The impact of soil water and nitrogen variability on the fitness and performance of *Neolema abbreviata* Larcordaire (Chrysomelidae) a biological control agent for *Tradescantia fluminensis*

by

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This thesis is dedicated to my parents Nobandla and the late Sikhulu Elijas

Mbande and my entire family

PREFACE

The research contained in this thesis was completed by the candidate while based at the Agricultural Research Council Plant Protection Research Institute (ARC-PPRI) and School of Life Sciences, College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg, South Africa. The research was financially supported by the Department of Science and Technology Professional Development Program (DST-PDP) through the ARC-PPRI.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

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DECLARATION: PLAGIARISM

I, Abongile Mbande, declare that:

(i)   the research reported in this dissertation, except where otherwise indicated or acknowledged, is my original work;

(ii)   this dissertation has not been submitted in full or in part for any degree or examination to any other university;

(iii)   this dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons;

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(v)   where I have used material for which publications followed, I have indicated in detail my role in the work;

(vi)   this dissertation is primarily a collection of material, prepared by myself, published as journal articles or presented as a poster and oral presentations at conferences. In some cases, additional material has been included;

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ABSTRACT

Tradescantia fluminensis Vell. (Commelinaceae) is a plant of Neotropical origin native to the southern parts of Brazil bordering Argentina. In South Africa, it is classified as a category 1B invader species in the National Environmental Management Biodiversity Act (NEMBA) owing to its incipient phase of invasion. The occurrence of naturalised populations of *T. fluminensis* has so far been confirmed in all provinces except the Free State, Northern Cape and North West. In cognisance of the devastating effects of invasive alien plants on native biodiversity, ecosystem health and ultimately provision of ecosystem services, several control methods have been employed with varying degrees of success. Classical biological control, which involves the release of exotic natural enemies (pathogens and herbivorous insects), is one such method widely-used because of its relatively low costs and minimal non-target effects. For *T. fluminensis*, *Neolema abbreviata* (Larcodaire) Coleoptera: Chrysomelidae) is one agent that is earmarked for release in South Africa following a successful introduction in New Zealand. However, little is known how novel environments presented by soil water and nutrient gradients may indirectly influence its herbivore performance and life-history through alterations in host-plant quality. In this era of global climate change where anthropogenic activities have led to changes in rainfall patterns and biogeochemical cycles of major elements such as nitrogen, investigation of species responses to such is important. Results from my study show that both water and nitrogen (N) variability influenced plant biomass accumulation, foliar N content and subsequent herbivore performance, and life-history traits of both adult and larval *N. abbreviata*. The longest vines were on plants that had optimal irrigation under excess fertiliser whilst severely water stressed plants that had excess fertiliser had the shortest vines. Foliar N content was highest in plants that had excess fertiliser under both pulsed and optimal irrigation whilst lowest foliar N content was in plants under optimal irrigation without any fertiliser. Optimally irrigated plants that received moderate fertiliser had their highest rate of egg deposition in both no-choice and multi-choice conditions suggesting quality-based host ranking behaviour in *N. abbreviata*. The consequent larval performance traits which included weight gain and time to pupation were superior in this treatment thereby providing support for the preference-performance hypothesis (PPH). Feeding patterns

between larvae and adults among plant treatments were largely similar suggesting uniform nutritional requirements across the life-stages. There were limited parental effects of plant quality on the life-history traits in both larvae and adults across F1 and F2 generations. In reciprocal diet transplant experiments, there were no significant responses to parental diet effects on larval weight, mortality, feeding damage, pupal weight and days to pupation. However, there were significant parental diet x test diet interactions with offspring from parents fed on high N plants generally performing better on low N test plants in traits such as larval weight gain and final pupal weights. Oviposition selection, feeding weight and longevity did not respond to the effects of parental diet nor its interaction with test diet, unlike the case with larval traits. There were significant correlations between pupal weight and number of days to pupation, pupal weight and eclosion success. I conducted a 3 x 3 full factorial experiment to determine the impact of water and fertiliser variability on the performance of *Neolema abbreviata* (and its host plant *Tradescantia fluminensis*. My results show differential responses to parental diet between larvae and adults of the same generation among an insect species with both actively feeding larval and adult life-stages. However, there was no correlation between adult weight and longevity. Overall, my thesis contributes to the growing body of literature on the impacts of anthropogenic global change on plant-insect interactions. It will also assist land managers when applying biological control of *T. fluminensis.* Furthermore, my results show the implications on the successful biological control (mass-rearing and field release) of *T. fluminensis* resulting from variable nitrogen and water conditions.

ACKNOWLEDGMENTS

LIST OF TABLES

LIST OF FIGURES

Table of Contents

Chapter 3: Ontogenic variation in transgenerational effects of parental diet in a specialist herbivore *Neolema abbreviata* under manipulated foliar nitrogen variability in *Tradescantia fluminensis*

CHAPTER 1: GENERAL INTRODUCTION

1.1 Biological invasions

The term 'invasive alien species' (IAS) is used to describe organisms with the capacity to rapidly increase and spread and impact ecosystems outside their native range (Juliano & Lounibos 2005; Novak 2007; Hulme 2009). In the current era of global climate change the pervasiveness of such invasions will likely increase due to the geographic range expansion of alien species in response

to warming temperatures (Chown et al. 2015; Hulme 2017). From the aforementioned, the threats posed by biological invasions have become a matter of great societal concern (Shackleton et al. 2007; Caley et al. 2008; Downey & Richardson 2016). Key paradigms such as human, animal, crop and environment are all at risk of perturbations caused by IAS ultimately resulting in poor human well-being (Lange & Van Wilgen 2010; Chown et al. 2015; Hulme 2017). Such IAS can be plants, animals or microbiota. However, for the sake of this study, only plants are regarded as IAS, unless otherwise stated.

The ways in which biotic invaders reduce the integrity and health of recipient ecosystems are several fold. Dominant invader plants are known to disrupt trophic interactions among indigenous ecosystems resulting in alterations in key ecosystem processes such as nutrient cycling and fire regimes (Gaertner et al. 2014). When severe, such ecosystem disruptions result in poor species diversity and ecological instability (Báez & Collins 2008) which can eventually lead to extinction of indigenous species due to extreme competition for resources. Unlike the case with extinction of animal species, the extent to which invasive plants contribute to the extinction of indigenous plants is poorly understood (Bellard et al. 2016; Downey & Richardson 2016). Hence there has been substantial debate on whether plant invasions contribute to indigenous plant extinctions (Sagoff 2005; Sax & Gaines 2008; Skurski et al. 2013; Davis et al. 2015). However, it is generally accepted that patterns of plant invasions and their subsequent impacts are largely dependent on both temporal and spatial scales (Pauchard & Shea 2006; Báez & Collins 2008). An in-depth meta-analysis by Powell et al. (2011) concluded that, unlike in higher spatial scales, larger declines in biodiversity loss are more prevalent at lower spatial scales. The current study therefore underlines the importance of considering scale when investigating the impact of IAS on local biodiversity. Typically, the invasion process by alien plant invaders follow a multi-step process (Fig. 1.1) which includes transportation, introduction, establishment, spread and impact (Novak 2007; Theoharides & Dukes 2007; Grarock et al. 2013). Although biological invasions follow several dispersal pathways, anthropogenic activities such as agriculture, aquaculture, recreation and transportation have been noted as key in both transboundary and local spread of various IAS across various spatial scales (Kolar & Lodge 2001; Novak 2007).

Transportation: involves both local and transboundary movement of invasive species with the aid of human modes of transport (Grarock et al. 2013; Theoharides & Dukes 2007). It is influenced by geographic distances and barriers (biotic filters) such as travelling distances and mode of transport (e.g. sea or road) (Grarock et al. 2013). For example, the introduction of invasive alien species in South Africa in the year 1600 is largely attributed to the arrival of European explorers and their ships in the then Cape of Good Hope (Zimmermann et al. 2004). Introduction: may be intentional, usually for economic purposes, or accidental due to species distribution through several pathways including anthropogenic activities (Larvegne & Molofsky 2007; Chown et al. 2014). This step follows natural dispersal or transportation of a species in the invasion process (Hulme 2009; Grarock et al. 2013). Hence for a successful invasion, it is imperative that the new alien species survive and establish in the new environment (Allendorf & Lundquist 2003). In most cases, the introduced species remain inconspicuous producing only enough propagules to sustain the invader population before successfully colonising the recipient ecosystem. In South Africa, the introduction of *Leucaena leucocephala* was a deliberate addition to agroforestry. However, it has since naturalized, become invasive and has been targeted for control (Egli & Olckers 2012). Establishment: describes the phase at which the invading species can sustain natural populations with the ability to increase their population (Theoharides & Dukes 2007; Grarock et al. 2013). During this process, biotic filters such as reproductive rates and resistance of native species are important as they constrain populations of introduced species. Therefore, the magnitude of both biotic and abiotic pressures on newly introduced species will determine their establishment (Larvegne & Molofsky 2007). Population Growth: depends on the establishment, dispersal ability and favourability of the new environment (Theoharides & Dukes 2007). Propagule pressure, that is the size of the invader population, is also recognised as a key factor mediating the rate and magnitude of population growth (Novak 2007).
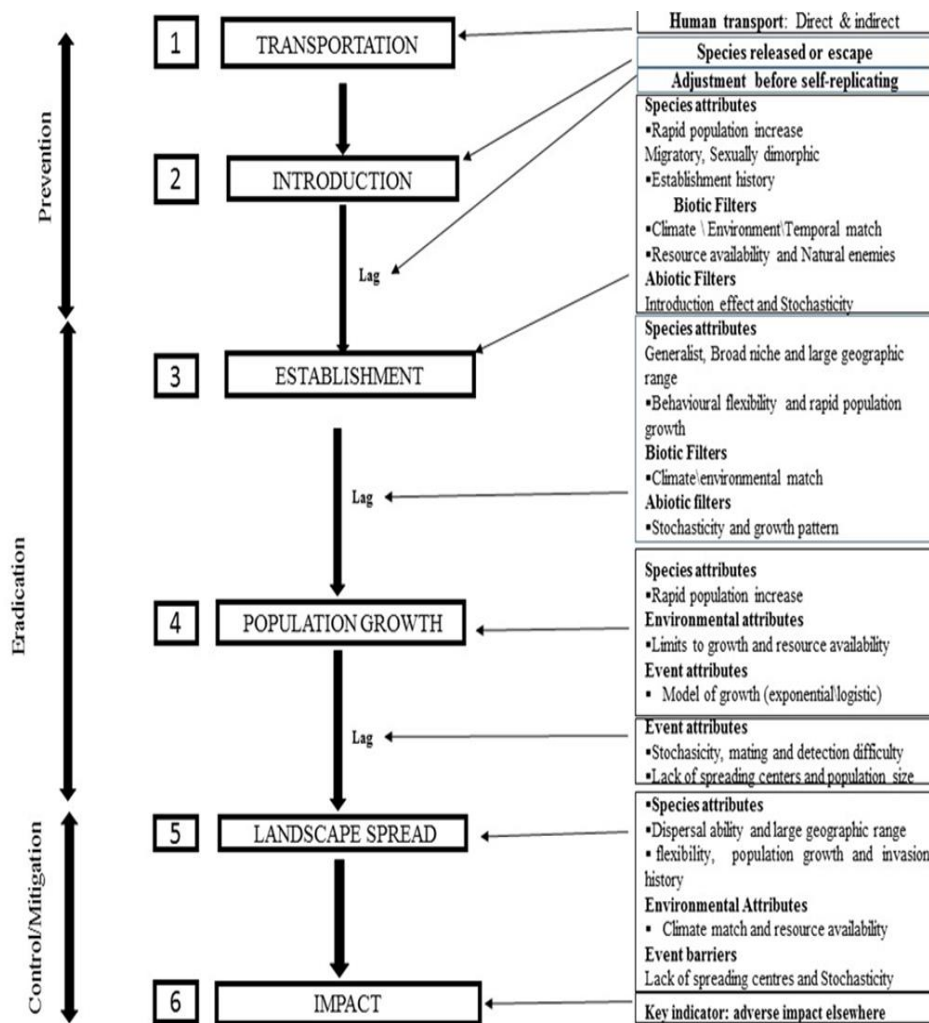
Figure 1.1: Conceptual model of the invasion process (redrawn from Grarock et al. 2013).

1.2 Impact of weeds on ecosystem services and human well-being in South Africa

Invasive plants affect societal benefits from ecosystem services with significant costs to the economy (Westphal et al. 2008) and general human well-being (Shackleton et al. 2007). In South Africa, the *Pinus* species which has invaded conservation areas not only displaced native species, but reduced surface water run-off and diminished water-flow in rivers (Moran et al. 2000; Le Maitre et al. 2000; Van Wilgen et al. 2001). Reduction in available soil water through higher consumption by alien tree invaders such as the Australian *Acacia* species has also been reported (Richardson et al. 2004; Shackleton et al. 2007). If these invasive alien plants were to realise their full potential of invasion in South Africa, the reduction in water is predicted to be increased 8 times (Van Wilgen et al. 2008). For a water-scarce country like South Africa, such water losses affect important sectors such as agriculture and ultimately result in poor economic growth. A recent economic valuation of losses from the economy due to alien plant invaders was estimated to be R6.5 billion per annum, which translates to 0.3% of South Africa`s GDP (Chamier et al. 2012).

Apart from water quantity, some invasive plants also compromise the quality of groundwater and freshwater bodies. Invasion by aquatic plant species such as the Kariba weed *Salvinia molesta* and water hyacinth *Eichhornia crassipes* in nutrient-rich aquatic ecosystems result in dense floating mats that disrupt water flow and reduce diffusion of air into the water. Consequently, there will be lower concentrations of dissolved oxygen in the water, which subsequently promote accumulation of sediments and eutrophication (Téllez et al. 2008). Increased eutrophication results in the rapid development of algae or cyanobacteria, typically recognised by bad odour, and discoloration of water (algal bloom). In addition, due to increase in oxygen depleted zones, coastal environments are increasingly subjected to aquatic hypoxia. This may consequently aid in the establishment of invasive alien species which become drivers of ecological change (Norkko et al. 2011). Hence, such modifications to aquatic habitats make the water toxic and unsuitable for native aquatic organisms such as fish and amphibians (Pieterse 1989; Hill 2003; Chamier et al. 2012). Invasive aquatic plants have also been associated with increased occurrence of gastropod vectors that transmit trematodes that cause schistosomiasis, commonly referred to as bilharzia (Masifwa et al. 2001). It is however,

important to note that several anthropogenic activities such as agriculture and disposal of household or industrial waste into water bodies also immensely contribute to eutrophication (Williams & Hecky 2005). Reduction in the nutrient pollution of water bodies will therefore undoubtedly help in reducing the invasion by aquatic weeds.

Terrestrial plant invaders mainly affect water quality via indirect effects. For example, aboveground fuel loads significantly increase as a result of increased biomass due to invasive plants (Brooks et al. 2004). In sensitive ecosystems such as the fynbos biome, these fuel loads increase fire frequency and the levels of fire intensity (Brooks et al. 2004; Jayiya et al. 2004). This high fire intensity results in hydrophobicity or water repellence in the soil. In turn, the water repellence results in poor infiltration accompanied with a high rate of sedimentation that increases nutrient levels and salinity and decreases soil pH (Smith et al. 2011; Chamier et al. 2012). Furthermore, fire alone may have an economic impact as it also can destroy important natural resource capital and infrastructure.

Despite all the adverse impacts of invasive plants, there are sometimes conflicts of interest among environmental managers and the population at large. For example, some of the invasive plants are utilised as fodder for livestock, firewood, ornamentals, and stabilization of sand dunes, barriers and hedge (Van Wilgen et al. 2001). For example, the black wattle (*Acacia mearnsii*) was introduced for agroforestry and fuelwood, and construction/ fencing poles in the Eastern Cape and yet it presented significant challenges when it rapidly grew outside designated areas where it had adverse impacts on native biota (Shackleton et al. 2007). In the Eastern Cape, prickly pear (*Opuntia ficus-indica)* fruits are consumed in many households and are also used for supplementing household income when sold as whole fruits or as processed jam, syrups, beer and traditional medicine (Shackleton et al. 2011). This therefore calls for meticulous evaluation of costs and benefits of invasive plants to harmonise management options and community needs.

1.3 Management and control of invasive alien species

Management strategies of plant invasions include prevention, eradication and control in order to restore and conserve ecosystems (Hulme 2009). Recognising the fact that many invasive alien plants were initially intentionally introduced, the most important aspect of managing invasive plants is

to prevent their introduction. Robust phytosanitary regulations that encompass risk analysis should therefore be in place to control the types of plants that enter all the ports within a country. If the plant is already in the country, regulations should also have provisions for restriction of movement of the plants together with prohibition of sale, where necessary.

Complete eradication of alien invasive species is possible if the weed is detected early (Mack et al. 2000; Buckley et al. 2004) and if it is a slow growing species (Hulme 2006). However, complete eradication may result in secondary invasions if not accompanied with rigorous ecological restoration measures (Hulme 2006).Control strategies involve long-term reduction in population size and biomass towards a level that is not harmful to local biodiversity (Buckley et al. 2004; Hulme 2009). There are three major methods that are used to control invasive species, namely: chemical, mechanical and biological control.

Chemical control

Chemical control involves the use of herbicides to suppress plant populations and insect pests (Simberloff 2014). Although largely effective, herbicides are not easily applicable to extensive areas due to their high costs (Wadsworth et al. 2000) and impacts on non-target species (Mack et al. 2000; Van Wilgen et al. 2000). The choice of herbicide and location of application is therefore restricted to minimise deleterious effects on non-target species (Wadsworth et al. 2000). Furthermore, prolonged usage of herbicides may also result in the evolution of resistance (Mack et al. 2000). For example, in Florida, the heavy usage of Flouridone on *Hydrilla verticillata* resulted in the evolution of a more resistant biotype (Puri et al. 2007). Hence, herbicide usage presents both economic and environmental challenges.

Mechanical control

Mechanical control methods involve the physical removal of plants. These include, hoeing, tilling, hand pulling, grubbing, bulldozing and chaining (DiTomaso 2000). Both hand pulling and hoeing are not practical on larger areas and woody plants (Sheley et al. 1999; DiTomaso 2000;

Buckley et al. 2004). Mowing can be used prior to flowering to avoid invasive plants building seedbanks for future populations (Benefield et al. 1999). Tilling can control annual weeds but can promote the spread of perennial weeds (Young et al. 1998). Chaining, Bulldozing, wood cutting, shredding, roller chopping and root–ploughing are used in the control of shrubs and trees (Rasmussen 1991). However, the use of such large machinery presents a form of ecological disturbance which often leads to reinvasions (Paynter & Flanagan 2004). For large infestations, mechanical control is therefore generally not feasible as it is labour–intensive, expensive and presents ecological challenges including spreading of propagules of other invasive plants (Van Wilgen et al. 2000). This is despite it being a method that receives less public criticism when applied in appropriate spatial scales (Mack et al. 2000).

Biological control

Due to the numerous challenges of the control methods discussed above, biological control is regarded as a relatively cost effective and environmentally sustainable way for area-wide management of invasive weeds (Buckley et al. 2004; Van Wilgen et al. 2004; Seastedt 2015). The main aim of biological control is to supress weed populations and reduce their impact on the environment (DiTomaso 2000; Hulme 2006, Hulme 2009; Seastedt 2015). Antagonistic invertebrates and pathogens, collected from the weed's country of origin, are released in the invaded range in what is known as classical biological control (Van Wilgen et al. 2000, Van Wilgen et al. 2004). The strategy stems from the Enemy Release Hypothesis, which posits that alien invasive plants are more successful in their colonised habitats due to the decrease in regulation by their natural enemies (Keane & Crawley 2002; Colautti et al. 2004; Liu & Stiling 2006). This is mainly because invasive plants rarely exhibit any signs of invasiveness in their native range due to antagonistic pressure from the natural enemies that coevolved with them (Van Wilgen et al. 2000). Hence, biological control aims to recreate this antagonistic relationship in the invaded range. Even though biocontrol may be effective, several drawbacks exist. These include its relatively slow mode of action and high initial costs and time consumed in safety tests prior to release (Coutchamp et al. 2002; Culliney 2005; Shaw et al.

2009). Several biotic and abiotic factors affect the efficacy of biological control agents. For example, Mack et al. (2000) noted that biogeographic ranges as key in determining plant-herbivore interactions. This is even more important in novel environments where classical biological control of weeds is often practiced (Lau et al. 2008; Shea et al. 2010). Habitat characteristics, rainfall and temperature can all determine the net impact of biocontrol agents on their respective weeds through their influence of magnitude and direction of herbivore-plant interactions (Seastedt 2015). Hence, both top-down effects such as predation and bottom-up (plant quality) effects due to variable nutrient availability and play an important role among different habitats. For mass-reared insects like biocontrol agents, diet can influence several evolutionary characteristics by both phenotypic plasticity and conferring genetic changes in various heritable traits (Triggs & Knell 2012; Colombo et al. 2014). The latter are sometimes referred to as transgenerational effects and several nutrient-mediated insect traits such as body size, developmental rates, fecundity and longevity have all been shown to be highly transmissible across generations (Townsend et al. 2012; Chidawanyika 2010; Chidawanyika & Terblanche 2011). Hence it is of importance to understand how host plant quality influences insect herbivore performance and life-history traits both within and across generations to enhance the efficacy of biological control programs.

1.4 Study plant

Tradescantia fluminensis Vell (Commelinaceae) is a perennial herb, commonly known as wandering Jew, that is native to Brazil and Argentina (Standish 2001; Standish et al. 2004; Fowler et al. 2013). It belongs to the Commelinaceae family, of which several deliberately introduced species that have since become invasive (Fowler et al. 2013). In South Africa, *Tradescantia fluminensis* is considered as being in the incipient phase of invasion and is listed as category 1B invader in the National Environmental Management Biodiversity Act (NEMBA Act) of 2004. Other countries where *T. fluminensis* has also naturalised and is considered invasive include Australia, New Zealand and North America, Portugal, Swaziland and United States of America (Burns 2004; Fowler et al. 2013). In all these countries, the plant invades high rainfall areas and riparian zones. Similar habitats have also been invaded in South Africa in several provinces (Fig. 1.2).
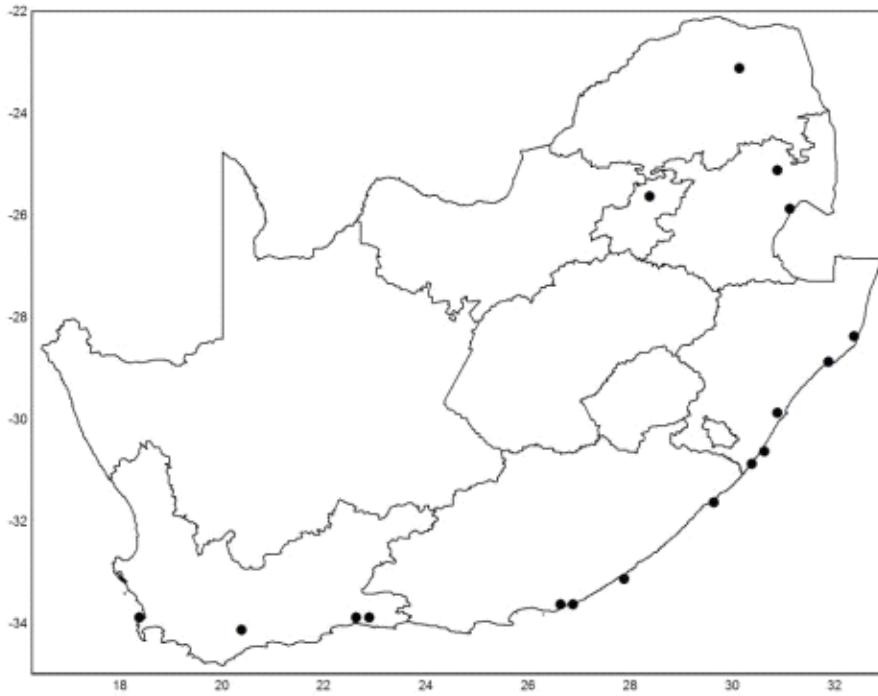
Figure 1.2: Map of South Africa showing the distribution of *Tradescantia fluminensis.* [Source: *Lesley Henderson ARC brochure*].

Large infestations of *T. fluminensis* form dense monospecific ground cover (up to 60 cm high) that lies flat on the ground (Fig. 1.3) with fragile stolons that can easily form new plants when broken (Standish 2001). Root formation occurs at any node, which enables rapid propagation of detached fragments under suitable conditions (Burns 2004; Hurrell et al. 2008; Jackman et al. 2015).

Figure 1.3: *Tradescantia fluminensis* infestation at a site in Pietermaritzburg [Photo Credit*: Dr. Frank Chidawanyika*].

The biomass accumulation of *T. fluminensis* can exceed $200 gm^{-2}$ out competing native seedlings and hindering forest regeneration (Kelly & Skipworth 1984; Toft et al. 2001; Fowler et al. 2013). Standish et al. (2004) reported that *T. fluminensis* invasions increase the soil nitrogen content, a growth limiting element, making the disturbed area prone to further invasion (Standish et al. 2004). *Tradescantia fluminensis* spread is limited by the availability of light and this also determines its impact on native forest regeneration (Standish 2001; Toft et al. 2001). In summary, *T. fluminensis* effects include alteration of litter decomposition, nutrient cycling, plant succession and invertebrate biodiversity (Maule et al. 1995; Standish et al. 2004) and therefore local species richness (Standish et al. 2001). In New Zealand, biological control of *T. Fluminensis* using *Neolema abbreviata* (Lacordaire) (Chrysomelidae) started in 2011 (Jackman et al. 2013) and preliminary investigation have revealed success (Chidawanyika, unpublished data).

1.5 Study organism

Biology of *Neolema abbreviata*

Neolema abbreviata (Lacordaire) (Chrysomelidae) is native to Brazil and was introduced into South Africa for host range studies in 2012 by Dr Frank Chidawanyika. Adults are slender, 2-5mm long and have yellow and black alternating longitudinal wing case stripes (Fig. 1.4). Females are generally larger than males and can lay over 100 opaque white eggs on the underside of leaves in their lifetime (Fowler 2015). Eggs hatch after 7-10 days at 27 °C into pale greyish-brown larvae and undergo 4-5 instars. Mature larvae accumulate moulted skins and secrete a protective cover for the cocoon (Fowler 2012). The cocoon is made from threads of white foam secreted from the mouth of the larvae and is stuck on the underside of a leaf or leaf litter (Fowler 2012). Complete development of larvae takes about 20-25 days feeding on leaves just like adults at under favourable conditions (Fowler 2012).

Figure 1.3: Adult *Neolema abbreviata* [Photo Credit: *Landcare Research New Zealand*].

1.6 Study rationale

Soil nutrient and water content are import bottom-up factors that influence plant growth, morphology, chemistry and subsequent interactions with organisms from other trophic levels (Showler & Moran 2003; Chidawanyika et al. 2014). For insect herbivores, plant quality, due to water and nutrient variability, has been associated with differences in oviposition and feeding preferences. The Optimal Oviposition Theory or 'preference-performance' hypothesis suggests that gravid females undergo high selection pressure when searching for oviposition sites in order to maximise the fitness potential of their offspring (Kohandani et al. 2017). Hence, factors such as absence of predators and abundance or quality of resources are all key factors that influence where and when to oviposit. Despite the potent effect on insect herbivore performance, the effect of water availability, remains poorly understood. Furthermore, despite several studies investigating the impact of water (Gutbrodt et al. 2011) or nutrient stress (English-Loeb et al. 1997; they are rarely tested in conjunction (e.g. Gutbrodt et al. 2011; Showler & Moran 2003; Chidawanyika et al. 2014) with other environmental stressors. In nature, however, multiple environmental stressors often occur simultaneously. Hence, studies focusing on single stress factors may miss additive or synergistic effects of stressful events that may not be apparent when investigating as single stress factors. For example, Han et al. (2014) reported that the interactive effects of suboptimal water and nitrogen content reduced the performance of the leaf miner *Tuta absoluta* (Meyrick (Lepidoptera: Gelechiidae) compared to when the stress factors where individually delivered. For biological control of invasive weeds, the understanding of the impact of such stressful events on insect herbivore performance is of importance as environmental heterogeneity influences herbivore performance and population dynamics. Furthermore, understating of the interaction between host plant quality and population dynamics of mass-reared insects may help to enhance mass-rearing protocols through manipulation of diet quality. Hence, the overall goal of this project was to unveil the impact of both water and nutrient stress on *T. fluminensis* and the life history traits of *N. abbreviata* within and across multiple generations. In chapter 2, I examined how soil water and nutrient variability affect foliar nitrogen content and biomass accumulation in *T. fluminensis* and how such changes in plant quality may influence life history traits and feeding and oviposition preference in *N. abbreviata* beetles. In chapter 3, I examined the transgenerational effects

of host plant quality among F_1 and F_2 generations in order to evaluate if there are fitness costs and benefits associated with matching or mismatches between parental and offspring diet.

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Chapter 2: Differential life-history responses of *Neolema abbreviate* to water and nitrogen gradients

Abstract

Due to their sessile nature, plants inexorably endure both transient and chronic environmental stressors with subsequent impact on their interactions with other trophic groups. Water and nitrogen variability are key abiotic factors mediating the interaction between plants and insect herbivores. For insects used in classical biological control of plants, such factors may determine extent of herbivory, population dynamics including field establishment and persistence. We conducted a full factorial experiment to determine the impact of water and fertiliser variability on the performance of *Neolema abbreviata* (Larcordaire) (Coleoptera: Chrysomelidae) and its host plant *Tradescantia fluminensis* Vell. (Commelinaceae). Water and fertiliser variability strongly influenced *T. fluminensis* biomass accumulation and foliar nitrogen content. These changes in host plant quality resulted in preferential oviposition selection by the beetles with optimally irrigated plants that received moderate fertilizer being most favoured. Larval performance, in terms of weight gain and time to pupation was fastest in this treatment providing support for the preference-performance hypothesis. There were similarities in feeding performance and subsequent life-history responses in some treatments suggesting uniform nutritional quality requirements among life-stages, with a few exceptions. Overall, my study reports on differential effects of paired abiotic stressors and performance on a herbivorous insect at both juvenile (larvae) and adult feeding life-stages. We therefore call for multi-trait and factor evaluation to understand the impact of plant quality on insect life history traits. My findings also have direct implications on mass-rearing protocols of *N. abbreviata* and its potential field performance.

Key words*: biocontrol, bottom-up effects, developmental plasticity, host selection, insect herbivory, preference-performance

fluminensis, under water and nitrogen gradients" by Mbande, A., Tedder, M. and Chidawanyika F. (doi:10.1007/s11829-018-9630-0)

2.1 Introduction

Environmental stressors such as soil water and nutrient variability affect plant quality with subsequent impacts on plant-insect interactions (Fischer & Fiedler 2000; Cornelissen & Stiling, 2006; Gutbrodt et al. 2011; Han et al. 2014). Severe water stress generally results in whole-plant biochemical and physiological responses, such as reduction in photosynthetic rates coupled with morphological changes such as leaf rolling and stomatal closure (Martin-StPaul et al. 2017) although, under mild or pulsed water stress, the impact of such water deficits can be ameliorated by acquired tolerance through acclimatisation (Valim et al. 2016). The mechanistic basis for which acclimatisation confers resistance to water stress is debatable, but the upregulation of various protein groups has been reported as a key defence strategy (Valim et al. 2016 and references therein). This can be accompanied by rapid amino acid accumulation due to catalysis of some proteins resulting in a marked rise in tissue nitrogen content (Mattson & Hack 1987; Helmberger et al. 2016). Other studies have also reported an increase in digestible carbohydrates or soluble sugars, and antioxidant enzymes that mitigate the deleterious effects of water stress (English-Loeb et al. 1997; Jithesh et al. 2006) through removal of free radicals within cells (Chaves et al. 2002; Lawlor & Tezara 2009). Such accumulated metabolites can determine plant quality, as they can be nutritive or anti-nutritive for herbivorous insects. When nutritive, stress-induced metabolic and structural changes result in improved insect herbivore performance due to increased plant nutritional value (Mody et al. 2009; Franzke & Reinhold 2011) or suppression of plant defence mechanisms (Olson et al. 2009; Gutbrodt et al. 2012) which all lead to increased host-plant utilization. On the other hand, anti-nutritive effects result in increased resistance to insect herbivory through factors such as reduced digestibility and increased production of toxins and repellents (Bernays & Chapman 1994; Olson et al. 2009; Pezzolla et al. 2017).

Due to the diverse water stress-related outcomes of plant-insect interactions, several hypotheses have been brought forward to explain such relationships. For example, the "Plant stress hypothesis" (PSH) posits that water stress results in improved insect performance due to increased amounts of available nitrogen and poor plant defence (White 2009; Huberty & Denno 2004; Franzke & Reinhold 2011). While, the "Plant Vigor Hypothesis" (PVH) assumes that insects prefer and perform better on fast growing, vigorous plants (Price 1991; Schowalter et al. 1999; Fritz et al. 2000, Santos et al. 2008). Though seemingly divergent views, it is now widely accepted that these hypotheses are complementary (Huberty & Denno 2004; White 2009; Gutbrodt et al. 2011; 2012) with outcome variations largely due to magnitude of stress and differences in feeding guilds among subject insect–plant systems (Rousselin et al. 2016). For example, sap sucking feeders require high water potential for efficient feeding from phloem cells (Douglas 2003; Guerrieri & Digilio 2008) whilst high water content is important for efficient protein assimilation among chewing insects (Huberty & Denno 2004; Sconiers & Eubanks 2017). This results in the same stressful event differentially affecting insects of two different feeding guilds. More recently, White (2015) further classified two sub-guilds: flesh-feeders and senescence feeders, which favour feeding on vigorous and stressed plants, respectively. Another hypothesis, the 'Plant Pulsed Stress Hypothesis' (PPSH), suggests that sporadic water stress favours insect herbivore performance, with supporting evidence mostly found among sap sucking phloem feeders (Huberty & Denno 2004, Rousselin et al. 2016, Sconiers & Eubanks 2017).

Soil nitrogen content is another important bottom-up factor that affects plant-herbivore interactions (Olson et al. 2009; Rousselin et al. 2016). However, the interactive effects of nitrogen and water availability on plant quality and, as a result, insect herbivore performance are more complex and remain poorly understood. At the plant level, water stress may result in poor nitrogen assimilation (He & Dijkstra 2014; Rousselin et al. 2016) with potential impact on subsequent trophic levels. For example, the 'Nitrogen Limitation Hypothesis' (NLH) posits that low nitrogen content in plants impedes the performance of herbivorous insects (White 1993). This may be attributed to insect inability to acquire transformable inorganic nitrogen or to utilise plant-derived amino acids important

for the formation of enzymes and structural proteins (Schoonhoven et al. 2005). An alternative explanation is that plants rapidly accumulate potentially toxic metabolites that also act as feeding deterrents during plant nitrogen deficiency (Le Bot et al. 2009; Larbat et al. 2012a, b; Royer et al. 2013; Han et al. 2014). Other studies have also reported a decreased attractiveness of herbivore induced volatiles to parasitoids due to changes in quantity and quality of the entire plant volatilome in response to elevated nitrogen (Olson et al. 2009) or water stress (Chidawanyika et al. 2015) thereby affecting plant indirect defence. In another study, Gutbrodt et al. (2012) reported that water stress enhanced plant constitutive and not induced defence. Hence, aspects encompassing host plant quality are broad, and cover both nutritional content and defensive capacity of the plants.

Herbivorous insects facing variable host-plant quality often display phenotypic plasticity among key behavioural and life history traits in order to improve performance. For example, oviposition selection and acceptance is presumably done on plants that ensure maximum offspring performance in what is generally referred to as the Optimal Oviposition Theory (OOT) (Jaenike 1978; Scheirs & De Bruyn 2002). Indeed, natural selection should be highly favourable for insects that select hosts that ensure reproductive success (Kohandani et al. 2017 and references therein). However, several studies have shown mismatches in maternal oviposition preferences and offspring performance (Thompson & Pellmyr 1991; Fréchette et al. 2006; Potter et al. 2012; König et al. 2016) resulting in ecological traps that arrest larval development and population persistence (Khan 2006; Midega et al. 2011, 2015). This is not surprising as, in nature, suitability of host plants may be determined by other factors such as risk of predation and density-dependent population dynamics (Williams 1999; Cornelissen & Stiling 2006, Davis et al. 2011; Chen & Chen 2016). Hence, insect oviposition selection alone maybe a poor indicator of host plant quality. Instead, other studies have included traits such as lifetime fecundity, larval mortality rates, pupal weight and adult longevity to determine the impact of plant quality on herbivore performance (Bauerfeind & Fischer 2005; Cornelissen & Stiling 2006; Bauerfeind & Fischer 2013; Chidawanyika et al. 2015). Developmental rate is another trait of high ecological relevance. Slower developmental rates on poor quality plants are known to result in increased exposure of juveniles to both abiotic and biotic mortality factors

(Benrey & Denno 1997; Williams 1999). Hence, the impact of plant quality on insect performance requires a multi-foci approach examining various traits in the entire life cycle.

Here, using *Tradescantia fluminensis* Vell (Commelinaceae), commonly known as wandering Jew or Brazilian spiderwort, is a monocotyledonous groundcover with soft hairless leaves and succulent stems. It is native to South East Brazil and Northern Argentina and has since naturalised and been declared invasive in many countries including Australia, New Zealand and South Africa (Standish, 2001, 2004; Fowler et al. 2013). Poor native seedling recruitment, which leads to poor forest regeneration, is the main adverse attribute of *T. fluminensis* invaded areas (Standish 2001, 2004). Mechanical control of large infestations of the plant is difficult as stem fragments can easily develop roots and form new plants. Chemical control is also perhaps unsuitable due to potential non-target effects in sensitive ecosystems, such as riparian habitats where *T. fluminensis* also grows. Biological control using arthropods and pathogens has therefore been employed as a sustainable measure to combat invasive populations (Fowler et al. 2013).

Native to Brazil, *Neolema abbreviata* Larcordaire (Chrysomelidae) is one of the biological control agents selected based on a feasibility study for in New Zealand (Fowler et al. 2013). It inflicts damage on plants by feeding on both young and mature leaves thereby reducing biomass accumulation and ultimately causing plant mortality. It was recently introduced into South Africa where it is earmarked for release in locations of variable climatic and soil fertility attributes. Due to the water and nutrient gradients present among these sites, it is expected that the performance and population dynamics of the beetles would be variable as previously reported for other biocontrol agents (Heard & Winterton 2000; Center & Dray Jr 2010; Bownes et al. 2013a, b). Hence, to understand the impact of soil water and nutrient gradients on the performance of *T. fluminensis* and subsequent life history traits and population dynamics of *N. abbreviata*, I investigated the within-generational effects of three nutrient and water levels in a full-factorial study. Specifically, I examined how variations in soil water and nutrient loads affect foliar nitrogen content and biomass accumulation. I also investigated how such changes in plant quality may influence oviposition preference, relative growth rate, herbivore performance and adult longevity of *N. abbreviata* beetles.

2.2 Materials and Methods

2.2.1 Study site

The study was conducted under quarantine facilities at Agricultural Research Council-Plant Health Protection (ARC-PHP), Cedara 29.5339° S, 30, 2733° E, KwaZulu-Natal, South Africa. Throughout the study, temperature and humidity ranged from 24 to 26.4 °C and 60 to 98 %, respectively, unless stated otherwise.

2.2.2 Beetle rearing

Beetles used in the current study were obtained from a culture that was established using ~ 300 beetles that were collected in the field in Auckland, New Zealand in 2014. The culture was maintained in mesh-covered steel cages (50 x 50 x 82 cm) which contained at least four potted *T. fluminensis* plants. In each cage, beetles were reared in high numbers (~80) to maintain high levels of heterozygosity. *Tradescantia fluminensis* plants used for feeding the beetles were propagated in potted loamy soils using cuttings under net shading in a glasshouse. These plants were maintained under drip fertigation for 2-3 weeks before introduction into the cages. Irrigation in cages was done using custom-made drip kits, without any further fertiliser application. Cages were changed at intervals of 2 weeks where adult beetles were introduced to new cages with fresh plants. The pots carrying defoliated plants were kept in 'eclosion cages' to allow for larvae and pupae to complete development until eclosion of new adults.

2.2.3 Plant treatment

Tradescantia fluminensis cuttings were propagated in a potted (25 cm high x 20 cm diameter) mixture of soil and vermiculite (1:1) and allowed to grow for a week with daily irrigation to allow for vigorous root formation. A full-factorial design consisting of three levels of both water and fertiliser (3^2 factorial design) was then established using these plants. Water levels comprised of no irrigation (severe water stress (SWS)), irrigation every 10 days (pulsed water stress (PWS) and irrigation every two days (optimal irrigation (OI)) for a period of 30 days prior to the introduction of beetles or onset

of the experiments. Fertiliser also comprised of three levels including no fertiliser (NF), 2.4g applied once a week (medium (MF)) and 5g applied twice a week (excess fertiliser (EF)). This fertiliser was a straight-N Multicote® ammonium nitrate (NH_4NO_3) slow release fertiliser (Haifa Chemicals, South Africa) currently used in culture for maintenance of the plants. Fertiliser was applied as a 300 ml aqueous solution. Hence, to standardise the potential irrigation effect of the aqueous solution, 300 ml of pure water was also applied to the non-fertiliser treatments whenever others received fertiliser. All the plants were grown under net shading in a glasshouse to mimic natural shading provided by the forest understory where *T. fluminensis* thrives best.

2.2.4 Determination of plant performance and quality

Thirty days after the onset of each treatment, prior to insect bioassays, length of parent vines and their branches was measured for five replicate plants from each treatment to determine plant performance. Furthermore, a single photon avalanche diode (SPAD) 502 Chlorophyll Meter (Minolta, Osaka 542, Japan) was used to determine plant condition. Previous studies have shown such SPAD readings as reliable indicators of leaf chlorophyll content (Uddling et al., 2007; Cowie et al., 2016). In the present study, a mean SPAD value derived from four randomly selected leaves per plant was recorded for the five replicate plants from each treatment. Foliar nitrogen levels were determined via the Dumas quantitative combustion procedure using a Rapid N Cube Elementar (Elementar Analysensystemme GmbH, Germany) connected to a computer that analysed the data using RapidN software (version 5.0.7 [1831be8]). Before nitrogen analysis, five replicate plants per treatment were cut and oven dried for 72 hours at 75°C (or to a constant weight) then finely milled through a 0.2 mm mesh size. Leaf water content was determined gravimetrically during that phase. Aliquots for each sample were prepared as 150mg pellets wrapped in tin foil and loaded onto the carousel of the auto-sampling platform on the analyser. The samples were then combusted at 960 °C and the resulting gases detected by a thermoconductivity detector that received gas mixtures via an electronic flow controller. Aspartic acid was used as the authentic standard for calibration with carbon dioxide as the carrier gas.

2.2.5 Choice and no-choice oviposition assays

1) No-choice assay

Plants from each treatment were individually placed in cages before introducing newly emerged beetles. Each cage was given a total of four beetles (2 male and 2 females), randomly selected from the main culture, and allowed to feed and females oviposit for 10 days. During the assay, plants maintained their respective treatment fertiliser and watering regimes. Thereafter, plant material was excised to allow for egg counting with the aid of a Leica stereomicroscope (model MZ7.5, Leica Microsystems GmbH, Wetzlar, Germany). This procedure was repeated five times to yield five replications for each treatment.

2) Multi-choice assays

Individual plants from each treatment were randomly placed in a walk-in cage (3 x 2 x 2 m) where forty newly eclosed adult beetles (20 females: 20 males) were released. The beetles were allowed to move freely and choose hosts for feeding and oviposition over a ten-day period. Care was taken to ensure that leaves of different plants were not in contact to avoid false positives. Plant parts were also excised to allow for egg counting under a microscope. Oviposition choice was recorded as the proportion of eggs oviposited on a plant in relation to the total number of oviposited eggs during each replication. This procedure was repeated five times.

2.2.6 Larval performance and development assays

To ensure naïve larvae without prior plant exposure were used, eggs from the main culture were placed in Petri dishes with moistened filter paper where they were allowed to hatch. The newly emerged larvae were used in subsequent assays. For each treatment, 5 larvae were placed in a Petri dish (1 replication) lined with moistened filter paper where they had access to excised plant material from each water nitrogen treatment. Petri dishes were sealed with Parafilm and each treatment was replicated 5 times (N=50 larvae). Performance of the larvae across the treatments was determined by evaluating *per capita* weight gain, mortality rates per dish, duration until 50% pupation and adult eclosion rate. To quantify herbivore damage, leaves were photographed against a calibrated

background before and after larval feeding using a high resolution digital camera (PowerShot G3X, Canon, Japan). Damaged leaf surface area was then derived from the digital photographs using ImageJ software (National Institute of Health, USA). Throughout the assays, Petri dishes were covered with foil and stored in a Labcon growth chamber (model: FSIM-RH20; accuracy: ± 0.2 °C; Labcon Laboratory Equipment, South Africa) set at 26 ± 1 °C. Filter paper and plant material for each treatment was changed every second day where performance traits for each treatment were also evaluated. Per capita weight for the larvae was determined using a Sartorius microbalance (model BP61, Sigma Aldrich, USA).

2.2.7 Beetle adult longevity

Newly emerged adults were collected from the main culture and individually placed in Petri dishes lined with moistened filter paper and placed in a growth chamber set at 26 °C. Petri dishes were randomly assigned to different treatments where beetles were given plant material from each respective treatment for feeding. Beetles were assessed every 2-3 days where filter paper was moistened and plant material replaced, if completely defoliated. The number of days until death was recorded and regarded as the influence of plant quality on survival. Preliminary trials did not show any sex differences in longevity among virgin adults so both virgin males and females (randomly) were used. Each treatment was replicated five times.

2.2.8 Data analyses

All data analyses were performed using R version 3.4.0 (R Development Core, 2017). Homogeneity of variance and data normality were assessed using the Bartlett and Shapiro-Wilk`s tests, respectively. A two-way ANOVA (aov function) was used to determine the impact of the water and fertiliser levels on plant parameters. Tukey`s *post-hoc* tests were done to identify significantly different groups. Data for oviposition acceptance and preference was non-parametric and heteroskedastic. Generalised linear models (GLM) assuming a Poisson distribution of errors with a log function were then used with water and fertiliser levels treated as the fixed factors. For each of the 9 treatment combinations, data for larval weight gain were first averaged and observations for each

Petri dish regarded as independent observations. This allowed the use of two-way ANOVA rather than a repeated measures ANOVA. Data for larval feeding damage and adult longevity met the assumptions of parametric tests and was analysed with a two-way ANOVA using the aov function in R. Due to data being non-parametric and heteroskedastic, larval mortality rates were analysed using GLM assuming a binomial distribution (dead or alive) with a probit link function and a single correction for over dispersion. Linear correlations were performed to test the relationship between foliar nitrogen content and insect performance traits among the three water treatments.

2.3 Results

2.3.1 Plant performance

The main effects of fertiliser level significantly affected total vine length, number of branches, foliar nitrogen content and SPAD values. Total vine length, number of branches, SPAD values and foliar water content were significantly affected by the main effect of watering regime. The interactive effects of fertiliser and watering treatments significantly affected all these plant performance traits except foliar water content (Table 2.1). Overall, optimally irrigated plants that received excess fertiliser had the longest vines (160 ± 10.4cm) whilst severely water stressed plants that had excess fertiliser had the shortest vines (15 ± 11.6cm). Plants that received moderate and excess fertiliser under optimal watering had the highest SPAD values (50 ± 5.2). Foliar nitrogen content was highest in plants that had excess fertiliser under both pulsed (6.3 ± 1.2%) and optimally irrigated plants (6.5 ± 1.2%) (Fig. 2.1). Under optimal irrigation without any fertiliser, plants had the lowest foliar nitrogen content (0.6 ± 0.4 %). All the summarised results for the impact of water and fertiliser on plant performance are presented in Table 2.1 and Figure 2.1.

Table 2.1: Summarised results of the impact of water and nutrient variability on *T. fluminensis* following a two-way ANOVA showing degrees of freedom (d.f.), F ratio (F) and significance level (P-value). Significant effects are indicated in bold

Source of Variation	d.f.	F	*P*-value
Total length of parent vine and branches			
Fertiliser	2	15.37	**<0.001**
Water	2	4.75	**0.031**
Fertiliser x Water	4	1.364	**0.026**
Residual	36		
Total	44		
Number of branches			
Fertiliser	2	23.97	**<0.001**
Water	2	5.43	**0.016**
Fertiliser x Water	4	4.08	**0.032**
Residual	36		
Total	44		
SPAD Values			
Fertiliser	2	67.69	**<0.001**
Water	2	17.41	**<0.001**
Fertiliser x Water	4	0.79	0.053
Residual	36		
Total	44		
Foliar nitrogen content			
Fertiliser	2	62.11	**<0.001**
Water	2	1.96	0.156
Fertiliser x Water	4	9.13	**<0.001**
Residual	36		
Total	44		
Foliar water content			
Fertiliser	2	1.896	0.541
Water	2	5.4	**0.021**
Fertiliser x Water	4	1.65	1.834
Residual	36		
Total	44		
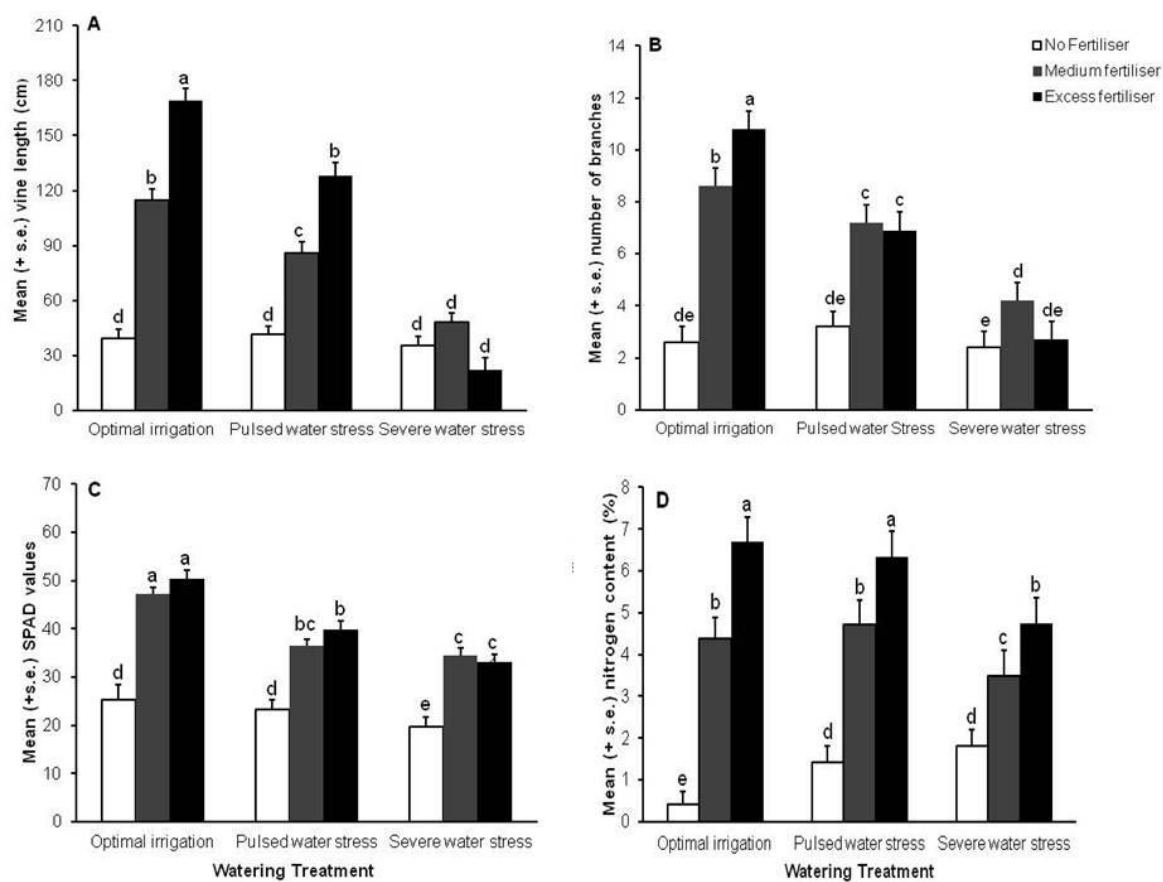
Figure 2.1: Impact of water and nitrogen variability on vine length (A), number of branches (B), chlorophyll (SPAD value) (C) and foliar nitrogen content (D) in *Tradescantia fluminensis*. Different letters above error bars denote statistically significant differences among.

2.3.2 Beetle performance

1) *Neolema abbreviata* larval traits

Larval leaf feeding damage was highest in OI plants without fertiliser ($7 \pm 1.2cm^2$) and was influenced by both water and fertiliser levels together with their interactive effects (Table 2.2). The main effects of fertiliser and water resulted in the lowest feeding in EF ($3.2 \pm 1.2cm^2$) and SWS ($3 \pm 1.2cm^2$) plants, respectively. Larval feeding damage negatively correlated with foliar nitrogen content

in OI (r(13) = 0.83, P < 0.001) and SWS (r(13) = 0.54, P = 0.03) but not PWS where there was no correlation (r(13) = 0.01, P = 0.74).

Only fertiliser treatments significantly affected larval growth rate (weight gain). The highest weight gain (0.092 ± 0.001mg) was observed in the MF plants whilst those that fed on the NF plants had the least (0.08±0.001mg). There was a positive correlation between foliar nitrogen content and weight gain among larvae feeding on OI r(13) = 0.80, P = 0.001 and PWS r(13) = 0.66, P = 0.001 plants. In SWS plants, there was no correlation between larval weight gain and foliar nitrogen content r(13) = 0.17, P = 0.55) (Fig. 2.3). Rate of larval development to pupation was fastest in the MF (9 ± 2 days) and EF (11 ± 2 days) plants with larvae feeding on NF (18 ± 2 days) plants taking longest time (Fig. 2.2). For these developmental rates both the main effects of fertiliser and its interaction with watering treatments significantly affects developmental rates (Table 2.2). There was negative correlation between foliar nitrogen content and rate of development to pupation in OI r(13) = 0.91, P < 0.0001 and PWS r(13) = 0.79, P = 0.001) plants whilst there was no such correlation in SWS r(13) = 0.17, P = 0.55) plants.

Larval mortality was influenced by the main effects of fertiliser alone together with their interaction with water levels (Table 2.3). Mortality was highest in EF plants (50 ± 15%). Larvae feeding on PWS plants with excess fertiliser had the highest mortality rate whilst those feeding on OI plants, at all fertiliser levels, together with those SWS with NF had the least mortality rates (Fig. 2.2). In all watering treatments, there was no correlation between foliar nitrogen content and larval mortality (OI: r(13) = 0.16, P = 0.57); PWS: r(13) = 0.41, P = 0.07; SWS(r(13) = 0.17, P = 0.55) (Fig . 2.3).

Table 2.2: Summarised results of the impact of water and nutrient variability on larval feeding damage of *N. abbreviata*, weight gain and mortality and adult leaf damage and longevity following a two-way ANOVA in R. Significant effects are indicated in bold

Source of Variation	d.f.	F	*P*-value
Larval leaf damage			
Fertiliser	2	59.73	**<0.0001**
Water	2	41.43	**<0.0001**
Fertiliser x Water	4	7.47	**<0.0001**
Residuals	36		
Total	44		
Larval weight gain			
Fertiliser	2	4.58	**0.01**
Water	2	2.16	0.27
Fertiliser x Water	4	1.7	0.2
Residuals	36		
Total	44		
Adult leaf damage			
Fertiliser	2	8.47	**0.01**
Water	2	1.3	0.24
Fertiliser x Water	4	5.74	**0.02**
Residuals	54		
Total	59		
Adult longevity			
Fertiliser	2	11.46	**<0.0001**
Water	2	4.25	**0.019**
Fertiliser x Water	4	3.7	**0.01**
Residuals	54		
Total	59		

Table 2.3: Summary output of a Generalised Linear Model (GLM) using a binomial distribution and a probit function, with a single correction for over dispersion, investigating the impact of fertiliser and watering regimes on the larval mortality rates of *Neolema abbreviata* showing degrees of freedom (d.f.), estimated marginal mean standard error (SE), Wald test statistic (Z) and level of significance (*p*). Significant differences are indicated in bold

Effect	d.f.	Estimate	SE	Z	P
Intercept	1	14.42	3.54	2.76	**<0.001**
Fertiliser	2	2.63	1.37	8.4	**0.034**
Water	2	0.51	0.49	1.04	0.29
Fertiliser x Water	4	4.52	2.25	6.8	**0.021**
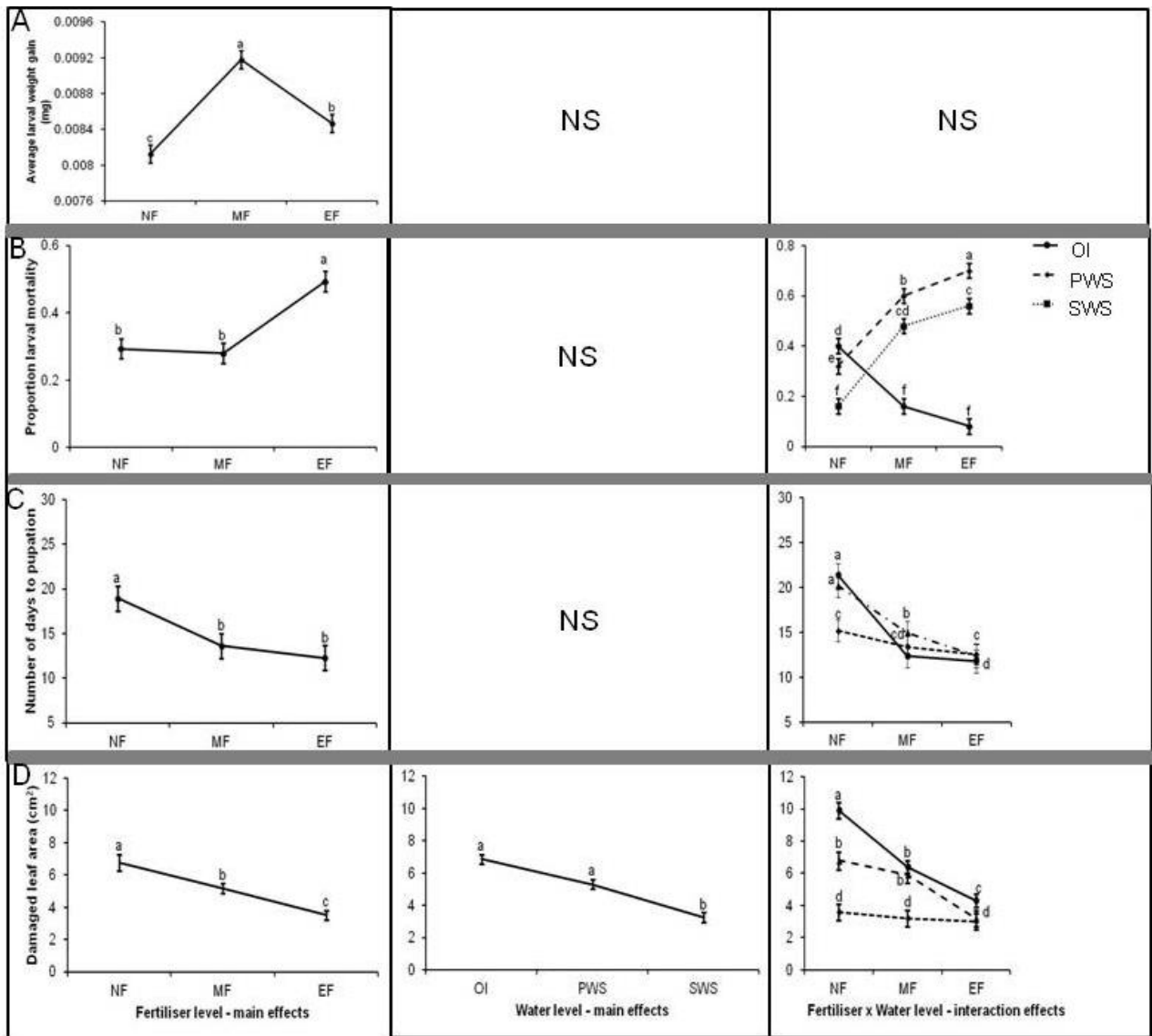
Figure 2.2: Main effects of 3 fertiliser levels [None (NF), medium (MF) and excess (EF)], 3 watering regimes [Optimal (OI), pulsed water stress (PWS), severe water stress (SWS)] and their interaction on A) weight gain, B) mortality, and number of days to pupation (developmental rate) D) leaf damage in *Neolema abbreviata* on *Tradescantia fluminensis* plants under different watering and fertiliser regimes. Different letters above error bars denote statistically significant groups. NS = not significant.

A
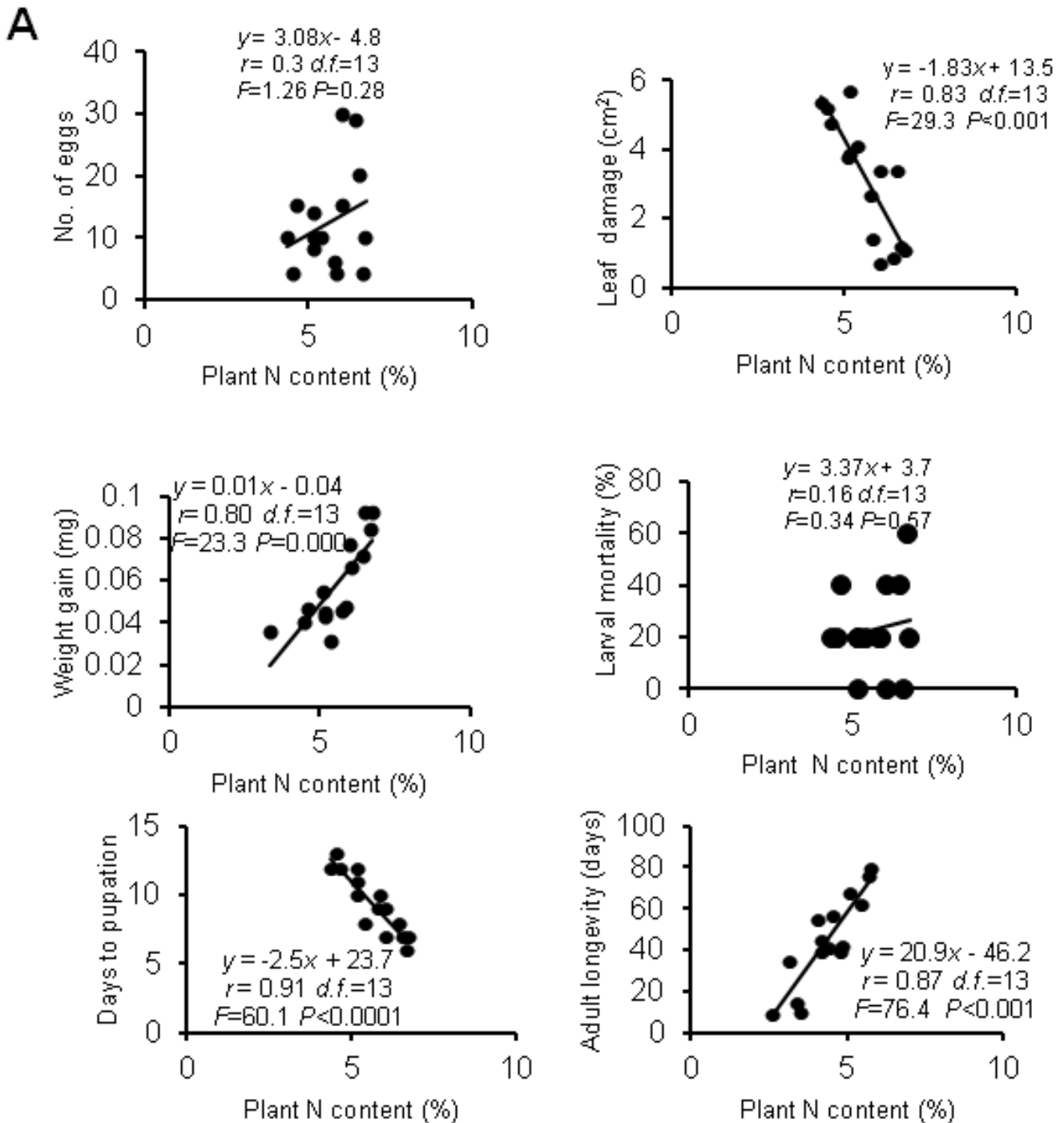
Figure2.3a: Relationships between nitrogen (N) and *Neolema abbreviata* life-history traits; oviposition selection (No. of eggs), leaf damage, larval weight gain, larval mortality, developmental rate (days to pupation) and adult longevity under optimal irrigation.

B

No. eggs vs **Plant N content (%)**
$y = 0.13x + 2.63$
$r = 0.08 \ d.f.=13$
$F=0.08 \ P=0.78$

Feeding damage (cm²) vs **Plant N content (%)**
$y = -0.64x + 6.6$
$r = 0.68 \ d.f.=13$
$F=11.3 \ P=0.01$

Weight gain (mg) vs **Plant N content (%)**
$y = 0.01x - 0.004$
$r = 0.66 \ d.f.=13$
$F=25.2 \ P=0.001$

Larval mortality (%) vs **Plant N content (%)**
$y = 7.59x - 12$
$r = 0.41 \ d.f.=13$
$F=3.71 \qquad P=0.07$

Days to pupation vs **Plant N content (%)**
$y = -1.74x + 21.5$
$r = 0.79 \ d.f.=13$
$F=22.4 \ P=0.001$

Adult longevity (days) vs **Plant N content (%)**
$y = 11.18x - 0.38$
$r = 0.54 \ d.f.=13$
$F=5.38 \ P=0.03$

Figure2.4b: Relationships between nitrogen (N) and *Neolema abbreviata* life-history traits; oviposition selection (No. of eggs), leaf damage, larval weight gain, larval mortality, developmental rate (days to pupation) and adult longevity under pulsed water stress.
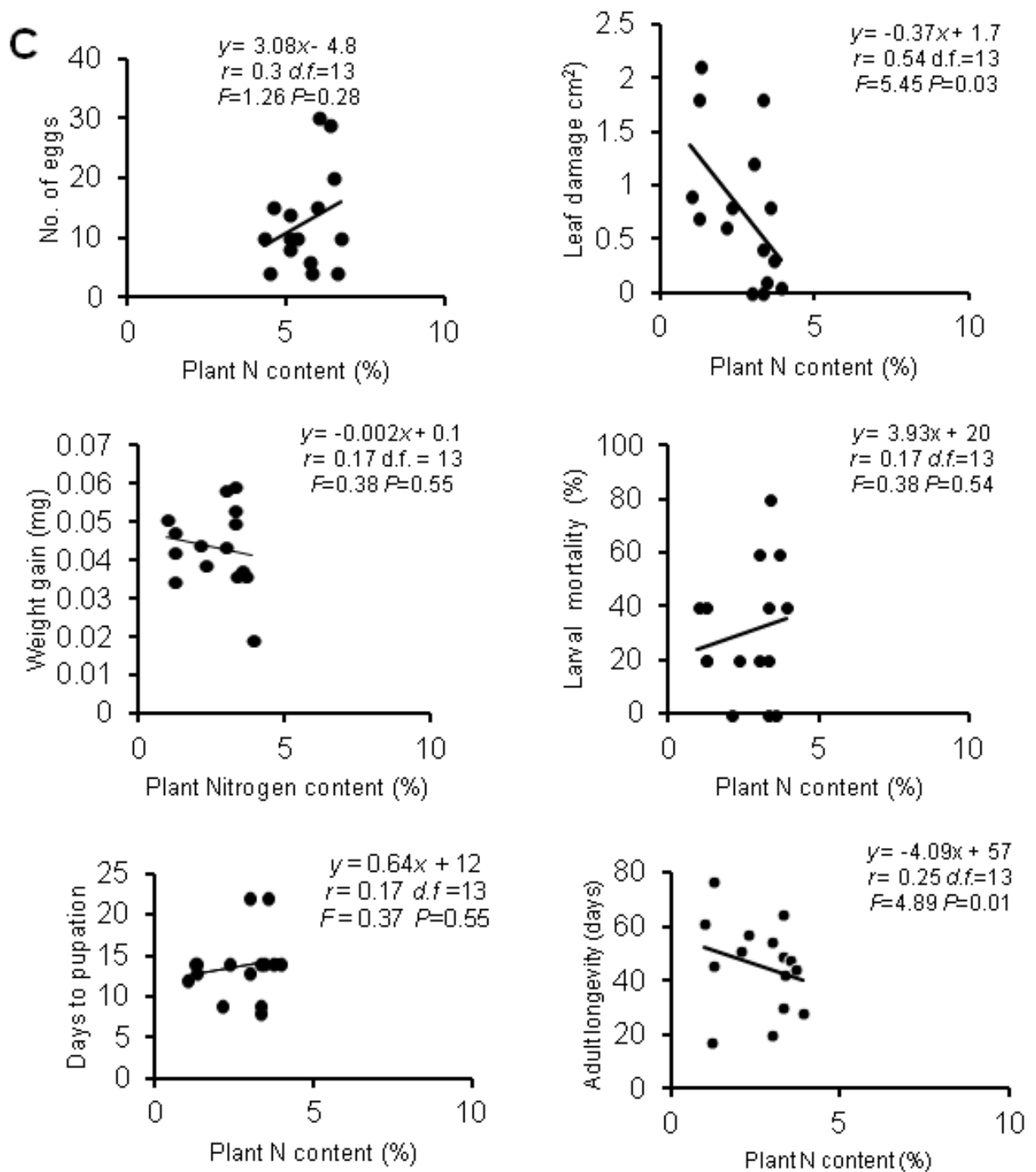
Figure2.5: Relationships between nitrogen (N) and *Neolema abbreviata* life-history traits; oviposition

selection (No. of eggs), leaf damage, larval weight gain, larval mortality, developmental rate (days to

pupation) and adult longevity under severe water stress.

2) Pupal traits

The main effects of fertiliser, water and their interaction significantly affected pupation success (Fertiliser: χ^2 = 27.2 df = 2, P < 0.001; Water: χ^2 = 15.1, df = 2, P =0.01; Fertiliser x Water: χ^2 = 4.71, df = 4, P = 0.02). For adult eclosion, only fertiliser and the interaction between fertiliser and water was significant (Fertiliser: χ^2 = 14.6 df = 2, P = 0.01; Water: χ^2 = 3.2, df = 2, P =0.1; Fertiliser x Water: χ^2 = 7.36, df = 4, P = 0.01). Beetles that developed on OI Plants under MF and those that developed on PWS and EF plants had the highest pupation (67 and 48%, respectively) and adult eclosion rate (62 and 41%, respectively). The lowest pupation (12%) and adult eclosion (10%) rate was on beetles feeding on OI plants under NF (Table 2.4).

Table 2.4: Rate of pupation and adult eclosion expressed as proportions of pupated and eclosed *Neolema abbreviata* for individual treatments comprising of three levels of both water and nitrogen. For all treatments insect performance was compared to those developing on the optimal group (moderate fertiliser with optimal irrigation) using Fisher`s Exact Test of Independence. For each column, the following asterisks combinations indicate significant differences from the control group: * P < 0.05, **P < 0.01, *** P < 0.001

Fertiliser level	Water level	Pupation rate	Adult eclosion rate
Moderate	Optimal irrigation	0.67	0.62
Moderate	Pulsed water stress	0.31***	0.19***
Moderate	Severe water stress	0.44*	0.34*
None	Optimal irrigation	0.12***	0.1 ***
None	Pulsed water stress	0.22***	0.2***
None	Severe water stress	0.24***	0.15***
Excess	Optimal irrigation	0.42**	0.35**
Excess	Pulsed water stress	0.48	0.41
Excess	Severe water stress	0.34***	0.28***

3) Adult traits

Only watering treatments significantly affected oviposition acceptance under conservative no-choice conditions. Under choice conditions, both water and fertiliser regimes, together with their interactive effects, significantly affected oviposition selection (Table 2.5). Overall, plants under optimal irrigation received the most number of eggs with those under MF receiving the most in choice conditions (20 ± 4.1 eggs). Under no choice conditions, OI plants received the most eggs and there was no significant influence of fertiliser at this particular water level (Fig. 2.4). When pooled together, there was no correlation between total number of eggs and foliar nitrogen content at all three watering treatments (OI: $r(13) = 0.3$, $P = 0.28$); PWS: $r(13) = 0.08$, $P = 0.78$; SWS $r(13) = 0.3$, $P = 0.28$) (Fig. 2.3). Adult leaf damage was highest in optimally irrigated plants with no fertiliser (12 ± 2.1 cm^2) and positively correlated with nitrogen content at OI ($r(13) = 0.64$, $P < 0.001$); and PWS($r(13) = 0.48$, $P < 0.001$). There was no correlation between adult feeding and foliar nitrogen content under SWS ($r(13) = 0.32$, $P = 0.07$). The main effects of fertiliser and water treatments, together with their interaction, significantly affected adult longevity (Table 2.4 and Fig. 2.4). Beetles feeding on PWS plants under MF survived longest (130 ± 7 days) whilst those feeding on plants from SWS under EF had the shortest life span (44 ± 5 days) (Fig. 2.5). There was a positive correlation between foliar nitrogen content and adult longevity in OI $r(13) = 0.87$, $P < 0.001$ and PWS $r(13) = 0.54$, $P = 0.03$. Surprisingly, this relationship was negative in SWS plants but relatively weaker ($r(13) = 0.25$, $P = 0.01$) (Fig. 2.3).

Table 2.5: Summary output of a Generalised Linear Model (GLM) assuming a Poisson distribution and a log function, with water and fertiliser levels as fixed factors and the number of eggs of *Neolema abbreviata* as the response variable in a no-choice (acceptance) and choice (preference) assay. Significant differences are indicated in bold

Effect	d.f.	χ^2	*P*
Oviposition acceptance			
Water	2	31.9	**<0.0001**
Fertiliser	2	6.1	0.201
Water x Fertiliser	4	8.2	0.791
Oviposition preference			
Water	2	32.4	**<0.0001**
Fertiliser	2	27.3	**<0.0001**
Water x Fertiliser	4	16.1	**0.03**
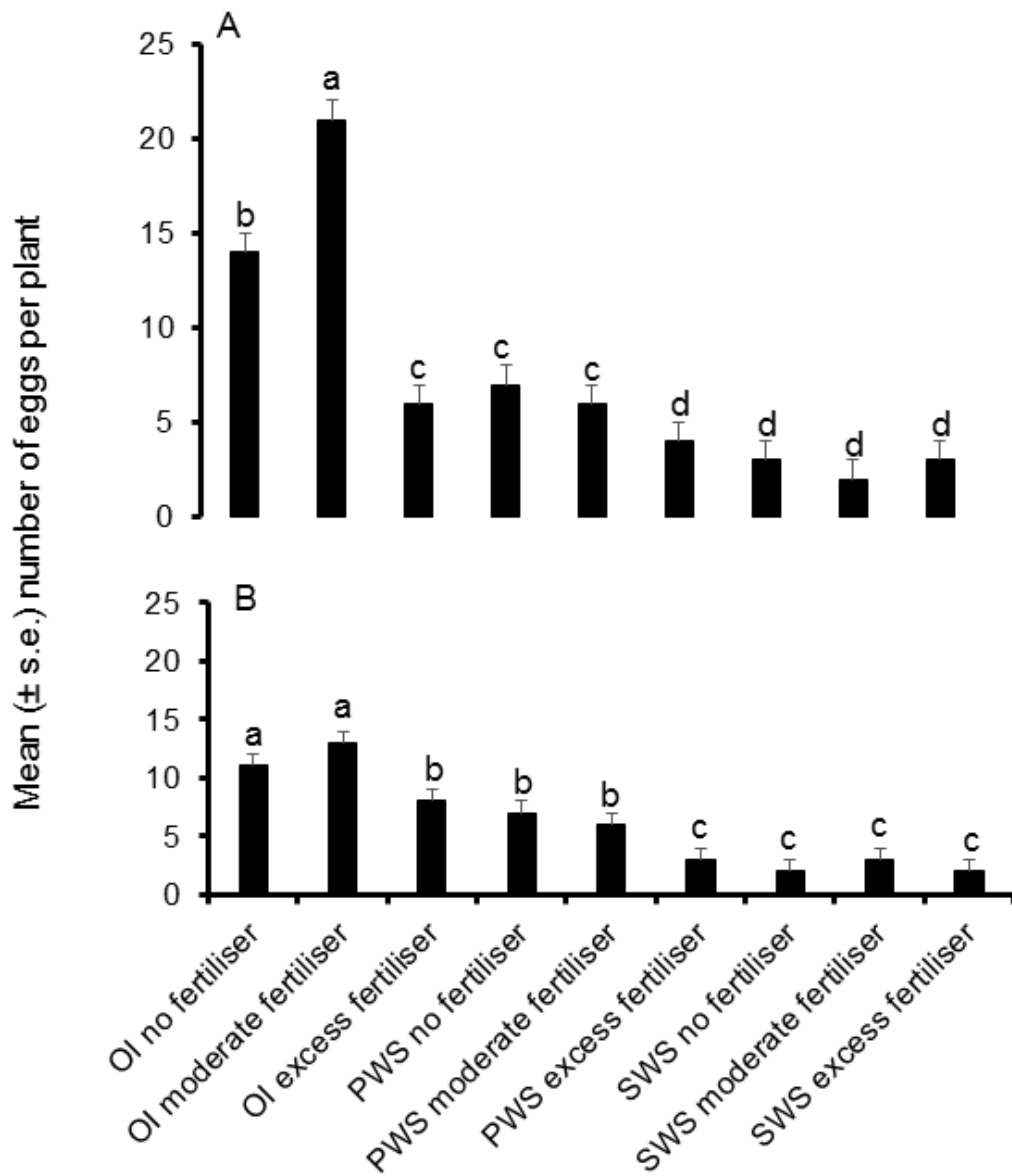
Figure 2.6: Impact of water and fertiliser variability on oviposition (A) preference and (B) acceptance of *Neolema abbreviata* under optimal irrigation (OI), pulsed water stress (PWS) and severe water stress (SWS).
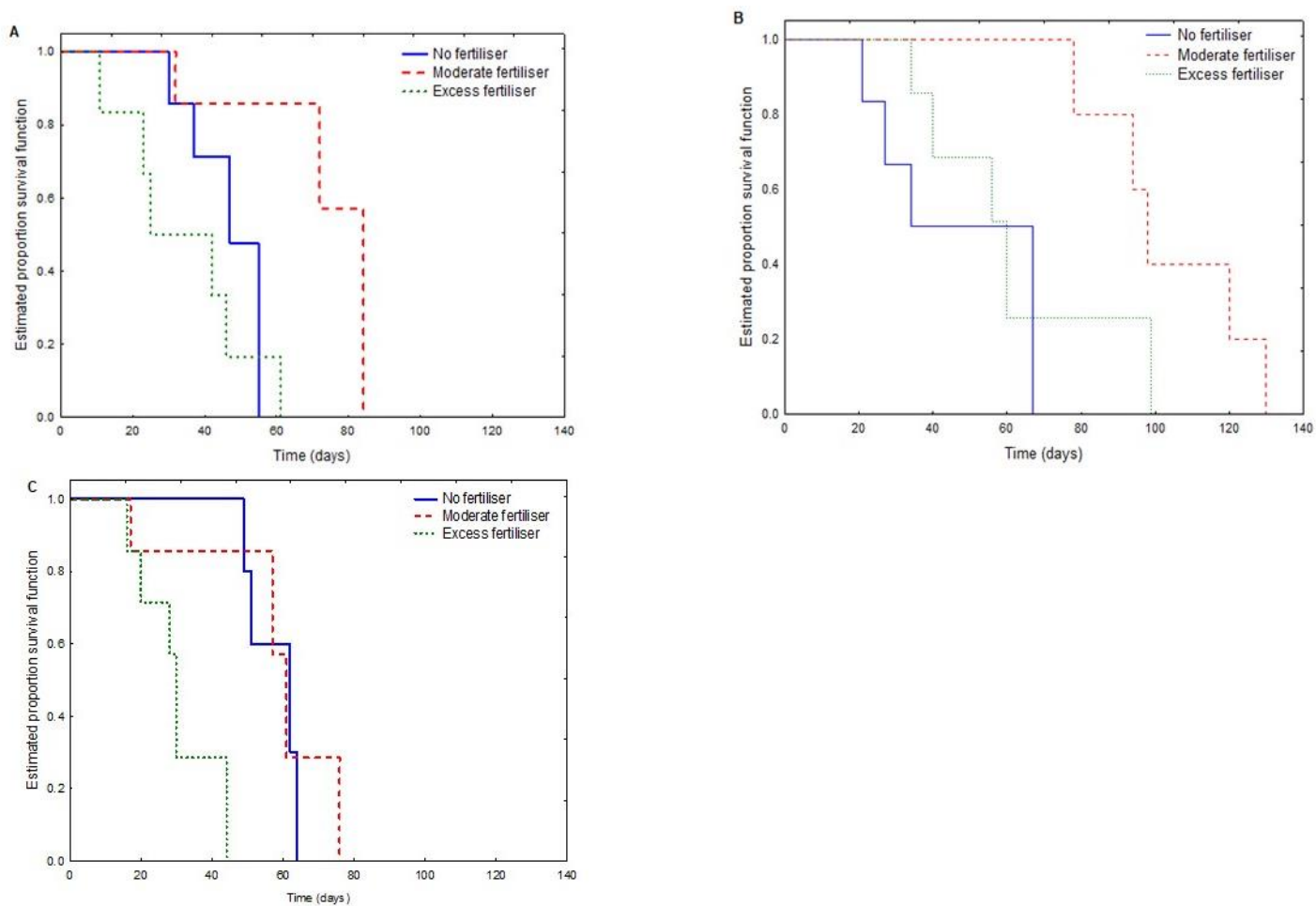
Figure 2.7: Survival curves of adult *Neolema abbreviata* reared on *Tradescantia fluminensis* plants of variable quality under optimal watering regime (A), pulsed water stress (B) and severe water stress (C) at fertiliser levels (none, medium and excess)

2.4 Discussion

Environmental stressors affect both plant biomass accumulation (Burns 2004) and intrinsic chemical composition (Gutbrodt et al. 2011; Pineda et al. 2016) with subsequent impact on their interactions with herbivores (Tariq et al. 2012; Chidawanyika et al. 2014, 2015; Han et al. 2014) and other trophic groups (Tariq et al. 2013). The current study revealed that both water and fertiliser influenced my proxies for plant biomass accumulation, total vine length and number of branches per plant. Total vine length and branching was highest in optimally irrigated plants that had high levels of nitrogen. This corroborated a previous study by Burns (2004) where biomass accumulation among invasive Commelinaceae positively responded to increased nutrients. In my study however, high levels of nutrients under severe water stress resulted in poor biomass accumulation indicating the importance of water in resource acquisition and performance of *T. fluminensis*. Foliar nitrogen content, even under SWS, was generally higher in all EF plants compared to MF treatments. Interestingly, plants that had no fertiliser under PWS and SWS regimes had more foliar nitrogen content compared to OI plants that had no fertiliser. Previous studies have attributed an increase in foliar nitrogen concentration during water stress to hydrolisation of proteins into free amino acid radicals and rapid accumulation of osmoprotectants in response to the low osmotic pressure (Huberty & Denno 2004; Sardans & Peńulas 2012). Since water is essential for nitrogen uptake by plants (Sardans & Peńulas 2012) I conclude, with caveats, that the higher nitrogen in all water stressed treatments in my study was partly due to this protein hydrolysis.

There was a general decline in SPAD values with decreasing water availability for all fertiliser treatments. Previously, SPAD values have been reported as good indicators of chlorophyll content with higher values denoting the superior condition of a plant (Uddling et al. 2007; Cowie et al. 2016). Although SPAD values in my study were generally in line with vegetative performance of the plants (vine length and branching), they were not always consistent with foliar nitrogen concentration in contrast to earlier reports by Nielsen et al. (1995) and Li et al. (1998). Such differential associations in leaf nitrogen content relative to SPAD readings have been attributed to variations in leaf position (Yang et al. 2014), environmental factors and leaf characteristics (Xiong et al. 2015). The current

study, did not tightly control for the position of the leaves when sampling for the SPAD values, thus it is possible that leaf position could have confounded the relationship between nitrogen content and SPAD values, there were similar SPAD values between MF and EF plants under PWS and under SWS, despite apparent differences in vine length. Overall, these results still demonstrate stress-induced physiological changes in the plant leading to intrinsic changes in quality and vegetative performance.

Larval feeding and survival were influenced by both water and fertiliser variability. However, only fertiliser influenced larval weight gain and number of days to pupation. For all watering treatments, larval feeding was highest in the plants that had no fertiliser, perhaps as a compensatory mechanism for inadequate protein among the host plants as attributed in other studies (e.g. Lindroth et al. 1991; Lee 2017). However, these high feeding rates in NF treatments did not reduce the number of days before pupation.

Several studies have attributed poor nitrogen content in host plants as one of the major factors leading to longer developmental times (Petersen et al. 2000; Chen et al. 2008; Bauerfeind & Fischer 2013). Indeed elevated nitrogen levels in my study resulted in shortened developmental time, increased weight gain but with relatively lower feeding rate. In nature, longer developmental times can be ecologically costly through increased larval exposure to mortality factors such as predation and abiotic stressors (Benrey & Denno 1997; Williams 1999). Furthermore, where sex differences exist, timing of pupation is of immense ecological importance as it may lead to the impairment of mating success, in particular among short-lived insects (Moreau et al. 2006). Even though I did not test sex differences in time to pupation it is highly unlikely that this could influence the population dynamics of *N. abbreviata* as they have a relatively long life span. However, other nutrient-mediated traits tied to developmental rates, not subject to this study, such as body size are known for influencing mate choice and concomitant mating success among insects (Hanks et al. 1996; Bangham et al. 2002; Cator & Zanti 2016). For all OI and PSW treatments, larval weight gain and number of days to pupation were all positively correlated with foliar nitrogen content. Rate of pupation and adult eclosion was lowest in the NF treatments suggesting nitrogen limitation in the development of *N. abbreviata.* My

study therefore is in keeping with several studies that demonstrate nitrogen limitation in the development of juvenile insects (Chen et al. 2004; Hwang et al. 2008; Han et al. 2014; Helmberger et al. 2016)

It has long been widely appreciated that insects discriminately select plants for oviposition in order to maximise offspring fitness and performance (Jaenike 1978; Renwick & Chew 1994; Awmack & Leather 2002; Martínez et al. 2017). However, several studies provide empirical evidence showing mismatches in host selection (Thompson 1988; Thompson & Pellmyr 1991; Mayhew 1997). Nevertheless, oviposition selection is likely to be even more important for specialist herbivores such as biological control agents in novel habitats, where host range is constricted, if the herbivore population is to persist. In my study, oviposition choices were influenced by both water and fertiliser regimes in choice assays. However, under no-choice conditions, only water availability affected host plant selection with optimally irrigated plants generally receiving more eggs. These results suggest that *N. abbreviata* exploits cues emitted by *T. fluminensis* to determine a suitable host under complex heterogeneous environments and water availability may be the most critical factor influencing oviposition selection. Previous reports have attributed such behavioural plasticity in host selection to be guided by visual (Goyret et al. 2008; Yv et al. 2015), olfactory (Bruce et al. 2005; Bruce & Pickett 2011; Schäpers et al. 2015), tactile (Goyret & Raguso 2006) and sometimes adult gustatory (Ômura et al. 2008) cues, in the case of actively feeding females. Although a large body of literature demonstrating host selection behaviour among phytophagous insects has largely been multi-plant species comparisons, it is also important within species where plant status is determined by abiotic and biotic factors, such as presence of conspecifics and pathogens, or risk of predation becomes important (Mappes & Kaitala 1995, Anderson & Anton 2014). It is likely that some of these factors may have acted in conjunction with the manipulated physical factors in the current study. In particular, for choice tests where discriminations could be made in the presence of eggs of conspecific beetles. Timing of the occurrence of stress in host plants has previously been reported as a factor influencing oviposition selection (Craig & Itami 2008; Helmberger et al. 2016). In the current study, both nutrient and water stress was simultaneously induced among similarly aged plants. I was

therefore unable to discern if timing of stress could have influenced oviposition selection in this study. Nevertheless, my results still confirm the potency of fertility-water interactions on insect oviposition selection.

Apart from developmental nutrition, adult nutrition is equally important in determining key life-history traits (Nevo & Coll 2001; Fontana et al. 2010; May et al. 2015). The current study revealed that host plant quality through manipulation of water and fertiliser availability influenced adult longevity. Beetles that fed on SWS plants with excess fertiliser had the shortest lifespan whilst those that fed on PWS plants with moderate fertiliser had the longest. This was surprising as I expected adult longevity to also be relatively high in SWS plants considering the high levels of nitrogen. It is therefore likely that poor water content in the plants may have affected resource acquisition of the beetles. My results therefore suggest similar optimal nutritional quality requirements for larval and adult stages of *N. abbreviata* to offset energetic demands and resource allocation across life-stages (Braby & Jones 1995; Boggs 2009). However, in some holometabolous insects, nutritional requirements between larval and adult life-stages vary (Geister et al. 2008). Furthermore, some studies such as Fox & Dingle (1994) and Aiken et al. (2016) investigated the role of diet quality (including plants) on adult longevity have been based on insects that are non-feeding during the adult phase. Hence, adult traits in such cases are mostly determined by the quality of the resources acquired during ontogeny (Dmitriew & Rowe 2011). In this study, the test subjects I used feed at both larval and adult phases making it more difficult to draw generalisations of the impacts of host plant quality on herbivore performance. Adults which were used in the longevity assessment all developed from larvae feeding on optimal irrigation and moderate fertiliser (culture conditions), it is possible that the mismatches presented by the environmental factors during adult phase could have impacted their survival. According to the environmental matching hypothesis, plastic responses acquired during larval development enhance adult performance when exposed to plants in similar conditions (Monaghan 2008; D'mitriew & Rowe 2011). This is in contrast to the 'silver spoon' effect where larval development under optimal conditions supports enhanced adult performance in both optimal and stressful conditions (Hopwood et al. 2014; Scharf et al. 2015). Even though I did not explicitly

test it, it is possible that *N. abbreviata* requires environmental matching to improve adult performance in otherwise adverse environments.

From the aforementioned, this study supports the preference-performance hypothesis as larval performance was generally in sync with oviposition preference. Furthermore, larval performance supported the PVH, in particular in the case of larval feeding, rate of development and pupation success. In the case of adult performance, longevity was highest in the plants that had pulsed water stress with moderate fertiliser in conformity with the PPSH. Correlation analyses revealed a positive linear relationship between nitrogen content and adult longevity. Previous studies (Huberty & Denno 2004) have associated pulsed water stress with elevation of soluble proteins. It is therefore plausible that the combined effects of elevated proteins together with that provided in the moderate fertiliser treatment was more favourable for adult longevity. This is in contrast to the feeding-guild explanation of the PPSH which asserts that plant recovery turgor pressure under pulsed stress favours sap sucking insects like aphids and not leaf chewers (Huberty & Denno 2004; Mody et al. 2009; Bauerfeind & Fischer 2013). In the current study, both larval and adult life-stages of *N. abbreviata* were assessed under both water and nutrient variability in contrast to most seminal work advancing the PPSH, PVH and PWS which were largely based on a single factor analysis in the form of water variability (White 1969, 1984; Mattson & Haack 1987; Price 1991; Huberty & Denno 2004). This is despite the knowledge that foliar nutrient levels, in particular nitrogen, play a key role in determining insect herbivore development and survival (Fischer & Fiedler 2000; Chen et al. 2008; Runagall-McNaull et al. 2015). It is therefore sometimes difficult to make outright generalisations of the impact of environmental variability on insect herbivore performance where multiple abiotic factors are considered. My study therefore demonstrates the importance of multivariate or multi-trait considerations when investigating insect-plant relationships (discussed in Moreau et al. 2006). This has even become of more importance in this era of global climate change where changes in climate and land use continue to alter species interactions.

In conclusion, the implications of my study on the mass-rearing of *N. abbreviata* and potential biological control of *T. fluminensis* are three-fold: Firstly, *N. abbreviata* exhibited plasticity in

oviposition selection as it could select for favourable host plants that ensured good offspring performance. Such plasticity could ensure population persistence under field conditions provided alternative host plants of superior condition are available. Secondly, host plant quality influenced larval development, pupation and eclosion success, and adult longevity suggesting the need for increased attention to plant quality during mass rearing to optimise production. Finally, fertiliser levels influenced feeding performance in both larval and adult stages. Biological control practitioners should therefore consider nutrient gradients among landscapes in order to release propagule populations or augment existing ones in order to inflict desirable plant damage. Here, my study was based on within-generational effects, future studies should endeavour to investigate transgenerational effects of nutritional history in line with the 'environmental matching hypothesis' in order to assess the feasibility of manipulating plant quality during mass rearing to optimise field performance under variable environments.

2.5 References

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Chapter 3: Ontogenic variation in transgenerational effects of parental diet in a specialist herbivore *Neolema abbreviata* under manipulated foliar nitrogen variability in *Tradescantia fluminensis*

Abstract

As global population continues to rise, alterations in soil and water nutrient loads due to anthropogenic activities such as agriculture and industrial waste disposal present major challenges to the environment. Nitrogen (N), a key element supporting life on earth, is by far one of the major elements with the most altered natural cycles resulting in significant nutrient gradients in both terrestrial and aquatic bodies. Differential host-plant quality is one major consequence of such elevated N levels resulting in changes in herbivore-plant interactions due to acute and chronic

responses in various traits. For insect herbivores, transgenerational effects of host plant quality remain relatively underexplored despite the potential insights they may give on their population dynamics under global change. Here, using a 3 x 3 x 2 factorial experiment, we evaluated how N enrichment in parental diets of *N. abbreviata* may impact life history and performance of F_1 and F_2 offspring under reciprocal experiments. We found limited transgenerational effects of foliar nitrogen variability among life-history traits in both larvae and adults. Larval weight, mortality, feeding damage, pupal weight and days to pupation were all not responsive to the main effects of parental diet. There were however significant parental diet x test interactions with offspring from parents under high N plants generally performing better under low N test plants in the case of larval weight gain and pupal final weights. Adult traits including oviposition selection, feeding weight and longevity did not respond to the effects of parental diet nor its interaction with test diet as was the case in the larval stage. Our results show conflicting responses to parental diet between larvae and adults of the same generation among an insect species with both actively feeding larval and adult life-stages. These results may have implications on the mass-rearing and field performance of *N. abbreviata* variable nutrient gradients.

Key-words: diet, early development, environmental matching hypothesis, fitness costs, host plant quality, life-history, parental effects, plant-insect interactions.

3.1 Introduction

Spatial and temporal environmental variability can shape community structure and function through changes in species abundance, composition and their subsequent interactions (Zizzari et al. 2016). For plants, abiotic factors are known to influence biomass accumulation, nutritional value and defensive capacity (Gutbrodt et al. 2011, 12; Karban et al. 2011, Pineda et al. 2016) thereby altering their interactions with herbivores. Such is the case between herbivorous insects and their host plants (Awmack & Leather 2002; Gibbs et al. 2012; Chidawanyika et al. 2014; Rousselin et al. 2016). Some studies have reported the effects of such variability in plant quality cascading to even higher trophic groups, such as parasitoids (Romo & Tylianakis 2013; Tariq et al. 2013; Weldegergis et al. 2015) through alterations in both foraging efficiency and resource utilisation.

Apart from water, nitrogen (N) variability is a major bottom-up factor determining host plant quality with differential consequences on herbivore performance (Chen et al. 2008; Han et al. 2014; Rousselin et al. 2016). For phytophagous insects, chronic suboptimal-quality diet can induce variable plastic life-history responses within and across generations (Boggs 2009; D'mitriew & Rowe 2011; Frago & Bauce 2014; Littlefair & Knell 2016) as it is true for non-phytophagous taxa (Valtonen et al. 2012; May et al. 2015; Emborski & Mikheyev 2018). Across generations, nutritional effects can be conferred to offspring via epigenetic (non-genetic) mechanisms (Youngson & Whitelaw 2008) in what is sometimes referred to as transgenerational plasticity or parental effects (Mousseau & Fox 1998; Monaghan 2008; Zizzari & Ellers 2014; Zizzari et al. 2016). Indeed, transgenerational plasticity among arthropods is perverse in nature and has been demonstrated among different insect taxa, against several environmental stimuli including temperature (Kristensen et al. 2008; Chidawanyika & Terblanche 2011; Guillaume et al. 2016), toxin exposure (Colombo et al. 2014), pathogenic infections

Such phenotypic plasticity, regarded as the variable phenotypic expression of a single genome in response to environmental context, is common among rapidly changing environments (Colombo et al. 2014; Taborsky 2017). The expression of beneficial phenotypes (adaptive phenotypic plasticity) therefore renders an immediate coping mechanism for organisms under change (Ghalambor 2007; Chevin & Lande 2010; Guillame et al. 2016; Taborsky 2017). Hence, adaptive phenotypic plasticity can delay the potential deleterious effects of stress thereby reducing mortality, and thus negate adverse density-dependent effects that discourage population persistence in otherwise unfavourable environments (Triggs & Knell 2012). Over evolutionary time scales, phenotypic plasticity can buffer populations against change by shifting the mean phenotype (Gibbin et al. 2017; Taborsky 2017). However, phenotypic plasticity can be maladaptive due to mismatches in phenotypic change and the future environment (Chidawanyika & Terblanche 2011; Guillame et al. 2016). More recently, it has also been reported that a strong phenotypically plastic signal can limit the potential for evolutionary response through maladaptive phenotype-environment mismatches thus limiting population persistence in the long term (Oostra et al. 2018). Nevertheless, phenotypic plasticity remains an

important determinant of individual fitness and population persistence among organisms in transient environments.

From the aforementioned, it is not surprising that a few hypotheses have been brought forward to explain these phenotype-environment interactions. For example, the 'environmental matching hypothesis' (EMH) argues that organisms developing under resource constrained environments (quality and availability) develop plastic responses that favour optimal performance under corresponding conditions for future offspring (Bateson et al. 2014; Scharf et al. 2015; Zizzari et at. 2016). This is in contrast to the 'silver spoon hypothesis' (SSH) which posits that parents that developed on good quality resources (e.g. diets) confer fitness potential that enables offspring to perform well even under poor-resource environments (Monaghan 2008; Hopwood et al. 2014). Whilst several studies demonstrated considerable support for the silver spoon effect, empirical evidence supporting the EMH remains scant (Frago & Bauce 2014; Scharf 2015). Regardless of such dissensions, it is widely accepted that there is a strong relationship between early-life nutrition and both adult and offspring fitness traits (Monaghan 2008; Monaghan et al. 2012; Hopwood et al. 2014). It is therefore of great importance to understand the consequences of resource allocation (even under variable diet quality) on life-history of organisms (Boggs 2009), partly because of the acuity it gives to the field of gerontology (Kulminski et al. 2009).

For arthropods, several traits including egg size, pupal weight, disease susceptibility (Triggs & Knell 2012; Shikano et al. 2015; Wilson & Graham 2015; Xia & de Belle 2016; Littlefair et al. 2017), development times, fecundity, offspring fitness and viability, and adult body size and longevity (Valtonen et al. 2012; Aiken et al. 2016) have all been found to be responsive to within and transgenerational nutritional effects (Frost et al. 2010; Boggs & Niitepold 2016; Xia et al. 2016). Due to their ectothermic nature, body size is of importance as it mediates several ecophysiological relations including resistance to environmental stressors such as desiccation and extreme temperatures (Angilletta et al. 2004; Chown & Nicolson 2004; Harrison et al. 2012; Chown & Gaston 2010; Klockmann et al. 2017). Furthermore, body size directly affects insect population dynamics due to its influence on mate-choice, copulative rate and success, female fecundity and thus ultimately

reproductive success (Honek 1993; Amarillo-Suarez et al. 2011; Helinski & Harrington 2011; French et al. 2014). Hence, resource acquisition during ontogeny and epigenetic effects on offspring is key for enhancing fitness traits and persistence of insect populations (Lee et al. 2008; Frost et al. 2010; Sahu & Kumar 2010).

Although there has been relatively wide research on the influence of diet on transgenerational effects, most have used artificial diets (Valtonen et al. 2012; Xia & Belle 2016) on model species such as *Drosophila*, perhaps due to their relatively short lifespan which enables rigorus tests that fully replicate evolutionary time-scales (Emborski & Mikheyev 2018). However, the need for such transgenerational studies is increasingly becoming more apparent in several other insect taxa, given the importance of such understanding in the face of global change (Woestmann & Saastamoinen 2016). To my knowledge, transgenerational effects of diet quality on long living insect herbivores such as Crysomelid beetles remain underexplored compared to Lepidoptera (e.g. Niitepõld et al. 2014; Woestmann & Saastamoinen 2016). Yet this can be of importance for mass-reared Chrysomelid beetles that are maintained under standardised diets for generations prior to field release in order to control invasive plants. This dearth of knowledge as to how nutritional conditions during rearing may influence life-history traits and ultimately field performance of herbivorous weed biocontrol agents may significantly affect the success of Invasive Alien Plants (IAP) control. Here, I investigated transgenerational effects of foliar nitrogen variability in the invasive herbaceous *Tradescantia fluminensis* on the Tradescantia leaf beetle *Neolema abbreviata* (Larcordaire) (Coleoptera: Chrysomelidae). Specifically, I tested parental effects of host-plant quality on life-history and herbivore performance across F_1 and F_2 offspring. In the literature, terms such as ancestral (Emborski & Mikheyev 2018), maternal or paternal (Guillaume et al. 2016; Triggs & Knell 2012) and grandparental diet (Monaghan 2008) have all been used to refer to transgenerational effects transmitted to offspring under variable diet29.5339. Here, I use the term parental diet to refer to the diets experienced by parents from F_0 through to F_2 generations prior to experimental tests.

Using reciprocal experiments, I investigated if parental diets influenced larval feeding damage, weight gain, mortality, pupal weight, developmental time to pupae and adult eclosion success

among F_1 and F_2 generation offspring. I also tested if parental diet influenced oviposition preference, feeding, weight and longevity among adults of both generations. I hypothesised that mismatches in diet quality among parents and offspring would result in poor performance following the EMH.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at Agricultural Research Council-Plant Protection Research Institute at Cedara 29.5339º S, 30.2733º E, Pietermaritzburg, South Africa.

3.2.2 Experimental design

Plant culturing

Loamy soil was first autoclaved to prevent soil pathogenic infections among culturing plants. The soil was then mixed with vermiculite (1:1) to improve soil drainage, aeration and promote root activity. Under shade-netting in a greenhouse, this mixture was potted in plastic pots (25 cm high x 20 cm diameter) where ~ 15 cm *T. fluminensis* stem fragments were planted and allowed to root for 5 days under irrigation every two days for 30 days. Thereafter, the pots carrying these propagules were immediately randomly placed into three groups based on manipulated soil nitrogen treatments (low, medium, high). In these treatments, plants continued with the same irrigation schedule for another 25 days before use in experiments. During this period, plants in the low treatment received no fertiliser whilst those in the medium and high treatments received 2.4g fertiliser twice a week and 5g fertiliser thrice a week, respectively. This fertiliser was a straight-N Multicote® ammonium nitrate (NH_4NO_3) slow release fertiliser (Haifa Chemicals, South Africa).

Insect culturing

Neolema abbreviata culture was originally established using 300 beetles that were field-collected in New Zealand. Newly hatched larvae were collected and independently reared in mesh-covered steel cages (50 x 50 x 82 cm) on the three different plant treatments to establish three adult cohort populations (~90 each), regarded as the parental generation. Thereafter, adults were moved and placed in newly-set cages containing plants of respective treatments and allowed to reproduce the F_1

offspring (Fig. 3.1). Whilst parental individuals were discarded, eggs and larvae that formed the F_1

cohorts were collected and used in subsequent experiments or allowed to develop for use in adult

bioassays. Remaining F_1 adults were collected and also reared on their respective plant treatments to

produce the F_2 offspring that was also used in assays as either larvae or adults under ambient
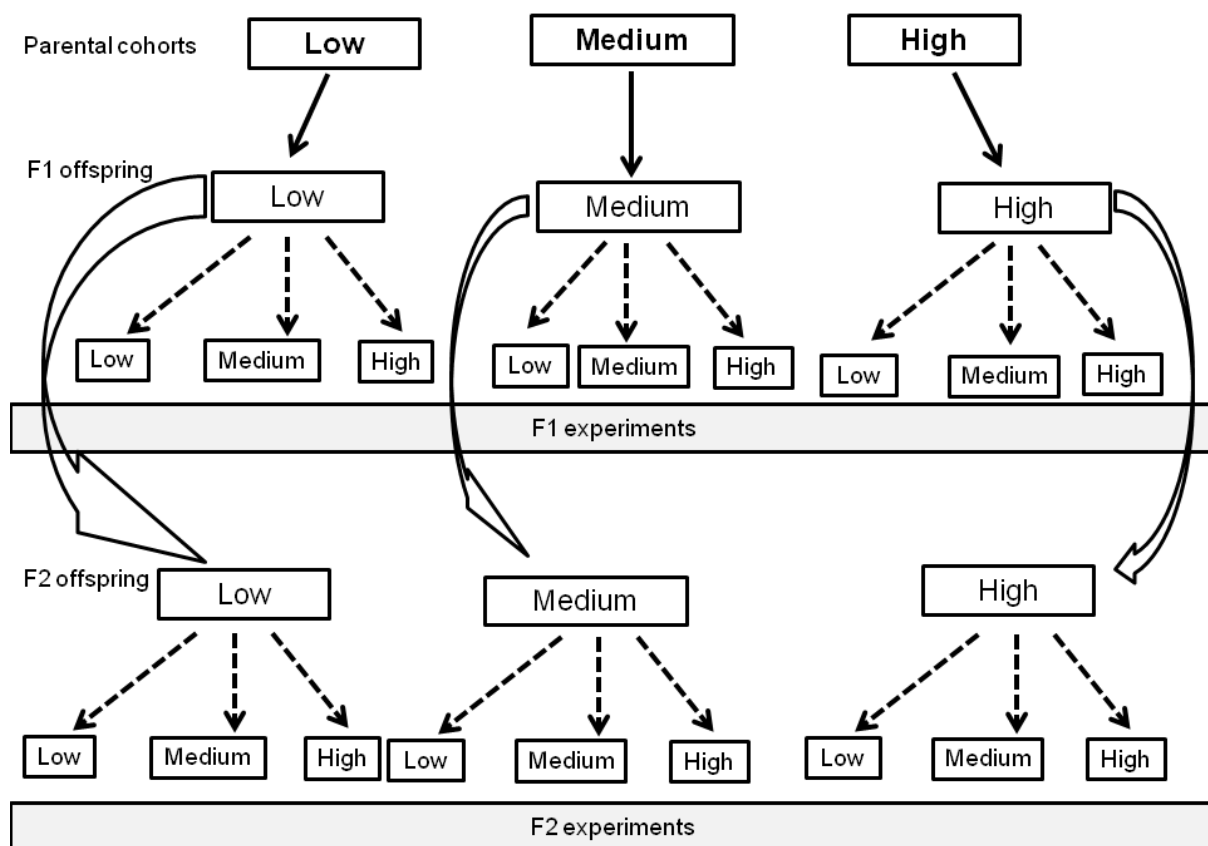
conditions (24 to 26.4 °C and 60 to 98 %RH).

Figure 3.1: Schematic diagram of the protocol that was used to prepare F_1 and F_2 cohorts of *N. abbreviata* under 3 different nutrient diets.

3.3.3 Determination of plant quality

Plant quality was assessed following (chapter 2). In brief, a Dumas quantitative combustion procedure using a Rapid N Cube Elementar (Elementar Analysensystemme GmbH, Germany) connected to a computer that analysed data using Rapid N software (version 5.0.7 [1831be8]) was used. Five replicate plants per treatment were cut and oven dried for 72 hours at 75°C (or to a constant weight) then finely milled through a 0.2 mm mesh size. Aliquots for each sample were prepared as 150mg pellets wrapped in tin foil and loaded onto the carousel of the auto-sampling platform on the analyser. The samples were then combusted at 960 °C and the consequential gases detected by a thermoconductivity detector that received gas mixtures via an electronic flow controller. Aspartic acid was used as the authentic standard for calibration and carbon dioxide as the carrier gas.

3.3.4 Experiment 1: effects of beetle parental diet on larval feeding, development rate, pupal weight and eclosion success in F1 and F2 offspring of *N. abbreviata*

To assess *N. abbreviata* parental effects on larval developmental rates F_1 larvae, collected from parental cages, and F_2 larvae collected from the F_1 parental line (for all treatments) were placed into Petri dishes containing filter paper moistened with 1 % sodium hypochlorite. A full-factorial design (Fig. 3.1) was then established by independently providing larvae of both generations access to excised plant leaves from the three plant treatments (low, medium, high fertiliser). Thereafter, all Petri dishes were covered with foil paper and placed in a Labcon growth chamber (model: FSIM-RH20; accuracy: 26± 2°C, 65 ± 4% RH, 12:12 hrs L:D; Labcon Laboratory Equipment, South Africa). Larvae were allowed to feed *ad libitum* and monitored daily to assess feeding, mortality and time taken to pupation. Both final instar larval and pupal weights were measured using a Sartorius

microbalance (model BP61, Sigma Aldrich, USA). Fresh leaf material was provided every 3 days or when feeding substrate was exhausted. Prior to placement in Petri dishes and upon retrieval after feeding damage, images of leaves were taken using a high resolution digital camera (PowerShot G3X, Canon, Japan) and later processed using ImageJ software (National Institute of Health, USA) to quantify the amount of leaf surface area that was fed upon. All treatments were replicated at least 9 times.

3.3.5 Experiment 2: Effect of beetle parental diet on oviposition selection adult feeding damage and longevity

F_1 and F_2 *N. abbreviata* larvae were allowed to develop until the adult stage on diets identical to parental treatments. Newly eclosed adults were then subjected to a factorial treatment similar to experiment 1 (Fig. 3.1). To evaluate parental effects on oviposition selection, 20 adults from the different treatments were separately tested in a walk-in cage with nine fresh intact plants. These plants comprised three plants from each treatment (low, medium, high N) randomly placed in the cage but ensuring that no plants were contact with each other. The beetles were then allowed to mate and oviposit on the preferred plants for duration of 8 days before egg counts were done. Feeding damage was also assessed under no-choice conditions using excised leaves in Petri dishes (5 adults per treatment/dish) placed in a growth chamber with similar conditions as in the larval experiments. Adults were allowed to feed for 5 days and feeding damage was quantified using ImageJ software. All treatments were replicated 5 times. For adult longevity, freshly eclosed F_1 and F_2 adults were exposed to the same factorial treatments to test parental effects on longevity under variable plant quality. Individual adults, replicated 7 times, were placed in Petri dishes, stored in a growth chamber, and allowed to feed. Plant material and filter paper were changed every 3 days whilst simultaneously checking for mortality. Both males and females were used in these assays as preliminary studies I conducted prior to experiments had shown no sex differences in longevity among virgin adults.

3.3.6 Data Analyses

As multiple individuals from different cohorts and generations were assayed, linear mixed effects models (lmer) assuming Gaussian distributions of residual error were used. The restricted maximum likelihood (REML) framework was followed for model comparisons. Fully complete models comprising of all possible fixed or explanatory variables and random effects were first tested before removing non-significant interactions and random effects (e.g. Quinn & Keough 2002). Parental diet, test diet and the generation were all initially included as categorical or explanatory factors together with their interactions and as random effects. The goodness-of-fit of all the models was then assessed using maximum likelihood for model fits (Zuur et al. 2009). In all cases after removing non-significant terms, models that comprised of 'Parental diet' and 'Test diet' as fixed factors and 'Generation' as the random factor had the most exploratory power and hence were applied for all analyses. An ANOVA was then run for the final model. Weight data for larvae, pupae and adults were first square-root transformed before analyses. In the case of larval weights, final weights were used and those that had died were excluded from the analyses. Data for oviposition choice tests were analysed using generalised linear mixed effects models (glmer) as we assumed a Poisson, and not Gaussian, distribution of residual errors with a log-link function. Similarly, glmer were used to assess the impact of parental and test diets together with their generation on larval mortality assuming a binomial distribution. All response variables were tested for normality and heteroscedastity before analysis and transformations were performed when necessary. Type I errors were minimised by adjusting the false discovery rate using the Benjamini-Hochberg procedure (Benjamini & Hochberg 1995). Linear correlations were determined to identify the impact of pupal weight on eclosion success, days to pupation and pupal weight, and adult weight and longevity. All data analyses were performed in R version 3.4.0 (R Development Core, 2017).

3.3 Results

3.3.1 Plant quality

Fertiliser levels significantly affected foliar N content ($F_{2, 14} = 18.8$, $P < 0.001$). As expected, the highest N content was in the plants from high ($12.1 \pm 1.4\%$) followed by the medium ($7.2 \pm 1.4\%$) and low ($4 \pm 1.4\%$) treatments (Fig. 3.2).
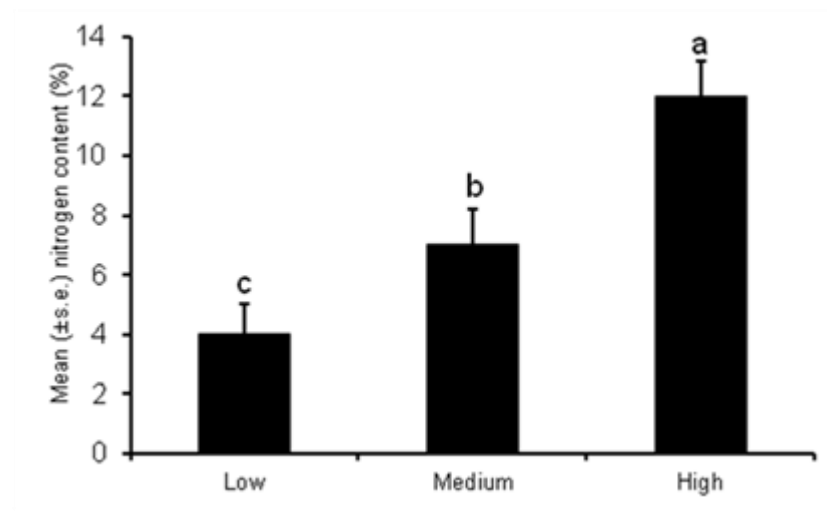

Figure 3.2: Impact of fertiliser variability on plant N content (%). Letters above error bars denote statistically different groups.

3.4.2 Larval performance

The main effects of parental diet did not influence, larval weight gain, mortality, feeding damage, pupal weight, number of days to pupation and eclosion success (refer to Table 3.1). Test diet

however significantly affected all these traits. Larval mortality and weight gain was responsive to the main effects of generation. Parental diet x test diet interactions significantly influenced larval feeding, mortality and weight, pupal weight, days to pupation and eclosion success. Larvae from high N parental diets performed better than those from low parental diet in both generations under the poor-quality test diet (Fig. 3.4). In pupal weights, there was significant statistical test of parental diet x generation interactions P=0.04. Larval feeding damage was generally higher (7.4 ± 1.9 cm^2) on the low N test plants regardless of generation and parental diet (Fig. 3.3). Larval weight gain was higher on the medium and high N test plants. Furthermore, F_2 generation larvae from medium and high parental diets gained more weight (0.03 ± 0.006mg) than larvae from low N parental diet treatments (0.02 ± 0.006mg) under the low N test diet suggesting some significant influence (P= of parental diet on offspring performance (Fig. 3.4). There was generally higher proportion of mortality on high N test plants (0.4 ± 0.1) regardless of parental diet and generation (Fig 3.4.). Despite lower mortality on low N plants, there was generally higher number of days taken to pupation regardless of parental diet and generation (Fig. 3.4). When considering only individuals that pupated, adult eclosion was generally higher on medium and high N test plants in comparison to the low N test plants, regardless of parental diet and generation (Fig. 3.4). After pooling data from all treatments, linear correlations revealed a negative relationship between pupal weight and number of days to pupation ($r(71) = 0.42$, $P = 0.01$). Conversely, there was a positive relationship between pupal weight and eclosion success ($r(69) = 0.56$, $P < 0.001$) (Fig. 3.5).

Table 3.1: Summary results on effects parental diet, test diet and generation on larval performance in

N. abbreviata with bold significant P-values

Source of Variation	d.f.	F	P-value
Larval feeding damage			
Parental diet	2	1.4	0.218
Test diet	2	6.5	**<0.001**
Generation	1	0.42	0.146
Parental diet x Test diet	4	3.27	**0.012**
Parental diet x Generation	2	0.23	0.711
Test diet x Generation	2	1.3	0.071
Larval weight gain			
Parental diet	2	4.47	**0.012**
Test diet	2	8.63	**<0.001**
Generation	1	0.79	0.621
Parental diet x Test diet	4	6.52	**0.001**
Parental diet x Generation	2	1.55	0.325
Test diet x Generation	2	0.43	0.721
Larval mortality			
Parental diet	2	6.85	**<0.001**
Test diet	2	1.42	0.214
Generation	1	4.78	**0.029**
Parental diet x Test diet	4	0.84	0.525
Parental diet x Generation	2	1.59	0.191
Test diet x Generation	2	1.23	0.293
Pupal weight			
Parental diet	2	1.52	0.22
Test diet	2	2.53	**0.039**
Generation	1	0.91	0.34
Parental diet x Test diet	4	2.33	**0.04**
Parental diet x Generation	2	4.07	**0.02**
Test diet x Generation	2	0.64	0.59
Days to pupation			
Parental diet	2	1.19	0.323
Test diet	2	10.52	**<0.001**
Generation	1	9.32	**0.003**
Parental diet x Test diet	4	2.34	**0.03**
Parental diet x Generation	2	8.6	**<0.001**
Test diet x Generation	2	5.62	**0.002**
Eclosion success			
Parental diet	2	1.49	0.227
Test diet	2	3.41	**0.009**
Generation	1	5.93	**0.018**
Parental diet x Test diet	4	1.86	0.116
Parental diet x Generation	2	0.49	0.618
Test diet x Generation	2	2.4	0.125
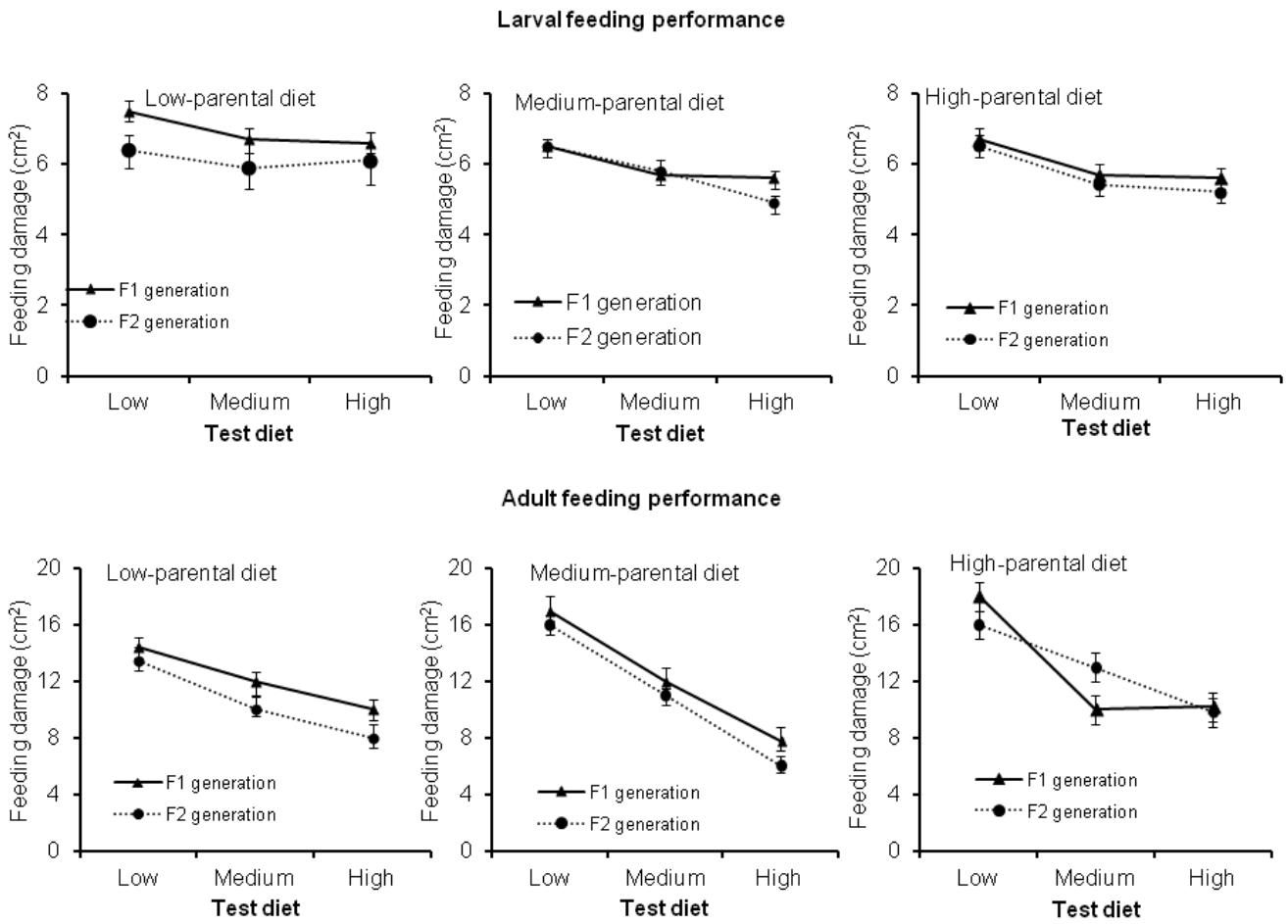
Figure 3.3: Effects of *N. abbreviata* parental diet in F_1 and F_2 generation on larval and adult feeding performance on test plants under three different N content (low, medium, high). Vertical bars indicate 95% confidence limits.
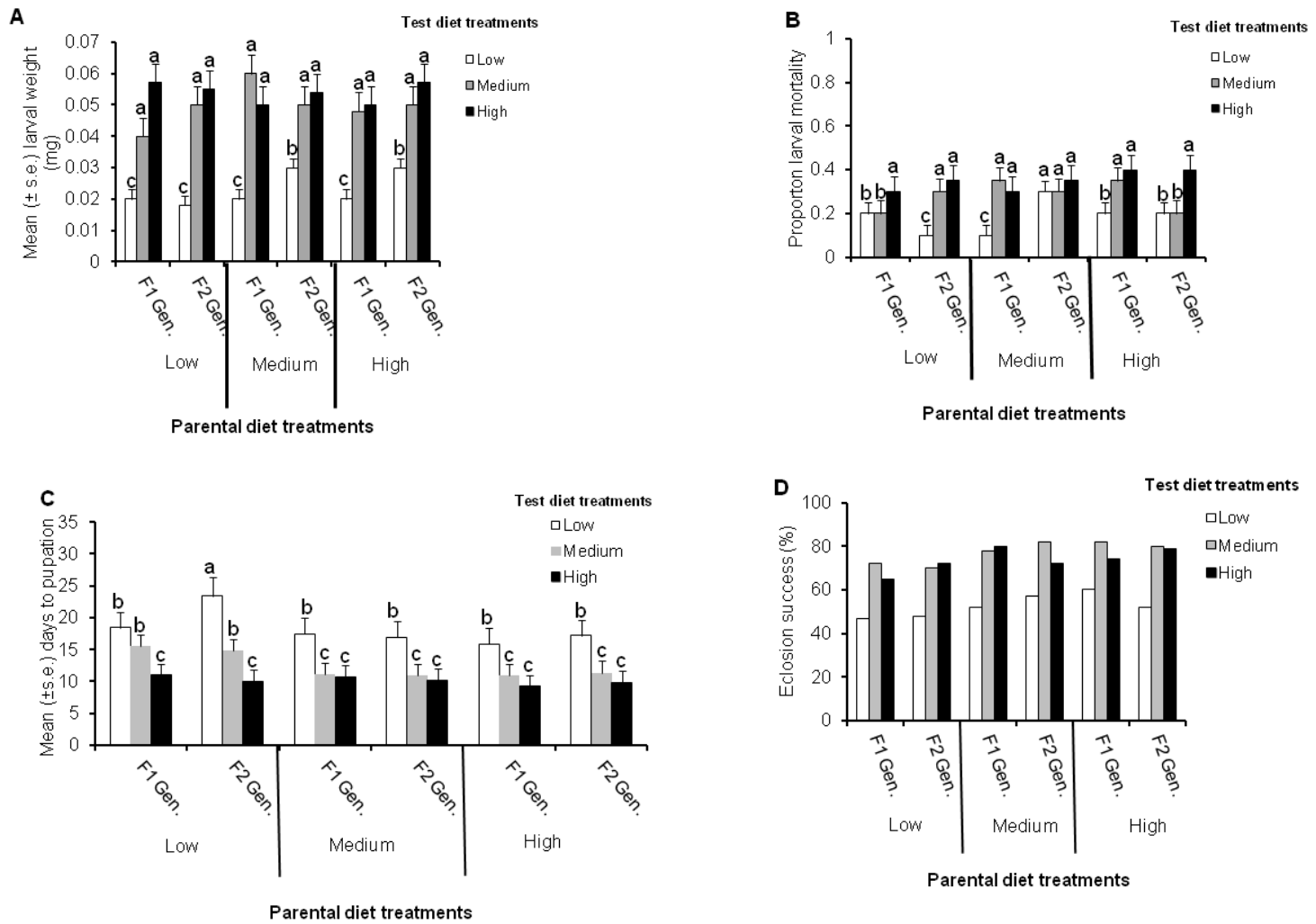
Figure 3.4: Impact of *N. abbreviata* parental diet and test plant quality on number of days to pupation A),proportion of larval mortality B), mean days to pupation C) and eclosion success D) in F_1 and F_2 generations of *N. abbreviata.*
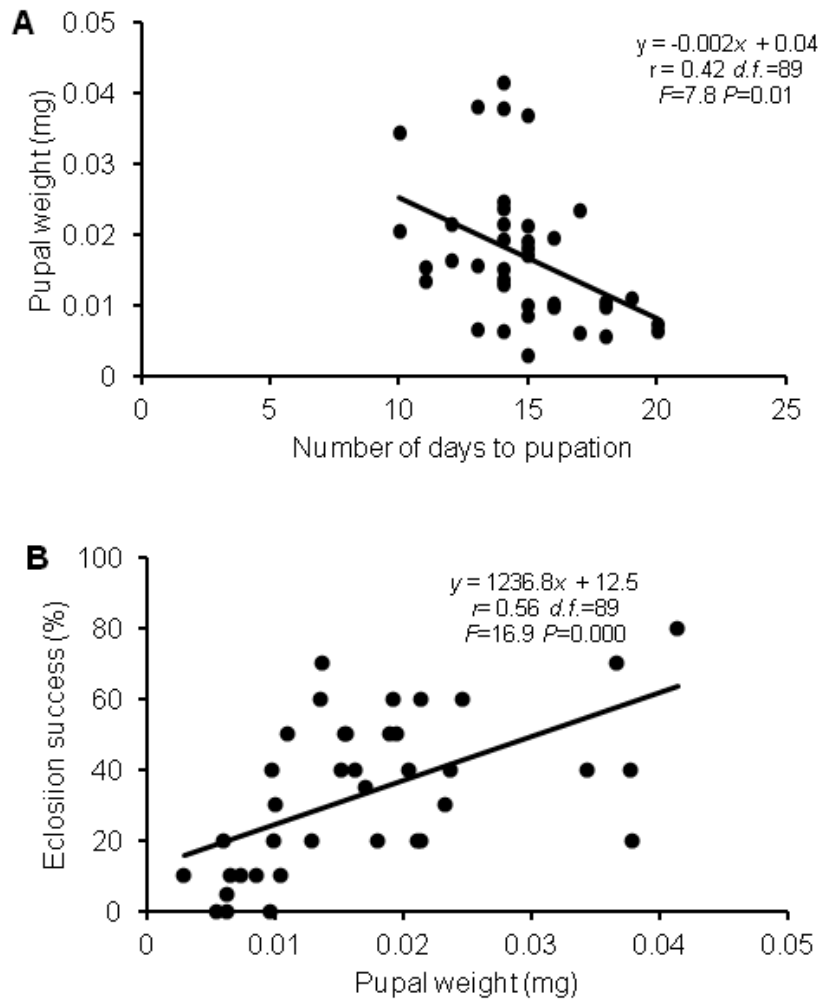
Figure 3.5: Relationships between number of days to pupation and pupal weight A) and pupal weight and eclosion success B) in F_1 and F_2 generation of *N. abbreviata*. Data for both generations and different parental and test diets were pooled together before computing a Pearson`s product-moment correlation coefficient across treatments.

3.4.3 Adult performance

Oviposition preference was only responsive to test diet with higher oviposition generally occurring in medium and high N test plants (Table 3.2, Fig. 3.6). As in larval feeding performance, adult feeding was higher by almost two-fold in low N plants compared to medium and high N plants, regardless of parental diet and generation (Table 3.2, Fig. 3.3). Only test diet and also its interaction with generation significantly influenced adult weight (Table 2). Feeding on medium and high nitrogen test plants resulted in higher adult weight (0.05 ± 0.2mg) compared to low N test plants (0.01 ± 0.02mg) among adults from all the parental diets and both generations (Fig. 3.7). Adult longevity was also higher in medium and high N test plants across all parental diets and generations. However, F_2 adults from low N parental diets had even shorter longevity under low test diet suggesting significant influence of parental diet and generation (Fig. 3.7). There was however no correlation between adult weight and longevity ($r(63) = 0.15$, $P = 0.082$).

Table 3.2: Summary results on effects of parental diet, test diet and generation on adult performance

of *N. abbreviata*

Source of Variation	d.f.	F	P-value
Oviposition preference			
Parental diet	2	1.98	0.144
Test diet	2	7.86	**<0.001**
Generation	1	1.26	0.322
Parental diet x Test diet	4	1.33	0.531
Parental diet x Generation	2	0.64	0.652
Test diet x Generation	2	0.78	0.547
Adult feeding			
Parental diet	2	1.29	0.238
Test diet	2	11.2	**<0.001**
Generation	1	0.42	0.146
Parental diet x Test diet	4	0.76	0.621
Parental diet x Generation	2	0.8	0.231
Test diet x Generation	2	0.2	0.641
Adult weight			
Parental diet	2	1.001	0.401
Test diet	2	5.58	**<0.001**
Generation	1	0.137	0.713
Parental diet x Test diet	4	2.1	0.326
Parental diet x Generation	2	0.42	0.658
Test diet x Generation	2	0.91	0.486
Adult longevity			
Parental diet	2	1.74	0.166
Test diet	2	15.48	**<0.001**
Generation	1	0.35	0.556
Parental diet x Test diet	4	0.84	0.525
Parental diet x Generation	2	6.78	**0.002**
Test diet x Generation	2	1.12	0.345

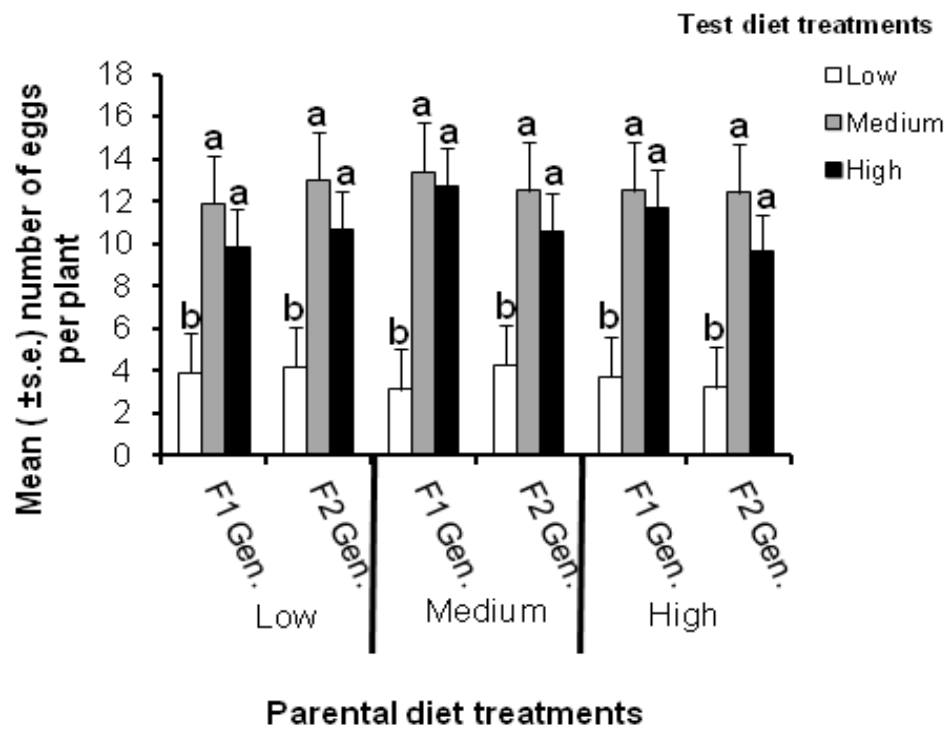**Figure 3.6:** Impact of parental diet and test plant quality on oviposition preference in F_1 and F_2 generations of *N. abbreviata.*
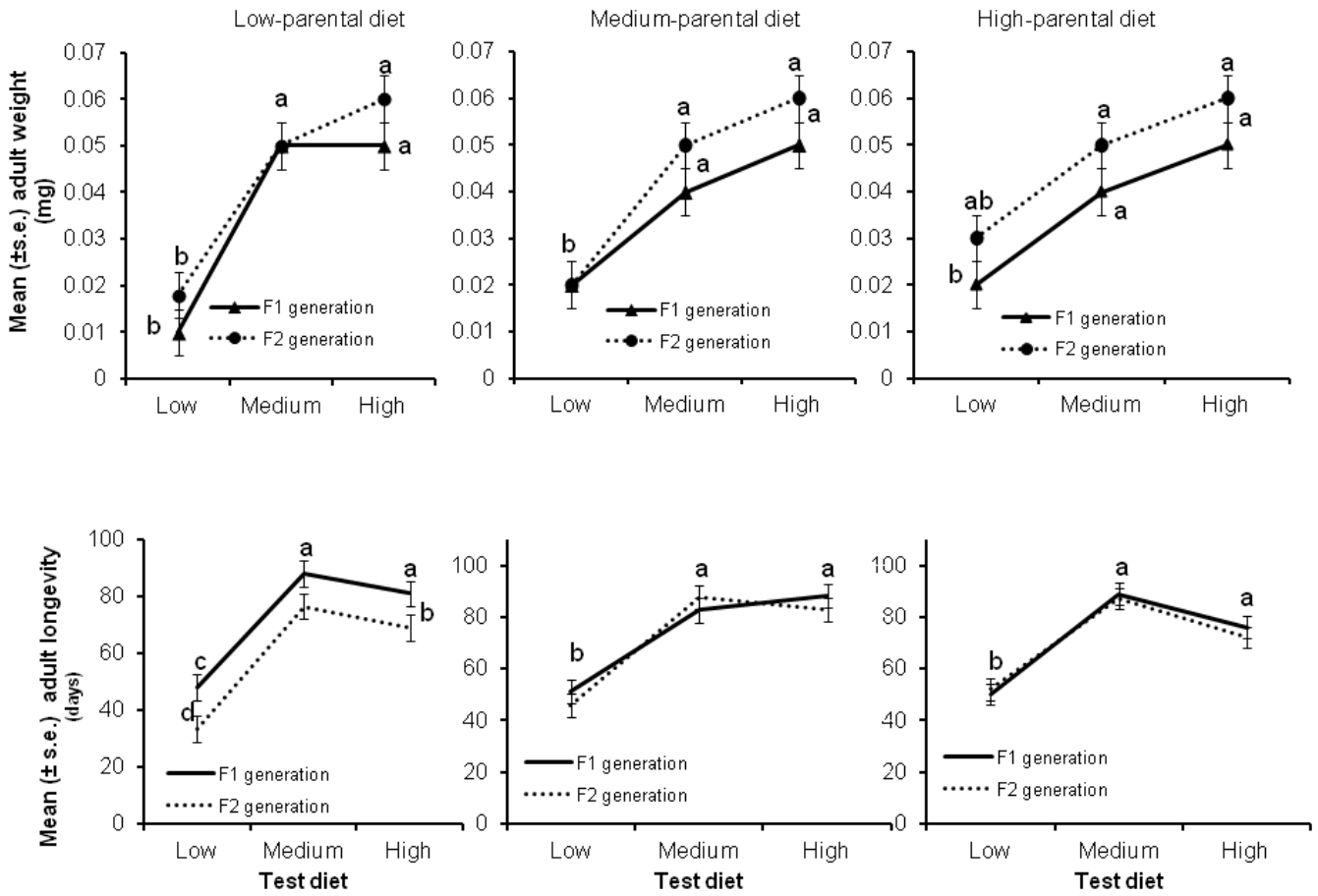
Figure 3.7: Impact of *N. abbreviata* parental diet on adult weight and longevity in F_1 and F_2 offspring under three different test diets comprising of low, medium and high foliar N content. Letters above error bars indicate significant differences.

3.5 Discussion

Host-plant quality is a key fitness determinant among phytophagous insects with potential to influence life-history traits across generations. My results showed limited capacity for transgenerational plasticity in response to host plant quality among several life-history traits. Feeding among both F_1 and F_2 larvae was generally more responsive to test diet than parental diet. On poor quality (low N) plants, feeding was high even though the larvae ended up with lowest larval and pupal weights. Larval mortality was however marginally higher in the medium and high N treatments. Several studies have previously reported on the impacts of N variability on larval performance in phytophagous insects, albeit mostly within single generations where growth both positively and negatively responded to N elevation (e.g. Fischer & Fiddler 2000; Chen et al. 2008; Baurfiend & Fischer 2013; Hwang et al. 2008; Safraz et al. 2009; Tao et al. 2014; Bownes 2018). In cotton plants *Gossypium hirsutum* L. (Malvacea), Chen et al. (2008) reported preferential feeding on high N plants compared to low N plants by *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) larvae leading to rapid ontogenic development. In the present study, all larval feeding assays were under no-choice conditions so I could not discern feeding preferences. Instead, the conservative conditions of no-choice experiments give insights into how feeding performance may be affected by soil nutrient conditions under fragmented habitats typical of where isolated populations of *T. fluminensis* grow in South Africa.

Bownes (2018) reported that there was no influence of N concentrations on larval mortality in the ephydrid fly *Hydrellia purcelli* Deeming (Diptera: Ephydridae). This was in contrast to my present study where mortality appeared to be influenced by N concentrations in test plants. It is not clear why higher mortality may have been present in a presumably nutrient rich diet. However, in another study, Tao et al. (2014) reported that feeding on high N plants resulted in increased mortality in the monarch caterpillar *Danaus plexippus* (Lepidoptera: Nimphalidae) due to increased production of defensive cardenolide compounds in the milkweed *Asclepias curassavica*. It is therefore plausible that *T. fluminensis* may have enhanced defensive capacity including growth of trichomes, such as surface hairs, which may have acted against *N. abbreviata* larvae under high N conditions. Nevertheless,

other larval traits in this study responded positively to increased N resulting in faster developmental rates and increased pupal weights leading to higher eclosion rates as reported in other studies (Huberty & Denno 2006; Bownes 2018). This therefore suggests that plant quality influences the growth trajectory in *N. abbreviata* and resource acquisition during ontogeny is vital as it directly influences adult eclosion and thus population persistence. In all likelihood, such superior eclosion rates may offset the increased mortality among natural populations in the field.

For many oviparous organisms such as holometabolous insects, the quality of oviposition substrate is of importance as it can confer fitness advantages to the offspring (Chidawanyika et al. 2014; Petit et al. 2015). In the case of phytophagous insects, host plant quality mediates offspring performance through both nutritive and anti-nutritive (defense) mechanisms (Mody et al. 2009; Olson et al. 2009, Franzke & Reinhold 2011; Gutbrodt et al. 2012). In the present study, oviposition selection was highest in high N plants despite initial high incidences of larval mortality. This oviposition selection was generally in sync with offspring performance in the case of rapid development to pupation and superior adult eclosion rates. It has long been appreciated that the larval stage is one of the most vulnerable life-stages among holometabolous insects as they are more prone to mortality factors such as predation, desiccation and thermal stress due to limited locomotory capacity (Schoonhoven et al. 2005; Harrison et al. 2012). Hence apart from production of high numbers of eggs to maximise reproductive success, rapid development to pupae is often considered as an adaptive evolutionary response to such potentially deleterious factors.

Apart from efficiency in growth trajectories, it has been argued that timing of adult emergence can maximise reproductive success through synchronisation with availability of favourable resources (Abrams 1996; Gotthard 2008). In this study, I did not explicitly test if there were any differences in pupal weights and time of adult eclosion. However, I did not find any correlation between adult weight and longevity. Adult weight has previously been linked with lifetime fecundity among holometabolous insects with larger adults generally laying more eggs, thus having higher reproductive potential (Gotthard 2008). I did not test how adult weight influenced lifetime fecundity due to logistical constraints. There was no significant relationship between adult weight and

longevity, however, the beetles from high N plants in this study tended to have higher longevity, a trait which can be of importance in promoting population persistence in the field. It is possible that there were other intrinsic changes in the body that could have promoted longevity as previous studies have reported the influence of changes in body composition on adult longevity (Emborski & Mikheyev 2018).

According to the 'Environmental matching hypothesis' (EMH) offspring perform best in environments similar to their parents. In this study, I did not explicitly find enough evidence to support or refute this among various larval and adult traits. Test plant quality appeared more pivotal in mediating performance in both F_1 and F_2 larvae and adults. For example, larval weights were lower or higher on low and high N test plants, respectively, regardless of parental diet history. This was also the case with adult eclosion success. Similarly, adult feeding was higher on low N plants, perhaps as a compensatory mechanism to acquire enough resources for somatic maintenance and investment in costly processes such as reproduction (Geister et al. 2008; Nestel et al. 2016). Nevertheless, this was also regardless of generation or parental diet. However, I found differences in longevity between F_1 and F_2 adults for individuals from low parental diets with the F_2 generation generally having a shorter lifespan. This indicated a strong parental diet x generation interaction where prolonged exposure to a high N diet resulted in longevity fitness costs for offspring under poor diet. Similarly, I found a significant parental diet x generation interaction in pupal weights where a low N parental diet elicited similar patterns of fitness costs among F_2 offspring under low test diet. These results are in line with studies by Smith et al. (2007) who demonstrated better offspring performance for individuals whose parents were reared in low N diet. Since individuals coming from low N diets had even lower longevity, this trait could not outrightly support the EMH.

There were significant parental diet x test diet interactions in larval feeding, mortality and weight, pupal weight, days to pupation and eclosion success. Larvae from high N parental diets tended to perform better than those from low N parental diets in both generations even under a low N test diet. However, larval mortality was apparently higher in all cases for offspring from high N diets. Nevertheless, these traits suggest a strong parental effect x test diet on offspring fitness. For adults,

oviposition selection, feeding, weight and longevity were all not influenced by parental diet x test diet interactions. From the aforementioned, my results indicate a significant impact of transgenerational effects on larvae but not adults. It is not clear why this was the case but I suspect sex differences could have confounded my results during adult life-stages. Previous studies have reported sex-dependent transmission of transgenerational responses with maternal and paternal environments differentially contributing to offspring phenotype (Bonduriansky & Head 2007; Lock 2012; Triggs & Knell 2012; Zizzari & Ellers 2014, Guillaume et al. 2016). Nevertheless, it is still highly unlikely that this alone could have influenced the differences between larval and adult performance in this study as all individuals used in my assays came from predetermined cohort populations without any parental treatment crosses. Instead, post-eclosion sex-dependent responses could have been influential as previous studies have reported sex differences in some of the traits I examined in this study, including adult feeding, weight and longevity (Fox et al. 2003; Chen et al. 2005; Knap 2016). In the case of longevity however, preliminary studies I conducted had shown no sex differences among virgin males and females of *N. abbreviata*.

Another bottleneck in this study was the failure to incorporate a split-brood design with full-sib when forming the parental cohort populations. This meant that I could not account for any potential population genetic diversity among traits of interest, which can influence fitness in variable environments. Nevertheless, this study shows differential fitness responses to parental diet among larvae and adults under variable environments. Overall, there was substantial support for the SSH compared to the EMH since offspring coming from high N diets did well on poor quality test plants. However, I make this conclusion with a caveat since I only managed to assess F_1 and F_2 generations which may have limited the scope of my inferences. Another study investigating *Drosophila melanogaster* (Diptera: Drosophilidae) reported transgenerational effects of diet after assessing up to five generations (Emborski & Mikheyev 2018). Indeed, the *Drosophila* species have been a strong model organism for studying transgenerational effects owing to their short life cycles which enable robust experimental designs that replicate evolutionary time scales. It is therefore possible that the limited transgenerational effects in some of the traits in this study may have not been due to their

absence but failure to assess longer evolutionary time-scales due to the relatively longer life cycle of the *N. abbreviata* beetles that I assessed. It has previously been reported that delayed transgenerational effects where phenotypic changes may not become apparent immediately can manifest in later generations (Remy 2010; Rechavi et al. 2014). It has however been argued that what is generally referred to as transgenerational effects in literature is where stable traits are conferred to offspring without any delays or diminishing effects, despite the dearth of studies showing this phenomenon (Rechavi et al. 2014; Ashe et al. 2012). Instead, delayed and diminishing transgenerational responses have been widely reported (Franklin et al. 2010; Walsh et al. 2015; Nystrand et al. 2016; Xia & Belle 2016; Emborski & Mikheyev 2018). It is not clear, if such plasticity is more prevalent in nature as it may lead to poor phenotypic adjustment under short-term environmental variability leading to compromised fitness. On the other hand, delayed or diminishing responses may also confer fitness advantages by avoiding induction of costly phenotypic changes under non-lethal ephemeral circumstances that may not require sustained phenotypic changes.

The current study has a few implications for biological control using *N. abbreviata* under field conditions. Developmental rates were higher on high N plants despite higher mortality rates which appeared to be offset by superior performance through higher eclosion rates. This suggests that high plant quality is key in order to rapidly produce numbers required for field release. Feeding rates were higher on low N plants despite lower developmental rates. This suggests that more effort in terms of rearing plants and time required to have numbers sufficient for field release may thus be required if plant quality under rearing conditions is not maintained. However in the field, low N plants may be effectively damaged by fewer beetles due to higher feeding rates although extrinsic factors such as predation may come into play. This is especially important where damage intensity may efficiently signal for higher parasitism (e.g. Girling et al. 2011).

In conclusion, the study demonstrates variable parental effects among juvenile and adult offspring. Future studies should endeavour to account for body composition and potential population genetic diversity through inclusion of split-brood designs to conclusively decipher the impact of parental diet on the offspring life-stages.

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Chapter 4: General Discussion and Conclusions

Environmental changes at various temporal scales present both direct and indirect challenges that arthropods have to endure through a range of plastic responses including behavioural and physiological adaptions in order to avoid population collapse and extinction. With the ongoing anthropogenic global change, there has been increasing call for studies incorporating multiple environmental factors simulating climate change to assess potential impacts on various levels of biological organisation (e.g. Holopainen & Gershenzon 2010; Zandalinas et al. 2017; Rizwan 2018). In my study, I manipulated both water and nitrogen (N) availability with dramatic changes in vegetative productivity and nutritional quality in the invasive plant *Tradescantia fluminensis*. Elevation of soil water and N levels resulted in increased biomass whilst limited soil water and N resulted in poor productivity through a decrease in both parent vine length and number of branches. In this regard, my study corroborated an earlier study by Burns (2004) where poor soil water and N resulted in poor vegetative performance in several plants of the Commelinaceae family. However under severe water stress, high levels of N did not result in increased biomass accumulation indicating the importance of water in N assimilation among plants as previously reported by Sardans & Peńulas (2012). Indeed, SWS led to a decline in SPAD values, a proxy for leaf chlorophyll content. This may further explain the loss in plant productivity under water stress in my study (Chapter 2).

The changes in host plant quality led to several changes in feeding performance and life history traits of larval and adult life-stages within and across generations of *N. abbreviata*. Within a single generation (F_0) elevated N levels shortened developmental times and increased weight gain but with a relatively lower feeding rate. Larval weight gain and number of days to pupation positively correlated with foliar Nitrogen content (Chapter 2). Pupation and adult eclosion was lowest in the low N treatments supporting the Nitrogen Limitation Hypothesis in the development of *N. abbreviata* in keeping with several previous studies (Chen et al. 2004; Hwang et al. 2008; Han et al. 2014; Helmberger et al. 2016). Across generations, I did not find support for the environmental matching hypothesis as both F_1 and F_2 larvae were more responsive to test diet than parental diet in several traits (Chapter 3). Feeding rate was higher on low N plants regardless of parental diet even though the

larvae ended up with lower larval and pupal weights as was the case with the F_0 generation. Similarly, larval mortality was also marginally higher on the medium and high N test plants as was the case with F_0 generation. However, of those larvae that developed to pupae, adult eclosion was higher on the N rich plants. This was surprising as I expected to get higher eclosion success where larval mortality was low. In addition, adult longevity was also higher on the N rich plants in contrast to larval survival. Gravid females seeking oviposition substrates were also able to track changes in host plant quality induced by the changes in water or nitrogen variability presumably to maximise offspring fitness potential and reproductive success (Renwick & Chew 1994; Awmack & Leather 2002; Price 2003; Chidawanyika et al. 2014; Adar & Dor 2018). Overall, my study highlights the complexities surrounding life-history responses of arthropods to environmental variability.

The implications of my study to biological control are several-fold:

1) *Neolema abbreviata* preferentially selected plants for oviposition based on quality. Such plasticity may enhance population persistence under field conditions by carefully depositing eggs where offspring fitness is high.

2) Since host plant quality influenced larval development, pupation and eclosion success, and adult longevity, care should be taken regarding the quality of plants used for mass rearing to optimise production.

3) Feeding damage intensity was highest on low N plants as opposed to high N plants. This therefore suggests that under field conditions, plant quality may influence efficacy of the control method. Apart from less feeding damage, high N plants had higher biomass and so in all likelihood, more beetles will be required to control the plants in high N habitats. However, population growth may also be higher on the high N plants since several traits associated with reproductive success positively responded to high N.

My study had several bottlenecks that I could not address due to logistical constraints. For example, I could not incorporate temperature, a known important driver for changes in biological systems under changing climates. In addition, I only managed to assess up to 2 generations when evaluating potential

transgenerational effects. This may have limited the scope of inferences I could make from my study as some organisms have been reported to exhibit delayed responses to parental diet. Future studies should therefore endeavour to include temperature, an important factor determining the productivity and quality of plants, and fitness of ectotherms. A few more generations can also be included to definitively assess the potential transgenerational effects. Nevertheless, the study still gives evidence of how host plant quality influenced by environmental variability may affect insect population dynamics.

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