region, for use as an additional agent for the biological control of water hyacinth. However, after two years, the exotic mirid *E. catarinensis* was apparently displaced in the laboratory culture by an indigenous mirid *Nycticapsus* sp (Hemiptera: Miridae).

The indigenous mirid *Nycticapsus* sp. is not a natural enemy of water hyacinth. It is widespread in tropical Africa, often found in luxuriant savanna and rain forests (Linnavuori, 1994; Akingbohungbe, 1996) before the inroduction of water hyacinth into West Africa. From specimens collected in our laboratory culture, it was identified by Professor A. E. Akingbohungbe (Department of Plant Science, Obafemi Awolowo University, Ile-Ife, Nigeria) during one of his visits to IITA-Benin in Cotonou. Eventually, it was observed that the indigenous mirid *Nycticapsus* sp. had displaced *E. catarnensis* in all the rearing cages. As a consequence, in May 2002, another culture of *E. cararinensis* was shipped from PPRI, South Africa to IITA, Benin.

In maintaining laboratory cultures of several biological control agents for the various projects executed by IITA in the past twenty years, major efforts are directed towards keeping the cultures free from contaminations. One of the first projects, the cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero, Hemiptera: Pseudococcidae) which started in the 1980s, about eight agents were

maintained which on request, were shipped to several countries across Africa (Herren *et al*., 1987; Neuenschwander, 2001). In the laboratory culture of these agents, some indigenous parasitoids and predators which originally attacked other mealybugs (Neuenschwander *et al*., 1987) were often found in rearing cages, apparently entering accidentally. In another project against mango mealybug, *Rastrococcus invadens* Williams (Hemiptera: Pseudococcidae) that started some years after the cassava mealybug project, some of the same indigenous parasites and predators found in cultures of the agents of the cassava mealybug were also found in the cultures of the mango mealybug natural enemies. This affected the quality and production of agents (Neuenschwander *et al*., 1989; Neuenschwander and Haug, 1992). Against this background, when *Nycticapsus* sp. was observed in the *E. catarinensis* culture, it was considered a serious contamination problem. In view of the planned shipment of *E. catarinensis* to other West African countries, it was important to have a clean culture since quarantine regulations require the shipment of agents without any form of contamination (FAO, 1996).

Damage to water hyacinth leaves by *Nycticapsus* sp. and *E*. *catarinensis* are similar (Ajuonu, pers. Obs.). This constituted a monitoring problem in Savalou, Southern Benin, were *E*. *catarinensis* was released in 2000. This experiment was therefore was conducted to determine if *Nycticapsus* sp. can out-compete the exotic mirid *E*. *catarinensis* in the laboratory, and therefore potentially in the field.

4.2 Materials and methods

4.2-1 Rearing of *Nycticapsus* sp. and *E. catarinensis*.

Nycticapsus sp. and *E. catarinensis* were reared on water hyacinth in the laboratory at IITA. The techniques for rearing and production of water hyacinth were similar for the indigenous mirid and the exotic mirid and have been described (see experiment 1, Chapter 2, section 2.2-2). Each water hyacinth

plant used for the experiment had 6-7 leaves.

4.2-2 Set up of experiment, design and data collection.

The experiment was conducted from August to December 2005 in the laboratory using wooden cages similar to those used in experiment one (Chapter 2, section 2.2-2). The conditions of temperature and relative humidity were similar to that of experiment one. The design consisted of the following insect treatments: (1) *E. catarinensis* only, (2) *Nycticapsus* sp. only and (3) *Nycticapsus* sp. combined with *E. catarinensis*. Each treatment consisted of two cages, each initially containing two water hyacinth plants and there were five replicates for each treatment, making a total of 10 cages for each treatment. The experiment was a completely randomised design with five repetitions in time duration

In treatment one (*E. catarinensis* only) and two (*Nycticapsus* sp. only), 12 adults of each species were introduced per plant (a total of 24 per cage). For the combined species, treatment three, six adults of *Nycticapsus* sp. and six adults of *E. catarinensis*, were introduced per plant, which is a total of 12 adults of each species in each cage. The infestation rates were based on existing data from field populations of *Nycticapsus* sp. (Ajuonu, unpublished data). To sustain the mirid populations, two fresh water hyacinth plants were added into each cage per week and as immatures and adults moved to newly introduced plants, old and dead plants were removed.

Two cages from each treatment were destructively sampled at 5, 8, 11, 14, and 17 weeks after introduction of the agents. From preliminary studies, the life cycle of *Nycticapsus* sp. is about 3 weeks, similar to *E. catarinensis*. Therefore, five weeks allowed for one generation and 17 weeks more than five overlapping generations. All adult mirids were collected with the aid of a mouth aspirator. Thereafter, counts of adult mirids and separation of species were carried out with

the aid of a binocular microscope.

4.2-3 Data analysis

Count data were log10(x + 1) transformed for normality before analysis. In the single species treatments, repeated measures ANOVA and polynomial models were used to assess populations of *E. catarinensis* and *Nycticapsus* sp. and to compare insect population trends. In species combinations, a paired t-Test was used to compare counts of adult *E. catarinensis* and *Nycticapsus* sp.

4.3 Results

4.3-1 Counts of adult mirids

In the single species treatments, counts of adult *E. catarinensis* taken at 5, 8, 11, 14, and 17 weeks following introduction of the agent were higher compared to those of *Nycticapsus* sp. (Figure 4.1). The weekly mean numbers of adults in the treatment with *E. catarinensis* were consistently higher (from 137.0 in week 5 to 1607.5 in week 17) than the treatment with *Nycticapsus* sp. (from 90.5 in week 5 to 1014.0 in week 17). Statistical comparison of adult counts (log10(x + 1)), indicated that the treatment with *E. catarinensis* was different (P <0.001) from the treatment with *Nycticapsus* sp. and that their trends over time, though similar (P =0.915), were showing a significant linear increase (P < 0.001).

In the treatment with both species combined, the mean after 17 weeks adult counts per cage for the three weekly intervals of the trial were 372.2 for *E. catarinensis* and 268.0 for *Nycticapsus* sp. (Table 4.1). The result of a paired t - Test to compare adult counts in the combined species treatment showed that the exotic mirid *E. catarinensis* was higher than the indigenous mirid *Nycticapsus* sp (Table 4.2).

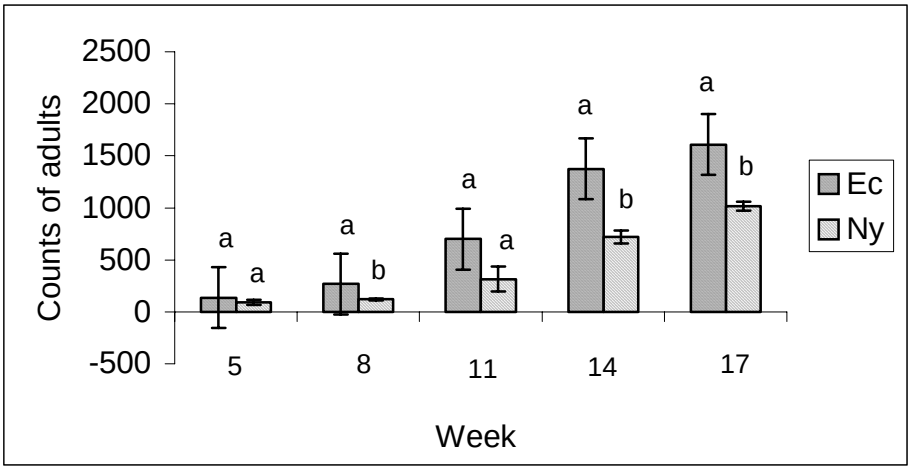

Figure 4.1. Mean counts of adult *E. catarinensis* (Ec) and *Nycticapsus* sp. (Ny) in in single species treatments taken at 5, 8, 11, 14 and 17 weeks after introducing agents. Bars represent means ±SE. Means across treatments followed by the same letter(s) above the bar are not different at *P*= 0.05 from (log10 (x + 1)) values.

Table 4.1. Mean counts per cage of adult *E. catarinensis* and *Nycticapsus* sp. in a treatment with both species combined.

Treatments	Mean	N	Mini	Max	Std error	Std Dev
Ec	376.2	10	56.0	1314	117.65	372.04
Ny	268.0	10	39.0	820	82.92	262.21

Table 4.2. Paired t-test of adult counts, *E. catarinensis* (Ec) and *Nycticapsus* sp. (Ny) in combined treatment species.

Variable	Mean	N	Std error	Std Dev	Pr > |t|
Difference (Ec-Ny)	108.20	10	46.13	145.88	0.0436
Log difference (Ec-Ny)	0.172	10	0.032	0.104	0.0005

4.4 Discussion

The exotic mirid *E. catarinensis* was displaced by an indigenous mirid *Nycticapsus* sp. after two years in the laboratory culture at IITA. In this present laboratory study, counts of adult *E. catarinensis* were, however, consistently higher than those of the indigenous mirid *Nycticapsus* sp. in the singe species treatments and in combined species treatment over time. This infers that reproduction by the exotic mirid *E. catarinensis* was superior to that of the indigenous mirid *Nycticapsus* sp. Therefore, this experiment did not show that the indigenous mirid was capable of displacing the exotic biological control agent.

In the laboratory culture and during rearing of *E. catarinensis* used for all the experiments, it was observed that both mirid populations remained high when new, healthy water hyacinth plants were supplied. A constant supply of healthy host at regular intervals was essential in maintaining natural enemies of the cassava mealybug (Neuenschwander *et al*., 1989). When a delay in replacement occurred incidentally and the quality of leaves deteriorated, populations of *E. catarinensis* quickly collapsed. Adults were particularly affected and in severe cases, surviving adults and immatures aggregated around the apex leaves of water hyacinth. In contrast, *Nycticapsus* sp. survived slightly better on relatively poor quality plants.

Over the course of this research study, mass production of large number of agents was one the most important tasks. Production of *E. catarinensis* was maintained for a period of 20 months. Also, rearing the indigenous mirid *Nycticapsus* sp. lasted for several months. Valuable rearing knowledge of both mirid species was gained during this period. For example, before *E. catarinensis* was displaced in 2001, water hyacinth plants from the outdoor culture were not washed with tap water before introducing them into the mirid rearing cages. In addition, cages used for rearing the exotic mirid are now cleaned and renewed more regularly than before. Practical knowledge is important in maintaining healthy natural enemies cultured at IITA insectary (Neuenschwander and Haug, 1992). Since the introduction of these practices, contamination of the rearing cultures by aphids and mites has been minimised. .

CHAPTER FIVE

CONCLUSION

The International Institute of Tropical Agriculture (IITA), Benin has successfully executed several classical biological control projects against arthropod pests across Africa (Herren *et al*., 1987; Herren and Neuenschwander, 1991; Neuenschwander, 2001). Among the successful projects, are biological control of weeds (water hyacinth, water lettuce and salvinia) which started in 1991 (Van Thielen *et al*., 1994; Neuenschwander *et al*., 1996; Ajuonu and Neuenschwander, 2003; Ajuonu *et al*., 2003; Mbati and Neuenschwander, 2004). For the biological control of water hyacinth in Benin, four agents were released at different dates (Neuenschwander *et al*., 1996; Ajuonu *et al*., 2003) and two agents established in the field (Cilliers *et al*., 2003) Therefore, Benin offered the ideal environment to conduct this study on the biological control of water hyacinth, using multiple agents.

How many bio-control agents should be used against a specific pest or host? This is a long standing question in the field of biological control (Myers, 1985; Denoth *et al*., 2002). In certain situations, additional agents have not contributed much, while in other cases, additional agents in specific niches have often been responsible for improved control (Bokonon-Ganta *et al*., 1995; Neuenschwander, 1996). The use of multiple agents could lead to synergistic effect, additive or inhibitory (Stiling and Cornelissen, 2005) and the research results of this study are important in view of the debate; multiple versus single species in weed biological control. Some examples are discussed bellow.

Nine exotic arthropods agents have been released against the cassava mealybug, *Phenacoccus manihoti* (Homoptera, Pseudococcidae) a pest of cassava (*Manihoti esculanta*; Euphorbiaceae) across Africa (Moore, 1988;

Neuenschwander, 2001). The parasitoid, *Apoanagyrus* (*Epidinocarsis*) *lopezi* De Santis (Hymenoptera, Encyrtidae) had established before the release of a second species, *Apoanagyrus* (*Epidinocarsis*) *diversicornis* Howard. The release of the second species was performed because at that time, *A. lopezi* was considered not to be effective (Neuenschwander, 1996). *Apoanagyrus diversicornis* eventually did not establish and *A. lopezi* controlled the cassava mealybug across many ecological zones of tropical Africa while two coccinellid predators, *Hyperaspis notata* Mulsant and *Diomus hennesseyi* Fürsch (Coleoptera, Coccinellidae) established only in few countries (Neuenschwander, 2001).

For the second pest of cassava, the green mite (*Mononychellus tanajoa*: Acari, Tetranychidae) also released across Africa, success was attributed to one agent, similar to cassava mealybug, but under circumstances different from that of the cassava mealybug. Eight phytoseiid mites were introduced (Herren and Neuenschwander, 1991) but failed to establish and not until 10 years later, when another phytoseiid *Typhlodromalus aripo* DeLeon introduced proved to be successful (IITA 1996).

The spiralling white fly *Aleurodicus dispersus* (Russell) (Homoptera, Aleyrodidae) is an insect pest of ornamentals, shade trees and food crops. In Honolulu, Hawaii, the introduction and establishment of the parasitoid *Encarsia dispersa* Polaszek (Polaszek *et al*., 2004) (*Encarsia sp. near haitiensis* Dozer) (Hymenoptera: Aphelinidae) and the predator *Nephaspis oculatus* Blatchley (*amnicola* Wingo) (Coleoptera: Coccinellidae) resulted in the successful biological control of the spiralling whitefly (Kumashiro *et al*., 1983). In West Africa, the two parasitods *E. dispersa* and *E. guadeloupae* Viggiani (Hym. Aphelinidae) had serendipitously invaded with their host, *A. dispersus* (Neuenschwander, 1994) and by the third year the population of *A. dispersus* had declined (D'Almeida *et al*., 1998).

The mango mealybug *Rastrococcus invadens* Williams (Homoptera, Pseudococcidae) is a pest of mango (*Mangifera indica*). Two parasitoids, *Gyranusoidea tebygi* Noyes, first released in 1987 and *Anagyrus mangicola* Noyes (both Hymenoptera, Encyrtidae) released in 1991, successfully controlled the mango mealybug in West and Central Africa, contrary to the predictions of an early model which predicted that the addition of *A. mangicola* would not be beneficial (Neuenschwander, 1996). Both parasitoids have separate niches and coexist. *Anagyrus mangicola* selects larger host instars than *G. tebygi* (Bokonon-Ganta *et al*., 1995).

For weeds, the control of *Sesbania punicea* (a leguminous weed in South Africa) is a good example where multiple agents have been used very successfully. Where the three agents, comprising a stem-boring weevil (*Neodiplogrammus quadrivittatus*) a flower feeding weevil (*Trichapion lativentre*) and a seed-feeding weevil (*Rhyssomatus marginatus*) are present, *S. punicea* is no longer problematic (Hoffmann, 2001).

Water hyacinth offers a particularly good example of this ecological question of the need for more agents in biological control. It differs from other aquatic weeds such as water lettuce, and salvinia where one agent proved very effective (Cilliers *et al*., 2003). In many countries, agents used against water hyacinth were released at different times over a period of many years (Chapter 1, Table 1). Two weevils, *N. eichhorniae* and *N. bruchi*, released in 1991 and 1992 respectively established but *N. eichhorniae* became more dominant even in sites where *N. bruchi* only was released (Ajuonu *et al*., 2003). In contrast, four agents were established in South Africa including the mirid, which was recently released, several years after the weevil had established (Cilliers *et al*., 2003). However, the weevil could preclude success of the mirid, or the mirid if well established could interfere with the weevil.

Different agents may be successful in different environments, and successful control may only be achieved in certain environments (Myers, 1985). Therefore the establishment and impact of water hyacinth control agents in West, East and South Africa may not be only due to direct or indirect interactions between agents. It could be attributed to the differences in environment such as variable climatic conditions, eutrophication of aquatic ecosystems (Hill and Olckers, 2001), level of salinity (van Thielen *et al*., 1994) and varying depths of lakes (Ajuonu *et al*., 2003).

The first experiment of this study investigated the co-existence of the mirid with the weevil in a situation where the weevil had long been established. The results showed that adult and immatures of *E. catarinensis* had high mortality on plants with high levels of old feeding scars from adult *N. eichhorniae* and *N. bruchi* which was significantly different from plants without scars and with fresh scars. Despite the negative effect of old scars on the mirid, they managed to survive on leaves with more than 200 old feeding scars made by the weevil due to their short life cycle. In contrast, on plants with recent feeding scars from *Neochetina* spp., or on undamaged plants the result showed that mirids survived well. Therefore it was concluded that the weevil, which had long been established, would not prevent the establishment of the mirid as additional biological control agent.

In the second experiment conducted out doors in a screen house, one of the aims was to evaluate the impact of combining both species. The results showed that the mirid reduced the fresh weight of water hyacinth by 35.2%, the weevil by 52.7% and both species together by 51.1%, compared to the control without agents. Although the mirid inoculation rate was by weight 75% less than the weevil, the decrease in water hyacinth fresh weight and new shoot production by the two agents together was similar to the treatment with the weevil alone and better than with the mirid only. In addition, the best impact on production of

flowers was in the treatment with the combination of the weevil and the mirid which would decrease the seed bank produced by the next generation of plants, without interfering with control by the weevil. This assumption should be tested in longer term field studies.

The third experiment determined if the indigenous mirid *Nyctcapsus* sp. could out-compete *E. caternensis* in the laboratory and therefore in the field. The results showed that *E. catarinensis* was reproductively superior (with a higher number of adults) produced compared to *Nycticapsus* sp. in all the treatments as well as over time, *Nycticapsus* is not likely to displace *E. catariensis* in laboratory culture as long as water hyacinth plants are washed with tap water before introducing them to the laboratory cultures and that fresh water hyacinths are introduced into rearing cultures at regular intervals.

Some fundamental issues in biological control include safety of agents. To prevent the risk of agents becoming a pest themselves in there new environment, host-specificity studies to determine if the agent will attack only the target host or other species are usually carried out. The mirid used in this study was released in South Africa against water hyacinth based on the results of host-specificity studies (Hill *et al*., 1999b) and subsequently in West Africa without further testing. In contrast, based on results of a similar study in Australia, the mirid was not sufficiently host-specific and was not considered suitable for release (Stanley and Julien, 1999). Therefore host-specificity results will however in some situations not provide absolute safety for a candidate agent across all ecological zones where related target weeds are at risk (Harley and Forno, 1992).

Efficacy testing before release is another issue. For water hyacinth control, *N. bruchi* was considered a better agent than *N. eichhorniae* based on a shorter generation time and faster rate of increase (DeLoach and Cordo, 1976). In the

same study, it was noted that *N. eichhorniae* tolerated higher temperatures compared to *N. bruchi* and this could explain why *E. eichhorniae* is predominant in tropical West Africa (Ajuonu *et al*., 2003). Similarly, for cassava mealybug control, the initial studies on *A lopezi* showed that it will not be efficient parasitoids because the life table parameters were not sufficiently higher than that of the target. However, the developmental biologies of the insect of the female in which (males are produced from small instars of the host and females from large instars) (Neuenschwander, 1996) and the searching capacity of the agent (Neuenschwander and Ajuonu, 1995), made *A. lopezi* a more efficient agent compared to the other three parasitoids released against cassava mealybug.

There is also the issue relating to the effect of multiple releases of agents, the focus of my study. Multiple agent introduction often after unsuccessful control efforts by previous agent or agents, has been described as ‘’lottery’’ (Myers, 1985) which could lead to synergistic effects, either additive or inhibitory (Stiling and Cornelissen, 2005). Even though my study has been an opportunistic “testing” post release, of the mirid, the results show that the mirid has positive synergistic/additive effects and therefore makes a contribution to the debate on single species versus multiple species in biological control.

In conclusion, predicting efficacy of biological control agents is difficult as field observations may sometimes vary from laboratory studies. The effort required to provide useful data for models is tremendous and can not be achieved by only one institution (Neuenschwander, 1996). In our rapidly changing environment with increasing concern on the effects of biological control on non-target organisms, adopting a precautionary approach and following simple guidelines are important in taking any decision (McEvoy, 2000).

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