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FACULTY OF SCIENCE

SCHOOL OF ANIMAL, PLANT AND ENVIRONMENTAL SCIENCES

The effect of temperature on the feeding and development of *Neochetina eichhorniae* larvae on water hyacinth (*Eichhornia crassipes*)

Ndongo Fyfy Bokotomba

A Dissertation submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science.

DECLARATION

I, Ndongo Fyfy Bokotomba, declare that this Dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



_____

Signature of the candidate

27 Day of March 2017

ABSTRACT

Water hyacinth (*Eichhornia crassipes*) is an example of an alien invasive plant, threatening ecosystems around the world. Taking into consideration all the negative impacts caused by the proliferation of water hyacinth, many control measures have been implemented to manage this weed. These include mechanical control, chemical control and biological control. But in recent years, the focus has shifted towards natural enemies for control (biological control) of water hyacinth because they have many advantages for environmental safety. However, in South Africa, success is limited in most parts of the country. Factors affecting the success of biological control of water hyacinth in South Africa have been attributed in part to weather conditions. Therefore, further studies and other strategies are needed to reinforce the biological control of water hyacinth. This is why this research investigated the influences of temperature on *Neochetina eichhorniae* larvae feeding and development on water hyacinth, to contribute to the future management of water hyacinth biological control.

To measure the effect of temperature on the feeding and development *of Neochetina eichhorniae* larva, experiments were conducted at four fixed temperatures (15 °C, 20 °C, 25 °C, and 30 °C). Larval feeding is expected to be greater and the development faster at warm temperatures compared with cold temperatures. The results of this study confirmed that, both feeding and larval development increased with an increase in temperature. The highest larval feeding damage was recorded at 30°C and the lowest at 15°C. Similar to the feeding of larvae, warmer temperatures were more favourable to the growth of larvae than cold temperatures; all larvae found at 30°C were in their third instars (with 0.72 mm being the biggest headwidth found) while all larvae at 15°C were in the first instars (with 0.28 mm being the smallest headwidth found).

Even if the optimal temperature for larval performance took place at 30 °C (with a high amount of biomass removal), it was at 25 °C that the larvae had a larger effect on the growth of water hyacinth. Larval feeding damage reduced the growth of water hyacinth by 30 % at 25 °C and only affected 24 % of water hyacinth growth at

30 °C. Therefore, for the biocontrol of water hyacinth, the ideal temperature to slow the progression of the weed will be recommended at between 25- 30 °C.

DEDICATION

This work is dedicated to my late father: Pierre Bokotomba wa Ndongo Lomboto, nothing in the world is worth the sacrifices you made to my well-being and my education. Find in this modest work the expression of these vows that you have always done in your prayers. May the Almighty God grant his mercy on your soul and welcome you in his Holy paradise.

And to my lovely daughter, Divine Ndongo Kahota, thanks for the joy that you make me feel. I love you and wish you a life full of success and happiness.

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1 CHAPTER ONE – INTRODUCTION

1.1 Problem statement

Nature has suffered considerable damage and loss of resources as a result of anthropogenic exploitation (Burgman and Lindenmayer, 1998). Many ecosystems have been degraded, polluted and even destroyed (Burgman and Lindenmayer, 1998). Alien invasive plants (AIP) represent the second most significant cause of species extinction worldwide after habitat destruction (MacDonald, 2008; IUCN, 2014). Alien invasive species are considered to be one of the key pressures on the world's biodiversity, shrinking the abundance and richness of local species and decreasing genetic diversity of their ecosystems (Saltman and Cliff, 1997; Rands *et al*., 2010; Zhang *et al.,* 2010; *Nature* Conservancy, 2014). Alien invasive species cause substantial economic losses estimated to be billions of US dollars annually, around the world (Pimentel *et al*., 2005; Kettunen *et al*., 2009). In South Africa, more than 0.3% of South Africa's gross domestic product (GDP) is used in invasive plant management (van Wilgen and De Lange, 2011; van Wilgen and Hoffmann, 2012).

Water hyacinth (*Eichhornia crassipes*) is one of the examples of alien invasive plants threatening ecosystems around the world. South African legislation has declared water hyacinth as a weed that must be controlled (NEMBA, 2014). The NEMBA regulations refer to more than 300 invasive alien plants. Water hyacinth has been identified by the International Union for Conservation of Nature (IUCN) as one of the 100 most aggressive invasive species (Téllez *et al*., 2008) and is recognized as one of the 10 worst weeds in the world (Pedigo, 1989; Shanab *et al*., 2010; Gichuki *et al*., 2012; Patel, 2012). In Africa, it is the most widespread and damaging aquatic plant species (UNEP, 2013). Water hyacinth is found across the tropical and subtropical regions worldwide. Its entry into Africa, Asia, Australia and North American was facilitated by human traffic from South America (Dagno *et al*., 2012). Water hyacinth plants have considerable environmental impact: they can easily dominate a water body and alter the species composition, structure, and function of indigenous communities (Langeland and Burks, 1998). Their large and dense mats can degrade water quality and choke waterways. Water hyacinth respiration and biomass decay can result in oxygen depletion, thus interfering with

water utilisation and other water activities such as fishing (Sterner and Elser, 2002; Masterson, 2007; Kateregga and Sterner, 2009).

1.2 Control of water hyacinth

Taking into consideration all the negative impacts caused by the proliferation of water hyacinth, many control measures have been implemented to manage this weed. These include mechanical control, chemical control and biological control. In recent years, the focus has shifted towards natural enemies for control (biological control) of water hyacinth. These include insects and pathogens because they have many advantages for environmental safety, such as:

- Long term management of target weeds;
- Limited side-effects on the environment;
- Sustainable;
- Low costs (Dagno *et al*., 2012; Villamagna and Murphy, 2010).

However, biological control takes more intensive management and planning than other techniques (Chaboudez and Sheppard, 1995; Hoffman, 1995; Moran *et al.*, 2013). There have been many successes around the world with regard to biological control of weeds, with dozens of weeds which have been successfully controlled using natural enemies (McFadyen, 2000). In South Africa, 59 % of target invasive weeds are totally or substantially under control by biological control agents and it is estimated that biological programs have already saved billions of Rand in weed control using natural enemies despite the high initial costs (Klein, 2011).

Factors affecting the success of biological control of water hyacinth efforts in some parts of South Africa have been attributed to the climate and water quality (Olckers and Hill, 1999; Hill and Olckers, 2001). According to Hill and Olckers (2001), many rivers and dams in South Africa receive water runoff which is highly polluted with fertilizers (nitrate and phosphate) from agricultural activities and sewage. This enriched water enhances the growth of water hyacinth and can affect the impact of biological control agents by making them less effective. Low temperature also affects biocontrol success by decreasing natural enemy populations (Byrne *et al*., 2010).

Detailed information concerning natural enemy establishment requirements, such as thermal conditions, are useful tools in understanding the behaviour of biological control agents (Vannote and Sweeney, 1979; Jervis and Copland, 1996; Obrycki and Kring, 1998; Jamieson *et al.*, 2012b). There are several natural enemies for water hyacinth and among them are the water hyacinth weevils (*Neochetina eichhorniae* and *N. bruchi*) (Deloach and Cordo, 1976). The control of water hyacinth using weevil such as *N. eichhorniae* and *N. bruchi* is considered successful in many countries, including some in Africa (Jones and Cilliers, 1999). In South Africa, the biological control of water hyacinth, using weevils, has been effective in certain areas such as New Year's Dam in the Eastern Cape (Jones and Cilliers, 1999), but remains hampered in other parts of the country. Therefore, further studies and other strategies are needed to reinforce the biological control of water hyacinth.

1.3 Way forward

This research investigates how temperature influences weevil feeding and development at the larval stage (Yang *et al.*, 1994; Zheng *et al.*, 2008). According to Deloach and Cordo (1976), weevils are most destructive to water hyacinth in their larval stage. Results obtained in the present study will contribute to the future management of water hyacinth biological control as well as for the construction of models predicting on the placement and timing of *Neochetina* weevils' releases in general and particularly for the *Neochetina eichhorniae* under different climate scenarios.

One of our teammates is investigating the influence of abiotic factors, including temperature, and turbidity on the performance of *Neochetina* weevils on water hyacinth in Eastern Africa. The results of this study may give some clarity to that work on the physiological relationship between water hyacinth and the *Neochetina* weevils.

2 CHAPTER TWO - LITERATURE REVIEW

2.1 Insects and temperature

The ability of a natural enemy to tolerate a wide temperature range is a critical factor in the evaluation of its suitability as a biological control agent (Jalali *et al*., 2010). Local temperature is a major environmental parameter influencing all the biological processes and functions of poikilothermic organisms, such as insects (Huey and Berrigan, 2001). Temperature is an important determinant of insect physiological and metabolic processes (Huey and Berrigan, 2001; Sanford, 2002). The metabolic demands increase linearly with temperature as well as consequently by food intake (Gillooly *et al.,* 2001; O'Connor, 2009; Lemoine *et al*., 2013) and consumption rates (Hillebrand *et al*., 2009; Vucic-Pestic *et al.,* 2011). Many studies show that herbivory by insect increases with rising temperature (Pakyari *et al.,* 2011; Jamieson, 2012). Parkyari *et al.,* (2011) found that total consumption by *Scolothrips longicornis* first instar larvae increased linearly when temperatures rose from 15°C to 37°C.

Bernays (1997) showed also that insect food consumption rates increase with temperatures between 20° and 30 °C, but decreased with further rise of the temperature. Despite these studies, herbivory rate of many insects remains unknown (Bernays, 1997).

Temperature is also a determinant in the physiological time of these organisms (Trudgill *et al*., 2005), especially for insect development rate research (Ratte, 1985; Honek, 1990).

Many methods of calculating thermal physiological time have been used to simulate the phenology of insects in biological scenarios as well as the biophysical model (Honek, 1996; Damos and Savopoulou-Soultani, 2012). The results of this project hopefully will contribute in the elaboration of a model for further water hyacinth biological control management. A linear relationship is expected to be found between temperature increase and the feeding damage of weevil larvae and their development (Jamieson *et al.,* 2012a).

2.2 Water hyacinth (Eichhornia crassipes)

2.2.1 Identification

Water hyacinth, also known as the water orchid (Figure), is a perennial and a free floating aquatic herb (Osei-Agyemang, 2002). The leaves of the water hyacinth are circular or ovate with thick petioles and dark roots (Figure.2) (Osei-Agyemang, 2002).



Figure 2.1: Water hyacinth *(Eicchornia crassipes*) (UNEP, 2013)



Figure 2.2: Water hyacinth plant from eutrophic water, showing long petioles and short roots (UNEP, 2013)

2.2.2 Geographical distribution

Water hyacinth is native to the Amazon basin in South America and it has emerged as a major weed in more than 50 countries in the tropical and subtropical regions of

the world, (Figure) with prolific and enduring impacts (Téllez *et al*., 2008; Shanab *et al*., 2010; Villamagna and Murphy, 2010; Patel, 2012;).

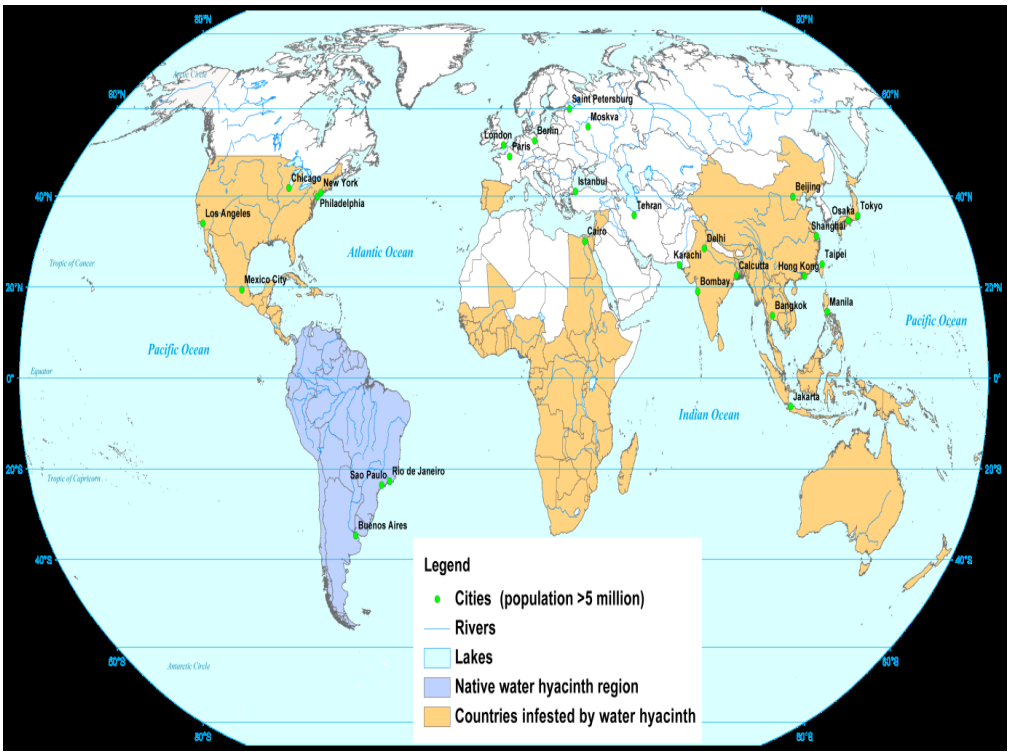


Figure 2.3: Global distribution of water hyacinth (Téllez et al., 2008)

Water hyacinth is mainly found in lakes, estuaries, wetlands, marshes, ponds, rivers, streams, and waterways rather than in saline water (Gopal, 1987; Coetzee and Hill, 2010). The plant is found at lower altitudes where growth is stimulated by the inflow of nutrient rich runoff waters from agriculture influence, as well as products of industrial or urban waste (Reddy and Tucker, 1983; Villamagna and Murphy, 2010). In Africa water hyacinth was recorded in Zimbabwe in 1937 (Mujingni, 2012), and Lake Victoria in 1989-1990 (Woomer, 1997). In South Africa, water hyacinth was first recorded in the Cape flats in 1908 (Stent, 1913) and subsequently spread country-wide (Hill *et al.*, 1999).

2.2.3 Reproduction

Water hyacinth can reproduce sexually and can flower throughout the year, with seeds living for many years (Albano Pérez *et al.*, 2011). Vegetative production is another way for water hyacinth to spread through the raceme (Albano Pérez *et al.*, 2011).

2.2.4 Productivity

Water hyacinth grows in all types of fresh water and its productivity is dependent on the environment in which it grows (Albano Pérez *et al.,* 2011).

There are five main factors limiting the growth of water hyacinth:

- Temperature;
- Nutrients;
- Disturbance;
- Natural enemies and
- Salinity (Wilson, 2001).

The main factor influencing the growth rate and spread of water hyacinth in South Africa is temperature and the water quality highly eutrophic (Albano Pérez *et al.,* 2011). Thus, the assessment of the performance of water hyacinth natural enemies at different water nutrient levels is being considered as a complement to this research by one of our team members. This study investigates temperature.

2.2.5 Management

Water hyacinth is difficult to control in all environments. Long term management should be carried out in order to reduce the infestation of the weed rather than eradication (Gopal, 1987), because the plant's reproductive capacity, makes it difficult to eradicate (Albano Pérez *et al.,* 2011). The different management methods for water hyacinth include:

a. Physical control

This consists of mechanical and manual removal of the plants. Mechanical control refers to the use of machinery designed to cut, shred, crush, press and remove plants from water bodies (Wangai, 2004).

b. Chemical control

Chemical control involves the application of herbicides to kill plants. In South Africa, herbicides are often regarded as the only option for water resource managers, needing a quick solution to a problem (Julien *et al*., 1996; Van Wyk and Van Wilgen, 2002).

Application of herbicides and mechanic removal of water hyacinth reduces not only the weed but also affects natural enemy populations consequently re-infestation of treated areas occurs soon after (Van Wyk and Van Wilgen, 2002).

c. Biological control

Biological control is based on the use of natural enemies of the weed to discourage the weed's development (Deloach *et al*., 1989). The most frequently used biocontrol agents for the weed are insects, mites or pathogens - such as fungi or bacteria (Charudattan *et al.*, 1978; Harris, 1991; Marlin, 2011). Biocontrol agents are specific with regards to their targets, thus the organisms attack only the species targeted for control (Terblanche *et al*., 2013).

The biological control of water hyacinth began in the 1960s and used the classical control strategy, which involves the importation of natural enemies from the point of origin of the weed to the invaded area. Biological control requires time for the assessment of the natural enemies, but it can offer a long term management solutions (Jimenez, 2003). In South Africa, the weevil *Neochetina eichhorniae* is used as a biocontrol agent for water hyacinth (Hill *et al*., 1999). This biocontrol agent was first released in 1973, but has had a limited impact on the weed (Hill *et al*., 1999). Therefore, this work will help to understand why the successful biological control of water hyacinth using *Neochetina* weevils is observed only in few parts of South Africa and its failure in other parts of the country.

2.3 Water hyacinth weevil (*Neochetina eichhorniae*)

Two South American weevils have delivered effective long term control of water hyacinth in many countries, notably Zimbabwe, Kenya, Benin, Papua New Guinea, USA and Mexico (Williams *et al*., 2007; Gichuki *et al*., 2012; Dagno *et al*., 2012). The weevils reduce water hyacinth vigour by decreasing plant size, vegetative reproduction, as well as flower and seed production (Reznick *et al.*, 2009; Venter *et al.*, 2013). This research will only assess *Neochetina eichhorniae*, the first biological control agent released and the most widespread or used in South Africa (Hill and Cilliers, 1999). The choice of *Neochetina eichhorniae* is based also on its successful performance in New Years Dam, Eastern Cape. And its apparent superior performance in autumn and winter compared with the other weevil biological control agent, *N. bruchi* (King, 2011).

2.3.1 Water hyacinth weevil life cycle

Adult water hyacinth weevils (Figure) are reproductive most of the year with average of 350- 400 eggs laid (Oberholzer, 2001).



Figure 2.4: Weevil beetle (*Neochetina eichhorniae*) (UPEN, 2013)

Female *N. eichhorniae* chew holes in the young leaf epidermis and then place eggs into those holes (Newete *et al.*, 2014). Eggs hatch in 7 to17 days and the three larval stages can take from 36 to 90 days, depending on the temperature. Newly hatched larvae (first instar) are about 2 mm long and grow to about 9 mm by the third instars (Gopal, 1987). Larvae tunnel into the petiole after hatching and usually arriving at the base of plant by the third instars (Deloach and Cordo, 1976).

2.3.2 Impact of the weevil (*Neochetina eichhorniae*) on water hyacinth

Both the adult and larvae of *Neochetina eichhorniae* impact water hyacinth (Cofrancesco *et al*., 1984). Adult feeding on the lamina reduces the epidermal surface area (Figure) and if the feeding is heavy, petioles are girdled and the lamina becomes desiccated (Cofrancesco *et al*., 1984).



Figure 2.5: An indication of the type of damage on the leaf surface caused by an adult *Neochetina* Weevil (Oberholzer, 2001)

Larvae feed on the meristematic tissue in the plant crown (Figure) then separate the growing tip from the rhizome and root (Cofrancesco *et al*., 1984).



Figure 2.6: The type of damage caused by *Neochetina* weevil larvae to water hyacinth (Oberholzer, 2001)

This literature review has examined the water hyacinth weevil as well as the impact of the weevil on the water hyacinth. With this information as a basis, I will now outline the aims and objectives.

2.4 Research aim

This study was designed to measure the damage caused by *N.eichhorniae* larvae on the water hyacinth growing in a hydroponic solution with a medium nutrient level at four different temperatures, to understand how temperature variation could impact on larval development when it is used in the biological control of water hyacinth.

2.5 Research objectives

1. To determine how temperature affects the feeding rate of *N. eichhorniae* larvae on water hyacinth;
2. To determine how temperature influences *N. eichhorniae* larval development.

2.6 Research questions

1. What is the relationship between temperature and *N. eichhorniae* larval feeding rate?
2. What is the relationship between temperature and *N. eichhorniae* larval development?
3. What is the relationship between *N. eichhorniae* larval feeding and its development?
4. What are the implications of these effects of temperature on the biocontrol and management of water hyacinth?

2.7 Predictions

Neochetina eichhorniae larval feeding behaviour changes with temperatures; larvae increase their feeding as temperature rises.

Neochetina eichhorniae larval development directly affects the biological control of water hyacinth.

3. CHAPTER THREE – MATERIALS AND METHODS

To measure the effect of temperature on the feeding and larval development of *Neochetina eichhorniae*, experiments were conducted at four fixed temperatures (15 °C, 20 °C, 25 °C, and 30 °C). From the 1st of August to the 10th of September 2015, the first inoculation took place, and the second inoculation took place from 12th of September to 24th 0f October 2015 (with the same plant material). Mean temperatures were 14.3 ± 0.30 °; 19.5 ± 2.61°; 25.1 ± 2.58° and 27.6 ± 0.55 °C in the 15 °C, 20 °C, 25 °C, and 30°C rooms temperature respectively for the first experiment. In the second experiment, mean temperatures were 14.7 ± 0.45°; 19.7 ± 1.16 °; 25.6 ± 0.83 ° and 29.5 ± 0.72 °C respectively. Experiments were done in the physiological laboratory at the University of Witwatersrand.

The second inoculation ("second experiment") was conducted after failing to collect larvae from the first experiment during plant dissections over three successive weeks. The objective was to reinforce the probability of finding larvae in experimental water hyacinth. It differed from the first inoculation in that oviposition and hatching of weevil eggs and larvae occurred directly into the water hyacinth (treatment plants only) by the weevils. However, with the first inoculation, oviposition and incubation were done externally and hatched larvae were then inserted into holes pierced in the petioles of water hyacinth. All other experimental steps remained the same for both experiments.

Water hyacinth plants and weevils were collected from a culture at the University of Witwatersrand (to minimize variation, the plants were taken from a single culture). Water hyacinth was grown in 5L buckets in a medium nutrient solution (2.8 mg/L of Nitrogen and 0.4 mg/L of Phosphorous) under controlled environmental conditions with a 12h00 light photoperiod.

3.1 Feeding damage assessment

The experiment comprised 22 5L buckets (11treatments and 11 controls) at each experimental temperature, 20 °C, 25 °C and 30 °C. Due to the fact that larval development was expected to be much slower at 15° C (King, 2011), 22 treatments and 22 controls buckets were utilised at 15 °C. All plants were acclimated for 7 days at their respective temperatures at the beginning of the experiment. Each 5L bucket

contained 3L of Hoagland's medium nutrient solution (2.8 mg/L of Nitrogen and 0.4 mg/L of Phosphorous) and a single water hyacinth plant. $NH_4H_2PO_4$, KNO_3, $Ca(NO_3)_2$, $MgSO_4$, H_3BO_3, $ZnSO_4$, $CuSO_4$, H_2MoO_4. H_2O, as well as Iron chelate were used as macro- and micro-nutrients, with distilled water, as recommended for Hoagland's solution 2 (Hoagland and Arnon, 1950). A total of 110 buckets, 110 *Eichhornia crassipes* plants and 110 *Neochetina eichhorniae* larvae were used for the first inoculation, and 45 adult weevils for the second inoculation. Plants were marked and their wet weight recorded at the start of experiment. Water hyacinth plants were placed on a perforated tray for at least three minutes to drain water before the weight was taken. Plants were placed on the scale (Ohaus Scout model, capacity 400 × 0.1 g) and their weight was recorded.

On a weekly basis, plant growth parameters such as wet weight, number of leaves, number of ramets, longest petiole length, were recorded for all plants. The nutrient solution was replaced weekly. To quantify damage by weevil larvae, their effect on growth of the weed was calculated. For example, with regards to the wet weight damage: the difference between mean wet weight change of controls and mean wet weight change of treatments gave the mean wet weight damage. To find the wet weight damage per larva per day, the mean wet weight damage was divided by number of larva found inside treatments on dissection per the number of days the larvae spent in the water hyacinth (= time in days) (Hauptfleisch, 2015) .

$$\text{Wet weight damage} = \frac{\text{Total control wet weight change} - \text{total treatment wet weight change}}{\dfrac{\text{Sample size}}{\text{Number of larva found} \times \text{time (days).}}}$$

The same calculations applied to:

$$\text{Petiole damage} = \frac{\text{Total control petiole length change} - \text{total treatment petiole length change}}{\dfrac{\text{Sample size}}{\text{Number of larva found} \times \text{time (days).}}}$$

Feeding damage for the first inoculation was not calculated due to the apparent absence of live larvae in the first dissections.

Only plants in good condition at the end of the second experiment were considered in calculations of feeding damage to a avoid bias. The second inoculation was interrupted because of the poor condition of the plants; the plants were dying in the majority of cases at high temperatures (25° to 30° C). These plants were damaged by the weevils and some were drowned in the solution (infiltration of water in to the plant could strongly influence the measurement of wet weight), so only plants in a suitable condition to be assessed were selected for the measurement of growth parameters. At 25° C, only 3 treatments were in a good enough condition to evaluate, so for consistency, at each temperature 3 treatments and 3 controls were selected for the evaluation.

3.2 Larval development

Every week one control and one treatment plant were removed from each temperature and were dissected to find larvae. If larvae were found, then larval survival and head width were recorded. Head widths were measured using an eyepiece micrometer on a stereo dissecting microscope (Wild M8 Microscope, Heerbrugg Switzerland) and larval development was then classified into one of three different stages, in accordance with the study conducted by Deloach and Cordo (1976). The stages were categorised as follow: first instar with a head width of up to 0.34 mm, second instar with a head width of up to 0.48 mm, and third instar with a head width from 0.49 mm and higher (Oke, 2009).

For the first inoculation, treatments were inoculated with two newly emerged larvae per plant. The culture of larvae was conducted as follows: adult males and females were placed in a 500 ml plastic box with green leaves of water hyacinth overnight for oviposition. Weevils were removed the day after and the water hyacinth leaves were left in the box for two more days and dissected on the third day. Eggs were dissected from the leaves under a stereo microscope (Wild Ms). Eggs removed from the leaves were placed in petri dishes with wet filter paper to maintain high humidity. Petri dishes were kept at 25° C for hatching, because according to previous studies (Deloach and Cordo, 1976 and Hauptfleisch , 2015), the optimal temperature for the development and survival of *N. eichhorniae* eggs is 25 °C, which was checked on a daily basis. The incubation period was recorded and the larvae were inoculated into the treatments on the same day they hatched. Larvae were carefully inserted into a pierced petiole of the first or second leaf with a small

wet paint brush. The reason for choosing the inner leaves was to avoid the early abscission of peripheral leaves when damaged by weevil larvae (Center, 1987).

In the second experiment (with the same water hyacinth plants used during the first inoculation) all plants were placed back into the 25 °C room for one week's acclimation. 45 adult weevils (males and females) were placed with water hyacinth leaves in a 500ml plastic box for a period of three days to ensure that a large number of the weevils were mated before inoculation. Treatment plants were inoculated on the third day by placing one male and one female from the mating box onto the treatment plants for one night only of oviposition, to ensure the same age of eggs. On the following day, the weevils were then gently removed (to not break the plant) from the water hyacinth. Using the hatching results found in the first inoculation (a maximum of 11 days for hatching at 25 °C) and counting from the first day of oviposition, all buckets were moved back to their respective temperatures on the eleventh day.

As for the larval dissection, larvae were dissected from the petioles under a stereo microscope (Wild Ms) and larvae found were placed in petri dishes with a moist filter paper. Head widths were measured under stereo microscope with an eyepiece micrometer (Leitz Wetzlar, Okular 10 ×). After head widths were recorded, larva wet weight was measured. Dry weight was found by placing a single larva in one small tube and larvae were dried for one day at 40 °C in an oven. Larvae were placed on scale (Swiss quality, Precisa 125 ASCS) and wet and dry weights were recorded. After 17 days (from the hatching date), the experiment was stopped.

Weight change of water hyacinth plants suggested a possible impact of the weevils on the treatment plants. Alternatively, the inoculation process itself (piercing the petioles) may have affected the treatment plants. Thus, the test on the inoculation process was conducted to check if the hole made in treatments affected their growth because difference between controls and treatments in the figure 4.1 was so visible at warm temperatures. In order to achieve this, 10 water hyacinth plants were used for the test (five as controls and five as treatments) grown under ambient conditions. All plants were weighed and the wet weight recorded. Subsequently, a hole was pierced in the treatments. A single plant was put in each 5L Bucket filled with 3L of medium nutrient which was changed weekly, as was done in the first experiment. After 22 days of observation (as for the first inoculation), wet weights were recorded and the growth of the controls and treatments calculated as described.

3.3 Statistical analysis

ANOVA with replicates was used to compare larvae feeding damage and the growth of plants (controls and treatments) of all measured parameters between different temperatures; and a t-test was used to compare the growth of the control and treatment plants at different temperatures. The statistical programme used to assess the results was statistical software and data analysis (XLSTAL, 2015).

Linear regression equations and correlation coefficients were used to determine relationships between feeding damage and development parameters to see if any positive relationship exists.

4. CHAPTER FOUR – RESULTS

The data were normally distributed at the beginning of the experiment, after the interruption of the second inoculation we had to select plants according to their conditions so the data were not normally distributed. And the time was accounted for, to calculate growth and damage per day; 22 days for the first inoculation and 17 days for the second inoculation.

4.1 First inoculation

For the first experiment, the calculation of growth of water hyacinth became a central question because the feeding damage and larval survival could not be measured due to the absence of larvae during the plant dissections. To find the feeding damage the growth of plant must be divided by number of larvae found. And for the survival of larvae, the number of larvae inoculated must be subtracted by the number of larvae found alive (or the number of larvae inoculated in the second inoculation was not known as it was for the first inoculation). Only, analyses on the growth of water hyacinth at four constant temperatures were conducted. The results showed that temperature influenced the growth of the plants. Wet weight change increased with raised temperature for both the treatment and control plants up to 25°C, and showed a decrease at 30 °C (Figure). The greatest weight change was found at 25 °C and the lowest at 15 °C for both control and treatment plants. The weight change of the control and treatment plants were not significantly different ANOVA ($F_{3,2} = 0.42$; $P = 0.74$), but the control plants gained slightly more weight than the treatment plants at the higher temperatures (20°, 25° and 30 °C). Overall, warm temperatures were favourable to all of the measured growth parameters for the controls.

Two others plant parameters showed the same trend as the biomass change; petiole length and leaf position increased up to 25 °C (Figure and Figure). Leaf numbers increased with a temperature, up to 30 °C (Figure). The number of ramets remained constant at three temperatures (15°, 20°, and 25 °C) and only increased at 30 °C (Figure). No significant difference was found for three measured parameters ANOVA ($F_{3,2} = 0$, $P = 0.89$, 21; $F_{3.2} = 2.07$, $P = 0.14$ and $F_{3,2} = 3$, $P = 0.06$) respectively for petiole length, leaf position and number of ramets) at four

temperatures, but a significant difference ($F_{3.2}$ = 4.79, P = 0.01) was found for leaf number.

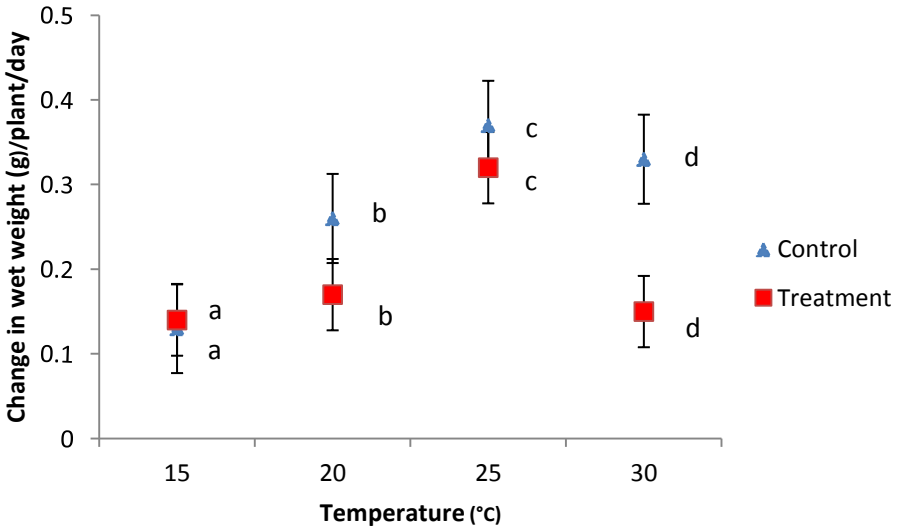


Figure 4.1: The effect of temperature on the change in wet weight of water hyacinth grown at four constant temperatures (15°, 20°, 25° and 30 °C). The error bars represent the standard error of the mean. Pairs of means with the same letter are not significantly different (t- test, α = 0.05) with P- Value = 0.94; 0.57; 0.92; 0.55 respectively at 15°, 20°, 25° and 30 °C. n = 6: 3 controls and 3 treatments at each temperature. Mean wet weight change found after 22 days of experiment.

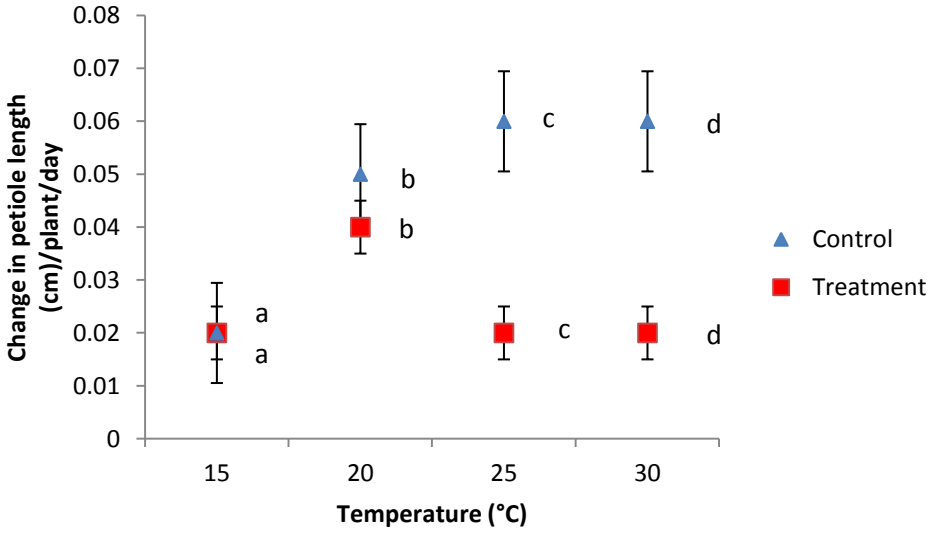


Figure 4.2: The effect of temperature on the change of length in the longest petiole of water hyacinth grown at four constant temperatures (15, 20, 25 and 30 °C). The error bars represent the standard error of the mean. Pair of means with same letter are not significantly different (t- test, α = 0.05) with P- Value = 0.80; 0.80; 0.59;

0.56 respectively at 15°, 20°, 25° and 30 °C. n = 6: 3 controls and 3 treatments at each temperature. Mean petiole length change found after 22 days of experiment.

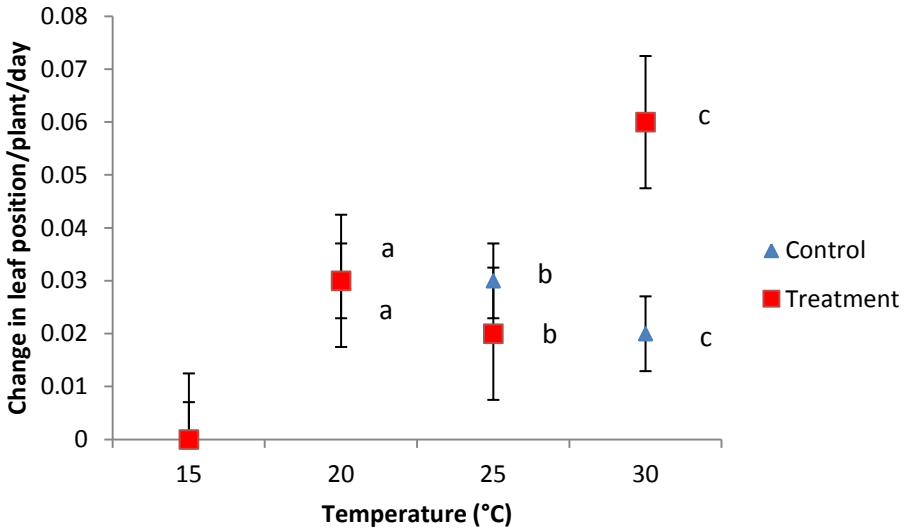


Figure 4.3: The effect of temperature on the change in leaf position of water hyacinth grown at four constant temperatures (15, 20, 25 and 30 °C). The error bars represent the standard error of the mean. Pairs of means with the same letter are not significantly different (t- test, $\alpha = 0.05$) with P- Value = 1; 0.52; 0.27 respectively at 20°, 25° and 30 °C. n = 6: 3 controls and 3 treatments at each temperature. Mean leaf position change found after 22 days of experiment.

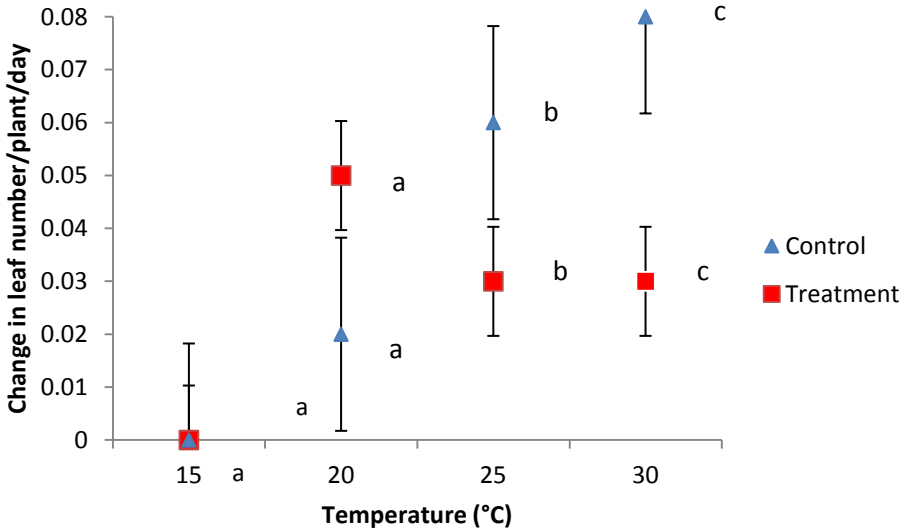


Figure 4.4: The effect of temperature on the change in the leaf number of water hyacinth grown at four constant temperatures (15, 20, 25 and 30 °C). The error bars represent the standard error of the mean. Pairs of means with the same letter are not significantly different (t- test, $\alpha = 0.05$) with P- Value = 0.18; 0.23; 0.27 respectively at 20, 25 and 30°C n = 6: 3 controls and 3 treatments. Mean leaf number change found after 22 days of experiment.

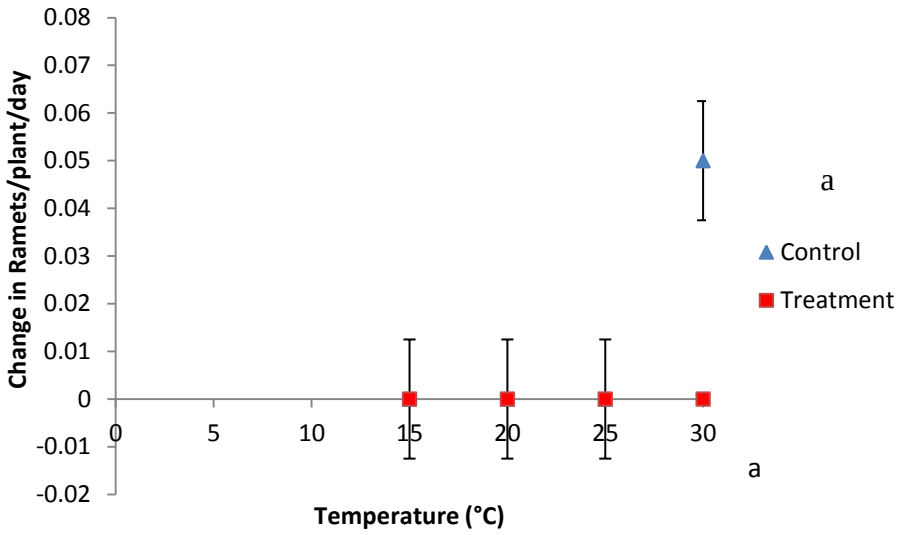


Figure 4.5: The effect of temperature on the change in the number of ramets of water hyacinth grown at four constant temperatures (15, 20, 25 and 30 °C). The error bars represent the standard error of the mean. Pairs of means with the same letter are not significantly different (t- test, $\alpha = 0.05$) with P- Value = 0.23 at 30 °C. n = 6: 3 controls and 3 treatments at each temperature. Mean ramets change found after 22 days of experiment.

As is the case in the first experiment, the statistical results for the experimental procedure revealed an insignificant difference between mean growth of controls and the mean of treatments (P = 0.92). However, the difference between the mean growth in wet weight of controls (50.98 g) and of the treatments (36.06 g) is clear. The test was conducted several times with different peoples and the same conclusion was found (no significant difference). Large variance and small sample size may explain the gap between the controls and the treatments not being statistically significant.

4.2 Second Inoculation

The effect of temperature on the performance of weevils on water hyacinth plants, at different temperatures showed that both larval feeding and rate of larval development increased with an increase in temperature.

4.2.1 Feeding damage

Larval feeding damage in terms of plant wet weight damage differed significantly at all four constant temperatures. The highest value was recorded at 30 °C and the lowest at 15 °C (Figure). The daily wet weight change of the controls and

treatments were significantly different at each experimental temperature ANOVA ($F_{3.2}$ = 3.3, P= 0.048).

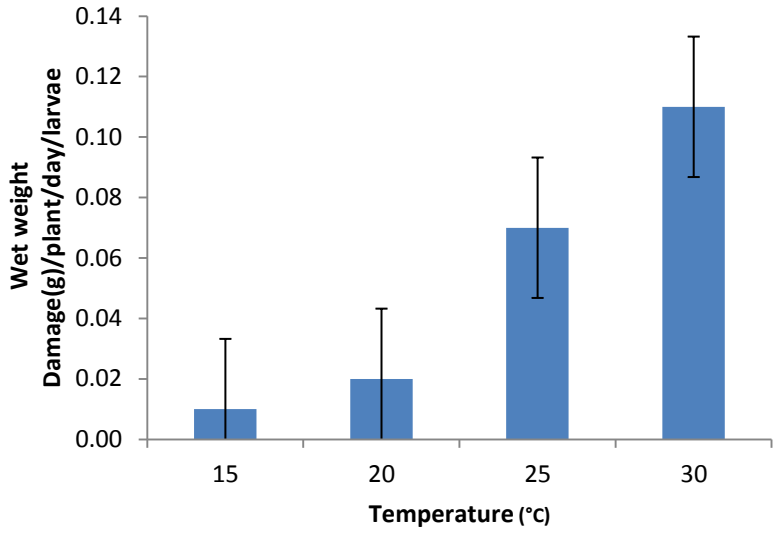


Figure 4.6: The effect of temperature on *Neochetina eichhorniae* larval feeding on water hyacinth wet weight at four constant temperatures (15°, 20°, 25° and 30 °C) in the second inoculation. The bars represent differences of mean wet weight change between controls and treatments plants (n = 6: 3 controls and 3 treatments at each temperature). The error bars represent the standard error of the mean. P - Value = 0.85; 0.89; 0.57; 0.35 (no significant difference) respectively at 15°, 20°, 25° and 30 °C (ANOVA, $\alpha = 0.05$).

A similar observation was made for the petiole length, with petiole damage increasing toward higher temperatures. The petiole damage recorded at 30 °C was the greatest and the lowest damage was recorded at 15 °C. However, at 20° and 25 °C, the same amount of damage was recorded (Figure). In Figure , the daily change petiole length of the controls and the treatments were significantly different ($F_{3.2}$ = 5.7, P = 0.007) at all four temperatures ANOVA. A positive relationship between wet weight damage and petiole damage was also found (Figure).

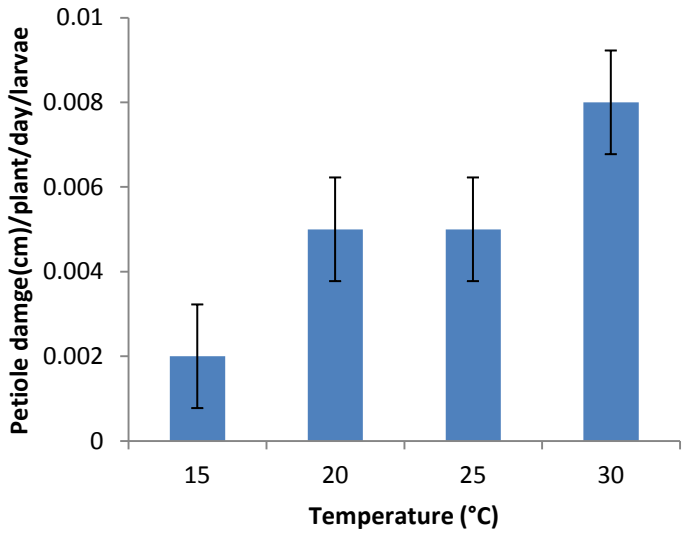


Figure 4.7: The effect of temperature on *Neochetina eichhorniae* larval feeding on water hyacinth petiole length, at four constant temperatures (15°, 20°, 25° and 30 °C) in the second inoculation. The bars represent the differences of the mean wet weight change between controls and treatments plants (n = 6: 3 controls and 3 treatments at each temperature). The error bars represent the standard error of the mean. P - Value = 0.89; 0.23; 0.68; 0.49 (no significant difference) respectively at 15°, 20°, 25° and 30 °C (ANOVA, $\alpha = 0.05$).

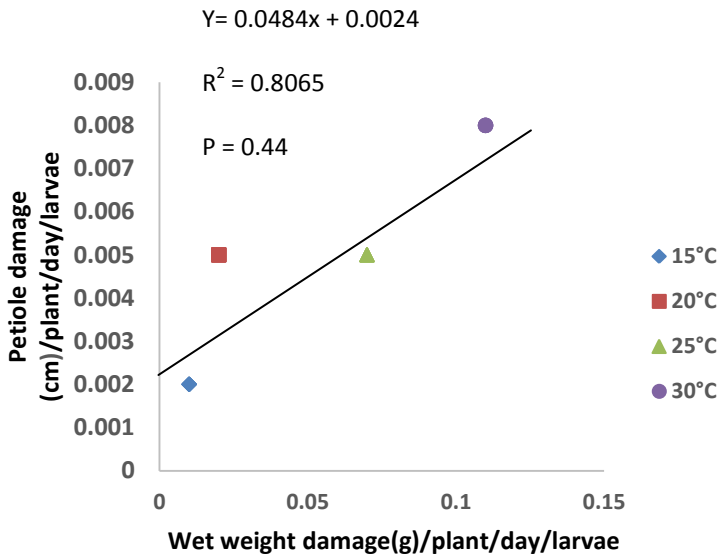


Figure 4.8: The relationship between daily wet weight damage and petiole length damage of water hyacinth by *N. eichhorniae*' larvae at four temperatures (15°, 20°, 25°and 30 °C). Wet weight and petiole length damage of water hyacinth are represented in the regression equations and the lines were fitted by linear regression analysis.

Comparatively with the results recorded by King (2011) at 20 °C, 8.42 spots per adult per day. Using the calculation provided by Deloach and Cordo (1976) with 4 mm^2 = 1 spot, and the formula provided by Hauptfleisch (2015) with 86.30 mm^2 /weevil/day = 0.015 g/weevil/day, one adult weevil feeding on water hyacinth leaf consumes an estimated of 0.007g/weevil/day at 20 °C. This feeding amount is less than 0.02 g/weevil/day found during this experiment at the same temperature.

4.2.2 Larval development

The results obtained from the development of larvae showed the influence of temperature on the growth of the weevil larvae. In general, the same age larvae are more developed at warmer temperatures than at colder temperatures. Larvae were found at the base of the petioles at 25 °C and 30 °C, while they were mostly found in the middle of the petiole at 15° and 20 °C. Some larvae were collected in the nutrient solution and a possible explanation for this could be that the larvae where trying to escape from injured petioles or the larvae may have fallen during their migration from the leaves towards the petiole.

a. Head width

In general, the larval head widths at warmer temperatures were larger than those from colder temperatures. After 17 days, all larvae found at 30 °C were in their third instar (with 0.72 mm being the biggest head width found) while all larvae at 15 °C were in the first instar (with 0.28 mm being the smallest head width found) in accordance with weevil larvae classification (Deloach and Cordo, 1976; Oke, 2009) (Figure). The results show that the development of larvae was promoted by high temperature. Suggesting that at warm temperatures *N. Eichhorniae* larvae will increase in size quicker than at cold temperatures.

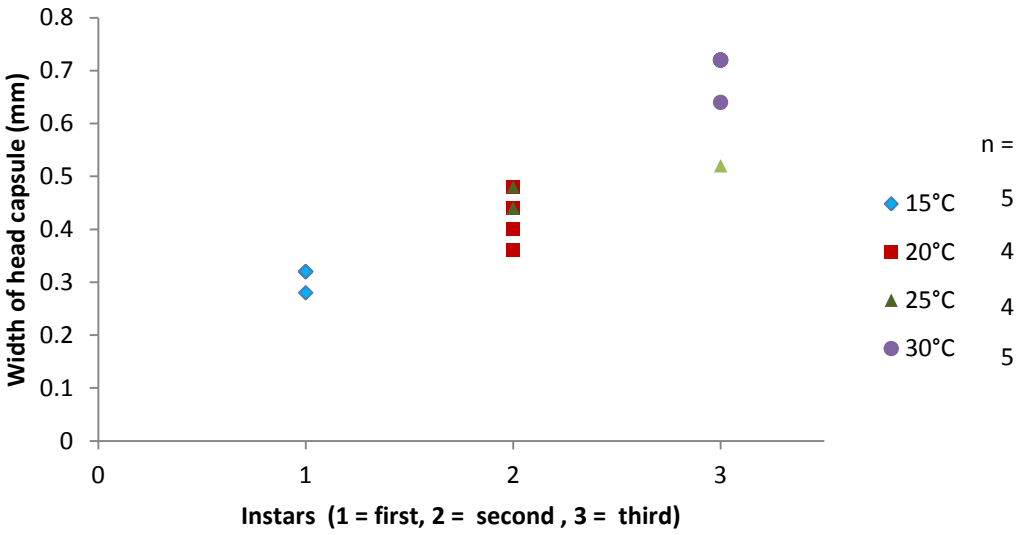


Figure 4.9: The effect of temperature on *N. eichhorniae* larval head width. The head width of *N. eichhorniae*' 17 day old larvae (from the hatching day) at four constant temperatures (15°, 20°, 25°and 30 °C).

b. Wet and dry weights of larva as influenced by the temperature

The relationship between larval wet weight and dry weight at four constant temperatures was examined. Wet and dry weights of larvae are strongly correlated ($R^2 = 0.92$) and increased with increasing temperature (

Figure). Wet weight and headwidth of larvae (Figure) also had a positive relationship ($R^2 = 0.92$).

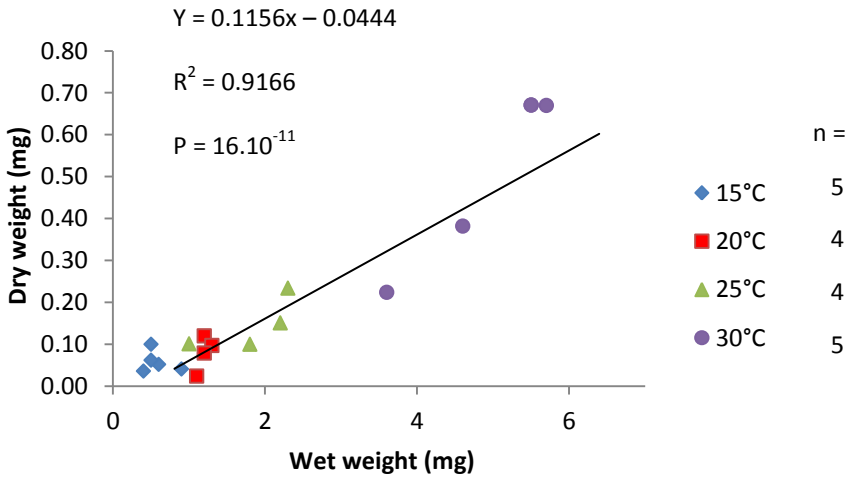


Figure 4.10: The relationship between wet weight and dry weights of weevil larvae reared at four constant temperatures (15°, 20°, 25°and 30 °C). Dry weight and wet weight of *N. eichhorniae*' 17 days old larvae (from the hatching day) are represented in the regression equation. The lines were fitted by a linear regression analysis.

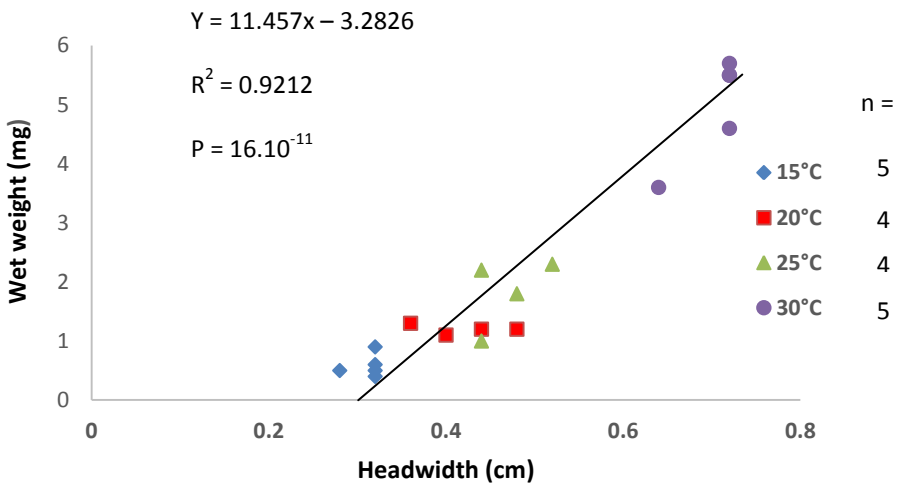


Figure 4.11: The relationship between wet weight and head width of weevil larvae reared at four constant temperatures (15°, 20°, 25°and 30 °C). Wet weight and head width of *N. eichhorniae*' 17 day old larvae (from the hatching day) are represented in the regression equation. The lines were fitted by a linear regression analysis.

4.2.3 Relationships between feeding damage and larval development recorded at four constant temperatures

The greatest larval feeding damage was recorded at 30 °C, where larvae were the most developed. Thus, higher temperatures increased the damage caused by weevil larvae on water hyacinth and simultaneously induced more rapid larval development (Figure). Thus, there is a direct effect of temperature on weevil development and its feeding on water hyacinth. Wet weight damage increased with increasing weevil development.

Overall, there is a positive relationship between damage to water hyacinth (wet weight damage and petiole length damage) and the development of the weevil larvae (headwidth) as illustrated in Figure and Figure . A position relationship was also found between treatment wet weight change and wet weight damage (Figure 4.14).

Thus, head width (larva development) affected the damage on water hyacinth plants. Damage was greater at higher temperatures where larvae were also most developed (with larger head widths). That was clearly demonstrated by the positive relationship found between the two measured parameters (Figure 4.11and Figure 4.12).

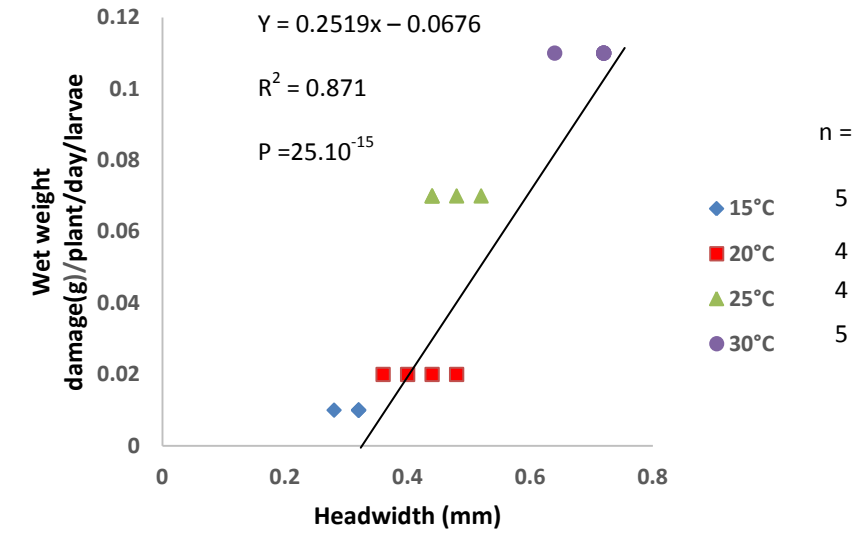


Figure 4.12: : The correlation between *N. eichhorniae* larvae head width and wet weight damage on water hyacinth at four temperatures (15°, 20°, 25°and 30 °C). The lines were fitted by a linear regression analysis**.**

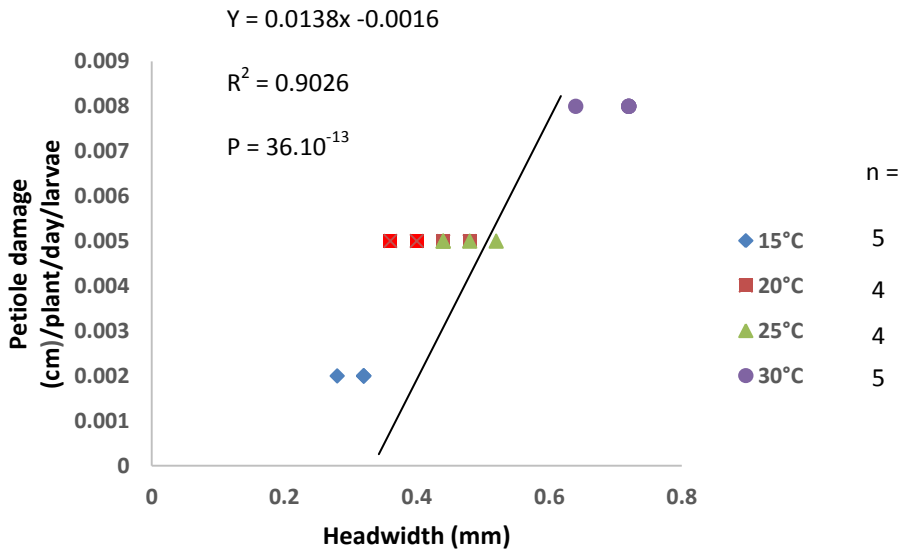


Figure 4.13: The correlation between *N. eichhorniae* larvae head width and petiole length damage on water hyacinth at four temperatures (15°, 20°, 25°and 30 °C). The lines were fitted by a linear regression analysis.

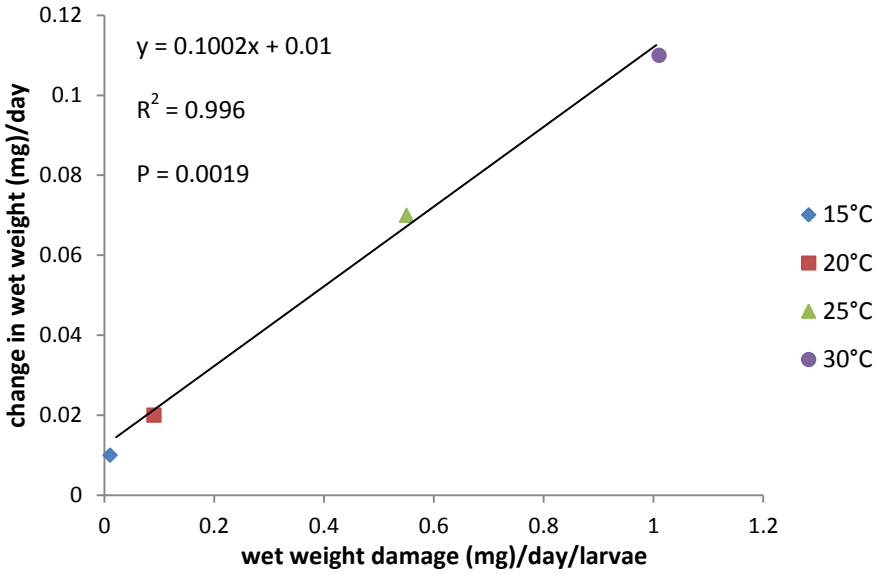


Figure 4.14: The correlation between treatment wet weight change and wet weight damage at four temperatures (15°, 20°, 25°and 30 °C). The lines were fitted by a linear regression analysis.

5. CHAPTER FIVE - DISCUSSION

Temperature is a major environmental parameter in the life cycle of insects (Stark and Goyer, 1983). Any variation in temperature will directly modify their metabolism since they are poikilotherms (Levesque, 2002). Several authors have shown that insects increase their metabolism with elevated temperature (O'Connor, 2009; Lemoine *et al*., 2013). Based on this knowledge, it was expected therefore that larvae of *Neochetina* (Cofranscesco *et al*., 1985) would increase their performance (measured in terms of feeding damage) and their rate of development (measured as head width and body size) when exposed to a higher temperature. Lemoine *et al*., (2014) suggested that the growth of insects (Peters, 1983; Bonner, 2006) will increase with rise temperature as the physiology of ectotherms is directly linked to the external temperature and their herbivory will increase also. The results found in this study support this prediction.

Previous studies have shown that plant growth is influenced by temperature and high temperature increases biomass whilst low temperatures depress growth (Sato and Kondo, 1983). This is also the case with water hyacinth (King, 2011). Experiments conducted by Sato (1988) confirmed (under the same temperature conditions of this study: 15, 20, 25, 30 °C), an increase in water hyacinth growth with increased temperature up to 25 °C and a subsequent decline of growth at higher temperatures (30 °C). These results are in accordance with those of the present work whereby wet weight gain increased with a rise in temperature up to 25 °C and then decreased at 30 °C (Figure). However the wet weight gain slowed at 30 °C. The warmer temperatures promoted the growth of the water hyacinth in the medium nutrient water.

Higher temperatures increased the wet weight gain of water hyacinth plants and the gain was slightly different between 25 °C and 30 °C for both controls and treatments. For instance, wet weight gain of controls at 25 °C was 1.2 times more than what was found at 30 °C. These findings are not to different from the 0.8 times found by Sato (1988) at the same temperature on the 20th day of Sato's experiment. The recorded wet weight gain of Sato (1988) at 30 °C was 5 times more than at 15 °C which is double the amount found in the present work (2.5 times). The reason for this could be the fact that the plants in Sato's experiment (1988) were housed in

larger containers which allowed an easier increase in size compared with the smaller containers used in this experiment – which could potentially restrict growth (Center and Van, 1989). All other measured growth parameters showed the same trend as the biomass change.

The results of the second experiment show the influence of temperature on the feeding and development of *Neochetina eichhorniae* larvae. The mean feeding of *N. eichhorniae larvae* per day increased in conjunction with a temperature rise up to 30 °C. Furthermore, the increase in larval consumption with the increase in temperature was positively correlated with its development. Others studies previously reported this correlation between larval feeding and development (Beck 1983; Stamp and Bowers 1990). When working with caterpillars of *Papilio glaucus L.* growing in plantain (*Plantago lanceolata*), Stamp and Bowers (1994) found that larvae at 30 °C had a consumption and growth rate up to two times higher than larvae growing at 20°C. Kingsolver and Woods (1998) also suggested the same with *Manduca sexta*, whereby the larvae increase consumption proportionally with their development at a range of temperatures between 14-34 °C.

The influence of temperature on insect herbivory is generally well documented (Gillooly *et al*., 2001; O'Connor, 2009; O'Connor *et al*., 2011; Marlin *et al.,* 2013). Several authors predict that phytophages will increase their feeding with elevated temperatures faster than the production of plant tissues and insect damage will affect the host plant biomass (Gillooly *et al*., 2001; O'Connor, 2009; O'Connor *et al*., 2011). In fact, plant metabolism consists of two processes: respiration and photosynthesis. But, plant growth is mainly as a result of the photosynthetic process rather than respiration (Farquhar *et al*., 1990; Dewar *et al*., 1998), and will be limited by carbon fixation (light) and extremes of temperature.

Conversely to plant growth, insect (ectotherm) consumption is linked to metabolic processes during cellular respiration (Taylor *et al*., 1982; Cossins and Bowler, 1987). Cellular respiration is more temperature dependent than photosynthesis (Lallana and Lallana, 1987; Allen *et al*., 2005; Padila-Gamino and Carpenter, 2007). Therefore, warm temperatures will favour insect food consumption more than plant growth (Padilla-Gamino and Carpenter, 2007). Weevil damage will be more severe at warmer temperatures compared with a low temperature-base, according to metabolic theory.

The above point is of paramount importance for the biocontrol of weed infestations because successful biocontrol of weeds is mostly about achieving substantial control, in which the agent is required to reduce the weed to acceptable levels, because extinction of the weed will be impossible to achieve (Hoffman, 1995). Control could be possible at higher temperatures where a herbivore will negatively affect the growth of its host plant by increasing its own performance above that of its host plant as demonstrated at 30 °C in this study. Hopefully, an increase in weevil effectiveness in the field could contribute to the biocontrol management of water hyacinth, at higher temperatures.

King (2011), when working on the performance of *Neochetina* weevils on water hyacinth at four constant temperatures (5°, 12°, 15° and 20 °C), found that temperature affected *Neochetina* feeding (His *et al.,* 1989; Douroudi *et al.,* 1999). He recorded the greatest weevil damage at 20 °C (which was the highest of his range of temperatures) and this diminished as the temperature decreased. Deloach and Cordo (1976) and Hauptfleisch (2015) also found a better performance of *N.eichhorniae* at an "optimum temperature" (25 °C). this suggests that warm temperatures will improve the weevil performance as metabolic theory suggests (Brown *et al*., 2004). Data for this study confirms King's results in that feeding damage measured in term of wet weight removal was greater at higher temperatures (30 °C) as compared with the lowest temperature of 15 °C. Petiole damage also has the same trend at different temperatures (Forno, 1981). King (2011) also reported that the damage at 20 °C was double than that reported at 15 °C; the same observation was made in the current study. However, the two studies differed in that King measured adult weevil feeding and this particular project focused on the feeding of weevil larvae. The recorded larval feeding in this study for the same temperature was three times as much as King's adult feeding (0.02 g/weevil/day at 20 °C) and is expected to be even more at higher temperatures. Note that the feeding at 30 °C (0.11g/weevil/day) was a considerable; five times more than that at 20 °C. Also, larval feeding damage reduced the growth of the host plant by 24% at high temperature and similar results were reported by Bashir *et al*.,(1984) when working with *Neochetina* weevils on water hyacinth, except that at 25 °C the damage by weevil larvae was greatest (30% of growth was affected negatively). Thus, confirming that larval feeding have more of an impact on water hyacinth compared with adult weevils, and their performance will increase proportionally to

temperature. Consequently, the management of water hyacinth by biocontrol agents needs to consider the life stage of the weevil and its interaction with warm temperatures for future implementation. Thus, the release timing of adult weevils in the field must be in accordance with the period in which weevil' larval performance is optimal, for most damage on water hyacinth and even result in plant death (Deloach and Cordo, 1976, Hauptfleisch, 2015).

Weevil larvae feeding on water hyacinth is known to be more efficient than adult feeding (Deloach and Cordo, 1976; Center *et al*., 1999). Larval damage show a strong relationship with water hyacinth stress (Center *et al*., 1999; Center and Van, 1989; Center and Dray, 1992) by affecting plant growth parameters (Deloach and Cordo, 1976) even resulting in plant death. The differences in the impact of feeding on the host plant between the two stages of the weevil may be related to their feeding behaviour: adults feed on the epidermis and the scars caused by their feeding do not penetrate deeply into the fleshy tissue of the plant (Deloach and Cordo, 1976). Contrary to adults, injuries by weevil larvae are deeper because larvae feed inside the petiole and can penetrate as deeply as the crown and thus the damage is more substantial (Deloach and Cordo, 1976). Thus, larvae movement towards the crown jeopardizes the meristem and could slow overall growth; therefore weevil larvae are potentially more harmful for the host plants. Focusing on the larval stage is thus recommended for better results in biocontrol by *Neochetina* weevils in general, and particularly for *N. eichhorniae*.

These results match with the range of feeding registered by Franceshini *et al.*, (2011). They found that one grasshopper (*Cornops aquaticum*) removed between 0.03g to 0.12 g of water hyacinth biomass on a daily basis.

Any biomass removal from the weed needs to be considered as positive in biocontrol and the weevil therefore always negatively affects water hyacinth health. Even a low level of weevil feeding could have negative impact on water hyacinth plants. In the current study, petiole damage was found to increase with temperature increase and a positive correlation between the plant wet weight damage and the petiole damage was established. Thus, a decrease in wet weight of water hyacinth due to the presence of weevil's larvae possibly affects the growth of several different parameters such as petiole length. Heard and Winterton (2000) mentioned petiole length among the parameters of water hyacinth that are affected by the weevil's presence in relation to biomass. They reported a decrease in the growth of

water hyacinth measured in terms of wet weight and petiole length with the presence of weevils, but the difference was not significant compared with plants without weevils. In the current work, weevil presence also affected the growth of petiole lengths and the damage was positively correlated with the loss of wet weight.

For the development of *N.eichhorniae* larvae, as with all insects, the development shows a positive relationship with rising temperatures within the favourable range (His *et al*., 1989). The effect of temperature on the development of an organism is a consequence of the increase in metabolic rate (reaction) at warmer temperatures and low consumption of energy at lower temperatures (Anger and Dawirs, 1981). Temperature operates on the metabolism by overall acceleration of all metabolic functions such as digestion (Rico-Villa *et al*., 2009). The influence of temperature on larval development in this study clearly demonstrated and corroborates with the work of His *et al*., (1989), which showed that larval development is stimulated as temperature increases (Doroudi *et al*., 1999) and the duration of larval development is shortened with increasing temperature.

The results of this study coincide with the above findings by His (1989) and Doroudi *et al*., (1999), even if the experimental period did not allow for pupation. The development of 17 day old *Neochetina* larvae at warm temperatures was quicker than at low temperatures. At 30 °C, the larvae were in the third stage of development, referring to the head width, whilst still in the first stage of development at 15 °C. The same observation was recorded for body size (fresh and dry weight), where the largest larva was found at a high temperature while the smallest was found at 15 °C, meaning that development at 15 °C was slower than at 30 °C. The difference in the development of larvae between temperatures, with fast development at high temperatures, could be explained by applying the same principles used in explaining an increase in feeding at high temperatures (stimulation of the metabolism at higher temperatures). Furthermore, temperature can affect the efficacy of certain enzymes involved in metabolism (Corraze *et al*., 1999). Therefore, using the weevil as a biological agent would be better if considering the temperature or place (tropical, temperate) where larval development is promoted and greater insect fitness is expected.

Biocontrol of water hyacinth by weevils has been successful in many areas with warm temperatures. For instance, Wangai (2004) demonstrated the effectiveness of weevils in the control of water hyacinth in Lake Victoria. Lake Victoria is situated

in a tropical zone with an equatorial climate all year-round. The mean temperatures for his studies varied from a minimum of 21.44 °C to a maximum of 23.78 °C. The author recorded higher damage caused by weevils during the growing season, suggesting that weevil's effectiveness will likely increase at that time of the year when temperatures are highest. Furthermore, the author also noticed the greatest performance of the larval stage on water hyacinth plants compared with adult weevils. Weevil larvae stopped the flowering of the plant and as the damage continued, the destruction of all plants occurred (Wangai, 2004).

In South Africa, the same observation was made by Hill and Ocklers (2001); successful biocontrol of water hyacinth by weevils was reported in New Years Dam Eastern Cape. Here a summer temperatures between 20 °C and 35 °C and a moderate winter temperature not much below 10 °C. In this dam, weevils reduced the infestation of water hyacinth by 70% only four years after their release (Hill and Ocklers, 2001). Four years is in the range of time for an ideal biocontrol success in tropical regions (Harley, 1990). Therefore, biocontrol of water hyacinth in South Africa is not impossible, however more inventive strategies and time needs to be allocated where weevils have failed to meet these positive results. Center *et al.,* (1999) suggested a more aggressive approach by undertaking several releases of weevil during the warm seasons to significantly reduce the probability of water hyacinth recovery (Tauber *et al.,* 1986). According to Cilliers and Hill (1996), stress inflicted on plants during warm periods is likely to increase the chance of plant damage and consequently will increase further temperature-related stress during the next winter.

However, it was demonstrated that water hyacinth experienced accelerated growth directly after the winter season (Byrne *et al.*, 2010). Unfortunately, weevil recovery takes longer than that of the water hyacinth and thus their recovery time does not directly correlate with the weed growth. Therefore, there is obviously a mismatch between growth-rates between the two organisms (King, 2011). In fact, it was discovered that during the process of introducing water hyacinth into new areas, particularly in South Africa, water hyacinth has shifted to a lower minimum temperature niche; thus suggesting that water hyacinth in South Africa has adapted to colder temperatures (Smit *et al*., 2016). This could explain why the plant accelerates its expansion in spring, and in winter water the growth is slowed but not stopped. Conversely for the weevil, its cold tolerance apparently remains the same,

suggesting the weevil did not adapt in the way that water hyacinth did. The explanation could be found in their metabolic processes, the growth of the plants will continue during the winter time, as long as the plant could fix carbon for the photosynthesis process while during the same season insect has a life in slow, all their activities stopped.

This idea substantiates claims of poorer results of weevils as biological agents in South Africa compared with warmer part of Africa. Thus; inundative releases may be the best option to improve water hyacinth biocontrol by weevils in South Africa. In addition, it is recommended that releases of the weevils take place at the beginning of growing season before the water hyacinth growth accelerates (Center *et al*., 1999).

Even if the optimal temperature for larval performance seems to take place at 30 °C (with a high amount of biomass removal), it was at 25 °C that the larvae had a larger effect on the growth of water hyacinth (the growth of treatment at 25 °C was 0, 49 times less than the growth of control compare to 0.45 times less at 30 °C). Therefore, for the biocontrol of water hyacinth, the ideal temperature to slow the progression of the weed is between 25- 30 °C.

6. CHAPTER SIX - CONCLUSION

In the many scientific studies on water hyacinth biocontrol by *Neochetina spp*, there have been few that quantify and address the impact of *Neochetina* larvae on its host plant. In this study, it was demonstrated that temperature influenced the performance and the development of *N. eichhornia* larvae on water hyacinth. Larval feeding on water hyacinth increased as temperatures rose whilst simultaneously, larval development accelerated. Warm temperatures facilitated high larval feeding damage compared with lower temperatures, and larvae, in warm conditions, were most developed and with a larger body size than larvae developing at cooler temperatures. It has been shown also that the feeding damage of larvae and their development on water hyacinth were positively correlated. The mass of tissue removed by the weevil larva was estimated, this amount will likely change with number of larvae present in the water hyacinth.

The implication of this study for biocontrol of water hyacinth is that larval feeding damage was greater at 30 °C, but it was at 25 °C that larvae impacted most on the growth of the weed. Therefore, based on the results of this study, it is recommended that a temperature of 25 °C must be considered as the temperature where weevil larvae are expected to greatly slow the expansion of water hyacinth infestation, when planning the biocontrol of this weed. However, given the lack of congruency between the weevil life cycle and water hyacinth growth in South Africa (King, 2011), it is recommended that there be inundative releases of *N. eichhornia* early in the growing season for the success of biocontrol of this weed. Further investigations concerning the utilisation of weevil larvae as a form of biocontrol agents of water hyacinth need to be addressed in order to determine if there is a way to combat the incongruence prevalent between the life cycle of water hyacinth and weevil.

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