The biology, behaviour and survival of pupating false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), a citrus pest in South Africa

A thesis submitted in fulfilment of
the requirements for the degree of

MASTER OF SCIENCE

of

RHODES UNIVERSITY

by

Claire Natalie Love

February 2015

ABSTRACT

Control of the citrus pest, false codling moth (FCM), *Thaumatotibia leucotreta* Meyrick (Lepidoptera: Tortricidae) is crucial for the South African citrus industry. The economic losses and phytosanitary status of this pest, coupled with increased consumer awareness and demands, has created a need for effective, IPM-compatible control measures for use against the soil-dwelling life stages of FCM. Promising developments in the field of microbial control through the use of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) have highlighted the need for research regarding pupation biology, behaviour and survival of FCM, as a good understanding of biology of the target organism is an important component of any biological control programme. The aim of this study was to improve the current understanding of FCM pupation habits through the manipulation of soil texture class, ground cover, shading, soil compaction, air temperature, and soil moisture in the laboratory. These findings would then be used to aid the biological control programmes using EPF and EPNs against FCM in the soil. Three soil texture classes (sandy loam, silt loam and silty clay loam) were obtained from orchards for use in the study. FCM larvae were allowed to drop into the soil of their own accord and the pupation behaviour that followed was then captured on film with pupae formed in the soil being kept in order to measure adult eclosion. In general, very few abiotic factors had a clear influence on FCM pupation. Larval wandering time and distance was short, but also variable between individuals. Distance did increase when soils were moist. Pupation depth was shallow, with pupal cocoons generally being formed on the soil surface. Depth of pupation was less than one centimetre for all abiotic conditions, with little burrowing into soil. Eclosion success was higher for sandier soils when these were dry and uncompacted, but the addition of both moisture and soil compaction increased FCM eclosion success. FCM was sensitive to desiccation when the soils were dry and temperature limits of 15 °C and 32 °C had a strongly negative impact on eclosion success. Preferences for particular abiotic conditions were limited to only certain moisture conditions when interacting with soil texture class and a preference for pupating in soil when it is available. Limited preference was found for particular soil textures despite this having a strong influence on eclosion success, but individuals did appear to pupate in close proximity to one another. Viable direct habitat manipulation for FCM control could not be identified. These results and all of the abiotic variables measured have important implications for EPF and EPN application, survival and persistence in the soil in order to improve the ability of these biological control agents to control FCM. These are discussed in each chapter.

Acknowledgements

This research would not have been possible without the support of a number of people and institutions. To everyone who has assisted with this work, both directly and indirectly, thank you so very much.

I could not have asked for more supportive and helpful supervisors than Martin Hill and Sean Moore. Your time, advice, insights and knowledge have been such a benefit to me and this body of work and I am extremely grateful to you both. My heartfelt thanks to both Martin Hill and Sean Moore for your assistance with regards to securing funding and for giving me exposure to the citrus industry, having your support with both of these has truly made all the difference.

Thank you to the citrus growers in the Sundays River Valley and Kat River Valley who were so willing to assist and allow for soil collection from their orchards. Your assistance with this made this work possible.

Field assistance was very kindly provided by the CRI staff, including Wayne Kirkman and Zongezile Zondi, as well as Kennedy Zimba, Candice Coombes, Veronique Chartier-Fitzgerald, Tamryn Marsberg, Mathew Goddard and Danielle Wiblin from Rhodes University.

FCM was kindly supplied by Craig Chambers and the staff of River Bioscience, as well as Mellissa Peyper from Rhodes University.

Laboratory equipment was always available for use thanks to the efforts of Tanya Pretorius, Mellissa Peyper and Candice Marshall. I am especially grateful to Terry Butterworth for advising and assisting in this capacity. The support staff at Rhodes University Zoology and Entomology Department is gratefully acknowledged, without whom we would not be able to do our research.

I am very grateful to the Rhodes University Geography Department, Gillian McGregor for soils advice and loaning me relevant materials, as well as Glynn Armstrong and Abe Ngoepe for assistance and equipment use.

A big thank you must be extended towards Tim Vink and Ryan Wasserman for their time and efforts with the VirtualDubMod and the ImageJ/Fiji software. Your willingness to help made this a much simpler task.

Statistical assistance was very kindly provided by Julie Coetzee, Philip Weyl and Candice Coombes during various phases of this work.

I am so very grateful to the other students who joined me on this postgraduate journey: Diane Smith, Megan Murison, Shana Mian, Dan Danckwerts, Megan Riddin, Jaryd Ridgeway, Izak Pretorious, Dan van der Vyver, Samantha Page, as well as Candice Coombes, Tamryn Marsberg, Tara O'Neill, the Rhodes postgraduate students and the other half of the Claire duo, Claire Faddel. The friendship, support, encouragement, academic discussions and comic relief when needed provided by you all has seen me through both good and challenging times. A special word of thanks to Candice Coombes for being so willing to help with all aspects of this work, from providing expertise to proofreading, your help has been so greatly appreciated.

Funding is a vital component of any postgraduate research and this work would not have been possible without the financial support of the National Research Foundation (NRF), the Ernst & Ethel Erikson Trust, Citrus Research International (CRI) and Rhodes University. The support provided by the postgraduate and undergraduate funding office staff members has allowed me to be in a position to pursue my postgraduate studies and for that I am very grateful. I would also like to acknowledge Jeanne van der Merwe for handling the financial matters over the duration of this work.

As I move onto the next phase this journey, I must thank all the staff of CRI and River Bioscience, as well as the students at CRI, Sean Thackeray and Sonnica Albertyn for being so welcoming and for giving me new insights into this work.

In conclusion, thank you to my parents, sisters, aunts, other family and friends for your support and interest from the early stages of my academic career to those who still continue to encourage me in these latter years. A tribute must be given to my grandfather, Reginald Sweet, a scholar and a true gentleman for having been such a wonderful influence on me in my younger years, both academically and personally. In particular thanks must be given to my mother, Bridget Love for your love and endless support. You have encouraged me since day one and none of this would have been possible without you.

.

Table of Contents

List of Tables

CHAPTER 2

List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 4

CHAPTER 5

CHAPTER 6

List of abbreviations

%	percentage
°C	degrees Celsius
CE	controlled environment
CGA	Citrus Growers' Association
CRI	Citrus Research International
DAFF	Department of Agriculture, Forestry and Fisheries
EPF	entomopathogenic fungi
EPN	entomopathogenic nematode
FC	field capacity
FCM	false codling moth
g	grams
GPS	global positioning system
IPM	Integrated Pest Management
kg	kilogram
kPa	kilopascal
L	litre
mbar	milibars
mg	milligrams
ml	millilitres
mm	millimetres
RH	relative humidity
RP	refill point
SIT	sterile insect technique
USA	United States of America
ZAR	South African Rand

1

GENERAL INTRODUCTION

PROBLEM STATEMENT

A number of advancements in the control of the soil-dwelling life stages of the agricultural pest species, false codling moth (FCM) in citrus have been made in recent years. However, this progress has highlighted the current lack of knowledge with regards to the biology, behaviour and survival of these life stages in the soil. This body of work will improve this knowledge through examining the impacts on pupating FCM when altering the abiotic soil environment. This will provide baseline biological information which will then be applied to both current and future control techniques for FCM in order to reduce the economic impact of this citrus pest.

1.1 SOUTH AFRICAN CITRUS

1.1.1 The Taxonomy of the *Citrus* Genus

The taxonomic classification of the *Citrus* genus places it into the tribe Citreae within the Rutaceae family and Aurantioideae subfamily (Webber 1967). The taxonomic relationships of the *Citrus* genus are complex and not universally agreed upon (Nicolosi *et al*. 2000). This is partially due to the sexually compatible nature of *Citrus* and other closely related genera, as well as modification in the bud due to mutations occurring and anthropogenic manipulation of the genus through artificial dispersal and cultivation (Nicolosi *et al*. 2000). Much controversy and disagreement surrounds the current classification of *Citrus,* however it is generally agreed that three standard species exist within the genus, namely the pummelo, *C. maxima* Merril, the citron, *C. medica* L. and the mandarin, *C. reticulata* Blanco (Scora 1975; Barrett & Rhodes 1976; Moore 2001). Various hybrid citrus fruits have been developed and these show an extensive range of diversity in morphological features such as colour, size, shape, time of maturity and seeds per fruit (Novelli *et al*. 2006). The difficulty with *Citrus*

classification is primarily as a result of facultative apomictic reproduction, where biotypes are able to produce both sexual and asexual offspring from the same seed (Barrett & Rhodes 1976). It is suggested that all other genotypes have been developed from the original three species (Nicolosi *et al*. 2000). *Citrus sinensis* Osbeck or the sweet orange is the most widely consumed type of *Citrus* and based on genetic analysis, appears to be a combination of predominantly mandarin or *C. reticulata* introgressed with the pummelo, *C. grandis* (Scora 1975; Nicolosi *et al*. 2000; Moore 2001; Novelli *et al*. 2006).

1.1.2 The South African Citrus Industry

Citrus production has a long history in South Africa, with the first citrus tree being introduced to the Western Cape region from St. Helena in the 1650s, which was well before the introduction of citrus trees into California in the United States (Dixie 1999; CGA Annual Report 2007). The export of South African citrus only began in the 1900s, with the majority of the fruit being exported to Britain initially and later to other European countries (Mather & Greenberg 2003; Mather & Rowcroft 2004; CGA Annual Report 2007). The consistency and the quality of the fruit proved to be problematic, with very little coordination and regulation existing within the industry during the early twentieth century (Mather & Rowcroft 2004). In order to address these problems, the South African Cooperative Citrus Exchange (SACCE) was established in the 1920s, which was also tasked with the marketing and promotion of South African citrus (Mather & Greenberg 2003; Mather & Rowcroft 2004). In 1927, the citrus industry began to operate separately from that of deciduous fruit and the Citrus Exchange was established (Mather & Greenberg 2003; CGA Annual Report 2007). While legally considered to be separate entities, a great deal of overlap existed between the Citrus Exchange and the SACCE due to many individuals working within both organisations (Mather & Greenberg 2003).

The Citrus Exchange became the single exporter of citrus produced in South Africa and began extensive research and extension programmes in order to improve citrus infrastructure and provide healthy trees for farmers (Mather & Greenberg 2003). The Citrus Exchange also chose to export citrus under the single brand name of Outspan, which made the brand synonymous with South African citrus (Mather 1999; Mather & Greenberg 2003; Mather & Rowcroft 2004; CGA Annual Report 2007). By the 1990s Outspan was not only the brand for

South African citrus, but was also involved in all aspects of its production, transport, distribution, marketing, research and extension (Mather 1999). In 1994, Outspan became a private company and was thereafter known as Outspan International Limited, which was largely as a result of the re-opening of foreign markets to South African citrus (CGA Annual Report 2007). This was due to the changes in the South African political climate and sanctions on the country being lifted (Mather 1999). In 1998, a merger between Outspan and Unifruco, the company which had previously been the only exporter of deciduous fruit in South Africa, resulted in the formation of Capespan, which made this company the largest exporter of South African fruit in the country and one of the largest in the world (Mather & Greenberg 2003; CGA Annual Report 2007).

However, the change in political climate post-1994 resulted in changes to the citrus industry and pressure to deregulate citrus increased, ultimately resulting in the Citrus Board being dissolved in 1997 and Capespan losing its exclusive export rights (Mather & Greenberg 2003; CGA Annual Report 2007). This in turn allowed for the development of alternative citrus export agents, which by 1999 had reached 160. Also in 1997, the Citrus Growers Association (CGA) was formed in South Africa which was tasked with registering all citrus growers and managing citrus research projects (CGA Annual Report 2007). The previous Outspan Citrus Centre became part of the Citrus Growers Association in 2001 and was renamed Citrus Research International (CRI). The development of the citrus industry meant that it had become a powerhouse of South African agriculture, generating a large amount of income for the country and in 2007 the industry marked its 100th anniversary of citrus exports from South Africa (CGA Annual Report 2007).

Currently, South Africa is ranked as the third largest exporter of fresh citrus fruit globally, which provides the country with an export income of over 4.5 billion ZAR per annum (CGA Key Industry Statistics 2012). Five different types of citrus are produced in South Africa: oranges, soft citrus (or ‘easy peelers’), grapefruit, lemons and limes, all of which are produced for both local and international markets. The majority of the citrus fruit produced by commercial farmers is, however, destined for the overseas export market which is due to the high quality of the fruit produced and the higher prices obtained in foreign markets (CGA Key Industry Statistics 2012). South Africa is able to produce citrus fruit in the winter to supply northern hemisphere markets with fruit during their summer season (Mather 1999). This counter-season production system (Mather 1999) is further enhanced by the development of different citrus cultivars which have varying maturity times within the citrus

types, thereby providing different citrus cultivars throughout the season. The Valencia orange is the cultivar most frequently produced for export with 941 695 tons of fresh fruit being passed for export out of the total 1 428 047 tons produced in 2011 (CGA Key Industry Statistics 2012). Navel oranges are the second most frequently exported cultivar followed by grapefruit, lemons and limes and finally soft citrus (CGA Key Industry Statistics 2012).

Citrus fruit is produced in seven of the nine South African provinces, namely the Eastern Cape, Kwa-Zulu Natal, Limpopo Province, Mpumalanga, Northern Cape, North West and the Western Cape (Fig. 1) (Mather & Greenberg 2003; CGA Annual Report 2014). The Limpopo Province has the largest area of land dedicated to citrus production, followed by the Eastern Cape and Mpumalanga provinces (Fig. 1) (CGA Annual Report 2014). The climate in these various areas also plays an important role in fruit production with the Eastern and Western Cape being considered as cooler climatic areas, making them suitable for production of Navel oranges, lemons and soft citrus or easy peelers (DAFF 2012). The warmer climate of the Limpopo, Mpumalanga and Kwa-Zulu Natal favours the production of grapefruit and Valencia oranges (DAFF 2012). Farm sizes in these provinces also tend to be larger and the fruit is often packed by privately owned facilities as opposed to the smaller farm sizes and large cooperative packhouses which are utilized by farmers in the Eastern and Western Cape (DAFF 2012). In the 2013 season, fresh oranges, grapefruit, soft citrus and lemons were inspected and passed for export to a number of countries across the globe (CGA Annual Report 2014). The largest overseas markets which import fresh South African citrus produce are Northern Europe which accounts for 22 % of all South African citrus exports, followed by the Middle East (18 %), United Kingdom (15 %), Russia (13 %), the Far East (12 %) and a number of other countries which import smaller volumes of citrus (Fig. 2) (CGA Annual Report 2014).

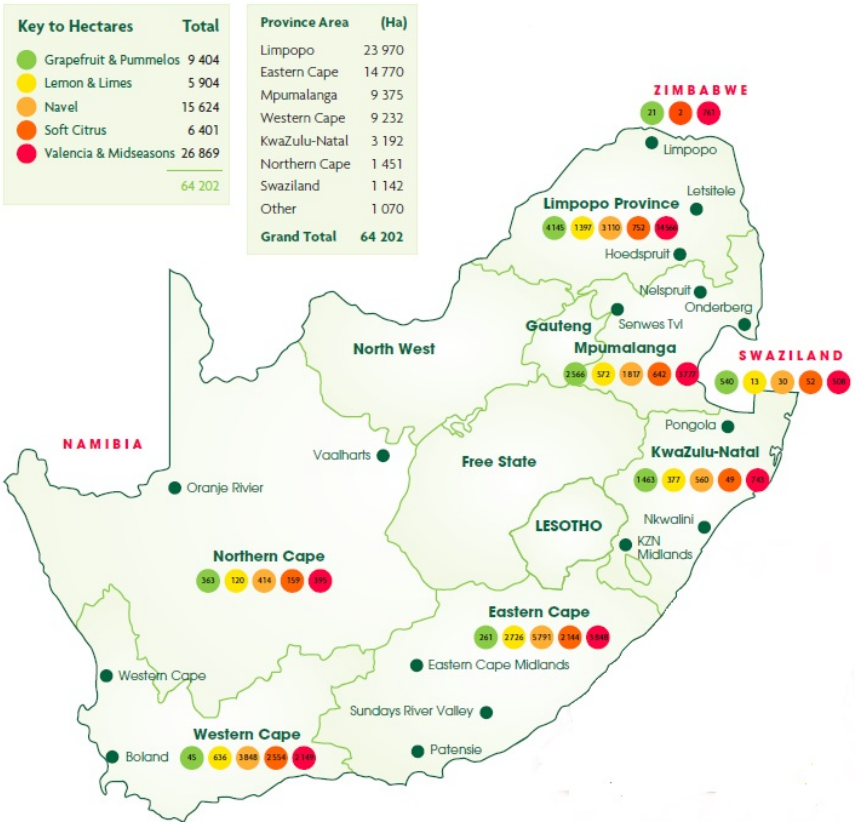

Figure 1.1 A map detailing the citrus producing areas of South Africa (Source: CGA Annual Report 2014).

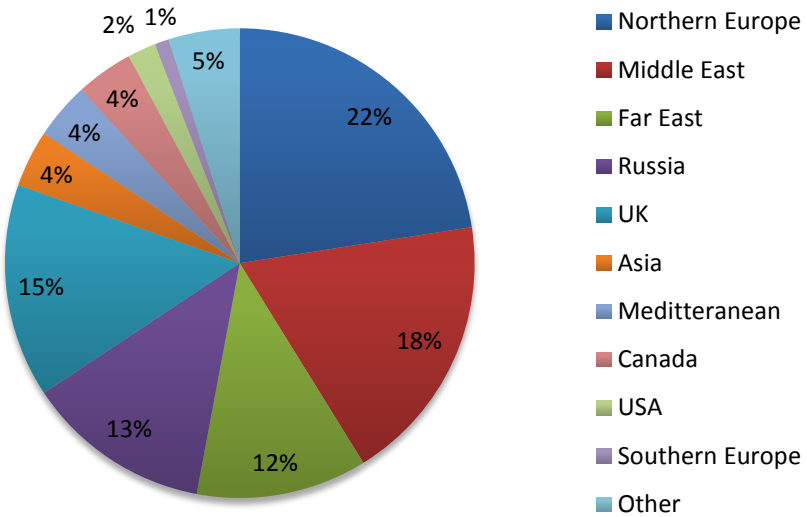

Figure 1.2 Export markets for fresh citrus fruit (including oranges, grapefruit, soft citrus and lemons) produced in South Africa (Adapted from: CGA Annual Report 2014).

1.1.3 Citrus Export: Sanitary and Phytosanitary regulations (SPS)

Food safety and security are important issues in the agricultural industry and are an important concern for the citrus grower. In order to ensure the international safety of food exports the World Trade Organization encouraged the implementation of Sanitary and Phytosanitary measures (SPS agreement) for all member countries, which holds exports to an international standard (Ndou 2012). Consumer demands for fresh fruit have increased due to the importance attributed to these foods in order to maintain a healthy lifestyle, which has become an increasingly popular trend in more recent times (Pollack 2001). The health benefits of citrus are well-known with bioactive compounds being found in citrus fruit which are involved in preventing degenerative diseases such as cancer, cataracts and cardiovascular diseases (Silalahi 2002).

A greater diversity and availability of fresh fruit has also allowed producers to cater far more to consumer preferences (Pollack 2001). A study conducted using soft citrus consumers in the United Kingdom showed that those surveyed felt that the aesthetic quality, fruit firmness and price were the most important external characteristics taken into consideration when selecting fruit for purchase (Poole *et al.* 2007). When examining the internal quality; the juice quantity, sweetness and acidity were deemed to be the most highly valued characteristics (Poole *et al.* 2007). Consumers also expect consistent fruit quality and supply (Ndou & Obi 2011). As a result of these factors, this growth in demand has resulted in the need for increased production of fruit in countries such as South Africa in order to supply citrus fruit to the northern hemisphere where citrus is out of season during the summer months (Mather 1999).

Despite the stringent regulations implemented by more developed countries, which are often the ultimate destination for South African citrus, South Africa has maintained and increased its fruit export to these areas (CGA Annual Report 2011; CGA Key Industry Statistics 2012). In addition, new and emerging markets have been developed in recent years such as those in Russia and the Middle East (Ndou 2012). South Africa has negotiated bilateral agreements or export protocols with China, the European Union (EU), Iran, Japan, South Korea and the United States of America, all of which have specific phytosanitary requirements for the importation of citrus fruit (DAFF 2010). In order to provide growers with increased export market options, negotiations to allow or improve market access for South African citrus produce in countries such as Indonesia,

South Korea and Vietnam are on-going (CGA Annual Report 2014). Since the onus is on national departments to provide safe, pest- and disease-free fruit for export, the Department of Agriculture, Forestry and Fisheries (DAFF) Directorate: Agriculture Products Inspection Services (D: APIS) is responsible for inspecting orchards and fruit to ensure their compliance (DAFF 2010). The requirements for food safety alter on a continual basis with changes in the Maximum Residue Levels (MRLs) allowed for pesticides used and changes in importing governments' food safety legislation (CGA Annual Report 2011). The citrus grower is therefore under pressure to provide a consistent supply of high quality fruit which is neither infested nor damaged by pests, while simultaneously reducing pesticide usage, which is at best an extremely difficult task.

1.1.4 Citrus Pests of South Africa

Citrus is known to suffer from attack by a wide range of pests on a global scale (Smith & Peña 2002). In South Africa, over 100 different citrus pest species have been recorded (Bedford 1998). This high number appears to be largely a result of the favourable climate found in South Africa, with a general global trend of increasing pest numbers in warmer, more humid areas (Urquhart 1999; Smith & Peña 2002). The majority of pests (approximately 30-60%) fall into the order Hemiptera, with pests such as scale insects, mealybugs, aphids, whiteflies and leafhoppers being most damaging (Smith & Peña 2002). These are followed by a large number of lepidopteran pests (fruit boring and piercing moths, leafrollers, and leafminers), but further damage to citrus fruit may be caused by mites, beetles, flies and thrips (Smith & Peña 2002).

Many pests, in addition to causing direct damage to the tree or fruit, are vectors of disease or may cause damage that renders the tree or fruit far more vulnerable to disease or fungus contamination (Smith & Peña 2002). Due to a rapid increase in both globalization and speed of transport, the risk of introducing new pests and diseases has increased dramatically, as can be seen in the global spread of a number of citrus pests over the last 18 years such as the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) (Albrigo *et al*. 2009). To date the spread of the Asian citrus

psyllid has been limited on the African continent to only Ethiopia, with South Africa as yet unaffected (Saponari *et al.* 2010; Bové 2014). Citrus pests are often region-specific, with each region potentially being dominated by a few major pests that occur most frequently and do the most damage (Smith & Peña 2002). However, the high demand for the unblemished fruit with an attractive appearance in the consumer market results in strict control measures also being implemented against pests that only cause cosmetic damage to fruit (Pimental *et al.* 1977; Pimental *et al.* 1993). In South Africa it has been established that the key problematic citrus pests are 1) *Aonidiella aurantii* Maskell (red scale), 2) *Scirtothrips aurantii* (citrus thrips), 3) *Trioza erytreae* Del Guercio (citrus psylla), 4) *Ceratitis capitatia* Wiedemann (Mediterranean fruit fly), 5) *Ceratitis rosa* Karsch (Natal fruit fly) and 6) *Thaumatotibia leucotreta* Meyrick (false codling moth – the topic of this thesis) with an additional 12 major pest species of less importance also being identified (Smith & Peña 2002).

1.1.5 Initial Pest Control and the Later Development of Integrated Pest Management

Pest control relies strongly on effective monitoring of pest population sizes in order to determine when action should be taken to control the pest, a strategy more regularly implemented on larger citrus farms than those with smaller orchards (Smith & Peña 2002). Control in the early stages of the South African citrus industry from the early 1880s involved the use of pesticides such as rainwash, lime sulphur, sulphur dust, nicotine sulphate, miscible petroleum and other oil emulsions, all of which were considered relatively safe to both humans and the environment, as these did not remain active long after application (Bedford 1998). Fumigation of trees using hydrogen cyanide (HCN) was also extensively used, largely to control scale insects, but was found to be highly toxic to humans (Bedford 1998). In order to improve control, the majority of these pesticides were replaced by the use of parathion from 1948 when the use of chemicals became the preferred mode of control (Bedford 1998). In the 1970s, the control provided by this pesticide was no longer sufficient and its use resulted in large scale pest outbreaks, due to insect resistance developing from overuse (Bedford 1998). These pest outbreaks were largely scale insects with red scale, *Aonidiella aurantii* Maskell (Hemiptera: Diapsidae) outbreaks being particularly high (Bedford 1998). A

movement toward pyrethroids then began, with chitin synthesis inhibitors and other insect growth regulating (IGR) pesticides also being used (Bedford 1998; Newton 1998). However, problems with resistance developing amongst pest populations, outbreaks of non-target pests, the threat of toxic pesticides to both human and environmental health and consumer demands to decrease pesticide residue levels all forced citrus growers to rethink their reliance on chemical control methods for pests (Bedford 1998; Urquhart 1999; Charleston *et al*. 2003).

In order to control these pests, many citrus growers in South Africa have changed from a largely chemical-based control strategy to an Integrated Pest Management (IPM) strategy (Urquhart 1999). Interest in the use of IPM has grown substantially since the initial research into this approach began in the 1960s (Urquhart 1999). IPM may be defined as "a dynamic, integrated approach involving a number of techniques to manage pest populations in an ecologically sound fashion" (Urquhart 1999). This approach focusses primarily on the biological control of pests by making use of their natural enemies and complementing this with the use of cultural control techniques (Smith & Peña 2002) Pesticide usage is limited as much as is possible (Smith & Pena 2002). Additional control techniques that are compatible with IPM are included. Notably, the concept of pest eradication is abandoned in favour of a more realistic pest management strategy (Charleston *et al*. 2003).

The implementation of IPM in South Africa has largely been as a result of three factors: 1) international pressure to reduce use of pesticides and reductions in MRLs of pesticides found on fruit while still producing high quality fruit, 2) increasing incidences of pest resistance to pesticides and 3) the promotion of being more environmentally friendly and taking greater responsibility for the environment (Urquhart 1999). Citrus has a long and extremely successful history of IPM in South Africa which began with the need to control ants and red scale in orchards (Bedford 1998). While initially enjoying a large amount of success, in the late 1960s to early 1970s, a move away from IPM with increased reliance on pesticides, resulted in a definite decrease in IPM use (Bedford 1998). This was until continued usage resulted in the 'pesticide treadmill' phenomenon beginning to occur where continued pesticide exposure resulted in pests developing resistance to the active ingredients. This in turn, required more frequent spraying or the use of other chemical control products (Bedford 1998). Once this trend and its negative impacts had been established there was a return to IPM and subsequently this has

become one of the most successful pest control programmes in agriculture (Charleston *et al*. 2003). Outspan International was also instrumental in the promotion of IPM amongst citrus farmers with support being provided for research and implementation (Mather 1999). While citrus farmers have largely moved toward the use of IPM for financial reasons, in order to retain their position in a highly competitive market, it has allowed for the development of IPM strategies in South Africa which are also of benefit to the environment (Urquhart 1999). This strong focus has resulted in a great deal of research being undertaken into alternative, non-insecticidal pest control techniques.

1.2 FALSE CODLING MOTH

1.2.1 Taxonomic Classification

False codling moth (FCM) has been most recently classified as *Thaumatotibia leucotreta* Meyrick 1912, (Tortricidae) (Newton 1998). The species was originally described by Fuller (1901), as a citrus pest in the Kwa-Zulu Natal area known as *Carpocapsa sp*. (Newton 1998). However, it was also later discovered to occur in the other north eastern areas of South Africa, where it became known as the orange codling moth (Newton 1998). Following the original discovery of what was later to become known as false codling moth, the taxonomic position of the species was altered on several occasions (Newton 1998). FCM was initially taxonomically described as *Argyroploce leucotreta* by Meyrick (1912); however the species did not remain there for long, being transferred by Clarke (1958) to the genus *Cryptophlebia* (van den Berg 2001). Komani (1999) subsequently removed the species *leucotreta* from the *Cryptophlebia* genus, placing it into *Thaumatotibia* where it has since remained (Venette *et al*. 2003). While species in the *Cryptophlebia* and *Thaumatotibia* genera have many external features in common, a list of those features which separate the two genera was produced by Komani (1999) and is also available in Venette *et al*. (2003).

1.2.2 Distribution and Host Range

FCM is considered to be native to the Ethiopian region of Africa (Catling & Aschenborn 1974). Records indicate FCM presence for the majority of sub-Saharan Africa, certain Indian and Atlantic Oceans islands and in general, most of the African continent (reviewed by Newton 1998; Stibick 2010). Notably exempt from this distribution are certain North African countries such as Morocco, Algeria, Tunisia, Libya and Egypt (Stibick 2010). In South Africa, FCM is known to be a serious citrus pest in all citrus-producing areas of the country (Newton 1998). However, the impact of the pest is known to vary as pest pressure is far lower in the far northern regions of South Africa which are much drier (Newton 1998; Moore 2012; Moore & Hattingh 2012). It has been suggested, based on the global distribution of FCM, that the moth species is closely associated with desert and xeric shrubland, tropical and subtropical grasslands, savannas, shrublands; and tropical and subtropical moist broadleaf forest (Venette *et al*. 2003).

The most recent survey of cultivated and native South African plant species which are able to host FCM reproduction and development by Kirkman & Moore (2007) indicated that approximately 24 cultivated and 50 wild species are host plants for FCM. The moth is particularly problematic on citrus; however it is also known to attack the heads of sorghum, cause damage to cotton in Zimbabwe and Central Africa, as well as the cobs and stems of maize in Central Africa (reviewed by Newton 1998). Losses due to FCM damage are also known to occur in the deciduous, subtropical and tropical fruit industries, on crops such as avocados, guavas, macadamia nuts and litchis (Newton 1989; Newton 1998; Erichsen & Schoeman 1992). Of the different citrus types, Navel oranges are most susceptible to FCM damage while soft citrus (mandarins) and grapefruit appear to be less susceptible (Newton 1998). Within Navel orange cultivars there is some evidence to suggest that in certain cases, female moths may exhibit preferences for particular cultivars over others (Newton 1990; Love *et al.* 2014). Lemons and limes are highly resistant to FCM damage as larval development is very rarely completed, which is thought to be due to the very high acidity and high juice content of these cultivars (Newton 1998).

1.2.3 Description of Life Stages

The egg of FCM is small in size and a very pale, almost translucent colour when it is first laid (Fig. 3a), darkening to red as the larva inside develops and finally becoming black just prior to the larva hatching (Daiber 1979a; Newton 1998; Moore 2012). First instar neonate larvae are diminutive in size (approximately 1.5 mm in length), but may be observed with the naked eye (Fig. 3b) (van den Berg 2001). They are cream to white for the first three instars with a dark brown head, with a change in body colour to a very characteristic pink-red occurring in the fourth and fifth instars (Fig. 3c) (Georgala 1969; Newton 1998; van den Berg 2001; Moore 2012). Once the fifth instar is reached the larva is ready to pupate in the soil in a silken cocoon (Newton 1998; van den Berg 2001). The pupae are dark brown (Fig. 3d) (Newton 1998; van den Berg 2001). The adult moth is fairly nondescript in appearance, being small in size with a wingspan of 16-20 mm with mottled dark grey wing colouration (Fig. 3e) (Georgala 1969; Newton 1998; van den Berg 2001; Moore 2012). The hind wings are fringed with small hairs and paler than the front wings (Newton 1998; van den Berg 2001). One is able to clearly differentiate between the two sexes as males are smaller than females, have a dense packed tuft of modified hairs on their hind legs and a scent organ near each hind wing (Newton 1998; van den Berg 2001).

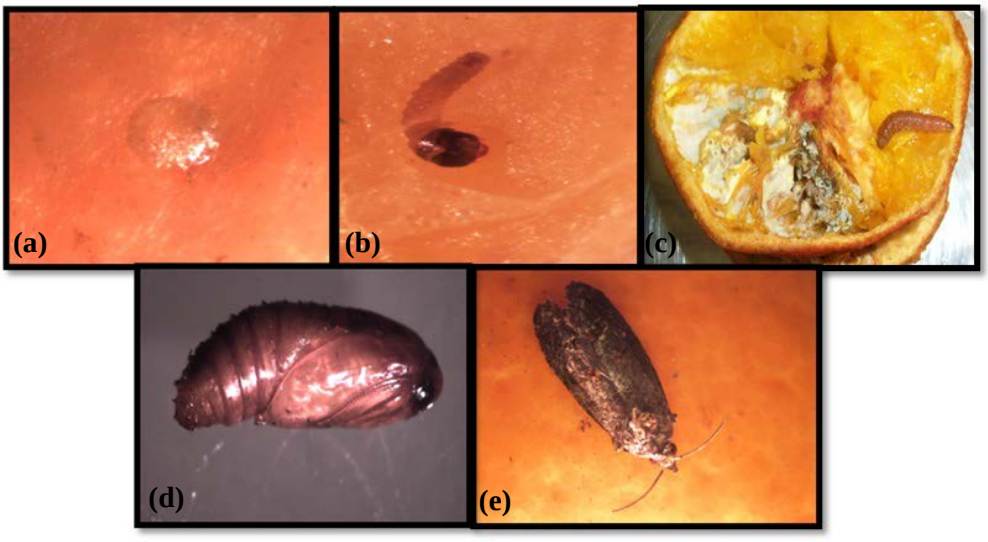

Figure 1.3 The false codling moth life stages: **a**) egg laid on an orange rind (approximately 1 mm in diameter); **b**) first instar neonate larva (1-2 mm in length); **c**) fifth instar larva with characteristic pink colouration and the fungal contamination of a Navel orange as a result of FCM entry; **d**) FCM pupa and **e**) adult female FCM (Images: C. Love).

1.2.4 General Life History of FCM

FCM is a nocturnal species, with female moths laying their eggs on the outer surface or rind of the fruit (Georgala 1969; Newton 1988; Newton 1989; Newton 1998). Very high numbers of eggs are laid over the early November to mid-December period and the survival rate of eggs laid during this time is extremely high (Catling & Aschenborn 1974). Fruit suffering from split damage or other injuries is preferred for oviposition, as well as any prematurely ripened citrus (Catling & Aschenborn 1974). Female moths do show oviposition preferences for the physical location of the fruit on the tree, with the number of eggs increasing as the height of the tree increases and more eggs being found on the side of the tree receiving the most sunlight (Catling & Aschenborn 1974). Once embryonic development is complete, hatching may occur at any point in the day (Newton 1998). The first instar neonate larvae which hatch typically suffer from high rates of mortality due to the delicacy of this life stage (Newton 1998). These first instar larvae penetrate through the rind of the fruit where development continues through five instars (Georgala 1969; Newton 1988; Newton 1989; Newton 1998). Larvae often enter through the most vulnerable areas of the fruit such as those areas which are already damaged or the navel end of Navel oranges (Newton 1998).

After the larvae have entered the fruit, wounding, premature ripening and fruit decay may occur either through direct larval feeding damage or through allowing fungus or disease entry into the fruit as the protective rind is now compromised (Fig. 3c) (Newton 1988; Newton 1989; Newton 1998). The successful penetration and development of FCM larvae inside the fruit has been found to be influenced by the specific Navel orange cultivar (Love *et al.* 2014). The larva burrows out of the fruit creating a clearly visible exit hole which is often filled with frass once the fifth instar is reached (Newton 1998). The larva drops from the fruit by producing a silken thread or it causes premature abscission of the fruit from the tree, after which the larva is able to pupate in the soil (Newton 1988). Once the larva reaches the soil, a silken cocoon is spun and pupation occurs in the upper layer of the soil (Georgala 1969; Newton 1988). Adult moths eclose from the pupae after which the wings dry and straighten before dispersal takes place (Georgala 1969; Newton 1998). Genetic analysis by Timm *et al*. (2010) of FCM at a population scale indicated that gene flow at a local scale and dispersal were limited. This provides further evidence for the relatively poor dispersal of FCM as previously indicated by field observations and mark-recapture studies (Newton 1998). The total developmental period has been estimated to be 2.5 to 4 months in winter and 1.5 to 2

months in summer (Newton 1998). Generations are notably poorly defined and overlapping, with approximately 5 to 6 occurring per year, making this pest an almost year-round threat to growers, a problematic feature of the FCM life cycle (Newton 1998).

1.2.5 The Pre-pupal and Pupal Life Stages of Lepidoptera

In order for holometabolous insects to change to their adult form by the process of metamorphosis they must enter into a life stage known as the pupal stadium (Gullan & Cranston 2000). This is a non-feeding form when the adult structures (most notably the wings, but also other internal organs such as the reproductive system) are formed (Gullan & Cranston 2000). The moult from the final instar larva into the pupa is a process known as pupation or the larval-pupal moult (Gullan & Cranston 2000).

The pupa has a closer resemblance to the adult than the larva, largely because all the features of the adult are visible in the pupal form (Chapman 2013). The final instar may be inactive for two or three days prior to pupal formation and in some cases a pharate pupa is formed during this time, which is often referred to as a pre-pupa, although this is not generally recognized as a separate morphological stage (Chapman 2013). Most insect pupae lack mobility and are therefore highly vulnerable to factors such as disease and predation (Chapman 2013). As a result of this, many insects pupate in a cell or cocoon (Chapman 2013).

On the completion of metamorphosis the pharate adult insect may enter into a period of rest within the pupal cuticle for a number of hours or possibly days, as environmental triggers may be required for eclosion to occur (Gullan & Cranston 2000). In current literature it is thought that eclosion is governed by both environmental factors such as temperature and light, as well as changes in hormonal levels through chemical signals (Gullan & Cranston 2000). Various studies have indicated that at least five hormones are involved in the process of eclosion (Gullan & Cranston 2000).

1.2.6 Pupation in False Codling Moth

The soil-dwelling life stages of FCM include the wandering larva (Fig. 4a), the pre-pupa (Fig. 4b) which then moults into a soft, beige coloured early stage pupa (Fig. 4c) with the colour gradually changing to a dark brown colour (Fig. 4d) as the chitin in the pupal case hardens to protect the insect developing inside (Daiber 1979c), the late stage pupa and the eclosing adult. Newton (1998) stated that "larvae pupated in loose soil, beneath the surface debris or in cracks in the soil" and that when pupating the larvae would spin a silk cocoon into which soil particles and "trash" would be incorporated. It is possible for pupae to be sexed as males have two small knobs found next to each other on the centre of the ventral side of the ninth (IX) abdominal segment, which female moths lack (Daiber 1979c). This particular abdominal segment is also larger in female FCM pupae (Daiber 1979c). Upon emergence, the pupa will begin to move out of the cocoon, after which the adult moth ecloses (Fig. 4e), leaving behind both the pupal case and cocoon which are often attached (Daiber 1979c). The average time taken for the pre-pupa to moult into a pupa was 2.1 days at 25 °C, with the average duration increasing as temperature decreased (Daiber 1979c). Development and eclosion of adult FCM was successfully completed at 25, 20 and 15 °C however 10 °C proved too low for this to take place (Daiber 1979c). Female FCM eclosion usually occurs first, taking an average of 13.9 days and males 14.0 days at 25 °C (Daiber 1979c).

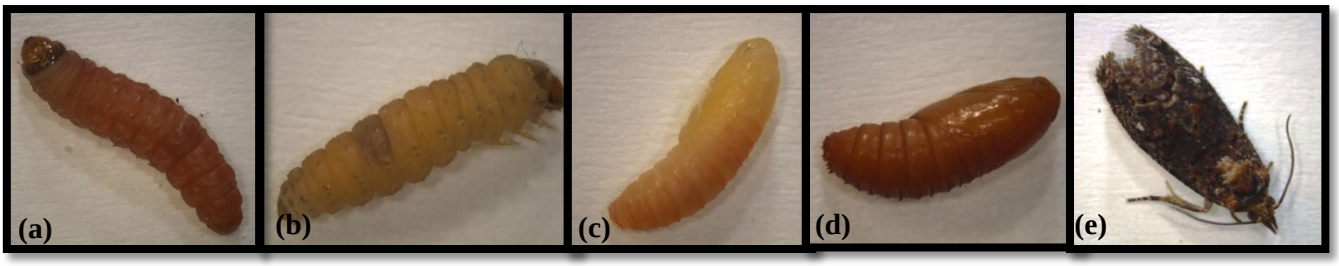

Figure 1.4 The soil-dwelling life stages of FCM **a)** the wandering fifth instar larva showing characteristic pink-red colouring, **b)** the pre-pupal stage which moults into **c)** the early pupal stage which hardens and darkens into **d)** the later pupal stage inside which **e)** the adult moth develops and finally ecloses, casting off the cocoon and pupal case (Images: C. Love).

1.2.7 The Economic Importance of False Codling Moth

Income loss for citrus farmers as a result of FCM has been estimated to be as high as 100 million ZAR (almost $86 00 000 at the current exchange rate) (Moore 2002) per annum, due to pre-harvest damage and phytosanitary concerns of South African export markets (Venette *et al*. 2003; Stibick 2010; Kirkman & Moore 2007; Moore 2012). Pre-harvest losses are caused by internal larval feeding and post-harvest fruit decay as the fruit is far more vulnerable to the entry of pathogens once the integrity of the protective rind has been compromised (Newton 1998; Kirkman & Moore 2007). Furthermore, the presence of larvae often results in both premature ripening and abscission of fruit from the tree (Newton 1998). False codling moth is regarded as a phytosanitary pest in all of the export markets for South African citrus, such as the USA and EU, as the pest does not naturally occur in these regions (Venette *et al*. 2003; Stibick 2010; Moore 2012). Should any indication of the moth be found in export citrus destined for a phytosanitary market, the entire shipment may be rejected resulting in great economic loss (Moore 2002; Moore 2012). The potential for establishment of FCM has been predicted for areas of the US (principally in the western and southwestern USA) using climate mapping (Venette *et al*. 2003; Stibick 2010) with the potential for economic loss being high should FCM become established (Venette *et al*. 2003; Stibick 2010). With an increase in global accessibility, it is important that this risk is managed and reduced wherever possible (Stibick 2010).

1.2.8 Control of False Codling Moth

1.2.8.1 Pre-harvest Control

Orchard sanitation through the removal of infested oranges is one of the principle recommendations for FCM control in citrus orchards, which while being an old technique, is still highly effective (Newton 1998; Moore 2012). However, this technique is only successful if the fruit is removed from the orchard and destroyed through the burying of the small, hard fruit in soil at least 30 cm deep and pulping of the juicy fruit with a hammermill (Moore 2012). A variety of options are available to citrus growers for monitoring FCM populations. Fruit drop surveys, where fallen fruit are dissected and investigated for indications of FCM infestation, is considered to be one of the most important methods for

monitoring FCM levels in orchards (Moore 2012). Guidelines for fruit drop surveys may be found in Moore (2012). Inspecting fruit on the tree for eggs is possible, but can be a time-consuming process as the eggs are very small and although visible to the naked eye, they are not readily apparent (Kirkman 2007). Useful monitoring products include controlled-release pheromone traps which are used by farmers to determine FCM presence, as it is preferable to keep the population size as close to zero as possible due to the phytosanitary status of FCM (Hofmeyr & Burger 1994; Moore 2012). In order to trap FCM to establish its presence or population size, a yellow delta trap or PVC pipe trap is available for use (Moore 2012). Three pheromone dispenser systems are currently available, which are the Lorelei, FCM PheroLure and Chempac FCM lure (Moore 2012). The delta trap is recommended for use with any of the three pheromone systems (Moore 2012).

A wide range of other control methods which target different FCM life stages are also available and these range from predominantly chemical control to a more IPM-based control approach. Historically, chemical controls for FCM which have ranged from dichlorodiphenyltrichloroethane (DDT) sprays to synthetic pyrethroids and insect growth inhibitors (IGRs) have been used extensively (Newton 1998). There are currently six chemical insecticides registered for FCM control in South Africa (Moore & Hattingh 2012). Alsystin® (Bayer, Germany) and Nomolt® (Cyanamid, South Africa) are both chitin synthesis inhibitor pesticides which inhibit larval development within FCM eggs (Kirkman 2007; Moore 2012; Moore & Hattingh 2012). Of the products registered, Meothrin® (Philagro, South Africa (Pty.) Ltd.; active ingredient, fenpropathrin) and Cypermethrin® (Agropharm, South Africa; active ingredient, cypermethrin) are both pyrethroid based pesticides (Kirkman 2007; Moore 2012; Moore & Hattingh 2012; Fullard & Hill 2013). The latest additions to the chemical FCM control assembly are Delegate® (Dow AgroSciences, Indianapolis) (active ingredient spinetoram), Coragen® (DuPont, South Africa) (active ingredient chlorantraniliprole) (Moore 2012; Moore & Hattingh 2012; Fullard & Hill 2013), and most recently registered is Runner™ (Dow AgroSciences, Southern Africa (Pty.) Ltd.) with the active ingredient methoxyfenozide (http://msdssearch.dow.com/PublishedLiteratureDAS/dh_08ed/0901b803808ed003.pdf?filepath=/011-10213.pdf&fromPage=GetDoc). These insecticides have been developed in order to address modern concerns through harmful residue reduction and being more environmentally-friendly (Moore & Hattingh 2012). This has been achieved by having low residue levels and very eco-friendly eco-toxicology profiles, which make this control technique more IPM compatible than chemical control has

been in the past (Urquhart 1999; Moore & Hattingh 2012). While chemical insecticidal sprays are very widely used by farmers and have been found to cause up to 75 % reduction of FCM in certain studies, there are limitations to their effectiveness in controlling FCM, primarily due to the almost continual egg laying of adult female moths and the limited access to newly hatched neonate larvae as they bore rapidly into the citrus fruit after hatching (Newton 1998; Stotter 2011).

Mating disruption products such as Isomate® and Checkmate® FCM-F are used to control the moths by releasing the synthesised female moth pheromone from dispensers placed in the orchard or in encapsulated form in a flowable spray (Mitchell 1975; Moore & Hattingh 2012). The male moth is disrupted by this pheromone, rendering it unable to find an actual female moth to mate with (Mitchell 1975). Attract and kill products work on a similar principle to mating disrupters, however in this case the male moths are attracted to the pheromone source which is coupled with an insecticide. Last Call FCM™ (Insect Science, South Africa) is the only product currently on the market which does this; however efficacy trials indicate that this method of control is best used in areas which experience low pest pressure or if used in areas which experience high FCM pressure, then only when activity levels of the pest are still low (Moore 2012; Moore & Hattingh 2012).

Another non-chemical option for the suppression of FCM is the use of the sterile insect technique (SIT), which works on the basis of supplying orchards with large numbers of gamma irradiated sterile male FCM that mate with wild females which then produce infertile eggs (Moore 2012; Moore & Hattingh 2012). SIT has been found to reduce both the fertility and fecundity of the moth population and was commercialised by XSIT (Pty) Ltd in 2007 (Stotter 2011; Moore 2012). The project was initiated in the Citrusdal area of the Western Cape and is now also being applied to areas of the Eastern Cape. A release ratio of ten sterile males for every wild male has been found to be most effective (Moore 2012). In addition, a study by Carpenter *et al*. (2004) showed that the sterile FCM eggs produced by females mated with irradiated males were still deemed as suitable for oviposition and development by the parasitiod wasp species *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae). Since this species is used for augmentative biological control, this indicates that SIT is also compatible with biological control as part of a larger IPM control programme. However, the impact of SIT is only apparent if an area wide approach is taken with many growers working together in the release of the sterile males (Moore 2012). Sterile

insect technique and mating disruption both ultimately result in a reduction of fertilized eggs being produced by the female FCM, which results in a reduction of subsequent generations of the pest.

Biological control has long been recognised as having potential for reducing FCM pest levels, with Catling & Aschenborn (1974) being one of the first to recommend the use of egg parasitoids in orchards. Further investigation into the natural enemies of FCM revealed that both the egg parasitoid, *T. cryptophlebia* and the larval parasitoid, *Agathis bishopi* Nixon (Hymenoptera: Braconidae) would provide some measure of control (Newton 1988; Newton 1998; Carpenter *et al*. 2004; Gendall 2007). Work done by Gendall (2007) showed that *A. bishopi* was able to reduce FCM populations by up to 13.27 % when parasitism levels were at their highest, however both viral and fungal contamination of the cultures limited the mass-rearing of this species. *Trichogrammatoidea cryptophlebia* has proved to be the more effective biological control agent, as releases on citrus farms in the Western Cape showed reductions of up to 54% of the FCM larval population in the first season and approximately 60% by the second season (Newton & Odendaal 1990). Currently, *T. cryptophlebia* is mass reared commercially by Du Roi IPM and Vital Bugs (Letsitele, South Africa) in order to provide augmentative inundative control (Moore & Hattingh 2012). The release rate in order to provide effective control is 100 000 parasitiods per hectare per season which shows that much like SIT, very high numbers of released organisms are required for these control techniques to be effective (Moore 2012; Moore & Hattingh 2012).

One of the most important developments in FCM control strategies was the development of microbial control. *Cryptophlebia leucotreta* granulovirus (CrleGV) is a pathogen found in sub-Saharan Africa which attacks FCM (Moore 2002; Kirkman 2007). There are three granulovirus products currently registered for use against FCM, Cryptogran® (River Bioscience, South Africa), Cryptex® and Gratham® (both Andermatt-Biocontrol AG Switzerland), all of which contain *C. leucotreta* granulovirus (strain CrleGV-SA) as their active ingredient (Moore 2012; Moore & Hattingh 2012; Opoku-Debrah *et al*. 2013; http://www.nulandis.com/wp-content/uploads/2013/03/GraTham-Label-Table-grapes.pdf). These viral products are applied as a full cover spray to the citrus trees and can highly efficient when used correctly as the virus is consumed by the neonate FCM larvae (Moore 2002). Furthermore, the virus is highly specific resulting in low risk of non-target effects on beneficial arthropods and is compatible with many other chemicals used on citrus (Moore 2002; Kirkman 2007; Moore 2012; Moore & Hattingh 2012). Although the risk of FCM

developing resistance to the products is considered low, proactive bioprospecting for potential replacement isolates has been conducted, indicating that there are a number of alternative isolates which may be used for product formulation in the future (Opoku-Debrah *et al.* 2013).

1.2.8.1.1. Entomopathogenic Fungi (EPF) and Entomopathogenic nematodes (EPNs) for FCM control

While the virus has been used to very good effect, only in more recent years has focus turned toward using entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) for control of the soil-dwelling life stages of FCM (Goble *et al.* 2011; Malan *et al.* 2011; Coombes *et al.* 2013). A number of management techniques exist for controlling the egg, larval and adult life stages, but control options were very limited for the pupal life stage of FCM. These soil-dwelling life stages include the late fifth instar wandering larvae, the pre-pupae, pupae and emerging adults (Malan *et al.* 2011).

The use of EPF to control these life stages is highly viable as the fungus reproduces in the soil where the moths pupate (Goble 2009). These EPFs are able to attack insects through the production of asexual spores or conidia which attach and germinate on the cuticle of the insect (Inglis *et al.* 2001). Following germination, penetration hyphae are able to pass through the cuticle to the haemocoel where hyphal bodies are formed, resulting in death of the affected individual through a reduction in nutrients, organ invasion, or toxic compound production (Inglis *et al.* 2001). Once death has occurred, further spread of the fungal species is aided by the growth of hyphae from the insect cadaver which produce conidia that are passively dispersed through abiotic means such as wind or water action (Inglis *et al.* 2001; Shah & Pell 2003).

A study performed by Goble *et al.* (2011) showed the virulence of four different strains of *Beauvaria bassiana* (Balsamo) Vuillemin against FCM, as well as the Mediterranean and Natal fruit flies with two of these strains being deemed as being worthy of further study. This work was undertaken by Coombes (2012) with re-screening of the 12 original isolates previously collected by Goble *et al.* (2011) showing that three of these had the highest potential for control of FCM. This was done through concentration-dose and exposure-time response bioassays where two of these three fungi were isolates of the *Metarhizium anisopliae* var. *anisopliae* species, and the third was an isolate of *Beauvaria bassiana*

(Coombes 2012). Semi-field persistence trials using these three non-commercial isolates, as well as two commercially produced isolates, *B. bassiana* Eco-Bb® strain R444 (Plant Health Products, South Africa) and *M. anisopliae* ICIPE 69 (Real IPM, Kenya), showed that all isolates tested were able to persist in the soil and had the ability to infect wandering fifth instar FCM up to six months after application to the soil (Coombes *et al.* 2013). The non-commercial isolates had superior rates of persistence in the soil when compared to the two commercialized isolates, with the two *Metarhizium* isolates performing the best overall (Coombes *et al.* 2013).The potential for using EPF against FCM was identified during this work and subsequent further research into the formulation, application methods and field efficacy have been undertaken (Coombes, C.A. pers. comm[1].).

Entomopathogenic nematodes are soil-borne roundworms which are able to parasitize their host species (Lacey & Georgis 2012; van Zyl & Malan 2014). These transparent worms are very small in size (0.4 mm - 1.1 mm), with the infective juvenile (IJ) life stage able to actively move through the soil environment to pursue their hosts through chemoreception and mechanoreception when host movement is identified (Riga 2004). Research attention has largely been placed on the Steinernematidae and Heterorhabditidae nematode families, with their mutualistic relationship with the *Xenorhabdus* and *Photorhabdus* bacteria being of particular interest (Gaugler 1988). The penetration of IJs into the body of the host does result in the death of the host, however the presence of the bacterium is able to speed up the process of host mortality through rapid multiplication in the haemolymph of the insect (Gaugler 1988). The EPNs are then able to feed, reproduce and develop within the liquefying host, with the bacterium providing a supply of essential nutrients to the nematodes (Gaugler 1988). However, even without the bacteria being present, IJs penetrating the host will still result in death, which will simply occur at a slower rate (Gaugler 1988). These EPNs are also excellent additions to IPM programmes, as studies have shown that they are able to work in combination with other control techniques, such as fungi, to improve the overall control of the pest (Lacey & Georgis 2012).

When examining the use of nematodes against FCM in South Africa, Malan *et al*. (2011) found that FCM larvae were susceptible to isolates of all six nematode species identified in a survey of South Africa, with *Steinernema yirgalemense* Nguyen *et al*. 2004 (Steinernematid) and *Heterorhabditis zealandica* Pionar 1990 (Heterorhabditidae) being found to be the most effective species against the soil-dwelling life stages of FCM. The life stage with the greatest susceptibility to the nematodes however, was found to be the pre-pupal or wandering larval

[1] Coombes, C.A. PhD student, Rhodes University.

instar (Malan *et al*. 2011). Additionally, infected adult moths may emerge from the soil only to die approximately 24 hours later which provides a natural and more sustainable spread of the control agent (Malan *et al*. 2011). More recently, research by Manrakhan *et al.* (2014) found that natural field infection of FCM by EPNs resulted in FCM reductions of up to 50% on average in citrus orchards, with recommendations being that naturally-occurring EPNs are either conserved or applied to orchards as part of the FCM IPM programme. The only commercially available EPN product in South Africa is Cryptonem™ (River Bioscience, South Africa) which contains *Heterorhabditis bacteriophora* Poinar which is imported from Germany in partnership with E-Nema (http://www.riverbioscience.co.za/news.php?nid=19). This product is very new to the market, having only become available in January 2015.

Both EPNs and EPF occur naturally in the soil and as a result are affected by soil conditions which need to be taken into account if they are to be used as biological control agents (Goble 2009; Lacey & Georgis 2012). Some of the abiotic factors that will influence the survival and infectivity of both of these groups are adequate moisture, temperature limitations, soil type and aeration (Goble 2009; Lacey & Georgis 2012). Mitigation to assist in reducing the impact of these factors can be undertaken, such as the selection of EPN strains which are more resistant to extreme and variable temperatures, as well as the use of correct application techniques where the time of year and temperature become vital factors for application decisions (van Zyl & Malan 2014). These factors will all ultimately impact the success of EPNs and EPF as biological control agents for the soil-dwelling life stages of FCM, which will in turn also have their own set of requirements of these factors.

1.2.8.2 Post-harvest Treatment

To date the most effective method of post-harvest treatment for FCM has been cold sterilization of the fruit which also provides phytosanitory control (Boardman *et al*. 2012). Due to the expense of cold sterilisation, this process is only justified for fruit which is destined for the most profitable export markets such as China, Japan and the USA (Kirkman 2007). Investigation into the use of nuclear irradiation has also been conducted, but despite showing some potential, is not used commercially in South Africa (Moore 2002; Kirkman 2007). The most recent developments in post-harvest technology have focused on the use of micro-focus X-ray technology which would allow citrus fruit to be scanned for the presence

of FCM (Kirkman *et al.* 2014). This work is extremely promising for potential use in the packhouse as the scan time was able to be reduced to just 34 seconds per fruit with 100 % accuracy when infestation occurred 8 to 16 days prior to scanning (Kirkman *et al*. 2014).

1.3 ABIOTIC FACTORS INVOLVED IN CITRUS PRODUCTION

1.3.1 Soil and Soil Management in Agriculture

Soil consists of both organic and inorganic constituents, with organic soils being principally composed of a very high amount of naturally accumulated organic matter (Fey 2010). These soils are classed separately from mineral soils which contain a very high proportion of mineral matter (Brady & Weil 2000; Fey 2010). The mineral composition of these soils is very high and can be divided into larger soil particles or rock fragments, which are mineral aggregates and small soil particles which usually only consist of one mineral (Brady and Weil 2000).

Soil plays an essential role in global agriculture without which the promotion of food security would be impossible (Mermut & Eswaran 2001; Barnard & du Preez 2004). The importance of soil and modern soil science was largely only recognised after the Second World War and it has subsequently become a highly managed natural resource in the agricultural sector (Mermut & Eswaran 2001). Good soil quality which may be explained as "a concept used to characterise the usefulness and health of soils" must be maintained and increased where possible to ensure that both crop quality and yield are sustained (Mermut & Eswaran 2001). The soil quality concept is considered to be a compound measurement of different factors (Mermut & Eswaran 2001). A wide variety of soil quality indicators may be used, for example measurements of organic matter, water infiltration, aggregation, pH, microbial biomass, forms of nitrogen available, bulk density, topsoil depth, conductivity or salinity and available nutrients (Karlen *et al.* 1997). All of these factors are known to have a strong influence on various soil processes and overall functioning.

One of the largest constraining factors to soil quality in Africa is moisture stress, with only 14% of Africa being considered to suffer from minimal or no water stress (Eswaran *et al.* 1997). Soil in Africa as a whole has suffered a severe degree of degradation, largely due to a lack of understanding and increasing human pressure on this resource (Dewitte *et al.*

2013). South Africa is considered to have a medium to high input soil system which includes the use of large-scale irrigation, fertilizer usage, control of weeds and pests is undertaken and the management of the soil environment requires the use of highly mechanized machinery (Eswaran *et al.* 1997). A significant amount of the country has soils which are classed as being part of prime agricultural land which produce crops of a high quality and are also able to be managed with relative ease. Should these areas become damaged or degraded, improvement of the soil management in these areas would allow them to rapidly return to their previous prime state.

1.3.2 The Role of Soil in South African Citrus

Soils are an essential part of ecosystems as they provide a medium for plant growth, recycle nutrients and organic wastes, provide a habitat for soil organisms and are involved in water supply and purification (Brady & Weil 2000). Various particles make up the structure of soil, with the principle components being sand, silt and clay particles (Brady & Weil 2000). In terms of crop production, soils are important for the absorption and infiltration of water, nutrient retention and cycling, weed and pest suppression, harmful chemical detoxification, carbon sequestering and finally the production of food and fibre (Cornell Soils Health Training Manual 2009). In South Africa, citrus trees grow at their optimum in light, well-drained soils which are generally sandy loam soils (Wellington 1955). Generally, soil mineral deficiencies are uncommon in South Africa, or are easily rectified through the addition of these minerals to the soil (Wellington 1955). A neutral to alkaline pH in the soil of orchards has been found to be optimal.

1.3.3 Other Abiotic Factors Influencing Citrus Growth

Citrus trees are able to grow in a wide variety of areas in South Africa (Wellington 1955; CGA Annual Report 2011). The temperature limitation for these trees ranges between 13 and 37 °C, with mature trees being able to survive temperatures as low as -5 °C when they are dormant, although growth of any kind is extremely limited at temperatures lower than 13 °C (Spiegel-Roy & Goldschmidt 1996; Wellington 1955). The inability to produce undamaged fruit at low temperatures is one of the most important factors that limit the geographical distribution of citrus trees (Spiegel-Roy & Goldschmidt 1996). The best quality sweet

oranges are however, produced within the temperature range of 22-31°C, which makes South Africa an excellent area in which to produce this tropical fruit (Wellington 1955). In addition to fairly warm temperatures, citrus trees require an extensive amount of water, particularly during the blossoming and fruit-setting stages of growth from August to November (Wellington 1955; Mather 1999). As a result of this, large scale production of citrus fruit is only possible in orchards which have irrigation systems as the summer rainfall at this time is unpredictable (Wellington 1955; Mather 1999). Access to water can be a very limiting factor for farmers, but it has also been noted that a slightly drier soil which is found to occur between February and June often results in improved fruit quality (Wellington 1955).

1.3.4 The Importance of Soil Abiotic Factors on Insects Pupating in the Soil

The value of soil-dwelling insect pupation studies examining the impact of various soil factors can be seen by the number of studies that address this topic (e.g. Eskafi & Fernandez 1990; Murray & Zalucki 1990; Rickelmann & Bach 1991; Ande 2004; Dimou *et al.* 2003; Ellis *et al*. 2004; Hulthen & Clarke 2006; Chen & Shelton 2007; Zheng *et al*. 2011). The majority of these deal with manipulating soil factors such as soil texture class, soil moisture content, soil compaction and temperature, either in isolation or in combination with each other (Eskafi & Fernandez 1990; Rickelmann & Bach 1991; Ellis *et al*. 2004; Hulthen & Clarke 2006; Chen & Shelton 2007; Zheng *et al*. 2011). Moisture impact studies are particularly prevalent as this appears to be one of the abiotic factors to have the most influence on insect pupation (Eskafi & Fernandez 1990; Rickelmann & Bach 1991; Ellis *et al*. 2004; Hulthen & Clarke 2006; Chen & Shelton 2007). Pupation depth and pupal survival are the most commonly measured variables (e.g. Alyokhin *et al.* 2001; Dimou *et al.* 2003; Hulthen & Clarke 2006; Chen & Shelton 2007), most likely due to the potential difficulty in observing certain pre-pupation behaviours such as larval wandering, and in some cases pupal cocoon formation. Larval choice for particular environmental conditions has been determined, such as shaded environments (Alyokin *et al.* 2001) and particular moisture levels (Rickelmann & Bach 1991) and these preferences are thought to influence pupal distribution in the field.The principle interest in this work is often in the influence of soil factors on the general biology and behaviour of the species. Most of the species investigated are pests of agriculture or horticulture (Murray & Zalucki 1990; Ellis *et al*. 2004; Hulthen & Clarke 2006; Chen & Shelton 2007; Zheng *et al*. 2011) where the goal is to improve the current

understanding of the pest biology and determine whether any of these factors may be used to provide additional cultural control for the pest. This information can however, be used for other purposes as seen in the work done by Ande (2004) where the pupation behaviour of *Cirina forda* Westwood (Lepidoptera: Saturniidae) was investigated as the pallid emperor moth is an important food source for many people in Nigeria and there was a need for the correct management of the species. The influence of these abiotic factors varies between species and will be discussed in more detail in each of the relevant chapters, as well as the potential implications of these abiotic factors on EPFs and EPNs for the control of FCM.

1.3 AIMS OF THE STUDY

The current knowledge on FCM behaviour, biology and survival of pre-pupal and pupal life stages is limited and knowledge of pest biology is a key aspect to any biological control programme (Georgis *et al*. 2006). An improved understanding of these factors would be highly advantageous in order to improve FCM control in citrus orchards. Since the focus of FCM control has turned more toward attacking the soil-dwelling life stage of the moth in recent times, a more comprehensive knowledge of pupation would be useful as a baseline for understanding FCM biology and could be used to great effect both for developing a direct control technique through soil manipulation, but also through determining where and when the use of the soil biological control agents (EPF and EPNs) is likely to be most effective for FCM control. This information would also be used to determine the importance of orchard sanitation in different soil environments.

The aims of the study were to measure 1) time wandered prior to pupation site selection, 2) distance wandered before site selection, 3) time taken to form pupal cocoon, 4) depth of pupa in soil, 5) the orientation of the pupa in the soil profile (horizontal or vertical), 6) the duration of the pupal life stage and 7) the survival of pupae or eclosion success, in order to better understand the behaviour, biology and survival of FCM. All of these factors were measured while manipulating the 1) soil texture class (Chapter 2), 2) ground cover and shading (Chapter 3), 3) soil compaction (Chapter 4), 4) air temperature (Chapter 5), 5) and soil moisture (Chapter 6). Therefore, this work will indicate the conditions preferred by FCM for pupation in the soil, as well as improve our understanding of how this biological process takes place in order to use information to better control this important citrus pest through answering the questions posed above.

2

The influence of soil texture class on false codling moth (FCM) pupation

2.1 INTRODUCTION

The nomenclature of soil taxonomy follows a very similar pattern to that of biological organism classification and a comprehensive review of which can be found in Brady & Weil (2000). The term ‘soil class’ is often used as an all-encompassing phrase, but more accurately refers to the soil phase, which is the equivalent of the species category in fauna or flora nomenclature. The phase of the soil applies more to field surveying, where basic soil identification is done using a ‘feel’ test prior to sending samples for a more accurate laboratory analysis. The soil phase is not an officially recognised group in its own right, but is rather a subdivision of the soil series. For the purposes of this study the various ‘classes’ of soil are more accurately referred to as soil texture classes. These are defined by using particle size analysis, which determines the various percentages of soil separates that comprise the fine earth faction (Brady & Weil 2000). Twelve major soil textural classes are recognised (Brady & Weil 2000). These are calculated by using the universally recognised soil textural triangle after the percentages of sand, silt and clay have been determined in the particular soil sample (Brady & Weil 2000). The value of improved knowledge of different soil texture classes in isolation has to do with the influence of the sand, silt and clay components on soil-dwelling insect pupation (Ellis *et al.* 2004; Ande 2004; Chen & Shelton 2007). Basic physical properties of sand, silt and clay have been provided by the International Society of Soil Science (Table 2.1).

Table. 2.1 Basic properties of soil separates in the fine earth faction (adapted from Brady & Weil 2000)

	Sand	**Silt**	**Clay**
Particle Size	< 2 mm; > 0.05 mm	< 0.05; > 0.002 mm	< 0.002 mm
Feel	Gritty	Smooth/silky	Sticky
Visibility of Individual Particles	Visible to naked eye	Not visible	Not visible
Specific Surface Area	Low	Medium	Very large
Drainage	Excellent	Medium	Limited
Cohesiveness	Non-cohesive	Limited cohesion*	Very cohesive

* Amount of cohesion is dependent on the amount of clay film which adheres to individual silt particles

A variety of agricultural insect pest species spend a portion of their life cycle in the soil (Villani & Wright 1990). Edaphic-dwelling insects whose larvae pupate in the soil often show a unique set of premetamorphic behaviours and characteristics when constructing the pupal cocoon or an even more complex microhabitat in which to pupate and ultimately, from which to successfully eclose (Dominick & Truman 1984; Danks 2002). Both internal (such as chemicals and hormones) and external (such as the abiotic environment) factors are known to influence such behaviours (Dominick & Truman 1984). Entomopathogens, such as entomopathogenic nematodes (EPNs), and entomopathogenic fungi (EPF) occur naturally in the soil environment and show potential to be effective biological control agents (Barbercheck 1992). However, the soil profile is a highly complex environment and its influence on both pest and agent is often not well-understood (Barbercheck 1992). In order to increase the potential for successful biological control, it is important to improve the understanding of the environment, the pest, the biological control agent and the potential interactions between all three (Villani & Wright 1990; Barbercheck 1992).

The first aim of the study reported in this chapter was to determine the influence of different soil texture classes on 1) false codling moth (FCM) larval wandering time, 2) distance wandered by FCM larvae prior to pupation site selection 3) time taken by FCM to spin the protective pupal cocoon, 4) depth of pupation, 5) orientation of pupation (horizontal or vertical in the soil profile), 6) amount of time taken to eclose and 7) eclosion success. The second aim was to determine whether FCM larvae would show a preference for a particular soil texture class when allowed a choice.

2.2 MATERIALS AND METHODS

2.2.1 FCM Insect Cultures

Jars of larval FCM were obtained from either the culture held at River Bioscience in Addo, South Africa, or from the Waainek Research Facility culture at Rhodes University. The larvae were raised on an artificial diet that has been specially formulated for FCM, the production of which was reviewed by Moore *et al.* (2014). The bulk diet dry mix consists of maize meal (2000 g), wheat germ (200 g), Brewer's yeast (200 g), Nestle®Nespray instant milk powder or casein (36.5 g), Nipagen (15 g) and sorbic acid (6.5 g). The Nipagen and sorbic acid have anti-microbial properties to reduce the possibility of microbial outbreaks in the cultures. The casein is usually replaced with milk powder due to the reduction in production costs and moth production being unaffected by this change. When preparing the diet for rearing FCM, 350 ml honey jars were used to which 47 g of diet and 45 ml of distilled water (unmixed) were added. Cotton wool was used to create a stopper for the jars. The jars were autoclaved before use, which simultaneously cooked and sterilized the diet.

2.2.2 Soil Collection and Analysis

Two fieldtrips were undertaken to obtain three different soil texture classes: one was to be high in sand content, another high in silt, and a third high in clay content. The first fieldtrip, in April 2013, was to the Sundays River Valley, Addo, South Africa and the second was to orchards in the Kat River Valley, in July 2013. Both sites are in the Eastern Cape Province of South Africa and are key citrus production areas where FCM is a prominent pest. Five orchards that were thought to contain one of these three soil texture classes required were visited. The soil was collected from directly beneath the citrus trees and the collection area was approximately 10 cm in depth. The soil was placed into large sealed plastic containers (30 x 50 x 34 cm). The GPS co-ordinates of the area from which each sample was taken were recorded, with sample one at 33.607745 °S; 25.66598 °E, sample two at 33.49373 °S; 25.69477 °E and sample three at 32.69407 °S; 26.61404 °E, as well as two additional samples not used in the study due to low clay content. In the laboratory, a 300 g sample of soil from each site was sieved, placed into a soil bag and was sent to SGS Laboratories in Somerset

West for particle size analysis. From the analysis results, the percentage of each soil component (sand, silt and clay) was provided and the soil texture class could then be calculated using the soil texture triangle. It was determined that three different soil texture classes were suitable for use in the study, namely: sandy loam, silt loam and silt clay loam.

Table 2.2 Soil texture class determined through particle size analysis by SGS Laboratories

Sample	Sample Area	Clay %	Silt %	Sand %	Soil Texture Class
Sample 1	Sundays River Valley	8	20	72	Sandy Loam
Sample 2	Sundays River Valley	16	59	25	Silt Loam
Sample 3	Kat River Valley	32	52	16	Silty Clay Loam

Once the soil texture class had been determined, it was possible to measure other soil properties such as pH, conductivity (electrical conductivity providing an indication nutrient availability) and salinity (soil salt content). All measurements were made using the Waterproof Multiparameter PCSTestr 35 (Eutech Instruments Pte. Ltd., Singapore). The soils were prepared in 1:5 ratio of soil to distilled water and thoroughly mixed for 30 minutes before the readings were taken (Table 2.3).

Table 2.3 Soil properties of the three different soil texture classes

Soil Texture Class	pH	Conductivity (µS)	Salinity (ppm)
Sandy Loam	7.80	136.7	68.7
Silt Loam	8.55	187.7	95.0
Silty Clay Loam	6.48	173.2	85.0

2.2.3 Preparation of Soils

The soils were brought back to the laboratory for preparation before use in the study. Each soil sample was labelled and kept in a Controlled Environment (CE) room at a mean of 25.3 °C (± 0.8 °C) and a mean relative humidity (RH) of 45 % (± 10.26 %). The soils were

allowed to air dry in this manner for a minimum of two weeks prior to the start of the first experiment. All soils were sieved using a 2 mm x 2 mm sieve to remove unwanted debris and break up large clumps of soil. Soil particles larger than 2mm in size are classified as gravel which was not required for this study (Brady & Weil 2000). The soil was then sterilized in order to eradicate any living organic matter or pathogens through the use of an autoclave (DaiHan Scientific Wiseclave® or Sturdy SA-300VL), which uses moist heat and high pressure for this process. Based on the recommendations of Trevors (1996) the soil was autoclaved at 121 °C for a minimum of 20 minutes at 1.1 atm in glass beakers covered with aluminium foil, or for up to an hour for soil weighing over 500 g. The previous sieving and drying of the soil improved the sterilization as this allowed the heat to penetrate the soil more thoroughly (Trevors 1996). The soil property measurements were taken using autoclaved soil, as this prepared soil would be what FCM larvae were exposed to. Post autoclaving, the soil was placed into plastic containers that had been sterilized using sodium hypochlorite (3.5 % concentration) and distilled water solution. The soil layer was 3 cm in depth throughout the container, measured using a standard ruler. Further air-drying of the soil occurred as the soil stood in the controlled environment (CE) room for a period of 24 hours prior to use in the experiment.

2.2.4 Preparation of Artificial FCM Diet

In order to determine the behaviour of FCM larvae when pupating, it was imperative to create as natural a setting as possible. To do this, individual vials of artificial FCM diet were prepared for each larva to simulate the development of the larvae inside a citrus fruit. This was the same bulk diet mix used for rearing FCM in the cultures, as previously described. Glass vials of the diet were prepared by adding 4.6 ml of distilled water (dH_2O) to 4.6 g of the diet. This combination was not mixed, but rather left to stand for 5 minutes to allow the water to filter through the diet. The vial was then sealed using a tinfoil cover and placed into the autoclave for a period of 20 minutes for sterilization of the vial and the diet. Once removed from the autoclave, the diet was allowed to cool before the larva was added. Each larva was taken from a larger jar of the same artificial diet which contained approximately 150 – 200 larvae. Early fifth instar or late fourth instar larvae were obtained and identified by their colouration and head capsule size (Daiber 1979b). In order to ensure that the exterior of

the larvae were uncontaminated, each one was rinsed in a 1:1 ratio of sodium hypochlorite (3.5 %) and dH_2O solution for three seconds, followed by a rinse in distilled water alone before being placed onto paper towelling to dry off. The larva was then placed onto the diet and the glass vial was sealed using a plastic stopper which had been cleaned in the 1:1 sodium hypochlorite and dH_2O solution to ensure its sterility. The vials were placed into the CE room and 24 hours later each vial was inverted as they would later be suspended above the soil in this way. The FCM larvae were allowed to develop within the diet. When the larvae showed indications of preparing for pupation by crawling out of the diet or, occasionally, starting to produce cocoon material, they were deemed ready for use in the experiments.

2.2.5 Pilot Studies

Pilot studies were essential in order to determine the most appropriate arena or tray size and the depth of soil to be used in the experiments.

2.2.5.1 Tray Size

For the tray-size pilot study three different tray sizes (small, medium and large) were selected for testing. The dimensions of the small tray were 28 x 20 x 5 cm, the medium tray, 38x27x8 cm, and the large tray, 40 x 30 x 9.5 cm. Soil, which had been prepared as described above, was placed into each of these trays to a depth of 5 cm. Test trials using mature fifth instar FCM revealed that the small tray was not large enough, with larvae often leaving the tray when in search of pupation sites. The medium tray was more suitable than the small tray, but the large tray provided the largest arena for larvae to wander, and also could be viewed in its entirety in the camera frame. This was an important consideration as larval behaviour was to be filmed. The large tray was selected for use as it allowed all the larvae in the pilot study to wander within the confines of the tray and thus the larval behaviour could be more clearly monitored within the frame of a video camera.

2.2.5.2 Soil Depth

Four different soil depths were tested for depth of pupation pilot studies. These were 1 cm, 3 cm, 5 cm and 10 cm depths. The studies were done using the medium-sized soil trays for the 1 cm, 3 cm and 5 cm trial experiments and using deeper circular plastic tubs for the 10 cm depth trial. In all four cases, FCM larvae pupated on the surface of the soil without burrowing into it. The pupal cocoons were formed in the upper 2 – 4 mm of the soil of each soil texture class. As such, the 5 cm and 10 cm depths were deemed as being unnecessarily deep. The 1 cm soil depth was considered to be too shallow, not allowing FCM larvae the possibility of burrowing into the soil for this or subsequent experiments where changes in other abiotic factors could potentially increase or decrease the pupation depth. The 3 cm soil depth was deemed to be most appropriate for these experiments.

2.2.6 Soil Texture Classes Experiment

2.2.6.1 General FCM Biology, Behaviour and Survival Experiments

All experiments were conducted in a CE room on a 12-hour day:12-hour night schedule with controlled temperature and relative humidity. For these experiments, a Canon Legria FS406 video camera was used to film the FCM biology and behaviour when dropping down into the soil, wandering, selecting a suitable site for pupation and formation of the pupal cocoon. The camera was set up on a tripod 40 cm from the edge of the plastic tray of soil, at a height of 60 cm and at an angle of 30 ° to ensure that the entire contents of the tray could be filmed. Once recording was initiated, the inverted individual jars of diet containing the mature fifth instar FCM larvae were placed into a retort stand 30 cm above the soil. The larval behaviour was then filmed up until the point that the basic pupal cocoon had been spun and the larval movement inside the cocoon was no longer visible. The position of the pupal cocoon was then determined either by eye or by using the video footage. A numbered aluminium foil marker was placed into the soil, close to the pupal cocoon to demarcate its position in the soil. Following checks at 24 hours and 48 hours after cocoon formation to ensure that the larvae had not abandoned the cocoon in favour of forming another cocoon in an alternate location, the pupal depth and orientation were recorded. The tubs of soil were

covered with mesh netting (to ensure that eclosed adult FCM could not escape) and were checked on a daily basis for evidence of eclosion. Successfully eclosed adults were easily captured using glass or plastic vials as they tended to stay close to the pupal cocoon after eclosion in order for the wings to dry. Once captured in the vial, the sex of the moths were determined and recorded. Female FCM moths are typically larger than males, but male moths have a distinctive anal tuft at the end of the abdomen and black tufts on the hind legs (Newton 1998; van den Berg 2001). These characteristics are clearly visible with the naked eye. The amount of time taken to eclose and eclosion success was recorded for each moth. At the end of the experiment, all non-eclosed pupae were examined to determine whether mortality had occurred at the larval or pupal stage and whether a possible cause of death could be established. Thirty larvae were pupated per soil texture class and each of these was treated as an individual replicate.

Observation of the video footage using VLC media player version 2.1.5 (2014) (and a previous version of the same player in 2013) was able to provide the data for the amount of time the FCM larvae wandered on the soil surface before selecting a pupation site and the time taken to spin the pupal cocoon; however, the distance travelled by larvae while wandering required additional processing. This was done by segmenting and cutting the relevant section of the video file which showed the larval wandering using the programme VirtualDubMod 1.5.10.3 (www.virtualdubmod.org). Once the section of video had been cut, it was saved as an uncompressed .avi file in compatibility mode. This simultaneously converted the video file from a .mod file to .avi file that could was then used in the programme Fiji (http://fiji.sc/Fiji). This free software is a package designed for processing images using Java, Java3D and a number of additional plugins. The package is an open source project which was previously known as ImageJ or ImageJ2 and was originally designed for use by those in the life sciences field for data processing and analysis. Fiji allowed the active tracking of FCM larvae using the programme plugin, MTrackJ. The measurement was calibrated using a standard 30 cm ruler that had previously been placed on the side of the soil trays for each video file. The known measurement of the ruler allowed for the programme to determine the number of pixels that were the equivalent of 10 mm. The movement of the FCM larva was then tracked through each frame of the segmented video file using the straight (segmented or freehand) line function. The length of movement of each individual track and the cumulative total was then provided by the MTrackJ measurement function and recorded for subsequent analysis.

2.2.6.2 Choice Experiments

In the soil texture class choice experiments, the possibility of FCM larval choice, when larvae were provided with the option of different soil texture classes in which to pupate, was explored. The medium-sized plastic trays were used but in this case, the trays were divided into two arenas by attaching 2 mm thick cardboard at a height of 3 cm to the trays using a hot glue gun. After the division of the trays into two clearly demarcated sections, each section was filled to the top of the cardboard divider with different soil texture classes. Three scenarios were provided: sandy loam versus silt loam, sandy loam versus silty clay loam, and silt loam versus silty clay loam. The FCM larvae were placed onto a plastic Petri dish that was suspended from the retort stand using an elastic rubber band, 20 cm above the soil. The Petri dish was suspended over the centre of the soil tray in such a way that the larvae would have equal opportunity to drop into either of the soil texture classes. Ten larvae were pupated per soil choice experiment and this was replicated three times for each of the soil choice experiments.

2.2.7 Statistical Analysis

The statistical analyses were performed using Statistica Version 10 (StatSoft, Inc. 2011. The datasets were tested for normality using the Shapiro-Wilk's W Test. When data transformation did not alter the normality result, the non-parametric Kruskal-Wallis ANOVA test was used to compare multiple independent samples. If a significant difference was detected, a multiple comparisons of mean ranks test for all groups was run in order to determine where these differences were occurring. These results were graphically represented using boxplots. Categorical data, including all choice tests were analysed using Chi-square tests. If a significant difference was found to occur in Chi-square tests with multiple categories, then each group was examined in order to determine where the significance lay.

2.3 RESULTS

2.3.1 General FCM Biology, Behaviour and Survival Experiments

The median distance wandered on the soil surface by FCM larvae prior to selecting a pupation site, was not significantly affected by soil texture class ($H_{(2,90)} = 0.310$; $p = 0.857$). Overall, the wandering distance was short, with the median for each of the soil texture classes not more than 20 mm; however, variability between individual larvae was high (Fig. 2.1).

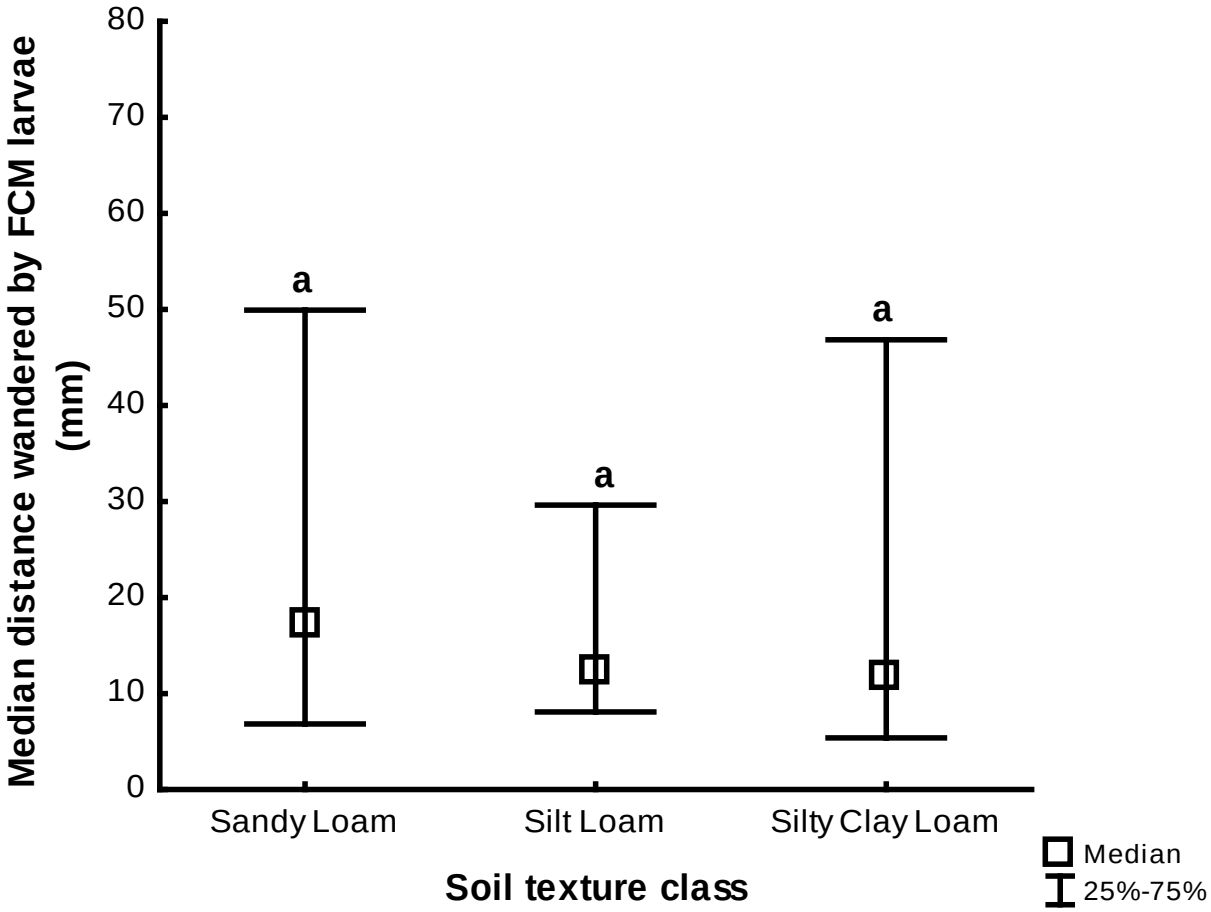

Figure 2.1 The median distance wandered by FCM larvae on the soil surface prior to pupation site selection for the three different soil classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A comparison of the three different soil texture classes and the median time wandered prior to selecting a pupation site revealed no significant difference between the three different soil texture classes ($H_{(2,90)} = 3.902$, $p = 0.213$). This indicates that soil texture class in isolation has no influence on the amount of time that FCM larvae spend wandering on the soil. Pupation sites were selected rapidly, with the median time wandered being less than three minutes (Fig. 2.2).

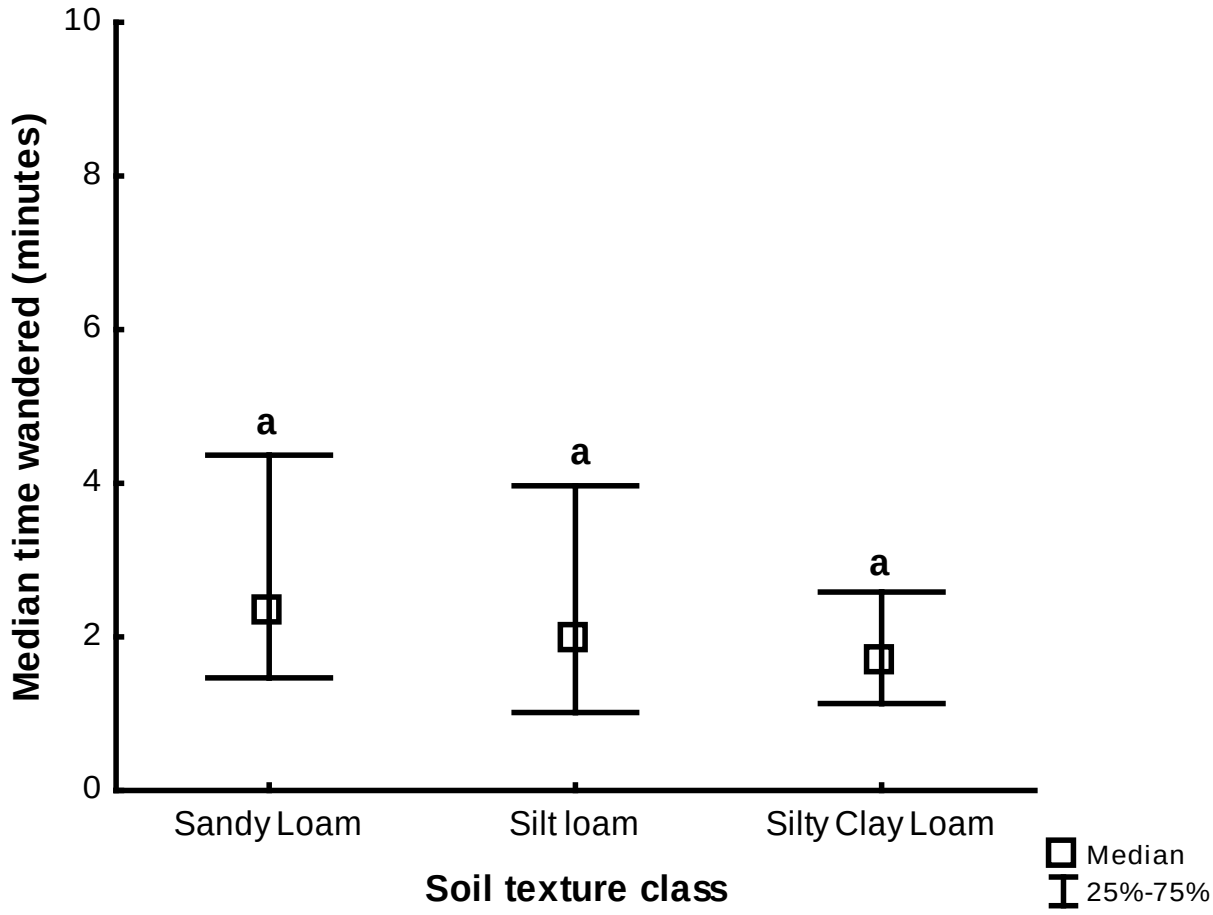

Figure 2.2 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection for the three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant differences were found in the length of time FCM larvae spent forming their pupal cocoons between the three different soil classes ($H_{(2,83)} = 7.092$; $p = 0.701$). Of the 90 larvae used in the experiment, only 83 formed pupal cocoons in the soil. The other six larvae formed naked pupae without a cocoon, hence the reduced sample size. The median amount of time taken to form the pupal cocoon was about 40 minutes (Fig. 2.3).

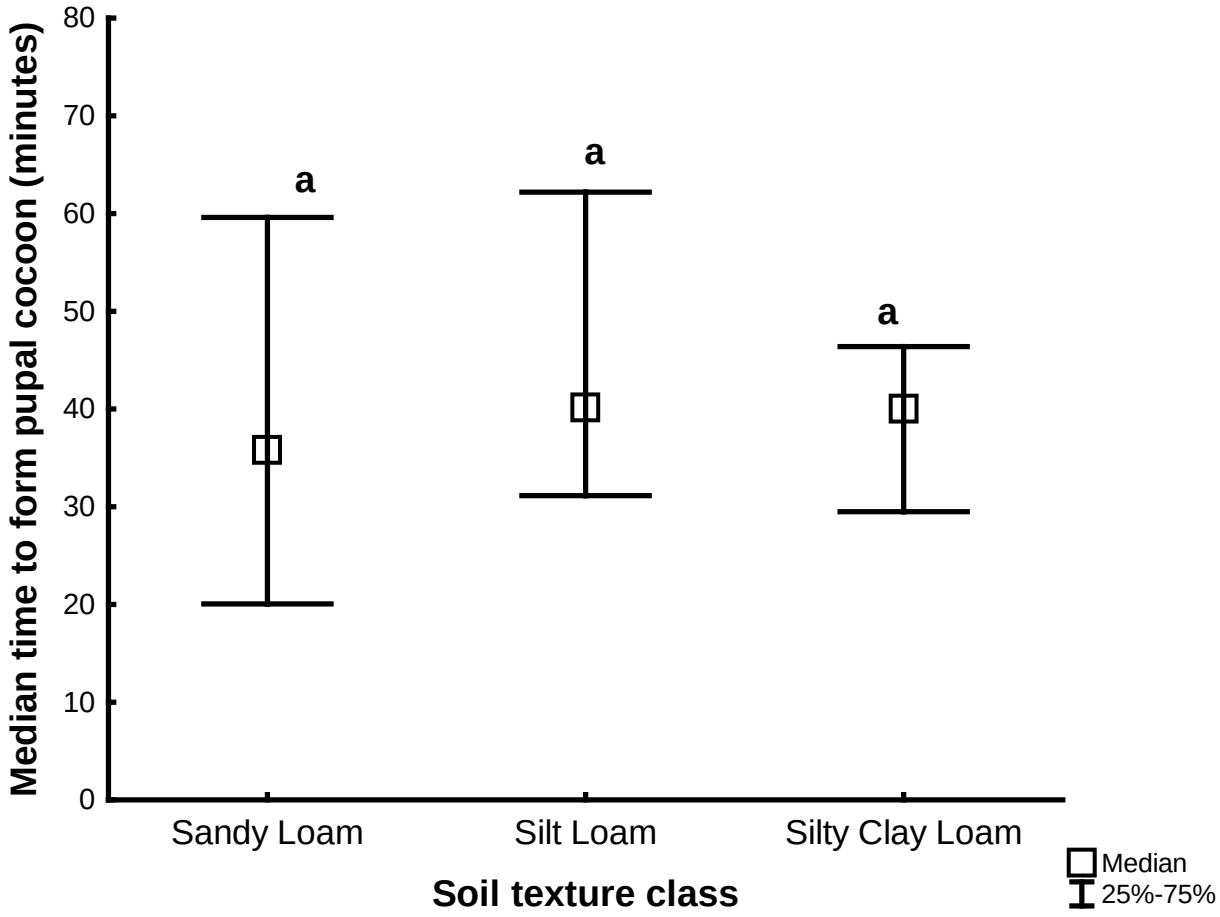

Figure 2.3 The median amount of time taken for FCM larvae to spin pupal cocoons for the three different soil texture classes (n = 83). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

FCM larvae either spun pupal cocoons when pupating or formed naked, exposed pupae on the soil surface. A significantly higher number of FCM larvae spun a pupal cocoon (Chi-square = 67.6, df = 1, $p < 0.0001$) (92 %) than pupated without one (8 %) (Fig. 2.4). Pupation without a cocoon resulted in a 100 % mortality of the seven pupae, compared with the 70 % of successfully eclosed FCM adults from pupal cocoons, regardless of soil texture class.

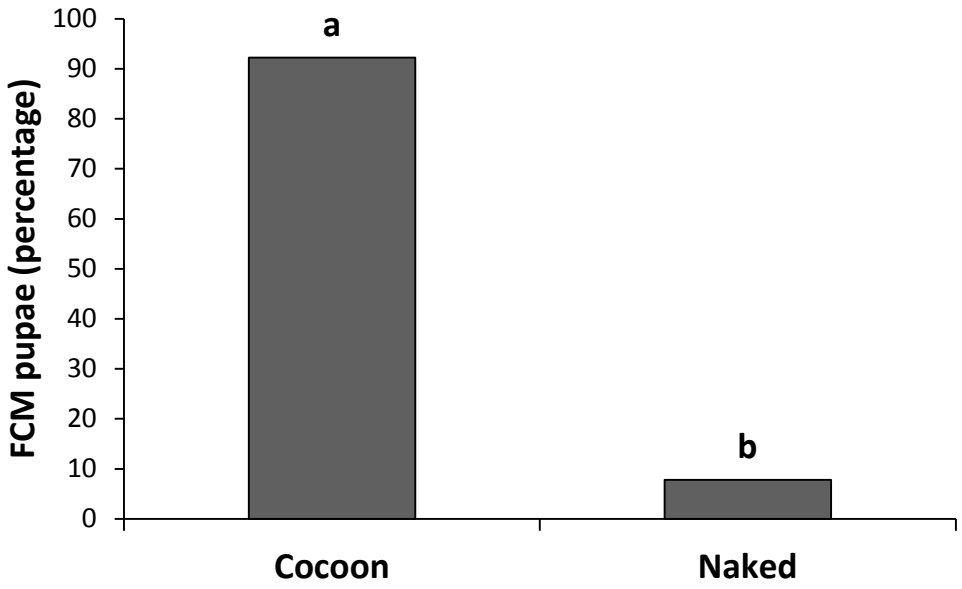

Figure 2.4 The percentage of FCM pupae that pupated inside a pupal cocoon compared with naked pupae with no cocoon (n = 90). (Different letters denote significant differences, Chi-square test, $p < 0.05$).

Both the depth of pupation and the orientation (horizontal or vertical) of the pupal case formed in the soil profile were measured for all three soil texture classes. The median depth of pupation was similar for all three of the soils, with a minimum of 0 mm and a maximum of 5 mm. Pupation depth was recorded for all pupae, regardless of whether a pupal cocoon was spun or not. A significant difference was found between the median ranking of sandy loam and silty clay loam ($H_{(2, 90)} = 8.074$; $p = 0.018$) (Fig. 2.5). However, despite a very similar ranking to silty clay loam, no difference was found between sandy loam and silt loam, or silt loam and silty clay loam. All pupal cases formed were horizontally orientated in the soil profile.

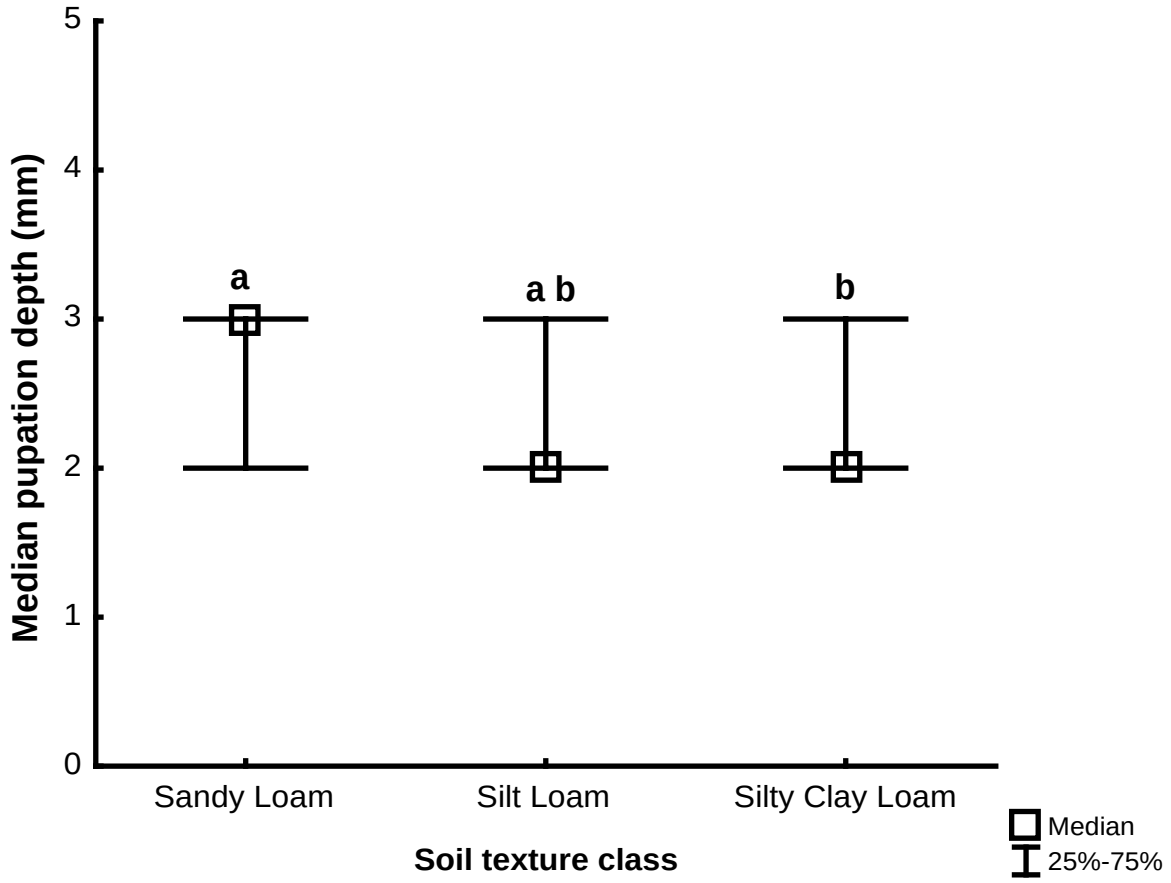

Figure 2.5 Median depth of FCM pupation in three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Only 64 of the original 90 FCM larvae which pupated were able to eclose successfully. The duration of FCM pupal development was recorded for the three different soil texture classes. A significant difference in the median time to eclosion was found between the silt loam and silty clay loam soil texture classes, with FCM adults from pupae formed in silty clay loam soil developing and eclosing significantly faster ($H_{(2,63)} = 17.311$; $p = 0.0002$). No significant difference was found in the median time to eclosion between sandy loam and the other two soil texture classes (Fig. 2.6).

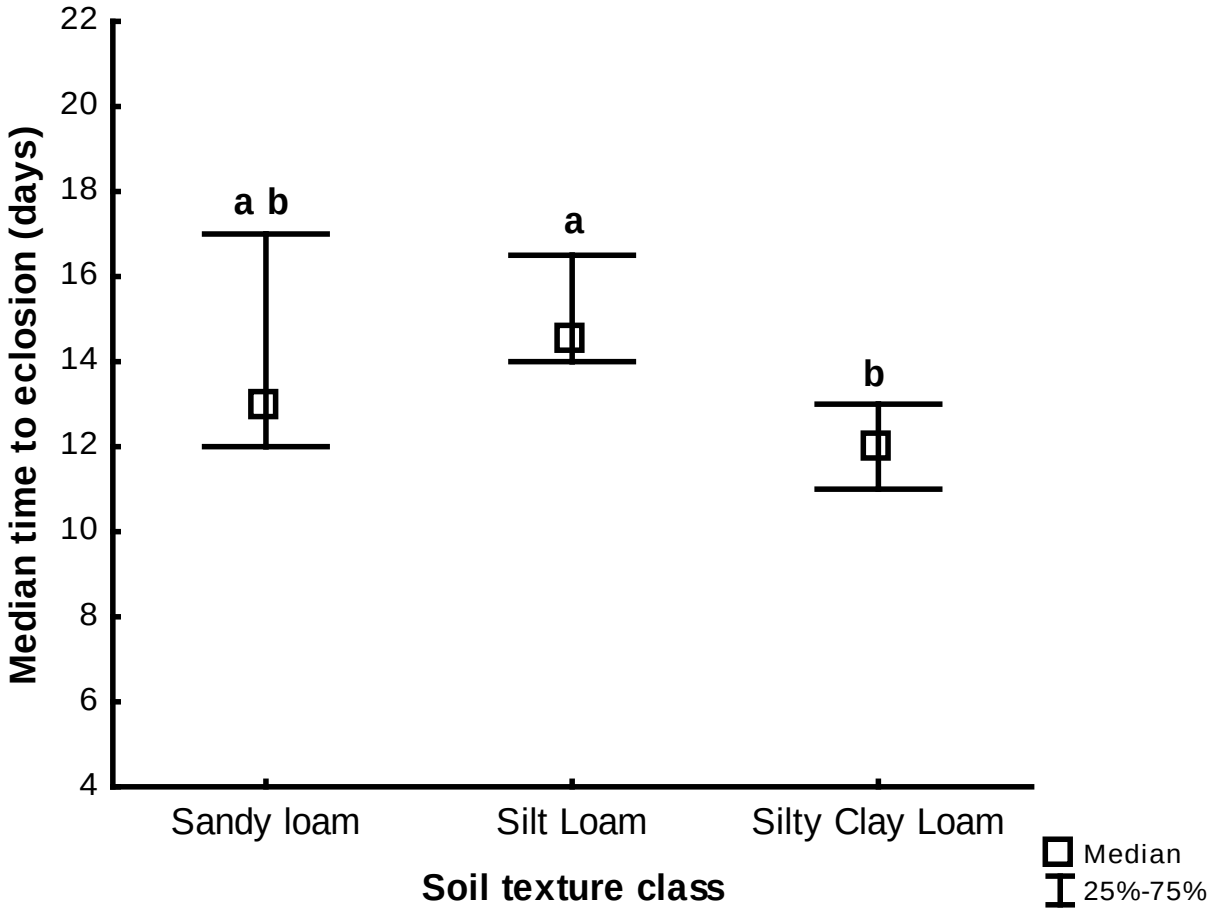

Figure 2.6 The median amount of time taken for adult FCM to eclose from the pupae formed in the three different soil texture classes (n = 63). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

During initial observations of eclosion behaviour it was noted the first moths to eclose from pupae of the same age were often female, thus it was important to establish percentages of males and females that had eclosed in order to determine a possible influence in the time to eclosion. The ratio of female to male FCM adults that were able to successfully eclose from the three different soil classes was examined: in the case of both sandy loam and silt loam more male moths than females eclosed while the reverse was found for silty clay loam (Fig. 2.7). When all three soil texture classes were combined, more male moths had eclosed (55.56 %) than female moths (44.44 %).

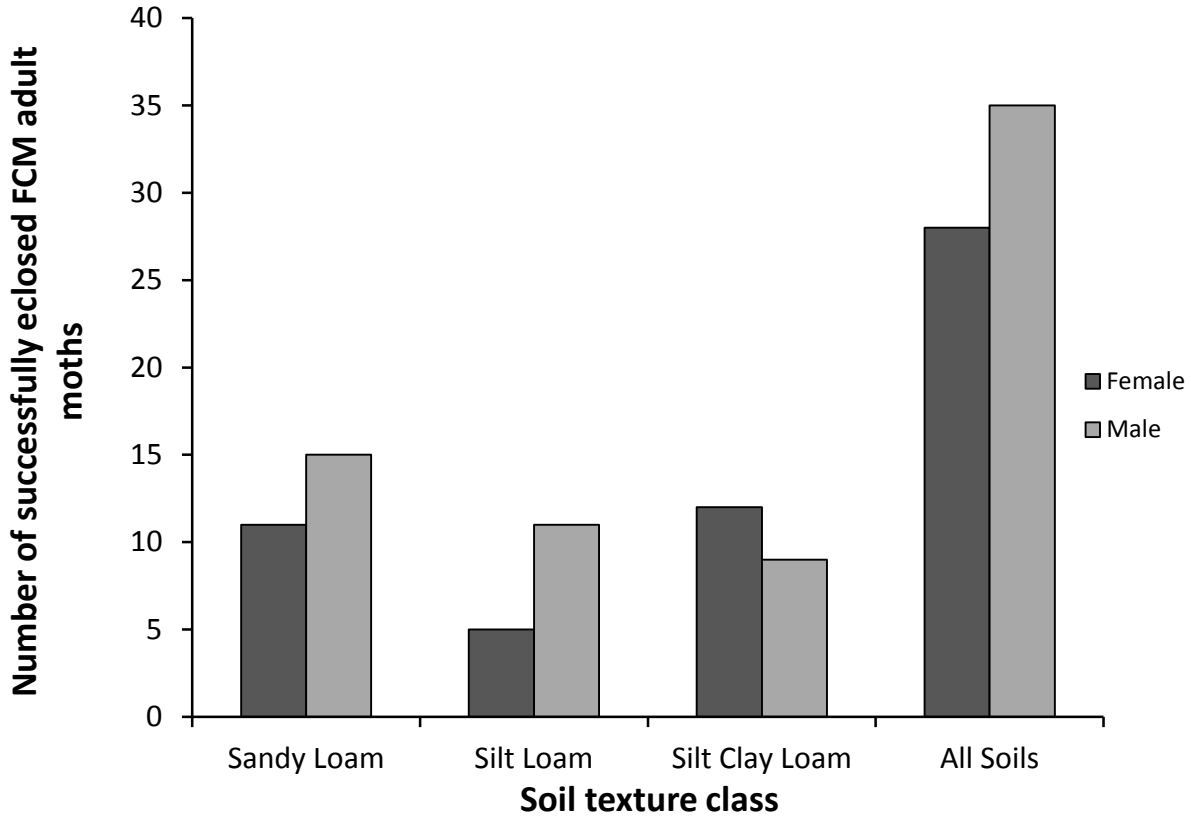

Figure 2.7 Comparison of male and female adult FCM successfully eclosed from the each of the three different soil texture classes and for all three classes combined (n = 63).

The median number of days to eclosion were compared for male and female FCM in order to determine if one sex eclosed more rapidly than the other. No significant difference was found between the two sexes (Mann-Whitney $U_{(28, 35)} = 416.0$, $p = 0.309$) (Fig. 2.8).

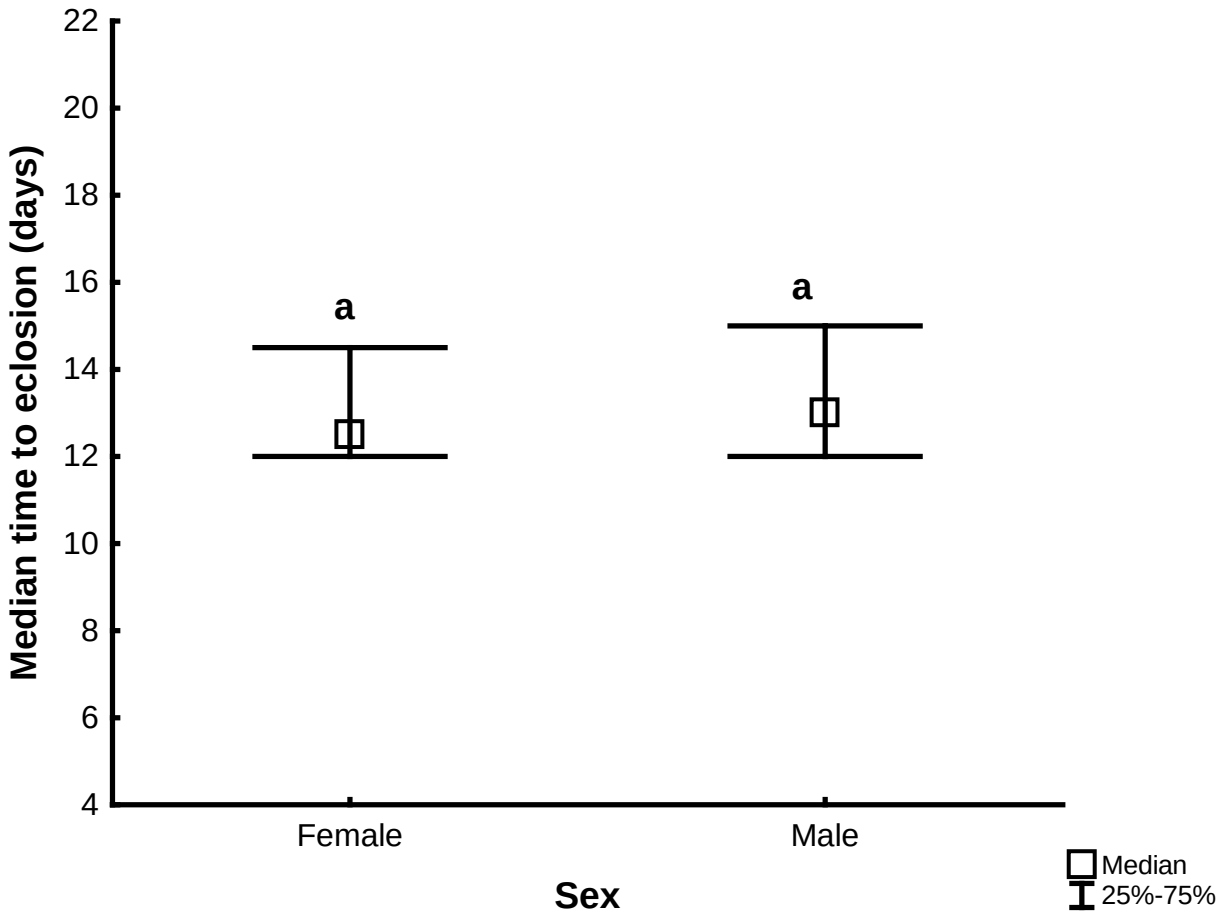

Figure 2.8 The median number of days to eclosion for male and female FCM (n = 63). Different letters denote significant differences (Mann-Whitney *U* Test, $p < 0.05$).

A significant relationship was found between soil texture class and eclosion success of FCM adults, with sandy loam having a significantly higher number of successfully eclosed adults than silt loam, but not silty clay loam (Chi-square = 10.058, df = 2, p = 0.006). Silt loam had the lowest eclosion success with only 57 % of the pupae produced in this soil giving rise to viable adult moths, while sandy loam soils had the highest amount of successful eclosion with 90 % and silty clay loam with 75 % (Fig. 2.9).

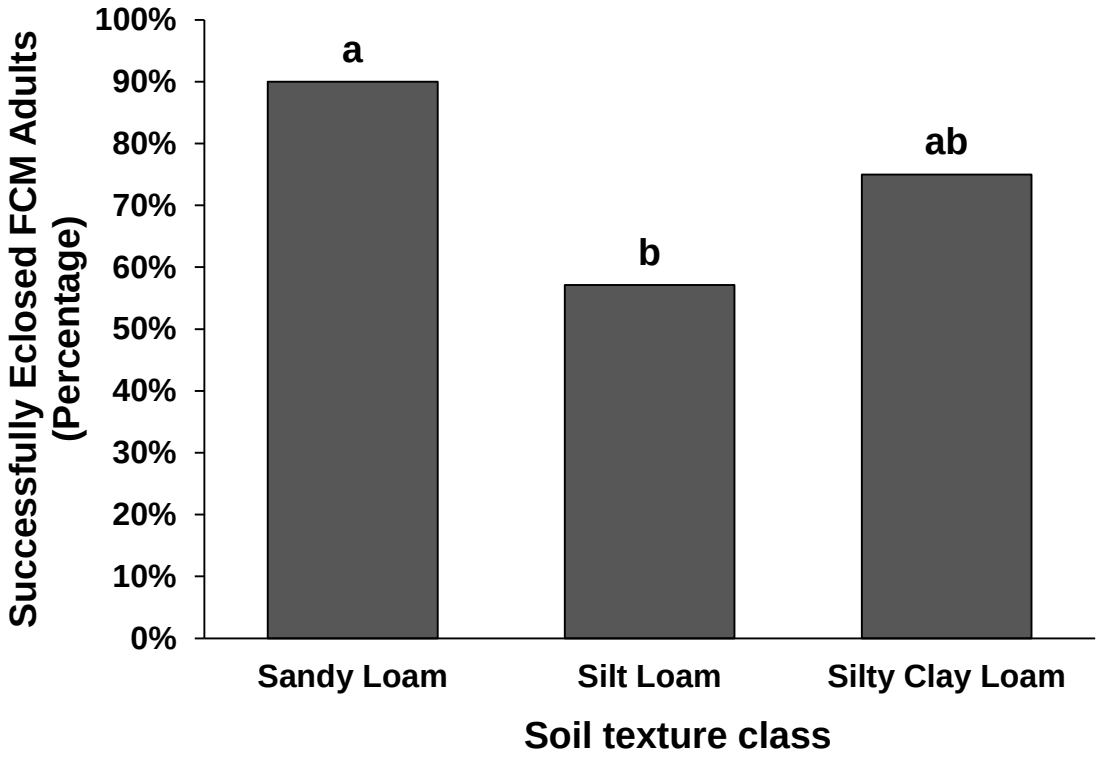

Figure 2.9 The percentage of successfully eclosed FCM adults from pupae formed in the three different soil texture classes (n = 63). Different letters denote significant differences (Chi-square test, $p < 0.05$).

2.3.2 Choice Experiments

These experiments allowed FCM larvae a choice of two soil texture classes. No preference was found when comparing sandy loam and silt loam (Chi-square = 0.133, df = 1, p = 0.715) (Fig. 2.10a). For sandy loam versus silty clay loam, the FCM larvae pupated significantly more frequently (70 %) in the latter soil texture (Chi-square = 4.800, df = 1, p = 0.028) (Fig. 2.10b). In the final comparison of silt loam and silty clay loam, no significant difference was found between the soil texture classes (Chi-square = 0.533, df = 1, p = 0.465) (Fig. 2.10c).

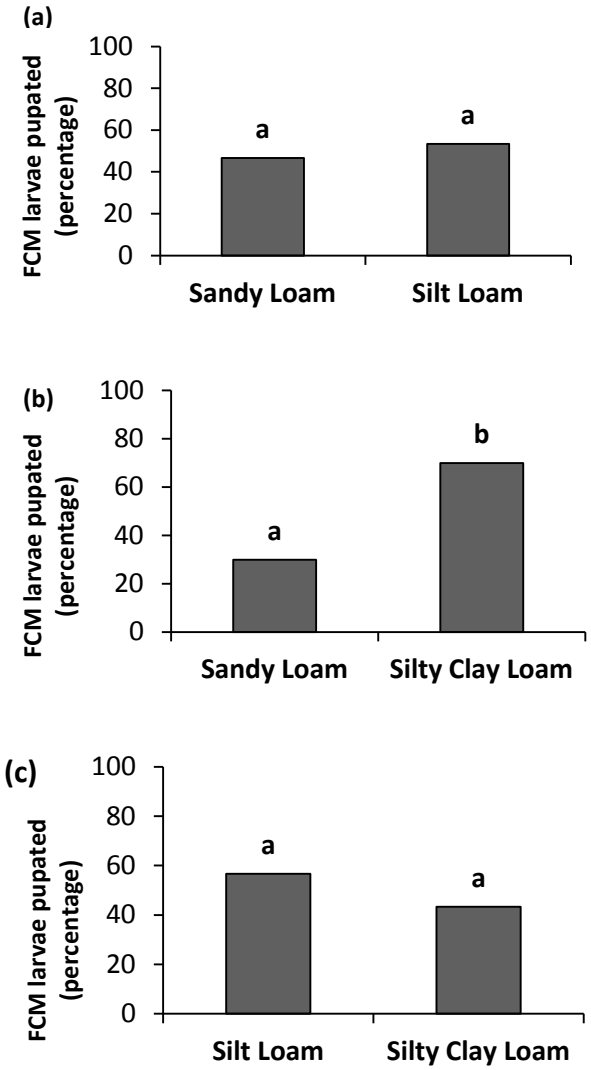

Figure 2.10 The percentage of FCM larvae pupating in soil texture class choice tests when allowed a choice between **a)** sandy loam and silt loam soils (n = 30), **b)** sandy loam and silty clay loam soil (n = 30), and **c)** silt loam and silty clay loam soils (n = 30). Different letters denote significant differences (Chi-square test, $p < 0.05$).

Larval wandering was noted in the choice study by determining the percentage of larvae that did so prior to selecting a pupation site. Overall, it was found that a significantly higher percentage of FCM larvae wandered on the soil surface before pupating than those that did not exhibit wandering behaviour (Chi-square = 21.511, df = 1, $p < 0.0001$) (Fig. 2.11). Larvae would either wander to a different soil texture class from the one they had dropped onto, or would wander on the surface of the original soils onto which they had dropped.

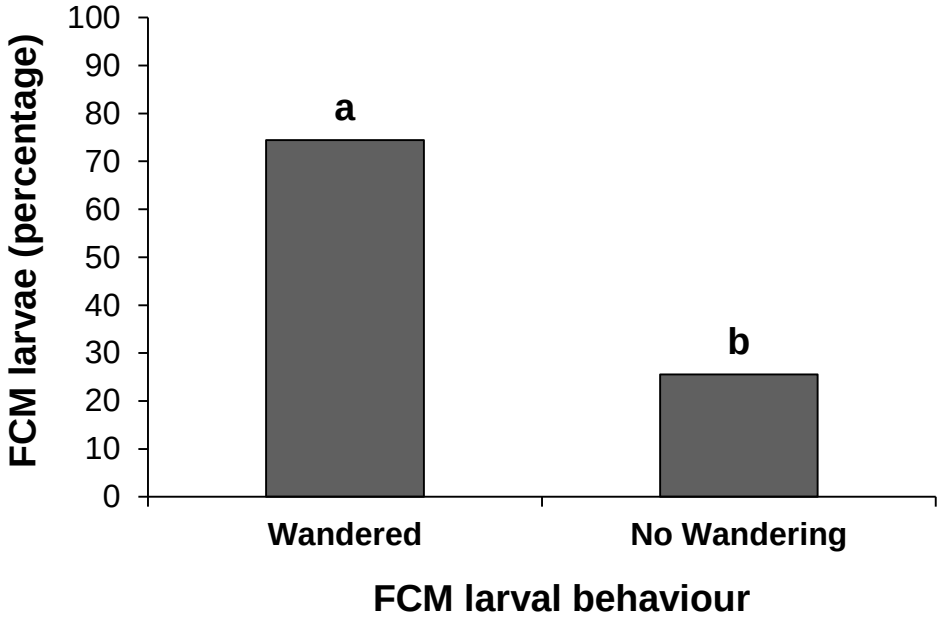

Figure 2.11 The percentage of FCM larvae that wandered on the surface of the soil prior to selecting a soil texture class for pupation when allowed a choice of two different soil texture classes (n = 90). Different letters denote significant differences (Chi-square test, $p < 0.05$).

For the choice experiments, no association was found between the soil texture class and eclosion success when comparing sandy loam versus silt loam (Chi-square = 0.182, df = 1, p = 0.67) (Fig. 2.12a). Sandy loam versus silty clay loam appears to be significant when observed visually, however statistically this was found not to be the case (Chi-square = 3.095, df = 1, p = 0.076) (Fig. 2.12b). No significant preference was found when comparing silt loam and silty clay loam (Chi-square = 0.40, df = 1, p = 0.841) (Fig. 2.12c).

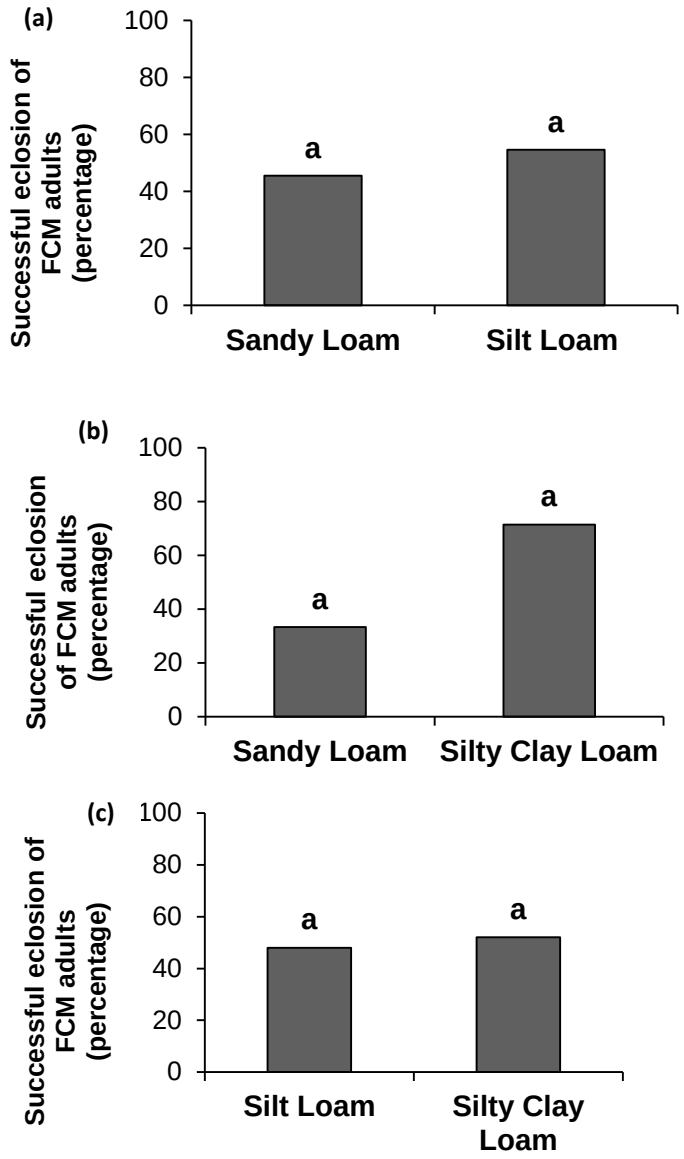

Figure 2.12 The percentage of FCM adults able to successfully eclose from pupae formed in soil texture class choice tests when allowed a choice between **a)** sandy loam and silt loam soils (n = 30), **b)** sandy loam and silty clay loam soil (n = 30), and **c)** silt loam and silty clay loam soils (n = 30). Different letters denote significant differences (Chi-square test, $p < 0.05$).

2.4 DISCUSSION

Soil texture class in isolation appears to have a fairly limited impact on the biological, behavioural, and survival variables measured for FCM. The short wandering distance of FCM larvae on the surface of the three soils would reduce the potential contact of the larvae with entomopathogens. However, this was variable with certain individuals wandering much further than the median. In the case of *Manduca sexta* L. (Lepidoptera: Sphingidae), this rapid cessation of wandering behaviour and moving on to the pupal cocoon formation phase has been recorded when larvae were able to detect a suitable substrate in which to pupate (Reinecke *et al.* 1980). Larval wandering could vary from hundreds of feet to almost instantaneous burrowing behaviour being initiated once the soil was reached (Reinecke *et al.* 1980), indicating that the natural variability from individual to individual is high. In the soil texture class experiment, FCM larvae were provided with a suitable pupation habitat and were mature enough to pupate. In the natural environment, it would be advantageous for the larva to only exit the fruit in which it was developing just prior to pupation in the soil in order to minimise the risk of predation, parasitism or disease (Danks 2002). However, it is possible that if the larvae did exit the fruit at a slightly earlier stage, the amount of wandering undertaken prior to pupation would be increased.

The observed limitation in both wandering time and distance of FCM larvae is likely to reduce the potential exposure of the insect to soil-dwelling entomopathogens, particularly EPF. Entomopathogenic fungi must come into direct contact with their host in order to have a chance of infection (Barbercheck 1992). For EPNs this is less problematic as the infective juvenile (IJ) life stage will actively seek out its target host (Georgis & Poinar 1983c; Riga 2004; van Zyl & Malan 2014). An important factor in host-finding behaviour in nematodes is the production of chemical cues which attract the nematodes to the correct host (Turlings *et al.* 2012). However, even within the different EPN species, different behaviours are likely to influence the effectiveness of these entomopathogens. Nematode host-finding behaviour is typically divided into cruiser or ambusher categories, where cruisers actively seek out hosts and ambushers wait more passively for a suitable host to move past (Lewis 2002). Controversy does exist around whether species show a greater tendency towards cruising or ambushing behaviour, with it being suggested that a species can demonstrate both sets of behaviour, given the correct conditions (Wilson *et al.* 2012). The only commercially available EPN species in South Africa is *Heterorhabditis bacteriophora* Poinar which is

known to demonstrate cruising behaviour (van Zyl & Malan 2014); however, should an ambush nematode species be identified for FCM control in the future, this potential behavioural limitation would need to be taken into account. Comprehensive application of EPF and EPNs to the soil will be required in order for coverage to provide the maximum level of FCM control.

The formation of the pupal cocoon is a process that has been well-documented in Lepidoptera, particularly in the case of the economically important silkworm species, *Bombyx mori* L. (Lepidoptera: Bombycidae) (Chen *et al.* 2012). The cocoon provides a level of protection against the natural elements, predators and disease, while simultaneously maintaining a more favourable internal environment for development of the adult insect (Chen *et al.* 2012; Horrocks *et al.* 2013). For silkworms, in particular, a great deal of energy is invested in the formation of the cocoon (Chen *et al.* 2012), but this also holds true for other lepidopterans (Danks 2002). An energy trade-off is likely to exist, with larvae investing time and energy into the formation of cocoons and gaining in the increase of potential survival and future reproduction of that particular individual (Danks 2002). In the vast majority of the FCM larvae that pupated, pupal cocoons were spun by the fifth instar larvae. As was previously reported by Newton (1998), soil particles were included into the cocoons. This added an element of crypsis to allow for improved protection of the FCM pupae, as it was notably harder to detect when soil particles were incorporated into the cocoon. This would likely be to camouflage the highly vulnerable pupae from predators occurring in the natural environment (Danks 2002). The vulnerability of the pupal lifestage to external threats is high as a result of the immobile nature and reduced response ability of the pupa (Horrocks *et al.* 2013).

Certain larvae did not spin a pupal cocoon, but rather formed pre-pupae and finally a naked pupa on the surface of the soil. This behaviour was limited to 8 % of the total number of FCM larvae that pupated. The production of naked pupae is not unknown. This can be seen in the case of the cat flea, *Ctenocephalides felis* Bouché (Siphonaptera: Pulicidae), where once disturbed, 43 % of larvae did not spin a second pupal cocoon and rather formed naked pupae (Dryden & Smith 1994). This was a much higher occurrence than for FCM, recorded in this study (although the FCM larvae were not disturbed after cocoon formation). For FCM, the mortality rate of the naked pupae was 100 %, with none able to successfully produce adult moths. In the case of the cat flea, it would seem that formation of a pupal cocoon is not an essential requirement for survival as 95 % of the adults successfully eclosed (Silverman &

Rust 1985; Dryden & Smith 1994). It is highly likely that the dry nature of the soil was the main cause of mortality as the exposed pupae were very vulnerable to desiccation in this environment during the study. The effect of soil moisture will be further investigated in Chapter 5 of this work. The pupal cocoon may provide some level of protection against moisture loss and subsequent desiccation and death.

FCM larvae exclusively pupated on the surface of the soil with no pupae being formed at a depth of below 5 mm. The pupation depth of FCM has been previously stated to be on the surface of the soil (Georgala 1969), as well as in cracks in the soil underneath organic debris that collects naturally on the surface of soils (Newton 1998). However, prior to this study, this depth had not been quantified. Daiber (1979c) stated only that the cocoons were spun on the ground; during the experiment successful moth eclosion was examined when pupae were artificially buried under a layer of 20, 40 or 60 mm of sand. Emergence was more successful when prepupae rather than pupae were buried. Overall, successful adult eclosion from both prepupae and pupae was 78.2 % when buried under 20 mm of soil, rather than the significantly lower emergence rates of 65.1 % at 40 mm and 68.6 % at 60 mm (Daiber 1979c). A reduction in adult emergence and an increase in time to eclosion has also been found to occur in the swede midge, *Contarinia nasturtti* Kieffer (Diptera: Cecidomyiidae) with these larvae pupating in the upper 1 cm of the soil (Chen & Shelton 2007). Habitat manipulation is therefore a possible control option, but this behaviour would need to be further investigated. By allowing the larvae to select their own pupation site, it is clear that very little burrowing into the soil takes place prior to spinning the pupal cocoon. Since all three of the soil texture classes had been sieved prior to the initiation of this experiment, burrowing into the soil would have been easily possible. However, natural cracks in the soil, which would typically be found in the orchard, were not present in the soil texture class experiment because the soil was dried and no additional moisture was added. This may have influenced burrowing behaviour, but will be examined in more detail in the chapters to follow.

It was thought that the rapid development and eclosion of pupae formed in silt loam compared to silty clay loam was possibly affected by the difference in the sex ratio of moths. Female FCM are known to eclose slightly faster than male FCM, with females eclosing in an average time of 13.9 days compared with the males' 14.0 days, when kept at a constant 25 °C (Daiber 1979c). However, this result was not tested for any significant difference between the two. As such, it was thought that the higher sex ratio of females to males in the silt loam soil

could be the reason for the more rapid rate of eclosion. A closer examination of the difference in eclosion time between males and females revealed that no difference could be detected and is therefore unlikely to influence rates of eclosion.

The high eclosion success rate of FCM in the sandy loam in the no choice experiments indicates that the pest may be more problematic in orchards with sandier soils. Historically, the Citrusdal area in the Western Cape had the highest amount of FCM pressure in South Africa (Moore, S.D. pers. comm.[2]), and this may now be possibly attributed to the sandy soils which are found there (Nieuwoudt 1987). The use of sterile insect technique (SIT) has substantially reduced the FCM problem in that area (Groenewald, S. pers. comm.[3]). Sandier soil areas may also be more suitable for successful FCM control using biological control agents applied to the soil. The retention of fungal conidia can differ with the soil texture class, where *Beauveria bassiana* (Balsamo) Vuill. conidia were more readily recovered from the surface layer of soils with high clay content than sandier soils and the opposite effect being seen for *Metarhizium anisopliae* (Metsch) conidia (Quesada-Moraga *et al.* 2007; Garrido-Jurado *et al*. 2011). Overall, the highest percentages of conidia were found in the surface of the soil (Garrido-Jurado *et al.* 2001). This, in combination with the surface soil pupation of FCM in loose soil, bodes well for FCM pupal control using EPFs. It may also be valuable to adjust the fungal isolate that is applied to the soil according to the predominant soil class in the orchard. The higher eclosion success rate of FCM in sandier soils is also unlikely to be problematic where EPNs are applied to the soil, as nematodes are known to move further distances and have increased infectivity in soils with a higher sand content (Kung *et al*. 1990a; Barbercheck & Kaya 1991). This has largely been attributed to large particle size and improved aeration of the sandier soils, which allows for easier nematode movement (Kung *et al.* 1990a; Brady & Weil 2000). While eclosion success was lower in silt loam and silt clay loam, both of which had higher clay content (Table 2.1), and the movement of EPNs is expected to be reduced in high clay content soils due to the small soil pore diameter (Georgis & Poinar 1983a & b). Control of FCM using EPFs and EPNs will be affected by both soil coverage with these organisms and soil texture class.

The identification of larval choice or preference for a particular habitat has been previously studied (Alyokhin *et al*. 2001; Ande 2004; Hulthen & Clarke 2006). The determination of any preferences that FCM may have would be useful, particularly for areas where different soil texture classes may be in close proximity. Preferences have been found in the case of species such as *Cirina forda* Westwood (Lepidoptera: Saturniidae), where

[2] Moore, S.D. Citrus Research International. South Africa.
[3] Groenewald, S. XSIT, South Africa.

loamy sand soils where preferred over soils with a higher clay content (Ande 2004). Surprisingly, the only time where a particular preference was noted for FCM was for silty clay loam soil over sandy soil. This and the lack of preference shown in the other choice trials may be due to the preparation of the soils as they were dried and sieved, making the differences in soil texture class properties less pronounced. Further investigation will be made into the possibility of FCM larval preference when allowed a choice of more moist and compact soils, as would be the case in an orchard. Wandering behaviour was noted for the majority of the FCM larvae. Larvae did investigate the soil area prior to selecting a pupation site, although the wandering behaviour often did not extend to investigating both soil texture classes. When choices between soil classes were allowed, there was no difference in the eclosion success percentage for any of the soil texture classes in the choice experiment; however, this may be due to the smaller sample size used for the choice experiments.

The results in this chapter provide important and useful biological information on FCM pupation and provide a baseline laboratory study into FCM pupation in soils from different soil texture classes. However, these results were obtained for soils which were bare, dry and had been sieved. In the field, soils are likely to be covered with organic debris, have varying moisture levels, varying air temperature and being more compacted. Therefore, there needs to be experimental testing of these variables.

3

The influence of soil texture class and ground cover on FCM pupation

3.1 INTRODUCTION

Mature FCM larvae have been recorded as incorporating materials which are in the immediate vicinity such as soil particles and organic ground debris when spinning the pupal cocoon (Daiber 1979c; Newton 1998; van den Berg 2001). In a natural orchard setting, these materials would be readily available to the larvae for use should they choose to do so. Organic soil ground cover in orchards is largely provided from the citrus trees themselves through leaf drop, but other plants or weeds nearby would also add to this ground cover, which can range from being several cm in depth to largely exposed soil. Increasing the diversity of cover crops in orchards has been encouraged in order to provide a more diverse and heterogeneous environment for the establishment of natural pest enemies to improve control and prevent pest outbreaks (Altieri & Letourneau 1982; Prokopy 1994; Bugg & Waddington 1994; Liang & Huang 1994; Gurr *et al.* 2003; Altieri *et al.* 2005). These plants all contribute to the ground cover and will be involved in biological interactions in the orchard.

Identification of preferred microclimates of insect pests for pupation has been recognised for its potential to improve pest control (Alyokhin *et al.* 2001). Insect preferences have been previously identified (Ande 2004) and a preference for pupation in shaded areas has been shown to exist for Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) (Alyokhin *et al.* 2001). This is thought to be in an effort to reduce pupal desiccation or to provide protection from larger vertebrate predators (Alyokhin *et al.* 2001). Pupae are known to be the most vulnerable life stage of the lepidopteran life cycle due to an inability to escape from unfavourable abiotic conditions, predation, parasitoides or microbes (Danks 2002). In

the citrus orchard, if FCM larvae showed a preference for a more shaded environment, application of the soil-dwelling biological control agents, entomopathogenic fungi (EPFs) and entompathogenic nematodes (EPNs) could be focused in these areas in order to reduce costs and possibly increase efficacy.

As such, the aims of the chapter were to firstly determine the influence of different soil texture classes and organic soil ground cover on 1) FCM larval wandering time, 2) the distance wandered by FCM larvae prior to pupation site selection 3) time taken by FCM to spin the protective pupal cocoon, 4) the depth of pupation, 5) orientation of pupation (horizontal or vertical in soil profile), 6) amount of time taken to eclose and 7) the eclosion success. The second aim was to determine whether FCM larvae would show a preference for shaded or open soil areas when allowed a choice.

3.2 MATERIALS AND METHODS

3.2.1 Ground Cover Experiments

The experimental design was very similar to that of the experiments run for the soil texture class experiments in Chapter 2. The three different soil texture classes were prepared as previously described and the FCM larvae were once again able to drop into the soil of their own accord, from the inverted vials of diet which were suspended above the soil. All larval behaviour was recorded using video camera footage.

The organic soil ground cover had been previously collected from each of the orchards where the soils had been collected. These were labelled and stored in plastic shopping bags in a fridge at 4 °C. Once the experiment was ready to commence, the ground cover was autoclaved in glass jars to ensure that it was free of pathogens and any other living organisms. The debris was spread on top of the soil, ensuring that at least 50 % of the soil was covered. This debris differed slightly, depending on the orchard from which it had been collected, but in general consisted of dried leaf litter, sticks and grass. Although in the orchard it is likely that more than 50 % of the ground would be covered with organic debris, it was still necessary to be able to view the FCM larval behaviour on film which would not have been possible had the coverage been higher. However, the larvae were always in close

proximity to ground cover once they had dropped into the soil and would easily be able to include it.

3.2.2 Shaded versus Open Choice Experiments

FCM larval choice of shaded versus open areas of the soil was also to be investigated. In order to do this, the methods used by Alyokhin *et al.* (2001) were followed, but slightly modified for use in the experiments. Cardboard grids of 35 x 20 cm were created with individual cells of 5 x 5 x 5 cm which were glued together. The grids were placed into the plastic trays to create a choice arena (Fig. 3.1). To provide the shaded environment, cardboard covers of 5 x 5 cm were made and attached to the cells using insect pins. Each cover was 3 cm above the soil grid and this was done in a checkerboard pattern (Fig. 3.1). It was important that the larvae still drop into the soil of their own accord, however in this case mature 5^{th} instar larvae were removed from the larger jars of FCM diet and placed onto a Petri dish which was suspended 20 cm above the soil arena. The diameter of the Petri dish allowed for larvae to have an equal chance of dropping onto a shaded or an open cell in the grid. When dropping onto the shaded cardboard cover larvae would then move down from these into the soil. From here a pupation site would be selected by the larvae. Once the pupal cocoon was spun, it was marked using an aluminium foil marker and checked both 24 and 48 hours later for any evidence of movement. The larval choice of shaded or open soil areas was then recorded and analysed.

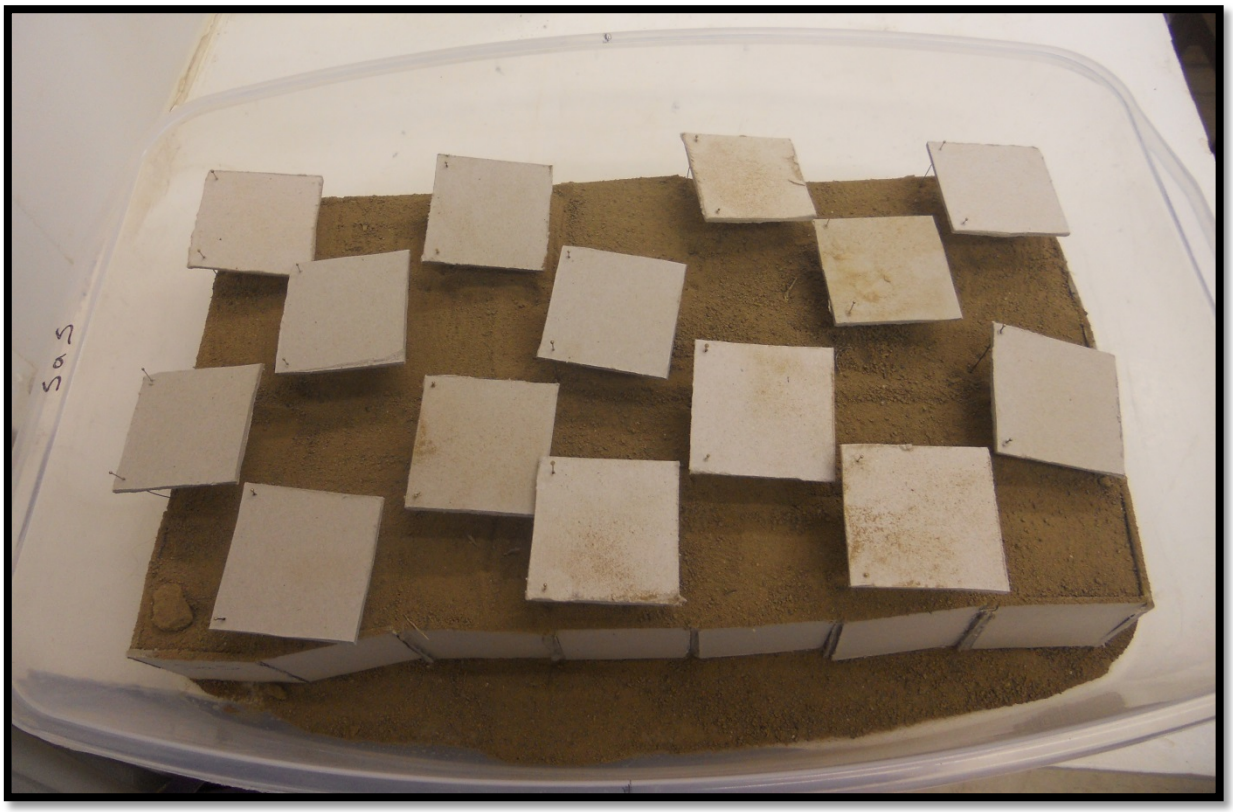

Figure 3.1 Image showing the layout of the grid arena, created for the shaded choice experiment

3.2.3 Statistical Analysis

The statistical analysis was very similar to that of Chapter 2, with the analyses being run in Statistica Version 10, 2011. Since the datasets were found to not be normal, non-parametric Kruskal-Wallis and Mann-Whitney *U*-tests were used for analysis. Where categorical data was compared, Chi-square tests were used.

3.3 RESULTS

3.3.1 General FCM Biology, Behaviour and Survival Experiments

When examining differences in FCM larval wandering distance where ground cover was added to the soil, no significant differences were found between the three different soil texture classes ($H_{(2,90)} = 0.679$, $p = 0.712$). For all three soil texture classes the median wandering distance was less than 20 mm, with high variability (Fig. 3.2).

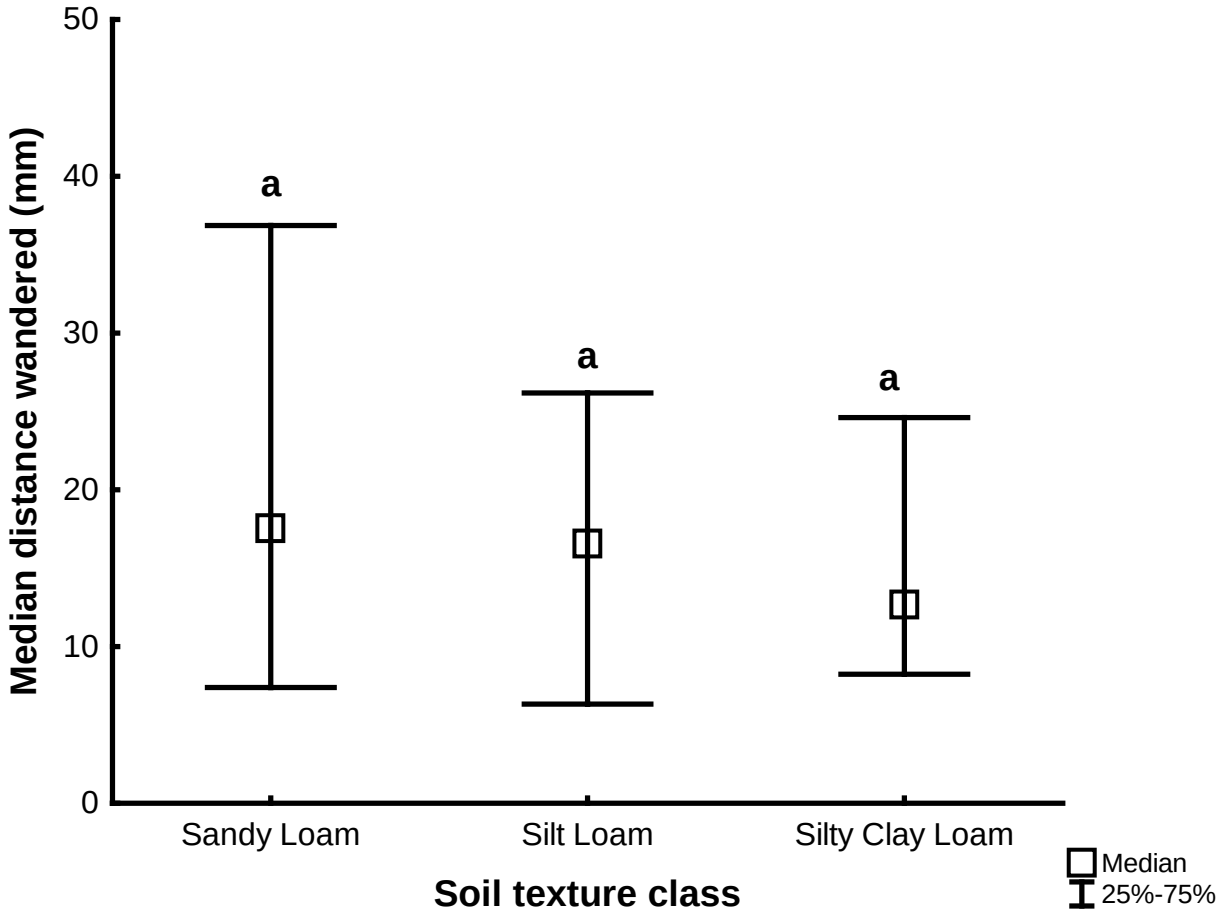

Figure 3.2 The median distance wandered by FCM larvae on the soil surface with ground cover, prior to pupation site selection for the three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A comparison of the median time wandered by FCM when ground cover was added to the soil surface showed no significant differences between sandy loam, silt loam and silty clay loam ($H_{(2,90)} = 0.199$, $p = 0.905$). A large amount of variability in wandering time was found for sandy loam and silt loam, but less so for the silty clay loam soil texture class (Fig. 3.3).

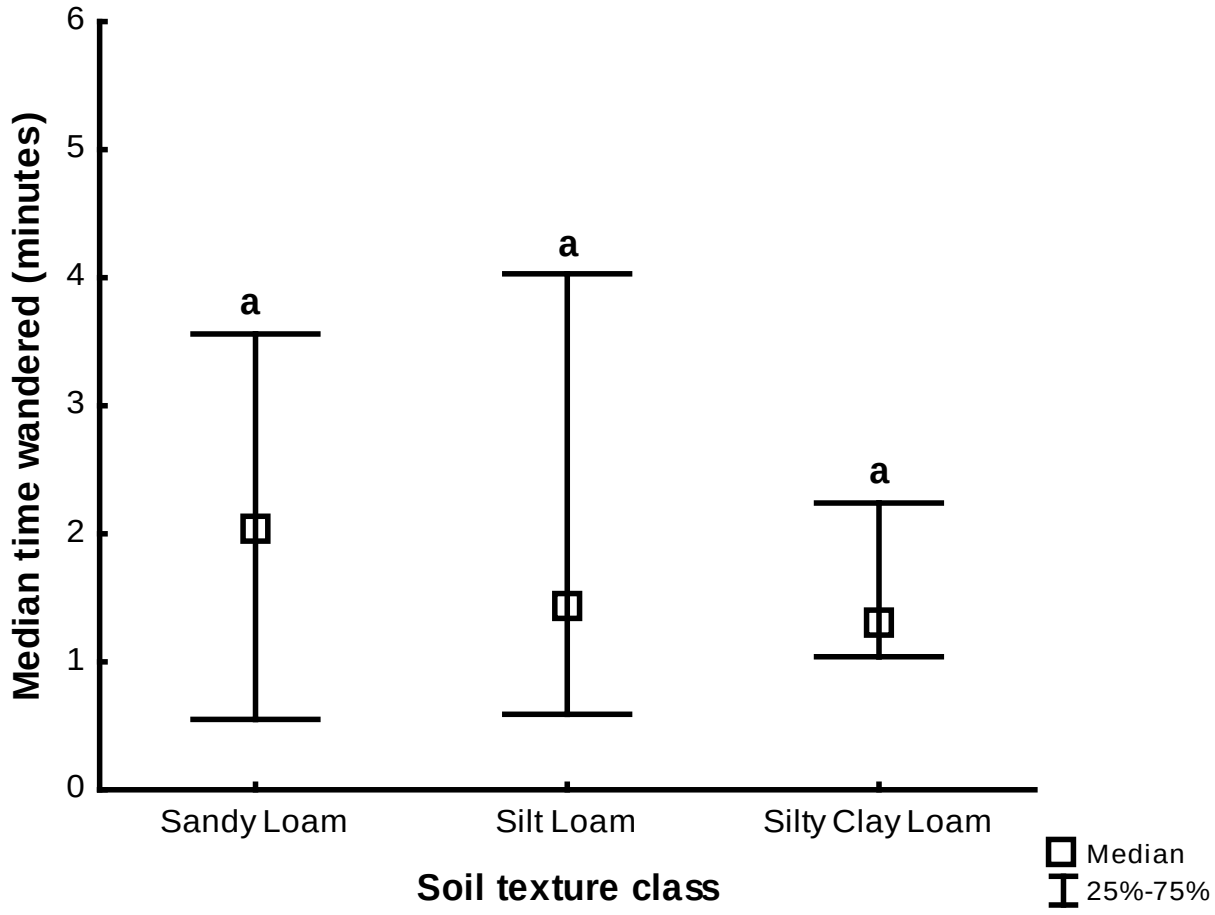

Figure 3.3 The median amount of time wandered by FCM larvae on the soil with ground cover, prior to pupation site selection for the three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time taken to form the pupal case revealed no significant difference between the three different soil texture classes when ground cover was added ($H_{(2,90)} = 4.739$, $p = 0.094$). Pupal case or cocoon formation was once again a rapid process, with no median being more than 45 minutes (Fig. 3.4).

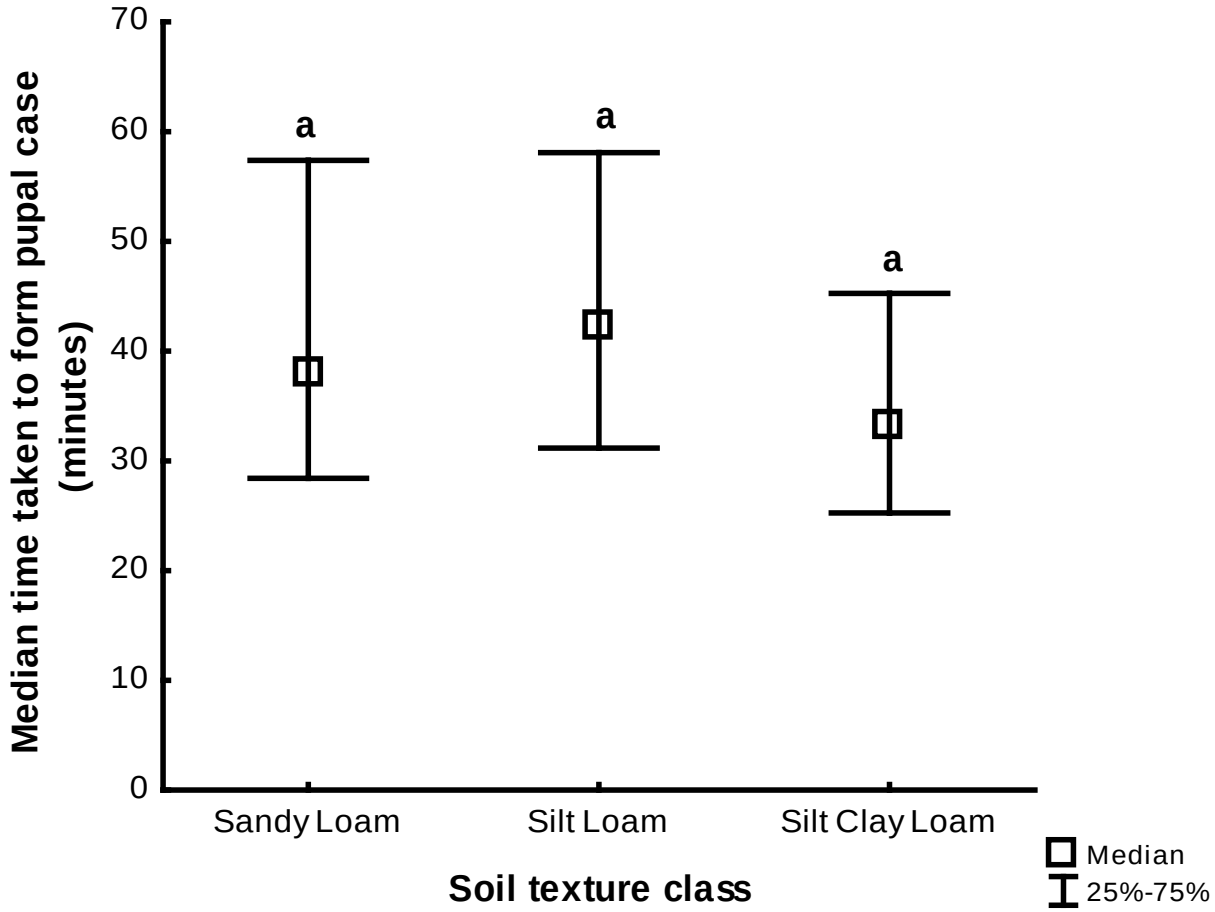

Figure 3.4 The median amount of time taken for FCM larvae to spin pupal cocoons for the three different soil texture classes soils with ground cover (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median depth of pupation was shallow with FCM larvae once again pupating in the very upper layer of the soil. Differences in soil texture class with ground cover had no significant effect on pupation depth ($H_{(2,90)} = 0.839$, $p = 0.657$). Almost no variation was found for silty clay loam (Fig. 3.5). The orientation of the pupae in the soil profile was also measured with all larvae pupating in a horizontal orientation on the soil surface.

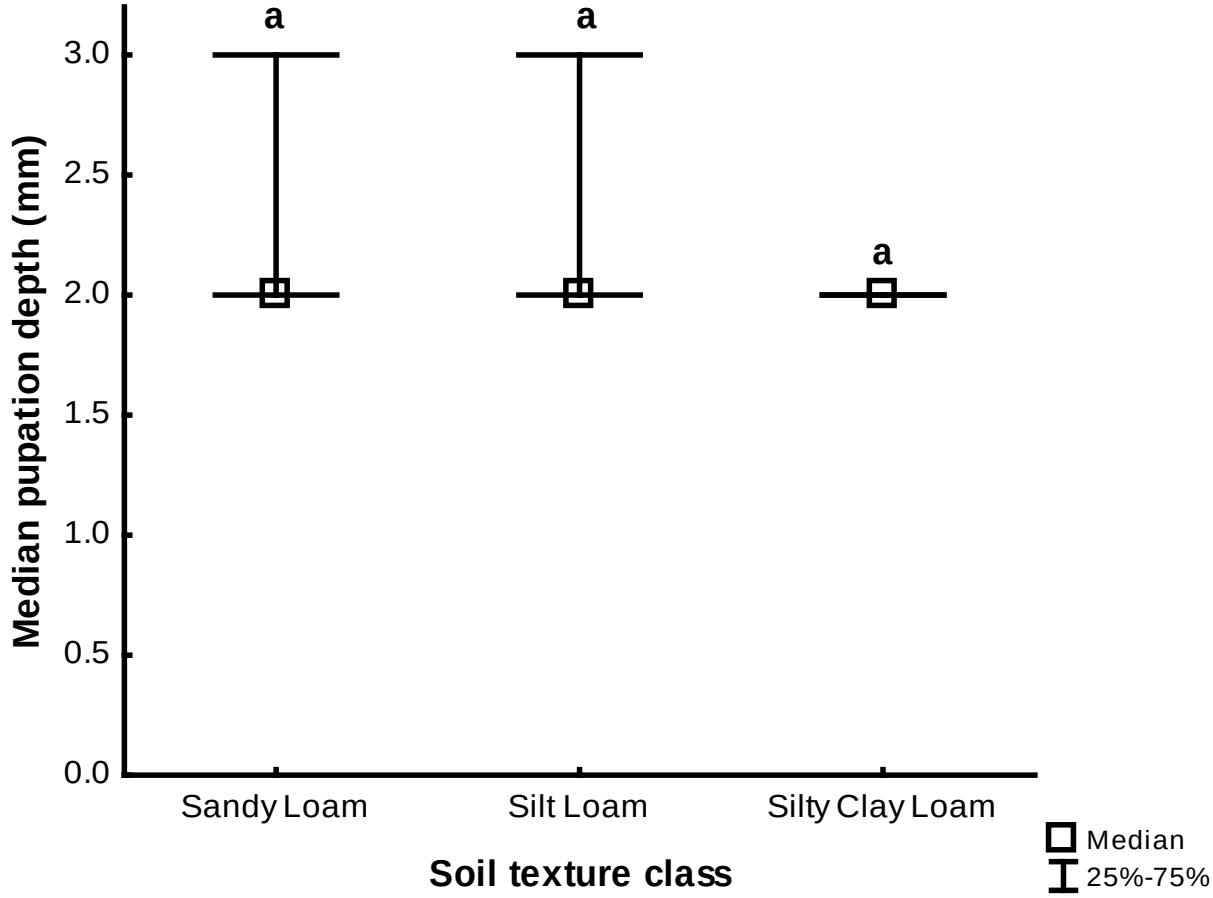

Figure 3.5 Median depth of FCM pupation in three different soil texture classes with ground cover (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time to eclosion for adult FCM revealed that moths which eclosed from the sandy loam soil with ground cover did so in significantly less time than those which had pupated in silt loam soil ($H_{(2,66)} = 8.296$, $p = 0.016$). No differences were found between sandy loam and silt clay loam or silt loam and silty clay loam soils (Fig. 3.6).

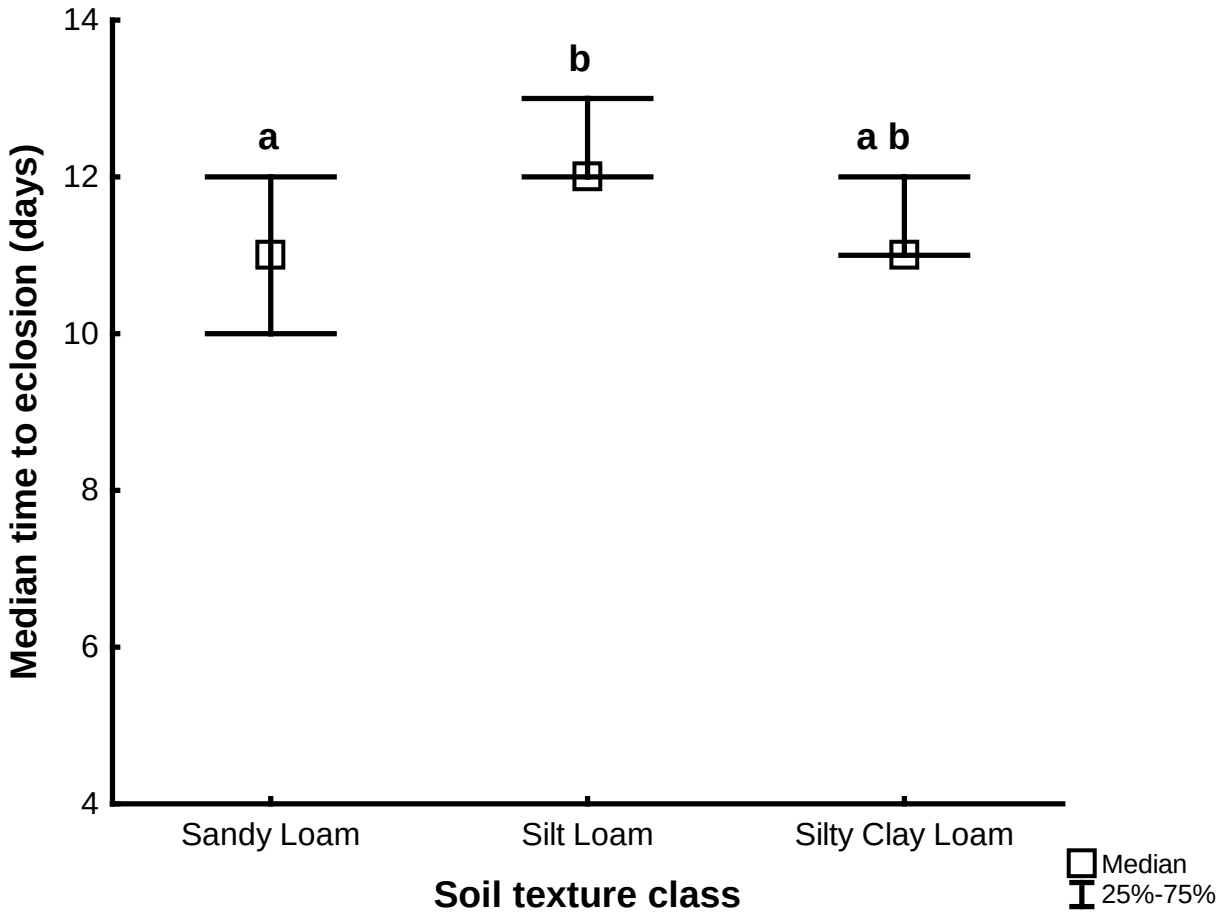

Figure 3.6 The median amount of time taken for adult FCM to eclose from the pupae formed in the three different soil texture classes with ground cover (n = 66). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A significant association between soil texture class with ground cover and successful eclosion was found, with sandy loam having a higher percentage of adult FCM eclosion (100 %) than silt loam (56.67 %) or silt clay loam (63.3 %) (Chi-square = 16.705; df = 2; p = 0.0002). No difference was found between silt loam and silty clay loam FCM eclosion (Fig. 3.7).

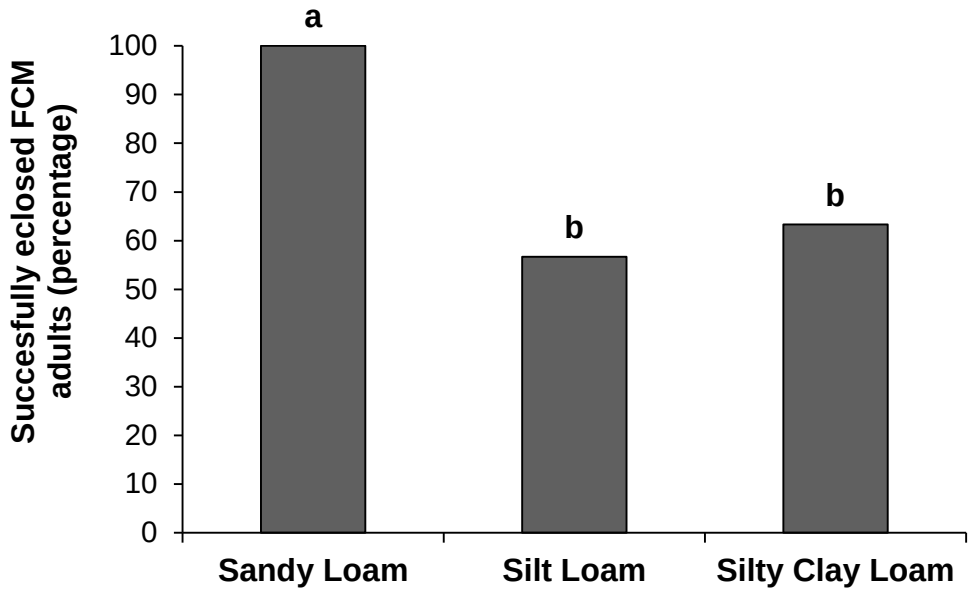

Figure 3.7 The percentage of successfully eclosed FCM adults from pupae formed in the three different soil texture classes with ground cover (n = 66). Different letters denote significant differences (Chi-square test, $p < 0.05$).

3.3.2 Comparison of Bare Soil and Soil with Ground Cover

When comparing whether the addition of ground cover would influence the wandering distance of FCM larvae, it was found that this addition had no significant effect on larval wandering behaviour when compared to bare soil results of Chapter 2 (Mann-Whitney $U_{(90, 90)} = 3921.0$, $p = 0.713$) (Fig. 3.8).

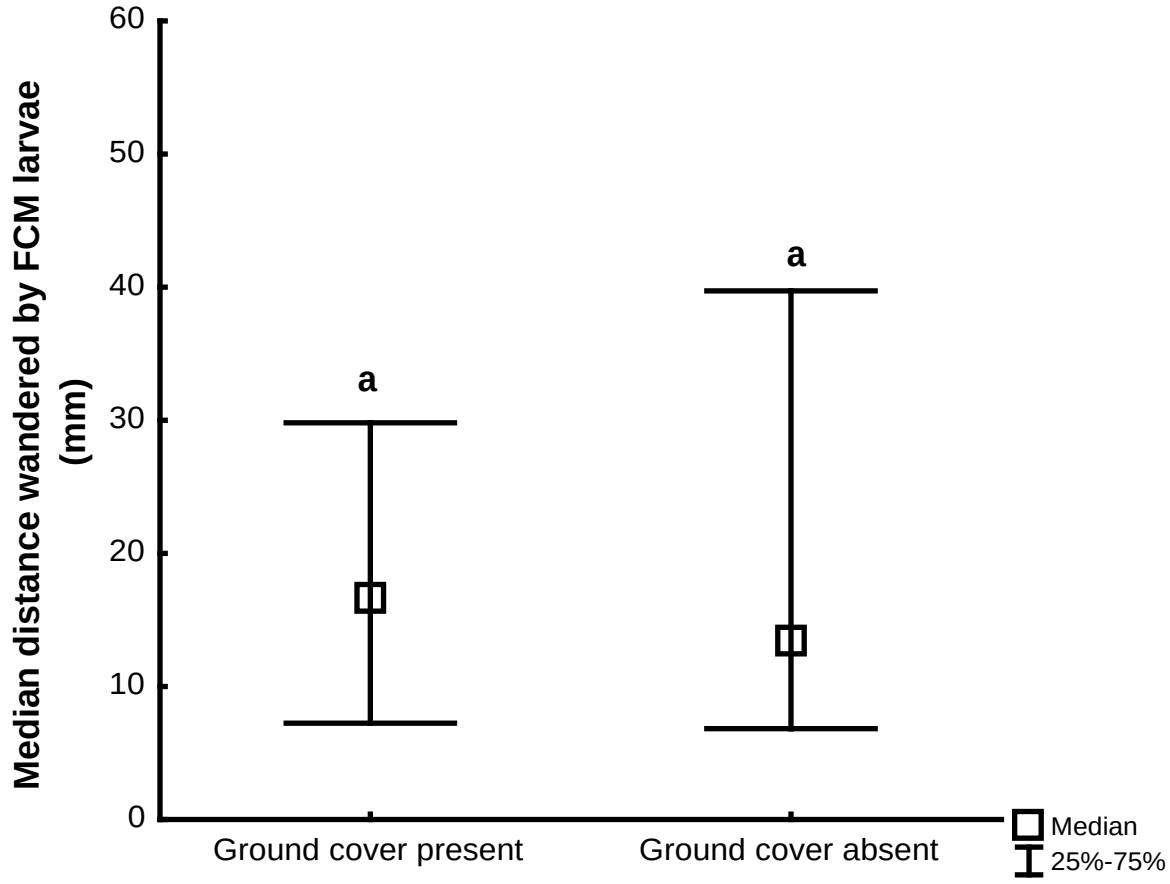

Figure 3.8 A comparison of median larval FCM wandering distance on soil with ground cover or bare soil (n = 180). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

The median wandering time was rapid for both soil with ground cover and bare soil, with neither having a median wandering time exceeding three minutes. The difference between bare soil and soil with ground cover revealed no significant difference between the two (Mann-Whitney $U_{(90, 90)} = 3461.5$, $p = 0.093$) (Fig. 3.9).

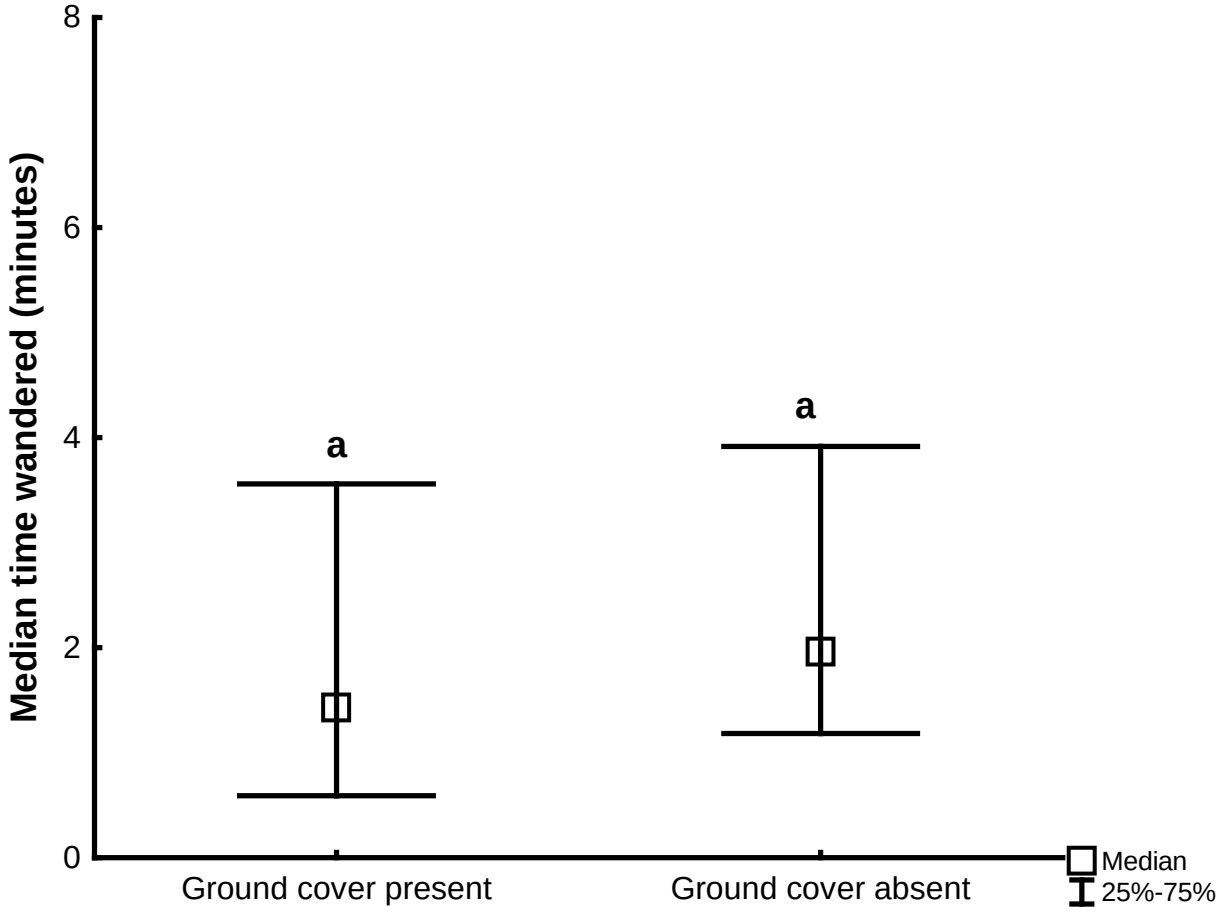

Figure 3.9 Median larval FCM wandering time on the surface of soil either with ground cover or bare soil (n = 180). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

The addition of ground cover to the soil had no significant impact on the median time taken to form the pupal cocoon, in comparison to cocoons formed in bare, open soils (Mann-Whitney $U_{(90, 83)} = 3575.0$, $p = 0.538$) (Fig. 3.10).

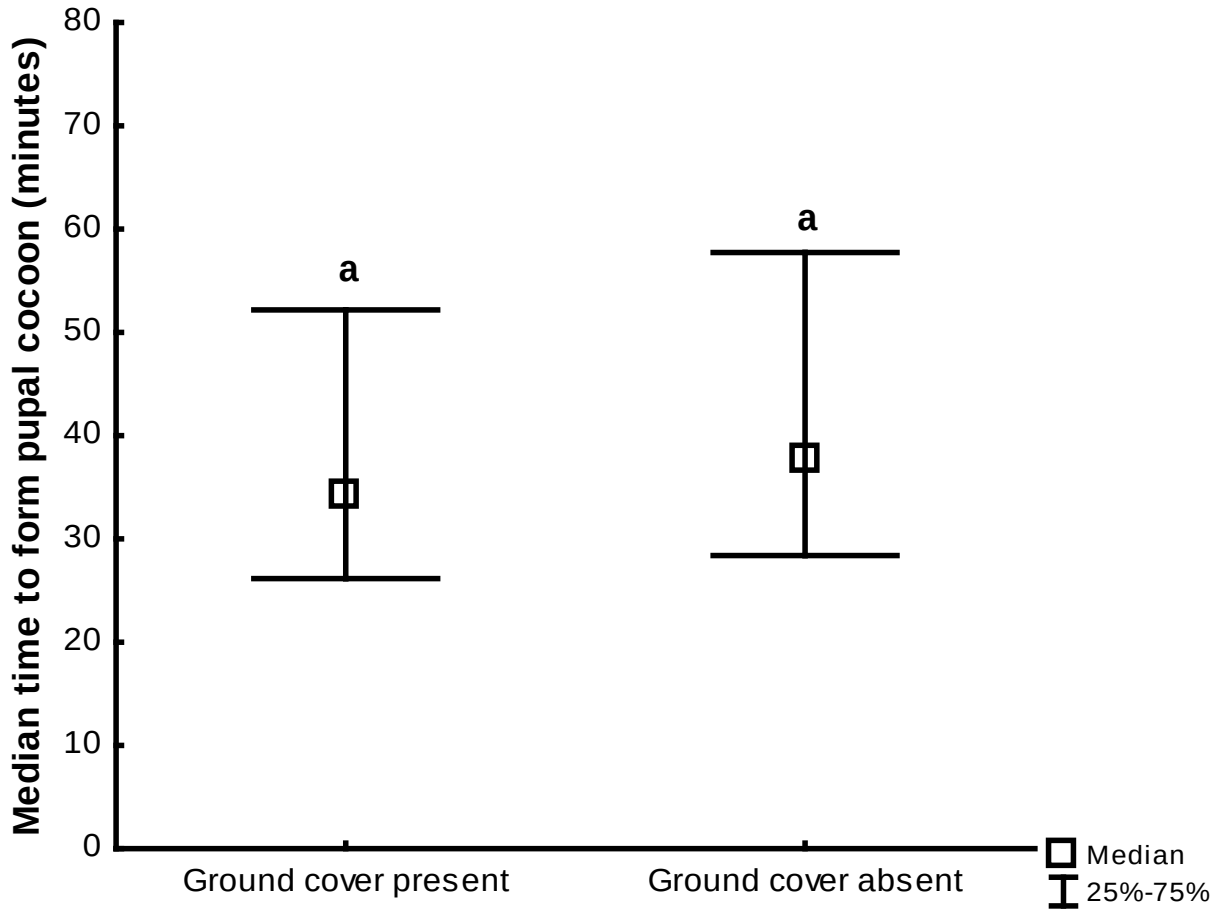

Figure 3.10 A comparison of the median time taken for larval FCM to form a pupal cocoon, either in soil with ground cover or bare soil (n = 173). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

When examining how many FCM larvae actually incorporated ground cover debris into their pupal cocoon it was revealed that the overall numbers were low. Only 40 % of pupae formed in sandy loam soil contained ground cover, with silt loam and silty clay loam having 31 % and 30 % of ground cover cocoons respectively. No significant effect of soil texture class with ground cover was found to be occurring for pupal cocoon with ground cover formation (Chi-square = 0.480; df = 2; p = 0.787) (Fig. 3.11).

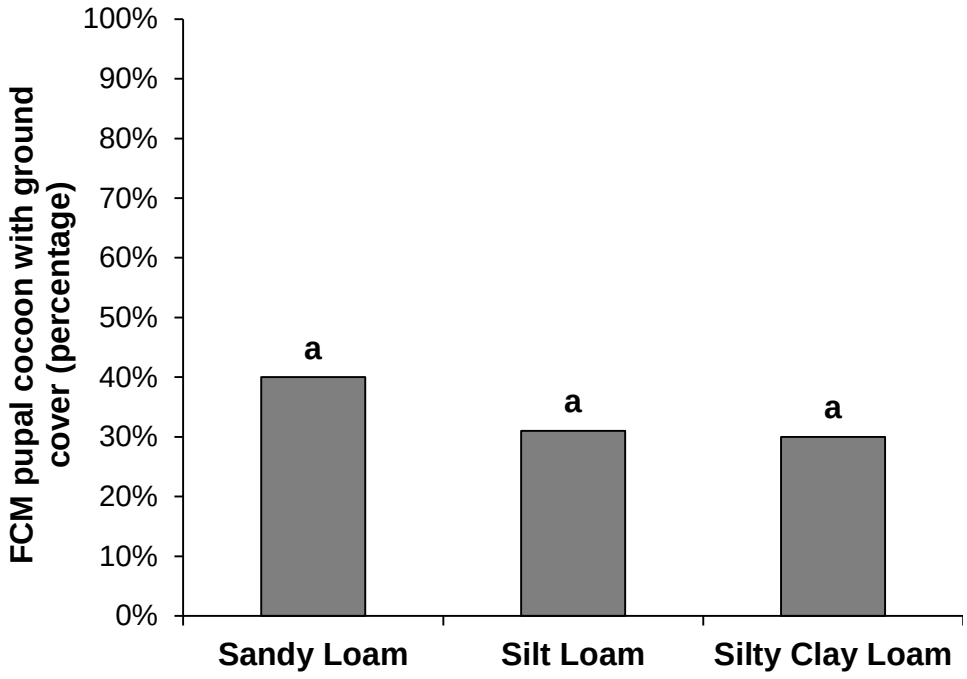

Figure 3.11 Percentage of FCM pupal cocoons incorporating ground cover debris for the three different soil texture classes (n = 90). Different letters denote significant differences (Chi-square Test, $p < 0.05$).

In order to determine whether ground cover influenced the eclosion success of FCM adults, a comparison of all three soil texture classes with and without ground cover revealed that the addition of ground cover did not affect the eclosion success of FCM (Chi-square = 0.031; df = 1; p = 0.861) (Fig. 3.12).

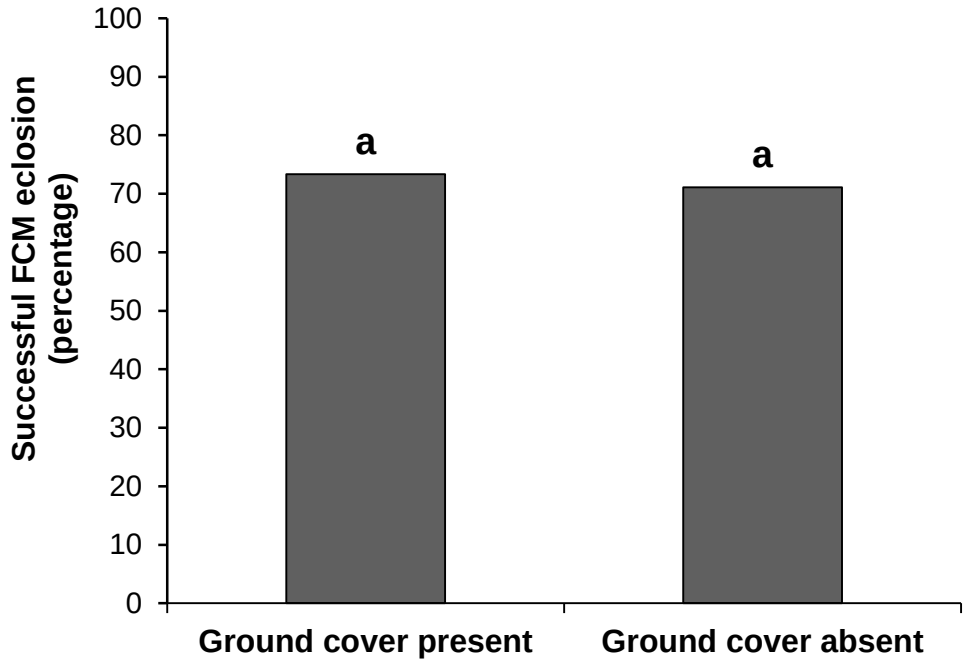

Figure 3.12 Percentage of successful FCM eclosion for all three soil texture classes grouped together, with ground cover being either present or absent (n = 129). Different letters denote significant differences (Chi-square Test, $p < 0.05$).

3.3.3 Choice Experiments

FCM larvae were allowed a choice of either an open, bare soil area or a shaded bare soil area for pupation. Data from all three soil texture classes were combined to examine the overall choice of FCM larvae. Analysis revealed that there was no significant relationship between the percentage of pupated FCM larvae and shading of the pupation site (Chi-square = 0.031; d.f. = 1; p = 0.861) (Fig. 3.13).

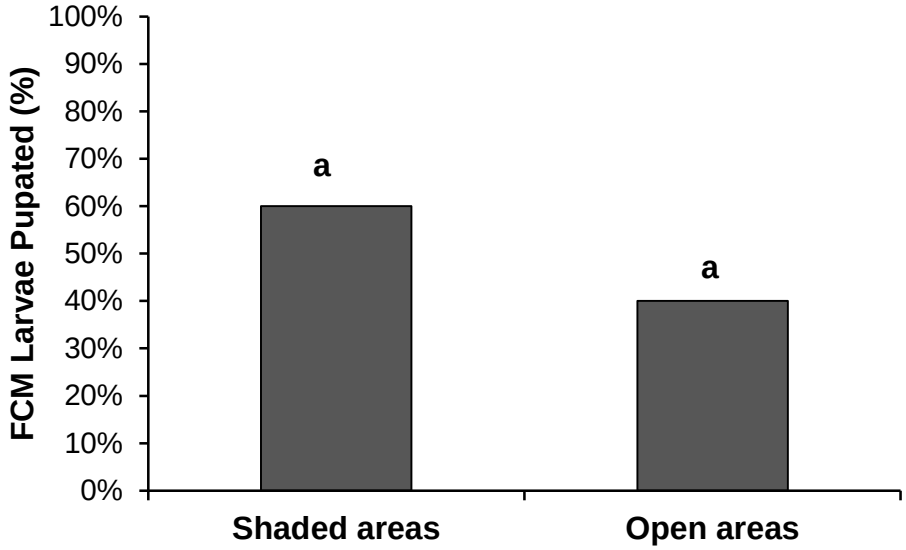

Figure 3.13 The percentage of FCM larvae pupating in shaded versus open areas when allowed a choice (n = 30). Different letters denote significant differences (Chi-square Test, $p < 0.05$).

3.4 DISCUSSION

The addition of ground cover to the soil had very little effect on the behaviour of pupating FCM larvae with no impact on the wandering distance, wandering time or time taken to form the pupal cocoon between the three different soil texture classes. Even in a more natural setting, FCM pupation habits appear to be unaffected by soil texture class. Again, larvae pupating in silt loam soils took the longest time to eclose, although for the ground cover experiments this was faster than that of pupae formed in sandy soils. Silt loam also had the lowest percentage of successfully eclosing adult moths, a pattern which was reflected in the

soil texture class experiments of Chapter 2. The unfavourable environment of the silt loam soils may be slowing the development rate of pupal FCM however this result does provide further support for the possible lowered threat of FCM in silt loam soils. When comparing distance wandered, time wandered and time taken to form the pupal cocoon it was noted that although one might expect larvae to wander further in a more complex environment and possibly take less time to form the pupal case due to the inclusion of ground cover, this was not the case with no differences being found between soils with or without ground cover.

Once again, the sandy loam soil had the highest number of successfully eclosing FCM adults, with 100 % eclosing in comparison to the 90 % which eclosed from bare sandy soil. This higher rate of survival was not reflected in either silt loam or silty clay loam. It was initially believed that the addition of ground cover may improve survival of FCM pupae due to protection from desiccation (Alyokhin *et al.* 2001), but this appears to have not been the case. Additionally, even though ground cover debris was readily available for FCM to make use of, only a limited number of FCM larvae actually included debris into their pupal cases with most preferring to use soil particles only. However, the ground cover may have encouraged more larvae to spin actual pupal cocoons, as no naked pupae were formed in this experiment. It is also possible that an interaction between soil moisture and ground cover would influence eclosion success.

It is likely that ground cover would be a positive addition for the biological control agents, in particular EPF, as the soil covered with organic debris would provide a more humid, moist environment and protection from UV rays, both of which are serious concerns when applying EPF to the soil (Barbercheck 1992; Fargues & Luz 2000). For EPNs, once the nematodes have moved down into the soil, ground cover is unlikely to have any negative effects. However, difficulty in moving into the soil when there is a thick layer of ground cover on top of it has been noticed in turfgass (Grewal *et al.* 200) and has the potential to impede EPN movement into the soil of citrus orchards. Moist, but not saturated soil conditions would also be positive as nematodes require high relative humidity levels to survive and EPN movement occurs in the film of free water between soil particles (Grant & Villani 2003). If the moisture content of the soil drops too low, EPNs become inactive and are no longer effective biological control agents (Grant & Villani 2003). Ground cover can assist in reducing soil moisture loss and protecting bare soil from high temperatures which would be advantageous for both biological control agents. Further impacts of temperature and moisture level will be investigated in more detail in Chapters 5 and 6.

FCM larvae did not appear to show a significant preference for shaded soil areas over open areas when allowed a choice. This was unexpected as shaded areas were thought to potentially provide a more suitable and less harsh microclimate, as has been shown for Oriental fruit fly where a very strong preference was found for a shaded environment (Alyokhin *et al.* 2001). Research conducted in which agricultural areas were shaded with a screen showed that while the maximum temperature was not highly affected, the amount of solar radiation and water loss from the soil was reduced (Möller & Assouline 2007). Despite this, FCM do not appear to show strong preferences with regards to the soil texture class or for shaded microclimates as demonstrated during the choice experiments for both Chapters 2 and 3. This may influence FCM distribution in the field and will require excellent coverage of EPF and EPNs in both shaded areas under the tree as well as more open areas to optimize FCM control.

4

The influence of soil texture class and compaction on FCM pupation

4.1 INTRODUCTION

Soil compaction occurs when soil particles are rearranged, being brought into closer contact with one another and increasing the bulk density of the soil (O'Sullivan & Simota 1995; Brady & Weil 2000). The principle causes of soil compaction in a cropping context, are the use of heavy farm machinery and trampling of the land by grazing animals (Hamza & Anderson 2005). Increased pressure and higher soil moisture causes greater levels of compaction and this will penetrate below the topsoil and into the subsoil below (Brady & Weil 2000). A certain amount of compaction may be beneficial to plants, particularly when seeds are first planted, as capillary water movement to the seeds is often improved (Kozlowski 1999) or to prevent soil erosion (O'Sullivan & Simota 1995). However, the general consensus is that soil compaction is largely undesirable and is a worldwide concern (Kozlowski 1999). Soil compaction often has a negative connotation and as such has been expressed as 'severe compaction' (Kozlowski 1999) or 'over-compaction' (Batey 2009). This is where the health of the soil has become compromised due to plants roots not being able to penetrate the soil as easily, aeration of the soil being poor, water and nutrient movement into the soil is reduced and toxic substance build-up from gases and root exudates is increased (Håkansson & Voorhees 1998; Brady & Weil 2000).

In order to better understand the influence of the abiotic environment on insect pupation habits in the soil, a number of studies have examined the impact of soil compaction on factors such as pupation depth, pupal distribution and pupal development (Roach & Campbell 1983; Hennessey 1994; Alyokhin *et al.* 2001; Dimou *et al.* 2003; Cammack *et al.* 2010). Since compacted soils have become more common in agriculture, this is the type of environment in which false codling moth (FCM) is likely to be pupating and the influence of this must be

established. A clear understanding of the pupal habits of FCM in compacted soils can then be compared to the impact that this would have on the biological control agents of interest, entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs).

Since it has been clearly established in a number of previous studies that soil compaction influences insect pupation habits, this was considered an important soil characteristic to investigate. The aims of this chapter were therefore to determine the influence of different soil texture classes and differing soil compaction levels on 1) FCM larval wandering time, 2) the distance wandered by FCM larvae prior to pupation site selection 3) time taken by FCM to spin the protective pupal cocoon, 4) the depth of pupation, 5) orientation of pupation (horizontal or vertical in soil profile), 6) amount of time taken to eclose and 7) the eclosion success.

4.2 MATERIALS AND METHODS

The first stage of the soil preparation was identical to that of the process described in Chapter 2. However, once the soil had been sterilized, it needed to be compacted once more. In order to do this, two tons of pressure was applied to the dry soil using a hydraulic press fitted with an SMF Svenska Manometer pressure gauge. While the soil was now more compact, it remained fairly loose. Previous fieldwork had determined that compaction levels in the soil surface could reach as high as 5000 kPa although this is also dependent on soil texture class which was not analysed for this field work. However, despite this it became apparent that the laboratory compaction levels were too low. The addition of moisture would assist in the compaction process, therefore the saturation levels of each of the soil texture classes was calculated. This was done by adding water to 100 g of completely dry soil held in fine mesh netting until the soil water capacity was reached, providing the amount of water required to saturate 100 g of each of the different soil texture classes. The plastic containers of prepared soil were weighed and the weight of the containers was subtracted from this to obtain the true soil weight. The amount of water required to provide a 50 % level of saturation as moisture is known to compact soil and this amount of moisture would compact such a shallow soil depth. The amount of water required was then calculated for each particular weight. Using a plastic graduated 1 L cylinder, the water was measured out and the

majority of it was poured evenly over the top of the soil. The remainder of the water was placed into a plastic spray bottle where the nozzle was adjusted to a fine spray mode. Any areas of the soil which remained dry were then moistened using the spray bottle. This concluded the initial compaction process.

The soil then needed to be dried as this experiment was only examining the impact of soil compaction and not moisture. The soils were placed into a drying oven for a period of 48 hours at 121 °C. After this time the soil moisture content was checked using a Blumat digital moisture meter or tensiometer and if not sufficiently dry (-40 kPa or higher), that particular soil was placed back into the drying oven until it had dried out sufficiently. This process made an immediate difference to the compaction of the soil, with each being visibly more compacted. In order to quantify the compaction level of each of the soil types, a standard pocket soil penetrometer with a maximum capacity of 5 000 kPa was used. The amount of pressure applied to the penetrometer in order to allow it to penetrate a marked distance (2.5 cm) into the soil was recorded. This distance was sufficient as the soil strength of the surface layer of the soil was most important for this study, based on previously determined FCM pupation depths. Four measurements from the centre of each container and four from the edges were taken from each of the soil containers in order to give an accurate measure of the compaction levels. Once prepared, the experiments continued as has been previously described in Chapter 2, with the larvae being allowed to pupate naturally in the soil and the filming of the pupation process for analysis.

4.2.2 Statistical Analysis

The statistical analysis, when comparing the influence of the different soil texture classes on FCM pupation habits, required Kruskal-Wallis tests, as the data were found to be non-parametric when tested for normality. When comparing overall differences between compacted and uncompacted soils, where soil texture class was not a factor, Mann-Whitney *U*-Tests were used. Data which contained categorical variables were analysed using a Chi-square test. All analyses were run using the statistical software, Statistica Version 10 (2011).

4.3 RESULTS

4.3.1 Soil Compaction of Different Soil Texture Classes

The compaction level or soil strength of the three different soil texture classes after the soils had been compacted revealed that the sandy loam soil was significantly less compacted than both the silt loam and the silty clay loam ($H_{(2,48)} = 31.517$, $p < 0.0001$). No difference was found between silt loam and silty clay loam, both of which also showed far more variation in soil strength than the sandy loam soil (Fig. 4.1).

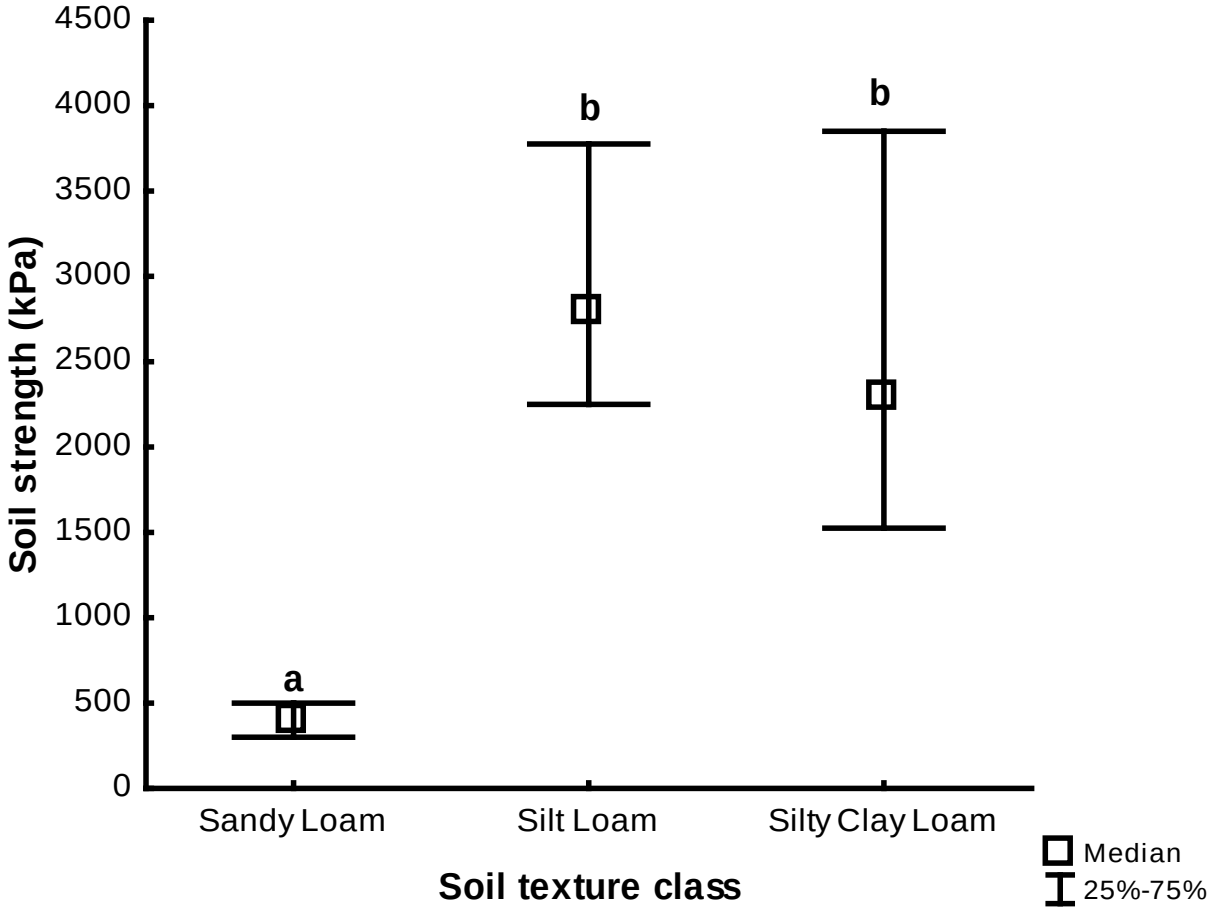

Figure 4.1 Soil strength penetrometer measurements for the three different soil classes used in the experiment (n = 48). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

4.3.2 General FCM Biology, Behaviour and Survival Experiments

Compaction of the soil for the three different soil texture classes (sandy loam, silt loam and silty clay loam) had no significant influence on the median amount of time that FCM larvae spent wandering on the soil surface prior to pupation site selection ($H_{(2,90)} = 1.5673$, $p = 0.433$). The variability in wandering time was high, particularly for the sandy loam soil (Fig. 4.2).

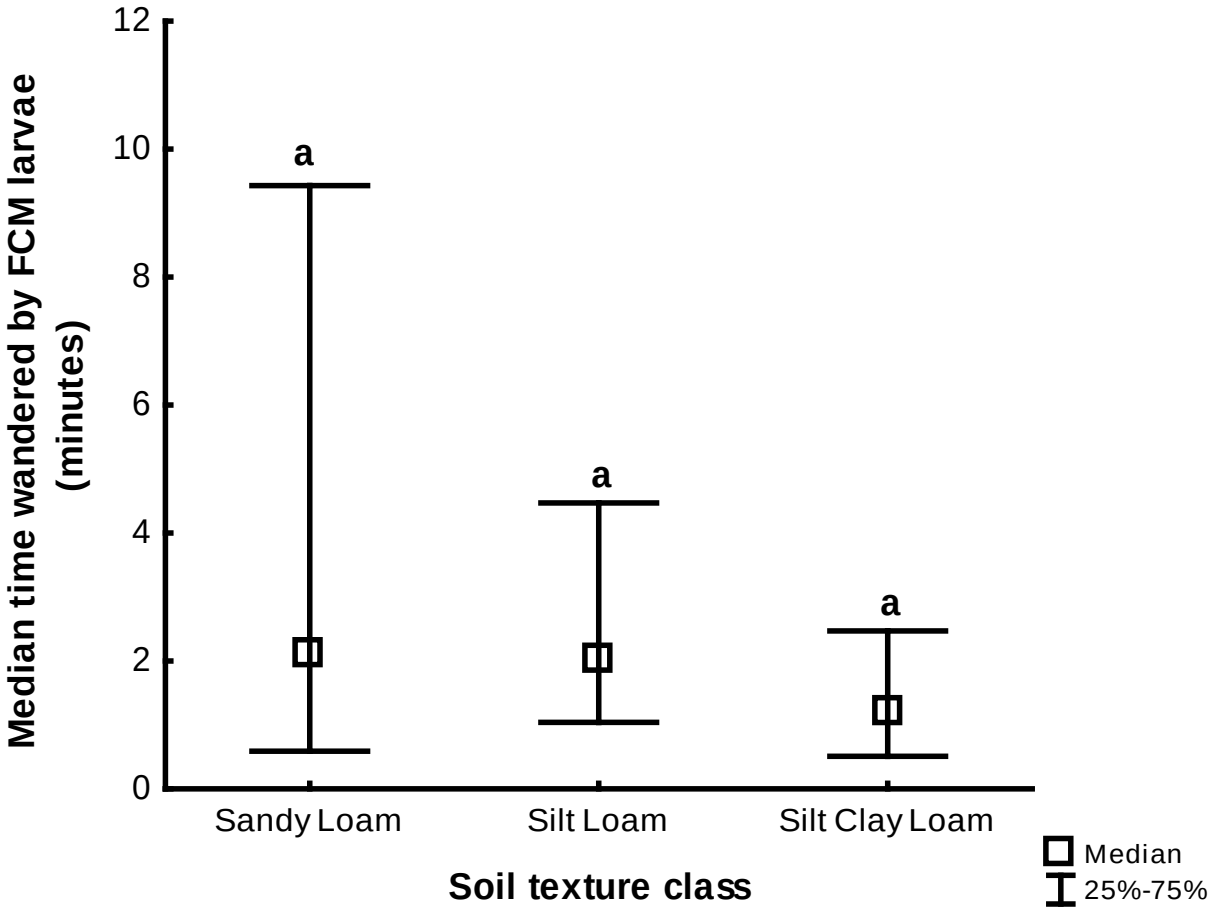

Figure 4.2 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection on compacted soil of the three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median wandering distance of FCM larvae was fairly short, with no significant difference being found between the three different compacted soil texture classes ($H_{(2,90)}$ = 4.217, p = 0.122). The median distance wandered was less than 30 mm for all of the soil texture classes. Variability was once again high however, while larvae wandering on sandy soil appeared to take a longer amount of time to locate a suitable pupation site, this did not translate to longer wandering distances (Fig. 4.3).

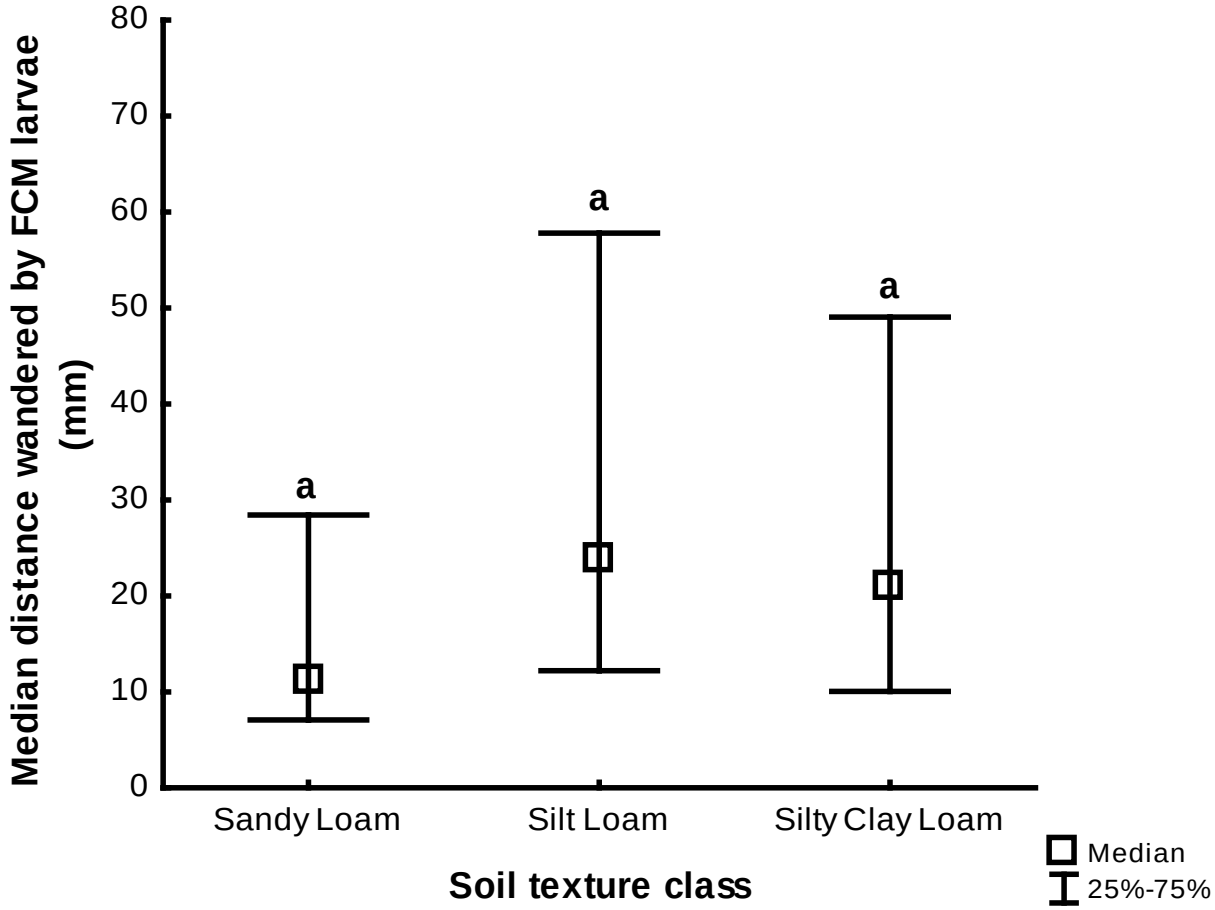

Figure 4.3 The median distance wandered by FCM larvae on the compacted soil surface, prior to pupation site selection for the three different soil texture classes (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time taken for FCM larvae to spin a pupal cocoon was in the range of 35 to 45 minutes for the three different soil texture classes, but no significant differences were found between these when the three different soil texture classes were compacted ($H_{(2,90)} = 3.048$, $p = 0.218$) (Fig. 4.4).

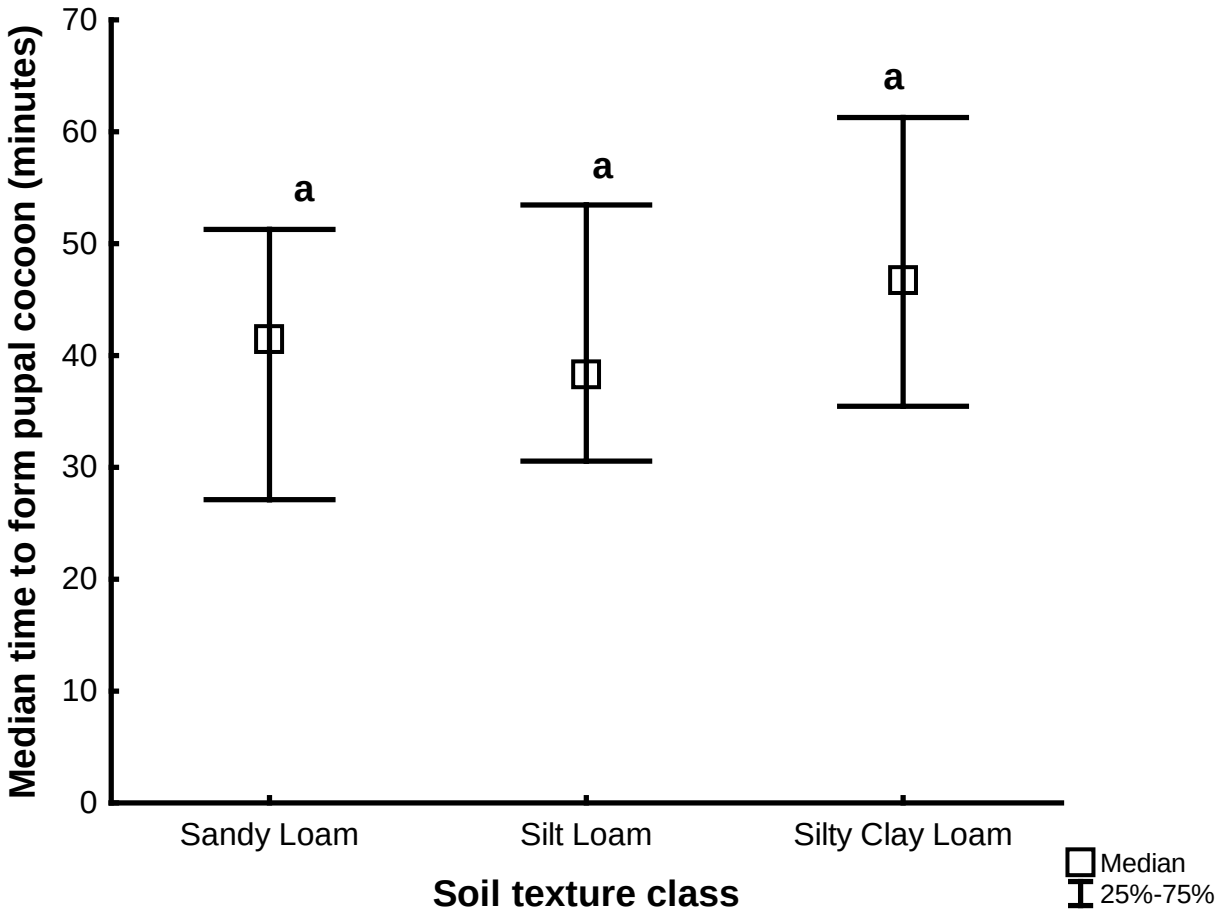

Figure 4.4 Median time taken for FCM larvae to form a pupal cocoon in compacted soil of the three different soil texture classes (n = 90). Different letters denote significant differences ($p < 0.05$).

Soil texture classes had no significant impact on the depth on FCM pupation depth when the soil was compacted ($H_{(2,90)} = 1.662$, $p = 0.920$) (Fig. 4.5). With the compaction of the soil, natural cracks appeared on the surface of the soil and extended deeper into the soil as well. Over a third of FCM did pupate in these soil cracks in all three soil texture classes and with this came the first appearance of vertically orientated pupae. This was in contrast to the soil texture class (Chapter 2) and ground cover (Chapter 3) experiments where the pupae were all horizontally orientated on the soil surface.

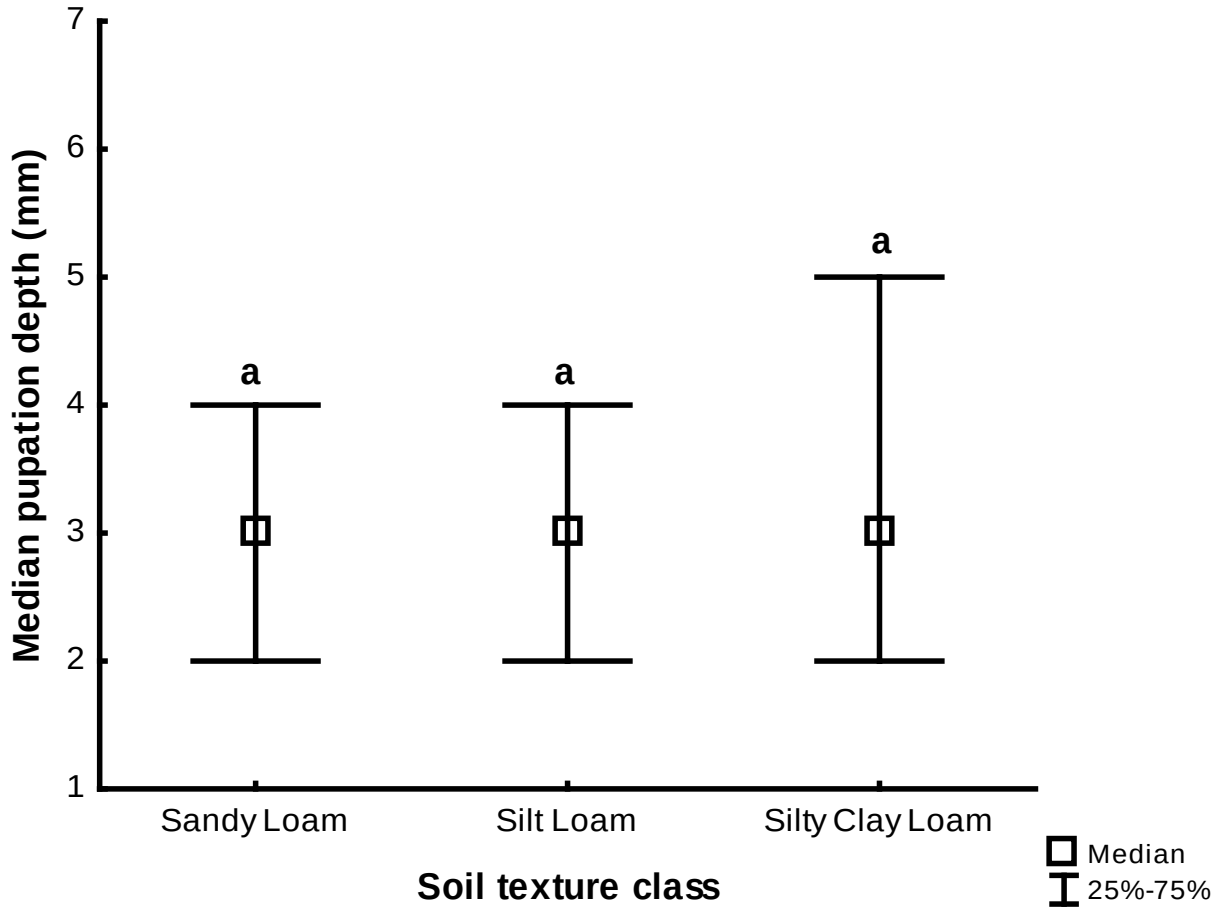

Figure 4.5 Median depth of FCM pupation in three different soil texture classes with compacted soil (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Compacted soil texture class had no significant effect on the median amount of time that was taken for adult FCM to develop and eclose ($H_{(2,75)} = 4.521$, $p = 0.104$). Adult FCM development generally took between 11 and 15 days to complete (Fig. 4.6).

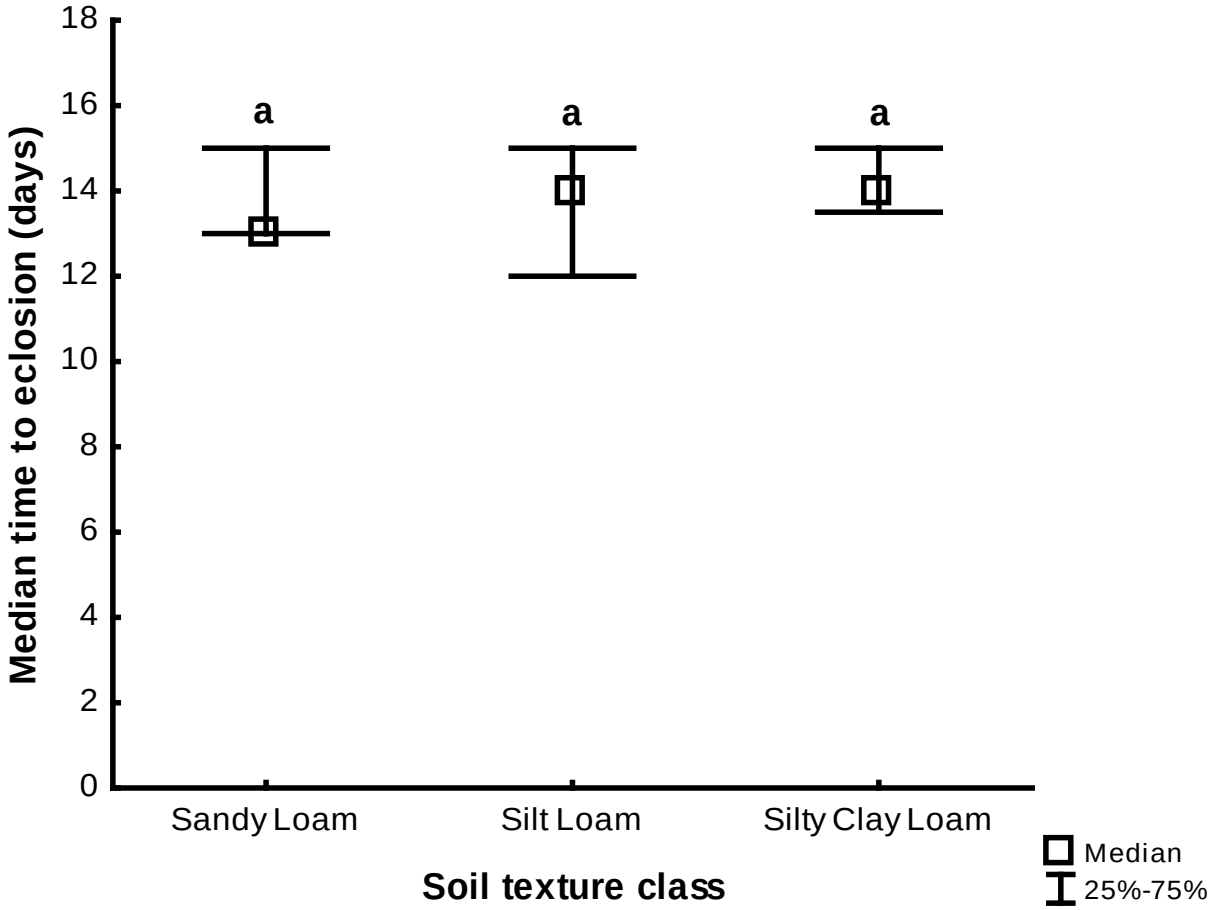

Figure 4.6 The median amount of time taken for adult FCM to eclose from the pupae formed in compacted soil of the three different soil texture classes (n = 75). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Overall, the percentage of successfully eclosed adult FCM was very high, with 87 % of adults eclosing from sandy soil, 83 % from silt loam and 80 % for silty loam soil, however no significant association was found between soil texture class and FCM eclosion (Chi-square = 0.480, df = 2, p = 0.787) (Fig. 4.7).

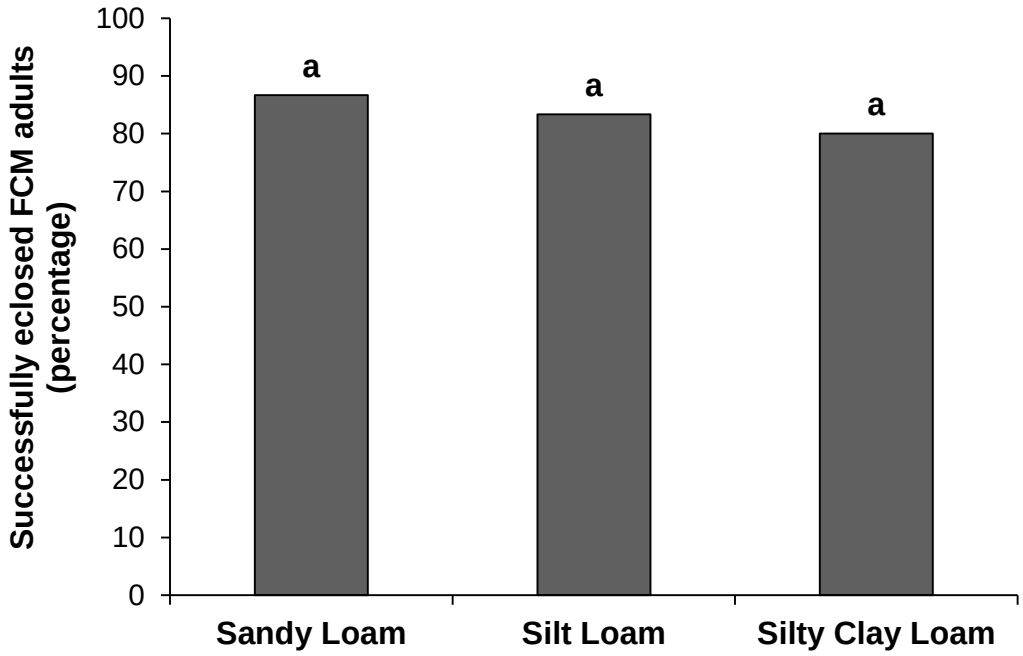

Figure 4.7 The percentage of successfully eclosed FCM adults from pupae formed in compacted soil of the three different soil texture classes (n = 75). Different letters denote significant differences (Chi-square test, $p < 0.05$).

4.3.3 Comparison of soil texture classes and soil compaction

When comparing each soil texture class and both compacted and uncompacted soil data from Chapter 2, there were no significant differences found between any of the treatments for FCM wandering time ($H_{(5,180)} = 6.839$, $p = 0.233$) (Fig. 4.8).

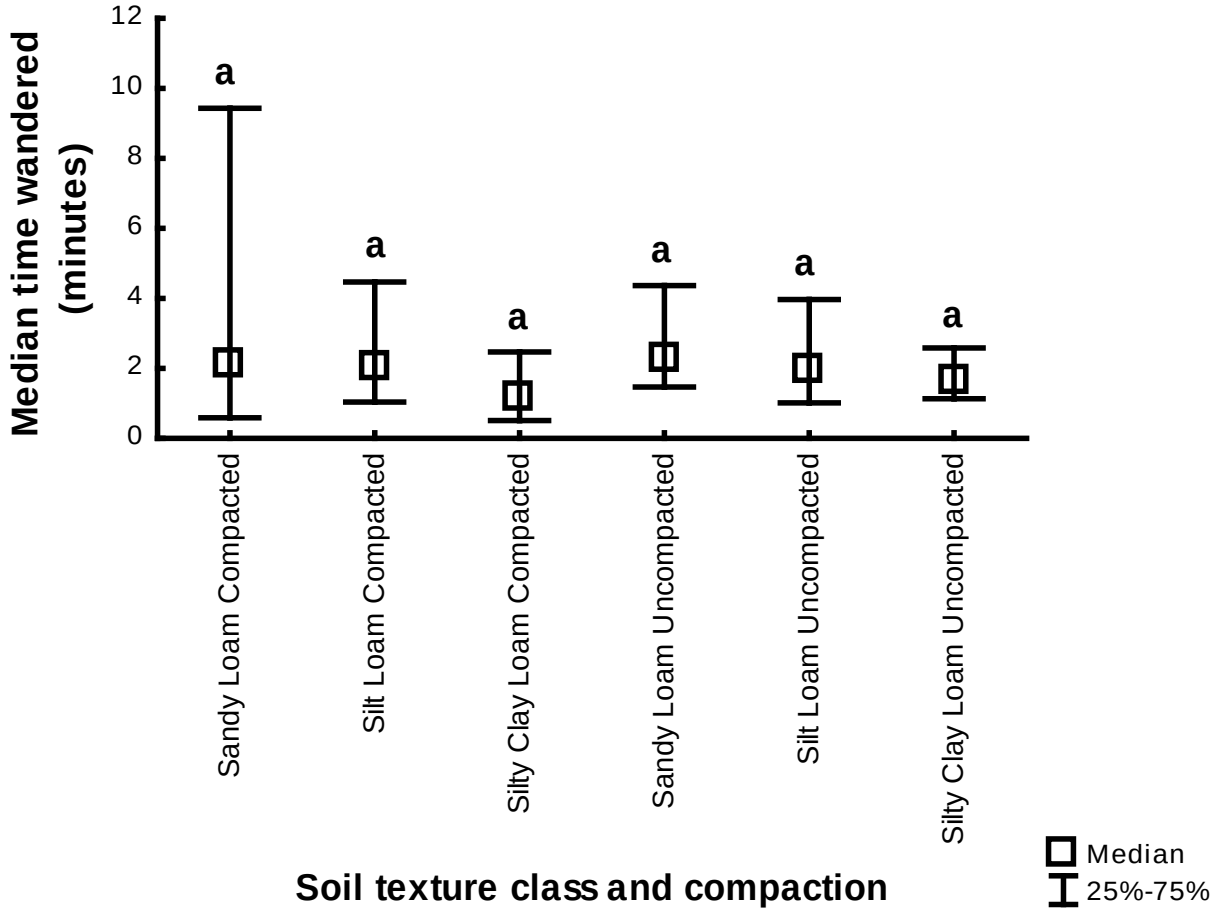

Figure 4.8 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection on compacted and uncompacted soil of the three different soil texture classes (n = 180). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant difference was found in the distance wandered by FCM larvae when comparing each soil texture class and both compacted and uncompacted soil ($H_{(5,180)} = 5.746$, $p = 0.332$) (Fig. 4.9).

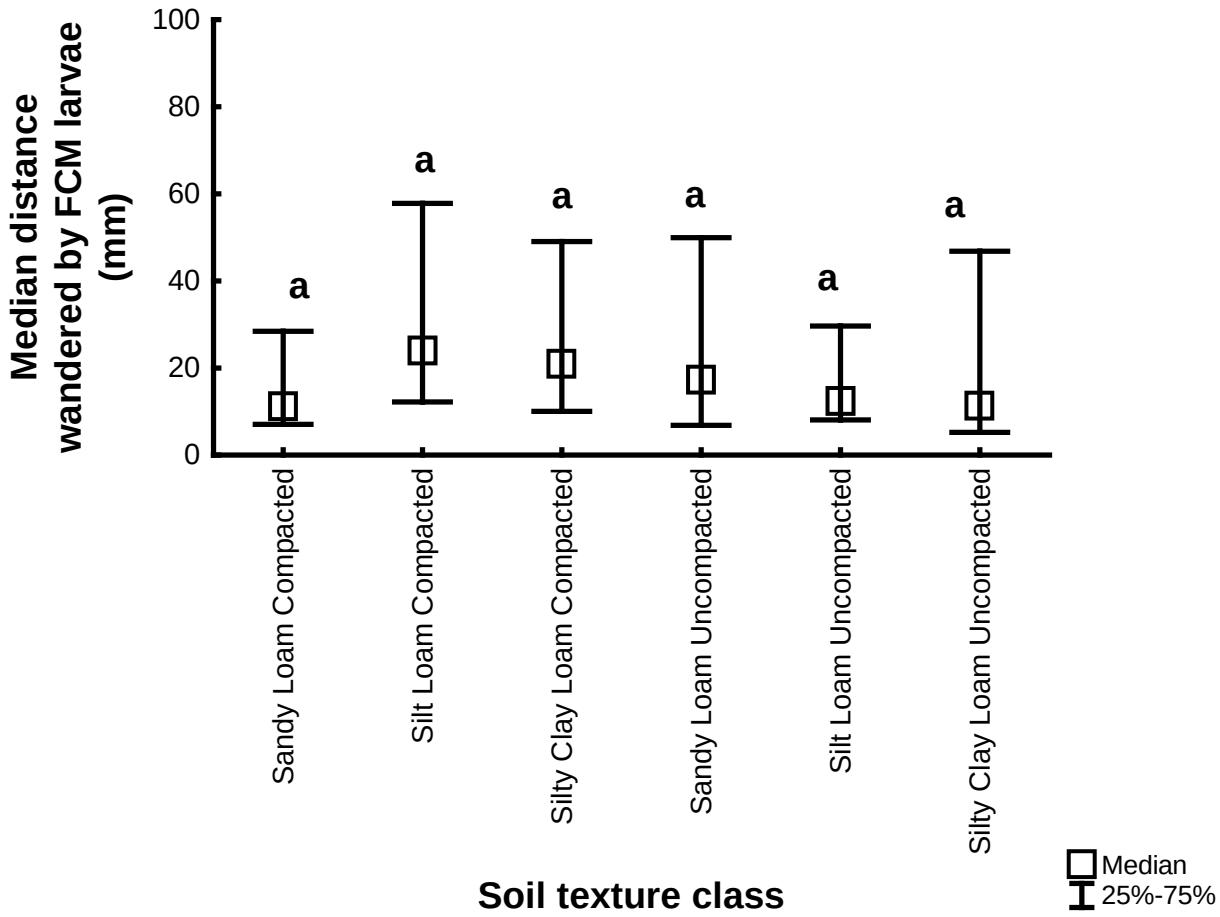

Figure 4.9 The median distance wandered by FCM larvae on the compacted and uncompacted soil surface, prior to pupation site selection for the three different soil texture classes (n = 180). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Pupal cocoon formation showed no significant differences found between any of the treatments for FCM wandering time for each soil texture class and compaction level ($H_{(5,173)} = 5.397$, $p = 0.369$) (Fig. 4.10).

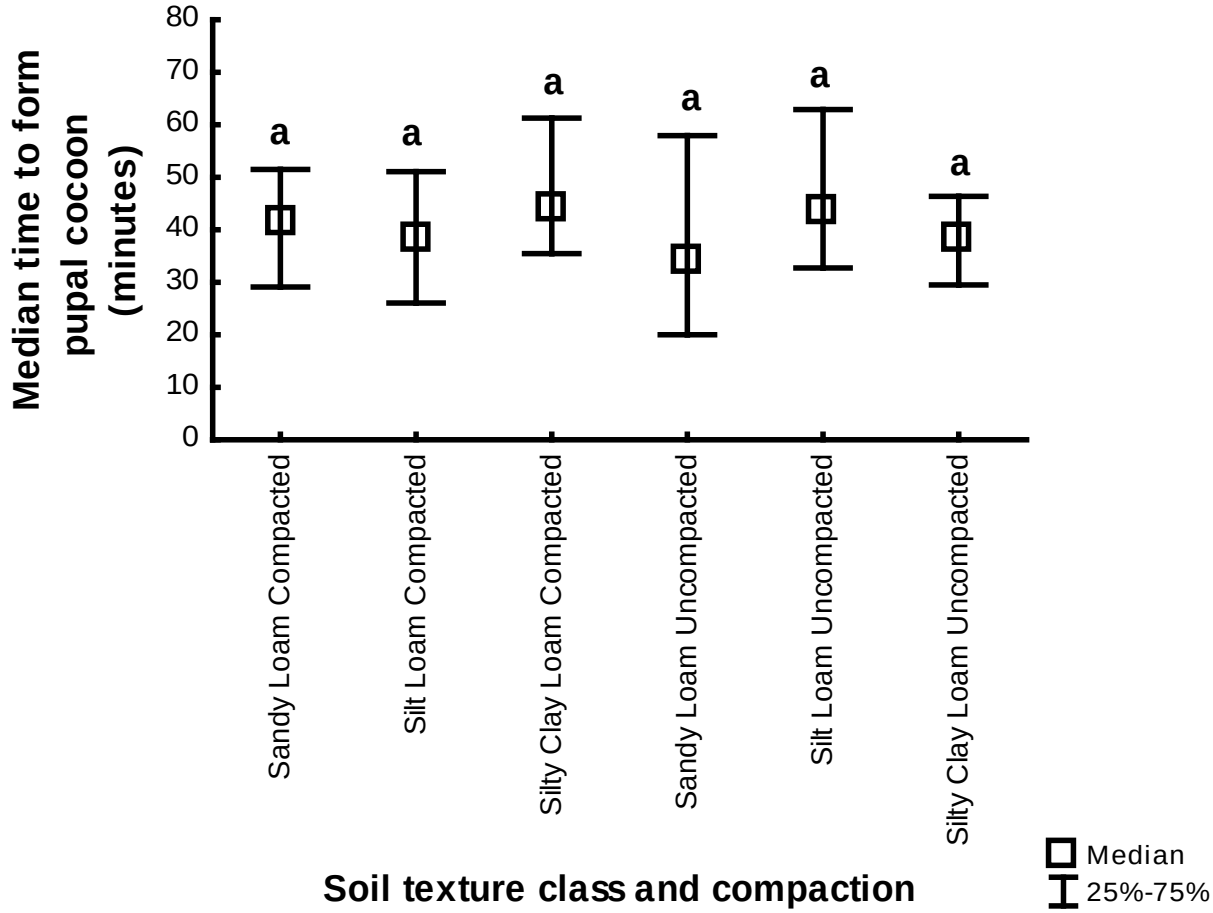

Figure 4.10 Median time taken for FCM larvae to form a pupal cocoon in compacted and uncompacted soil of the three different soil texture classes (n = 173). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Pupation depth was significantly shallower in the uncompacted silt loam soil and silty clay loam soil (with a median of 2 mm) when compared to the sandy loam uncompacted soil treatment and all three compacted soil treatments (medians of 3 mm) ($H_{(5,180)} = 29.54$, $p < 0.0001$) (Fig. 4.11).

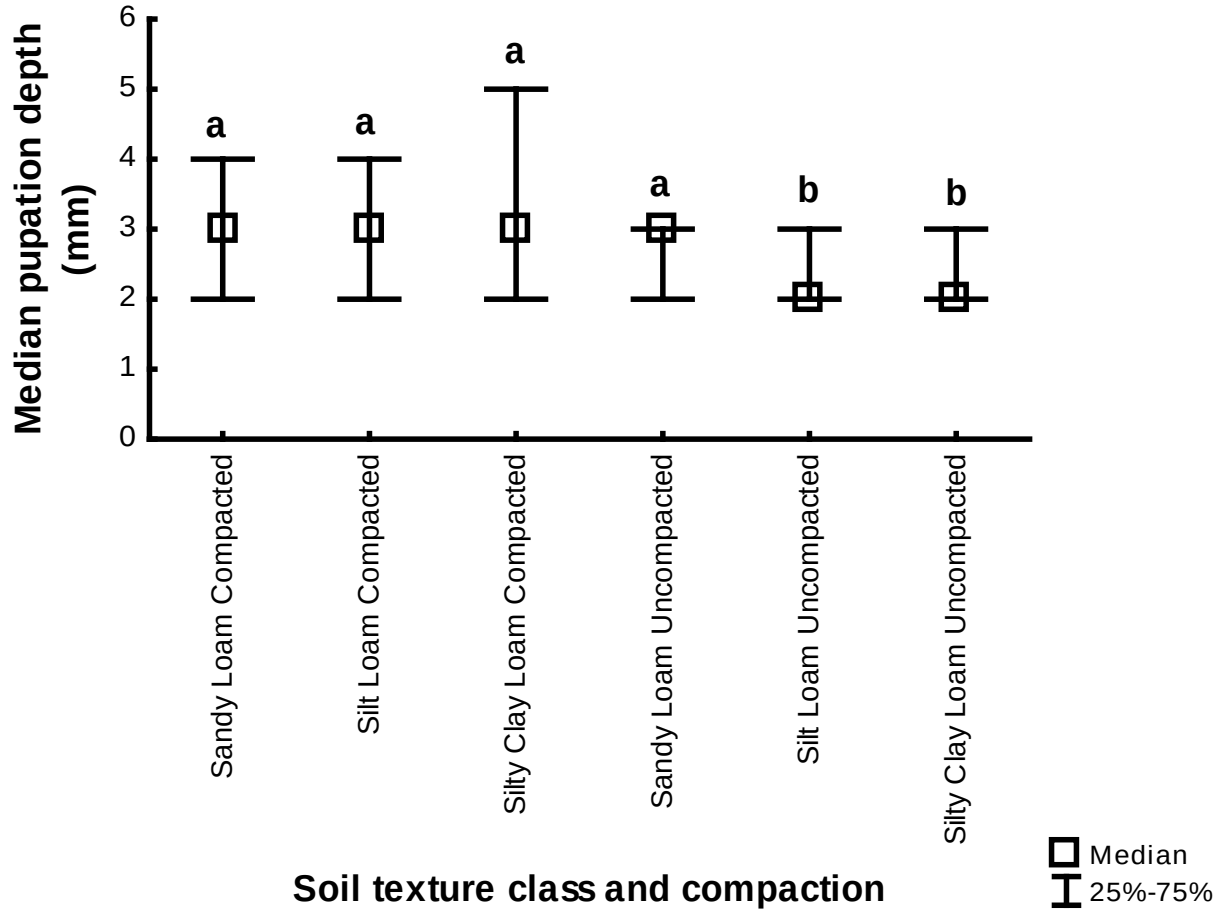

Figure 4.11 Median depth of FCM pupation in three different soil texture classes with compacted and uncompacted soil (n = 180). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time to eclosion showed some significant differences between the treatments when comparing each soil texture class and both soil compaction levels ($H_{(5,138)} = 30.195$, $p < 0.0001$). The eclosion time of pupae formed in silty clay loam uncompacted soil was significantly faster than that of all three compacted soil texture classes and the uncompacted sandy loam treatment, but not from the silt loam uncompacted treatment (Fig. 4.12).

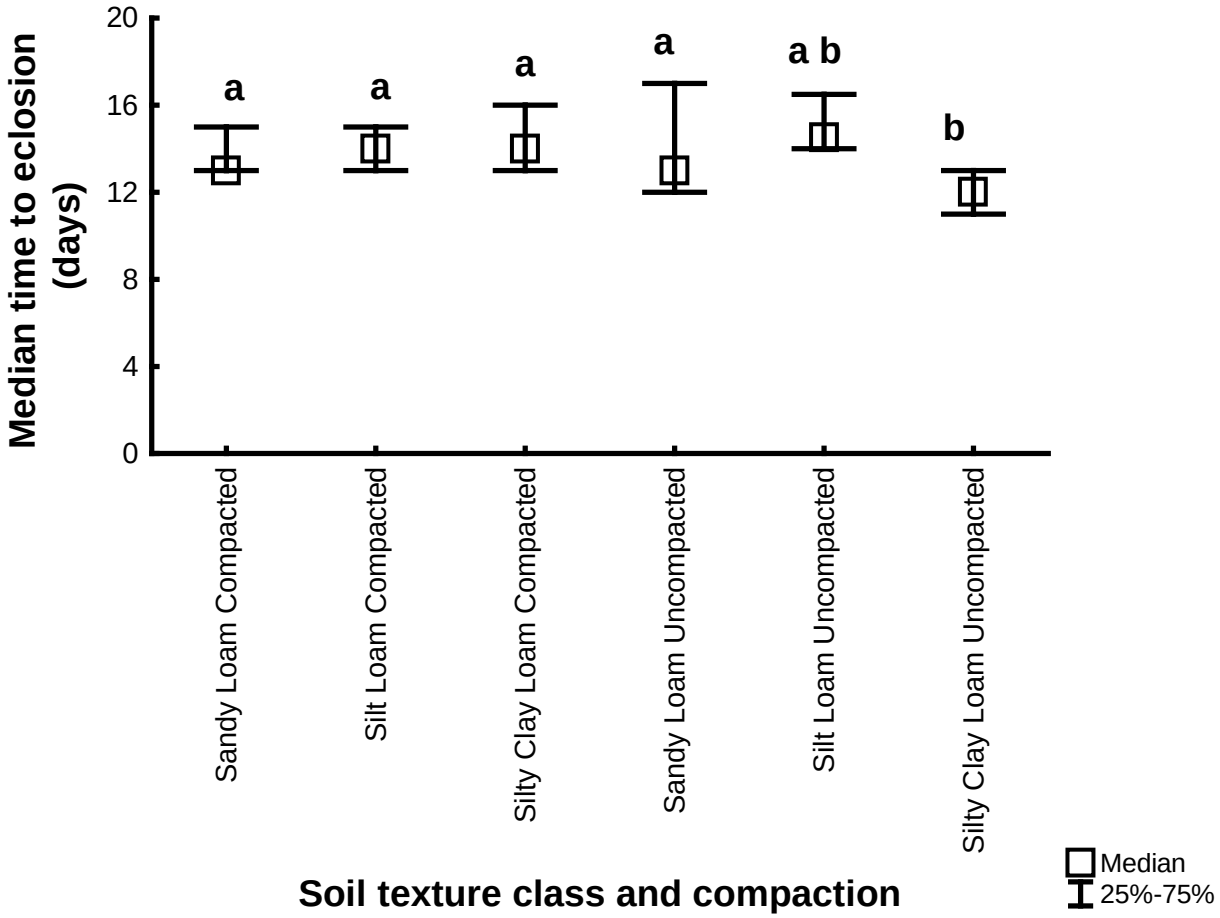

Figure 4.12 The median amount of time taken for adult FCM to eclose from the pupae formed in compacted and uncompacted soil of the three different soil texture classes (n = 138). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The compacted silt loam and silty clay loam soil treatments showed a higher percentage of FCM eclosion success than the uncompacted soil treatments. Statistically, the silt loam uncompacted soil treatment had significantly lower eclosion success of FCM adults when compared to the three compacted soil treatments and the sandy loam uncompacted treatment (Chi-square = 12.676; df = 5; p = 0.027) (Fig. 4.13).

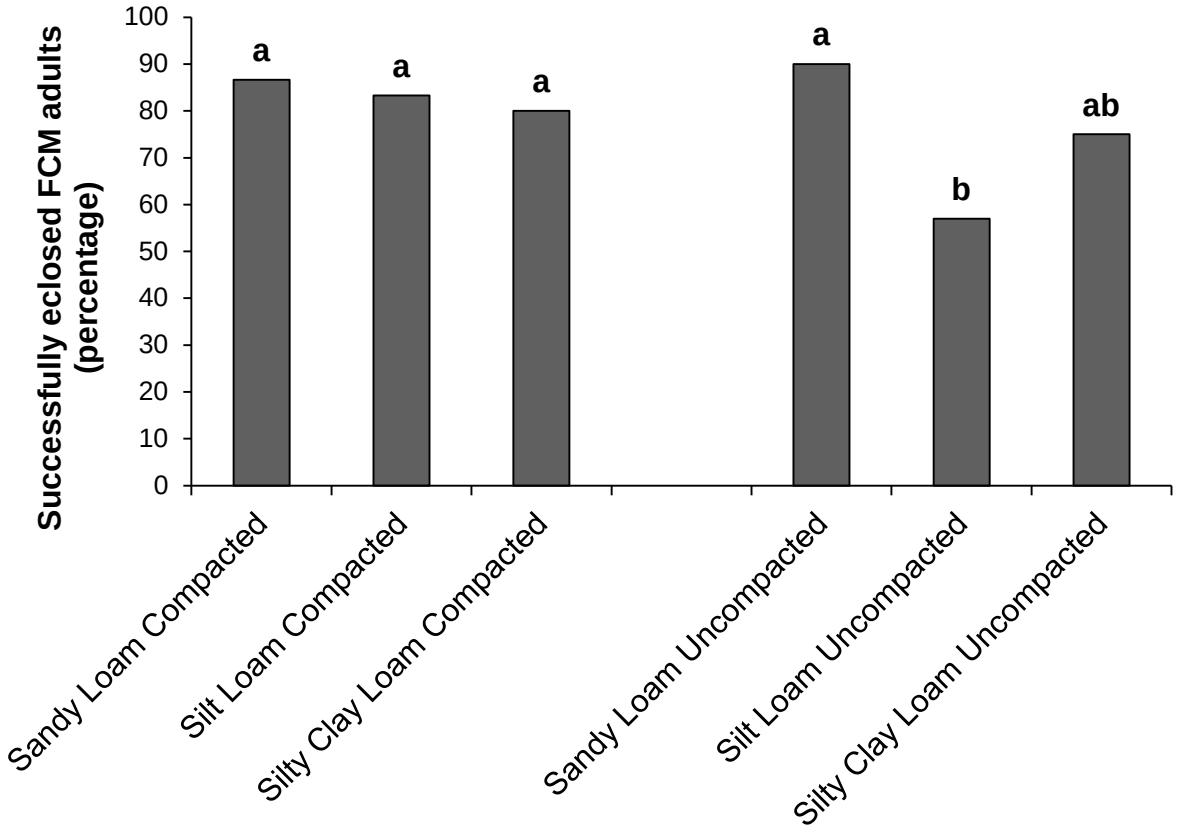

Figure 4.13 The percentage of successfully eclosed FCM adults from pupae formed in compacted or uncompacted soil of the three different soil texture classes (n = 138). Different letters denote significant differences (Chi-square test, $p < 0.05$).

4.3.4 Overall Comparison of Compacted and Uncompacted Soil

When grouping all of the soil texture classes together in order to compare the overall impact of soil compaction, no significant difference was found between the amount of time that FCM larvae wandered on the surface of compacted in comparison to loose soil (Mann-Whitney $U_{(90,90)}$ = 3572.0, p = 0.172). The wandering time remains short, whether in compacted or uncompacted soil (Fig. 4.14).

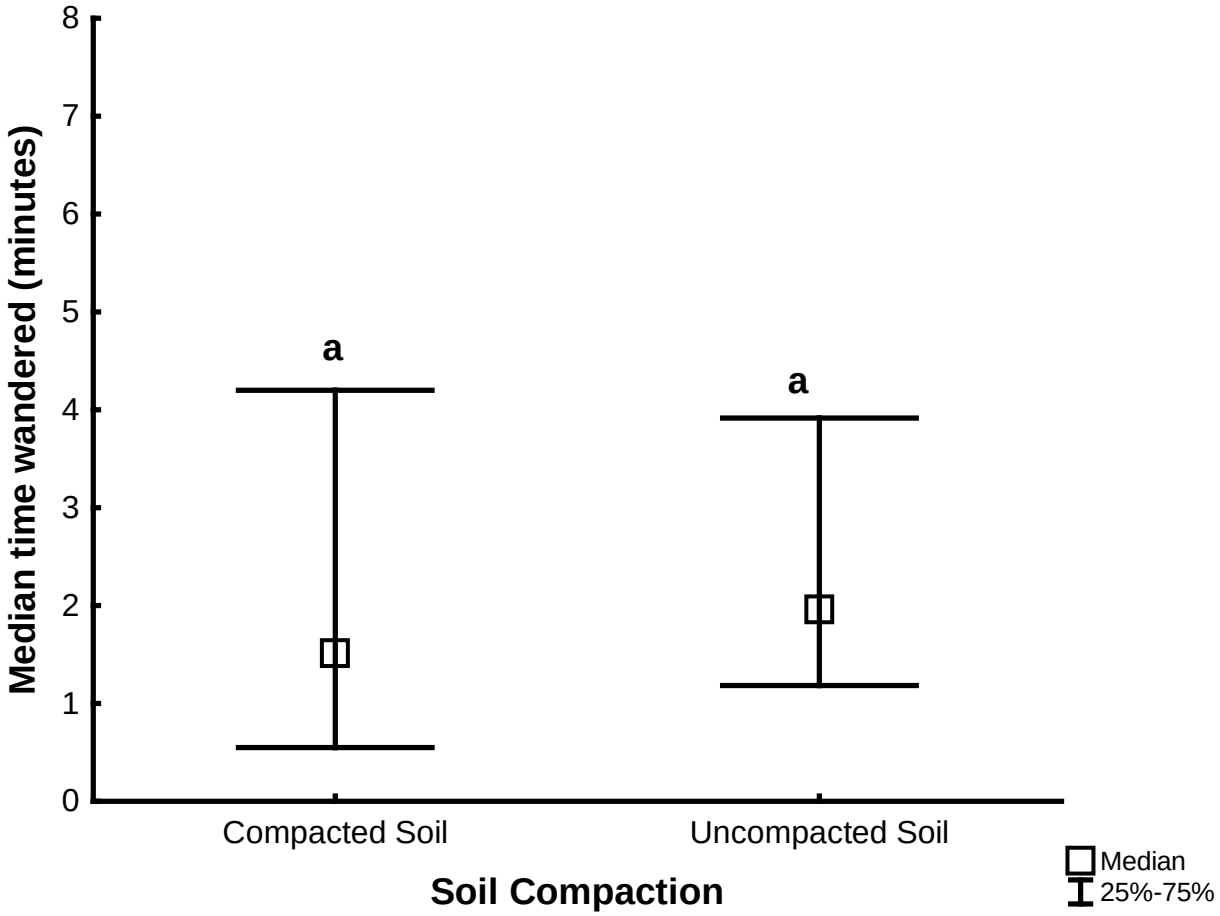

Figure 4.14 A comparison of median larval FCM wandering time on compacted and uncompacted soil, with all three soil texture classes (sandy loam, silt loam and silty clay loam) combined (n = 180). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

No significant relationship was found to occur between the median distance wandered by FCM larvae and soil compaction (Mann-Whitney $_{(90,90)}$ U = 3607.5, p = 0.206). The variability of wandering distance is high in both compacted and uncompacted soil (Fig. 4.15).

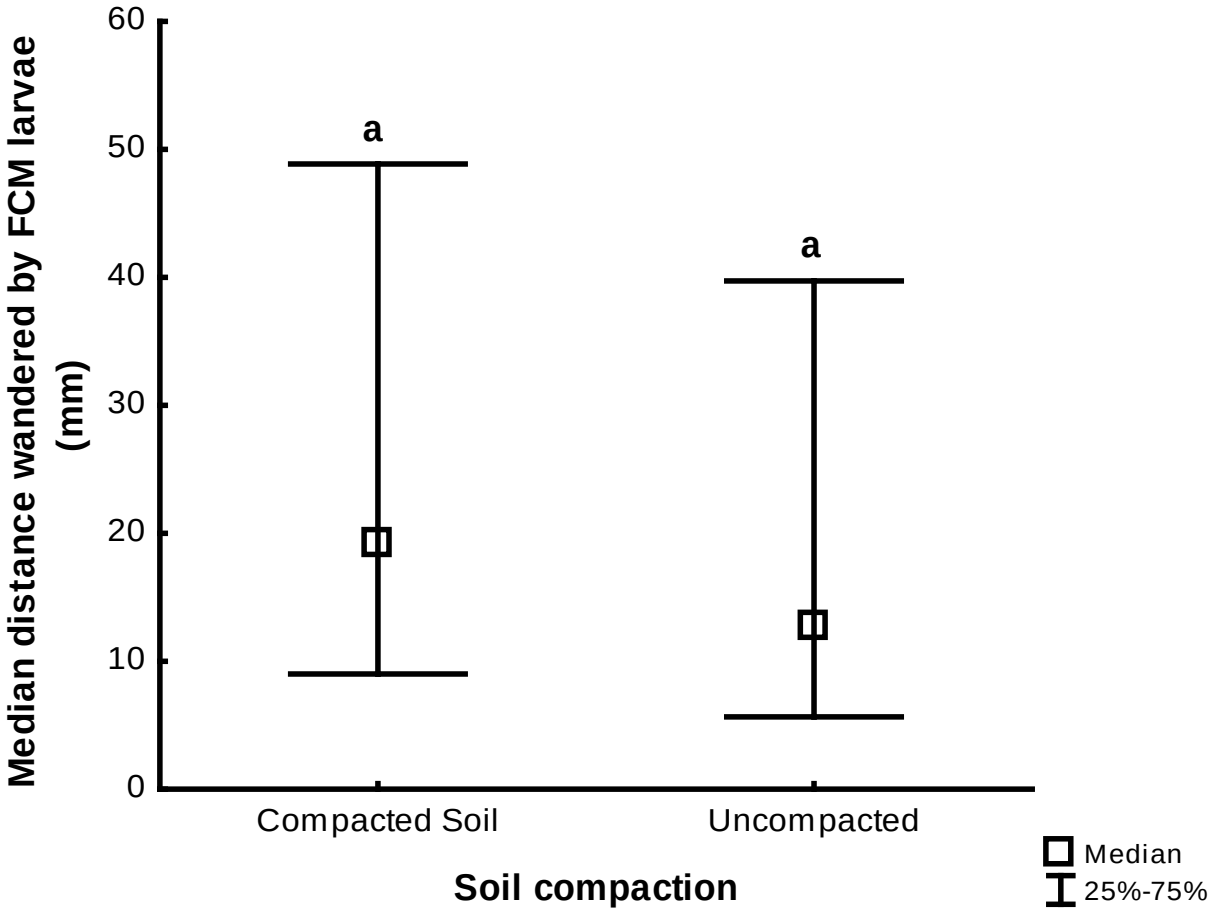

Figure 4.15 A comparison of median larval FCM wandering distance on the surface of compacted and uncompacted soil, with all three soil texture classes combined (n = 180). Different letters denote significant differences (Mann-Whitney *U*-Test, p < 0.05).

There was no significant relationship between the median time taken by FCM larvae to spin the pupal cocoon and soil compaction (Mann-Whitney $U_{(90,83)} = 3595.0$, $p = 0.672$) (Fig. 4.16).

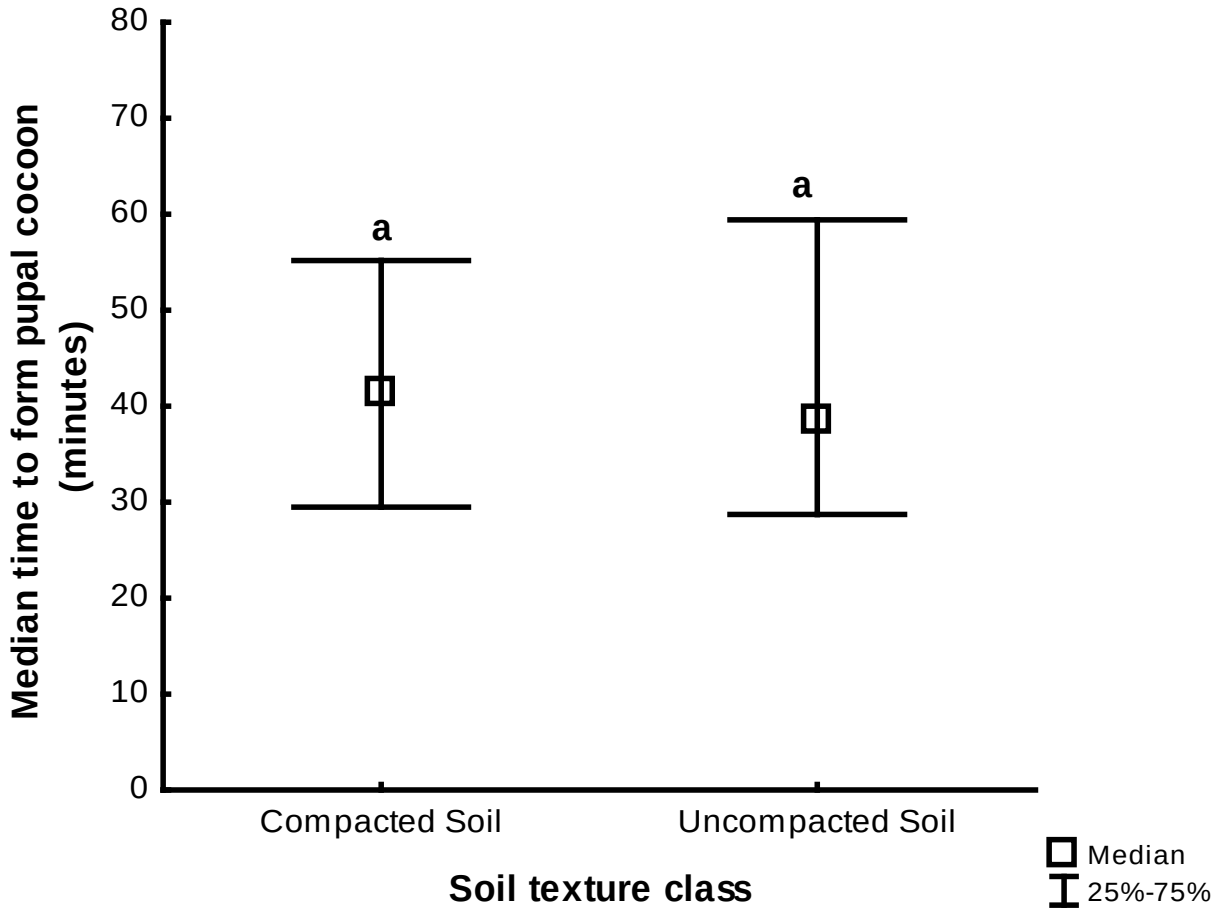

Figure 4.16 A comparison of median amount of time taken by FCM larvae to spin the pupal cocoon in compacted and uncompacted soil, with all three soil texture classes combined (n = 173). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

Median pupation depth did reveal a significant difference between compacted and loose soil (Mann-Whitney $U_{(90, 90)} = 2476.0$, $p < 0.0001$) with compacted soil resulting in a deeper pupation depth than when compared to uncompacted soil (Fig. 4.17).

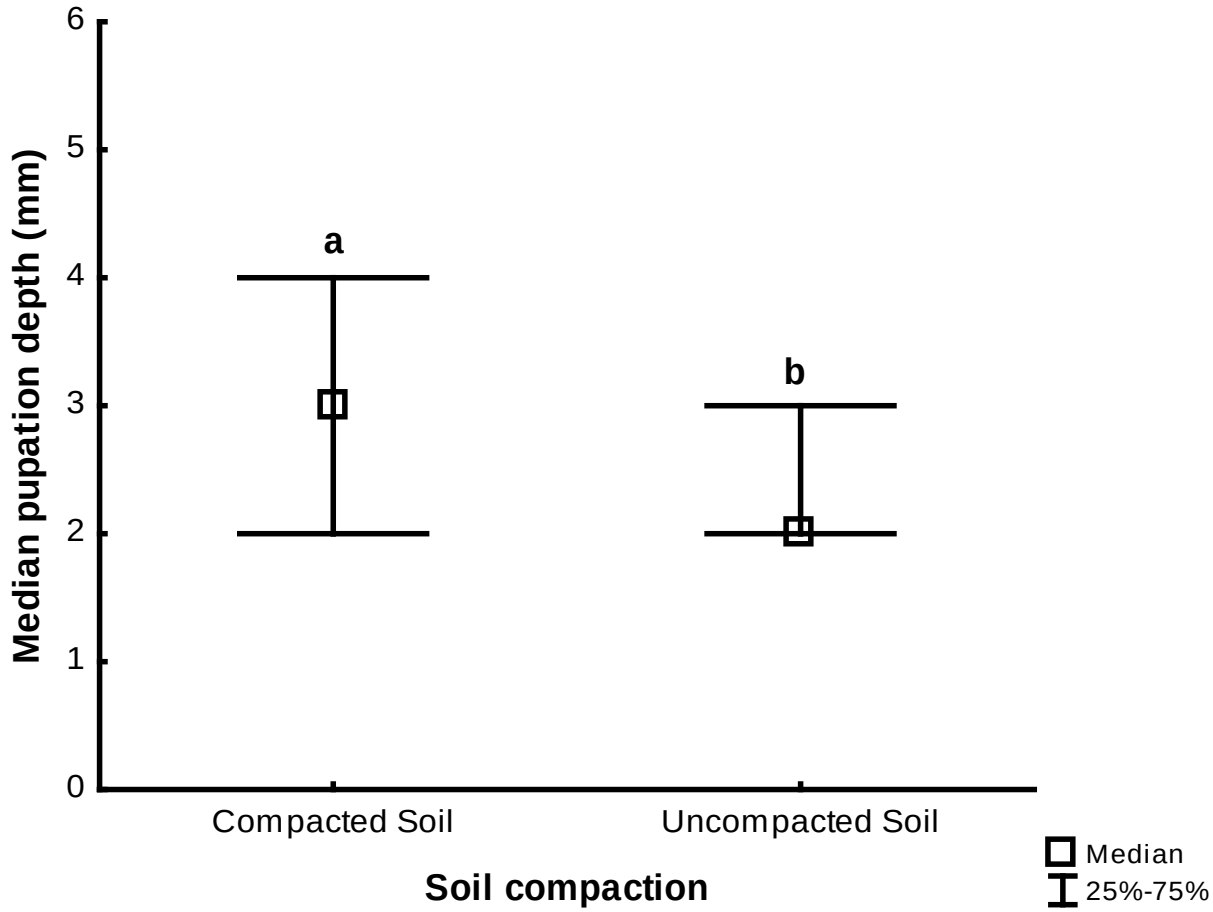

Figure 4.17 Median FCM pupation depth in soil that was either compacted or uncompacted, with all three soil texture classes combined (n = 180). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

The association between soil compaction and median time taken for adult FCM to develop and eclose from their pupae was very close to being a significant result where time to eclosion being longer in compacted soil than uncompacted soil, statistically this was found to not be the case (Mann-Whitney $U_{(75,63)} = 1935.0$, $p = 0.046$). In general, eclosion time is not very variable in both compacted and uncompacted soil (Fig. 4.18).

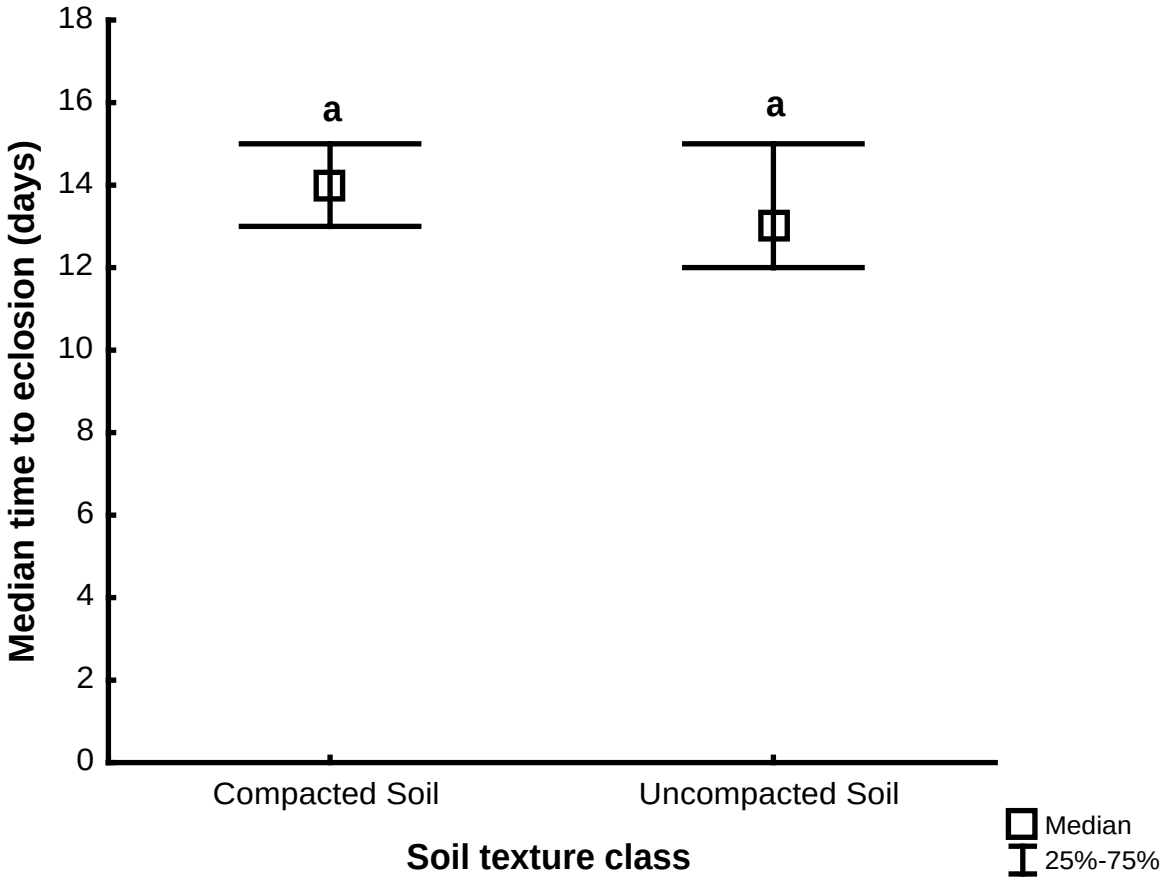

Figure 4.18 A comparison of median time to eclosion of FCM adults from pupae formed in compacted and uncompacted soil, with all three soil texture classes combined (n = 138). Different letters denote significant differences (Mann-Whitney *U*-Test, $p < 0.05$).

A comparison of the percentage of successfully eclosed adult FCM from compacted soil (83 %) and uncompacted soil (71 %) showed no significant association between soil compaction and adult eclosion (Chi-square = 2.93; df = 1; p = 0.087) (Fig. 4. 19).

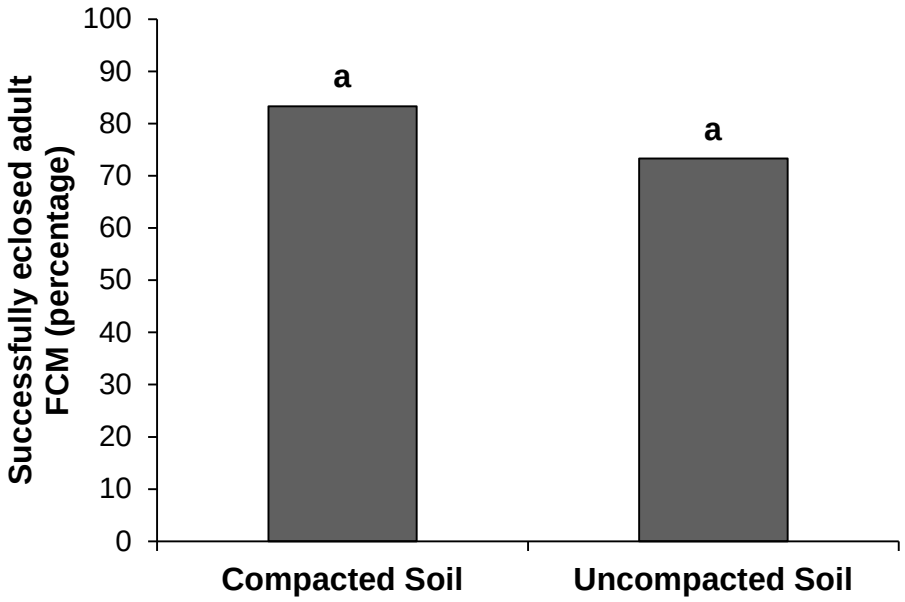

Figure 4.19 The percentage of successfully eclosed adult FCM from pupae formed in either compacted or uncompacted soil, where all three soil texture classes were combined (n = 138). Different letters denote significant differences (Chi-square test, $p < 0.05$).

4.4 DISCUSSION

Compacted soil is a common feature of the agricultural soil landscape (Kozlowski 1999; Brady & Weil 2000). Compaction level or soil strength is highly influenced by soil texture class, as indicated by the three different soil texture classes used in the study. In this study, sandy loam was by far the soil texture class which was least susceptible to compaction, most likely due to the large size of the soil particles resulting in these not being able to come into as close a contact with one another as the smaller sized silt and clay particles (Brady & Weil 2000). Silt and clay particles were more common in the silt loam and silty clay loam soils. As a result of this, both of these soils were far more compacted with no difference in soil strength between silt loam and silty clay loam being found, which may indicate the

importance of low sand particle content (25 and 16 % respectively) when examining soil compaction levels.

Surprisingly, the compaction of the soil made very little difference to the biology, behaviour and survival of FCM when comparing between the three different soil texture classes. No significant differences were found for any of the variables measured: wandering time, wandering distance, the amount of time taken for pupal cocoon formation, time to eclosion or eclosion success. Wandering time and distance were expected to increase, as the compacted nature of the soil may have made suitable pupation sites more difficult for the larvae to find but this was not the case. Based on real-time and video observations, once FCM larvae found a suitable area with (even a limited amount) of loose soil particles, the pupal cocoon would be formed. The eclosion success was very high overall, with the eclosion not dropping below 80 % for any one soil texture class.

The overall comparison of each soil texture class and compaction level showed only a deeper pupation depth for silt loam and silty clay loam soils and a faster eclosion time for FCM pupae formed in silty clay loam soil. Eclosion success was also higher for all three compacted soil texture classes when compared to the silt loam uncompacted treatment. No trends in wandering distance or time variation were found. The comparison of compacted soil and uncompacted soil revealed that not only did soil compaction not have a strong influence on larval FCM wandering time and distance, but the overall influence of compaction on these factors was minimal. Soil compaction did influence FCM pupation depth and and the result for the developmental time inside the pupae was very close to being significant. The influence of soil compaction on insect pupation depth varied. For the wild olive fruit fly, *Bactrocera (Dacus) oleae* Gmel. (Diptera: Tephritidae) soil compaction appeared to have no influence on pupation depth, although this was attributed to the researchers not selecting suitable compaction levels (Dimou *et al.* 2003). Increasing compaction levels have been shown in studies on bollworm, *Heliothis zea* Boddie (Lepidoptera: Noctuidae), to result in reduced pupation depth from an average of 35 mm in looser soil to 18.8 mm in compact soil (Roach & Campbell 1983). Hennessey (1994) similarly found that the depth of pupation of Caribbean fruit fly, *Anastrepha suspensa* Loew (Diptera: Tephritidae) was deeper in less compact soil. This was not the case for FCM where compaction resulted in larvae pupating deeper into the soil than in uncompacted soil. The formation of cracks in the soil which were produced during the compaction process are likely to be the cause of this as these cracks allowed larvae to pupate more deeply in the soil without having to physically burrow into it.

FCM larvae are known to pupate in soil cracks (Newton 1998). This also allowed for the larvae to pupate vertically in the soil, which occurred almost exclusively when the pupal cocoon was formed in a soil crack. This increase in pupation depth may be advantageous to FCM as the immobile pupae would be less vulnerable to predation (Danks 2002), while not compromising on oxygen availability which is reduced in compact soil (Barbercheck 1992; Brady & Weil 2000; Cammack *et al.* 2010).

The length of time to eclosion of FCM adults was expected to increase with increasing soil compaction, however this result was only just found to not be statistically significant. An increase in insect development time with high soil compaction has been found for *Lucilia sericata* Meigen (Diptera: Calliphoridae) (Cammack *et al.* 2010). This is thought to relate to the pore size and soil temperature where the larger pore size in uncompacted soil allows for more air availability (Barbercheck 1992). Soils which are less compacted and have better aeration are warmer than uncompacted soil which is poorly aerated and therefore cooler (Cammack *et al.* 2010). This is due to the specific heat of air being lower than that of soil (Brady & Weil 2000). Compacted soil conditions are therefore expected to be cooler and although it appeared visually that FCM development was slowed, this was not able to be statistically confirmed. No statistical difference was found in the number of FCM adults which were able to eclose from compacted soils rather than the looser soil. For bollworm moths, it was found that eclosion success reduced as compaction increased (Roach & Campbell 1983). The shallowness of FCM pupation in general may be the reason for this lack of difference in eclosion success.

Highly compact soils are likely to have varying influences on the soil-dwelling biological control agents of FCM. For entomopathogenic fungi, provided that the environment remains sufficiently moist and the spores are protected from UV radiation (Ignoffo 1992), high soil compaction may have a less negative effect on fungal pathogenicity as the majority of conidia have been found to be retained on the soil surface (Garrido-Jurado *et al.* 2011). If the current shallow pupation depths which have been determined in the laboratory were also found to occur in the field, it is highly likely that EPF would remain a viable control agent for FCM despite compacted soils. For EPN infective juveniles (IJs), soil compaction may be more problematic as IJs must be able to move through the soil in order to actively seek out their hosts (Riga 2004). The higher soil strength of compact silt and clay dominated soils would result in smaller pore spaces of these soils, reducing EPN movement and dispersal (Kaya &

Gaugler 1993; Brady & Weil 2000). This should be less problematic in compact sandy soil as the soil strength is lower and larger pore spaces would be available.

Soil compaction levels will also influence soil aeration and soil temperature (Brady & Weil 2000), both of which will impact EPF and EPNs. Low oxygen levels of 20 % or less negatively influenced both the survival and infectivity of *Steinernema carpocapsae* (Steiner) and *Steinernema glaseri* (Weiser) in sandy loam soils (Kung *et al.* 1990b). The impact of air temperature will be discussed in more detail in Chapter 5 however, the cooler soil temperature of more compacted soil may be advantageous for fungi as thermal death occurs for the EPF at temperatures of above 50 °C (Barbercheck 1992). Thermal influences on EPNs are highly variable and species specific, however, less extreme high temperatures are likely to be a positive influence for them (Barbercheck 1992). The slower development time of FCM pupae in compacted soil may also be able to compensate for the reduced mobility of EPNs as they would have more time to reach the pupae prior to FCM adult eclosion. However this would only be possible if soil moisture levels were sufficient, as EPNs require soil particles to be surrounded with a film of water in order to disperse (Kaya & Gaugler 1993).

5

The influence of soil texture class and air temperature on FCM pupation

5.1 INTRODUCTION

Soil temperature is an important abiotic factor, which impacts all soil-dwelling fauna and flora (Schaetzl & Anderson 2005; Osman 2013). The temperature of the soil is largely regulated by three factors: firstly, the amount of net heat energy which can be absorbed by the soil; secondly, the amount of heat energy needed to change the soil temperature; and thirdly, the amount of energy needed for evaporation and other soil surface processes (Brady & Weil 2000). Heat provided by the sun is the principle way in which soil is heated, even though only approximately 10 % of this solar energy reaches the earth's surface (Brady & Weil 2000; Osman 2013). The amount of solar energy which reaches the soil is influenced by the geographic location of the soil, climate of the area, as well as edaphic and topographical features of the area (Osman 2013).

Soil temperature is variable and changes throughout the day and night, also varying with depth (Osman 2013). Soil does heat more quickly than the air found in the interface between the two and this generally results in the soil surface temperature being between 1 and 5 °C higher than the air temperature (Osman 2013). Ground cover plays a very important role in soil temperature, as the ground debris tends to assist in moisture retention and reflecting solar radiation keeping soils cooler, but it also can reduce evaporation and radiation which warms soils (Osman 2013). Soil which has no ground cover will experience far more temperature variability, both warming and cooling in a short space of time (Brady & Weil 2000). As such, the soil surface can be a highly variable environment.

Any insects which spend a portion of their life cycle in the soil are strongly influenced by soil temperature (Villani & Wright 1990). Behavioural adaptations to temperature have been found, such as in the case of the woolly-bear caterpillar species *Gynaephora rossii* Curtis (Lepidoptera: Lymantriidae) found in Canada and Alaska where pupae were specifically orientated in a northwest-southeast direction in order to maximise sun exposure while reducing heat loss from the winds (Kevan *et al.* 1982). The ability of certain insects to diapause is often advantageous for cold temperature survival, as demonstrated by Morey *et al*. (2012) where diapausing corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) pupae had a lower supercooling temperature at which the body fluids freeze, than non-diapausing pupae.

A number of studies have examined the influence of cold temperatures on FCM biology (Stotter & Terblanche 2009; Boardman *et al.* 2012; Terblanche *et al.* 2014). Low temperature tolerance of adult FCM was found to be very limited, with poor survival and little indication of rapid cold-hardening at temperatures below 0 °C for periods of up to 10 hours (Stotter & Terblanche 2009). Boardman *et al.* (2012) found that FCM larvae were unable to survive the freezing of their body tissues (freeze-intolerant) with larval mortality being initiated at -8 °C and were chill-susceptible. Larval activity levels and feeding declined with decreasing temperatures, with FCM larvae entering into a state of chill-coma when temperatures reached between 7 and 3 °C, although the temperature that initiated this behaviour depended on whether the larvae had been allowed to feed and have water 24 hours prior to the experiment. There is also little evidence to suggest that FCM is able to diapause (Terblanche *et al.* 2014), therefore pupation of FCM is likely to be strongly influenced by lower temperatures. The majority of the temperature focus has been on the cold temperature tolerance of FCM, therefore less is known about the impacts of higher temperatures on FCM. To date, the influence of temperature on the pre-pupal and pupal life stages of FCM has not been examined. The value of understanding the influence of temperature on insect behaviour (Villani & Wright 1990) as well as on pupation (Simmons 1993; Mahroof *et al.* 2003; Bernier *et al.* 2014) has been noted. In some cases the effect of soil moisture and air temperature are combined (Eskafi & Fernandez 1990; Zheng *et al.* 2011). Temperature will also play an important role for potential FCM biological control agents, entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) (Barbercheck 1992; van Zyl & Malan 2014). The duration of FCM pupal development at the constant temperatures of 10, 15, 20 and 25 °C was previously determined Daiber (1979c). This study will add to this work by determining the

effect of the three selected constant temperatures (15, 25 and 32 °C) on FCM pre-pupation behaviour and pupal survival for the three different soil texture classes (sandy loam, silt loam and silty clay loam).

As such, the aims of this chapter were to determine the influence of different soil texture classes combined with different air temperatures on 1) FCM larval wandering time, 2) the distance wandered by FCM larvae prior to pupation site selection 3) time taken by FCM to spin the protective pupal cocoon, 4) the depth of pupation, 5) orientation of pupation (horizontal or vertical in soil profile), 6) amount of time taken to eclose and 7) eclosion success.

5.2 MATERIALS AND METHODS

In order to test the effect of temperature on FCM pupation, the larvae were allowed to pupate under three different constant temperature conditions. This was done at a high air temperature, an optimal development air temperature (25 °C) and a low air temperature. All experiments were run in Controlled Environment (CE) rooms where the temperature and humidity could be kept constant. For the 25 °C study, the FCM pupation data and temperature data as given in Chapter 2 were used where the mean air temperature was 25.3 °C (±0.8 °C). Previous research by Daiber (1979b, 1979c) had established that the theoretical lower threshold temperature for FCM larval development was 11.6 °C and pupal development was 11.9 °C although successful eclosion occurred at temperatures as low as 10.4 °C. Pupal development was slow, but still possible at 15 °C (Daiber 1979c), and therefore this was used as the low temperature treatment.

A temperature of 32 °C was selected for the high air temperature treatment. The air temperature was monitored using a Thermochron iButton® (Maxim Integrated Products, USA) which was placed into the CE room and readings were taken hourly. The soil surface temperature was monitored by burying an iButton just below the surface (approximately 3 mm in depth) of the soil in order to measure the soil temperature. The iButton was only added to the soil during pupal development and adult eclosion to reflect the temperature which the pupae were exposed to. The iButton was moved between the different soil texture classes to obtain an overall account of the impact of air temperature on soil temperature. These data

were then analysed using the ColdChain Thermodymanics software where hourly temperature measurements could be compared. All soils were placed into the CE rooms at the appropriate temperature at least 48 hours prior to the commencement of the experiment to allow the soil temperature to be reflective of the air temperature. The three different soil texture classes (sandy loam, silt loam and silty clay loam) were prepared in the same way as previously described in Chapter 2, with filming of FCM larval behaviour also being done in the same manner. Both the high and low temperature experimental results were compared with the data previously collected at 25 °C as presented in Chapter 2. The larvae were allowed to drop down into the soil of their own accord and the subsequent behaviour of 30 larvae per soil texture class was captured on film.

5.2.2 Statistical Analysis

The data were found almost exclusively to be non-parametric when testing for normality. Thus Kruskal-Wallis tests were used with a multiple comparison of mean ranks test being performed for statistically different data. In one case the data were found to be normal, however, a subsequent Levene's test showed that the variances were found to be significantly different therefore the non-parametric Kruskal-Wallis test was used. Categorical data were analysed using a Chi-square test to test for significant associations. The statistical software Statistica Version 10 (2011) was used for all of the analyses.

5.3 RESULTS

5.3.1 Air and Soil Temperature

The iButton temperature data recorded shows that the average temperatures maintained throughout the experimental period when FCM pupation was being filmed were fairly consistent with the temperatures which were required for the experiments (Fig. 5.1). Temperatures were recorded on an hourly basis.

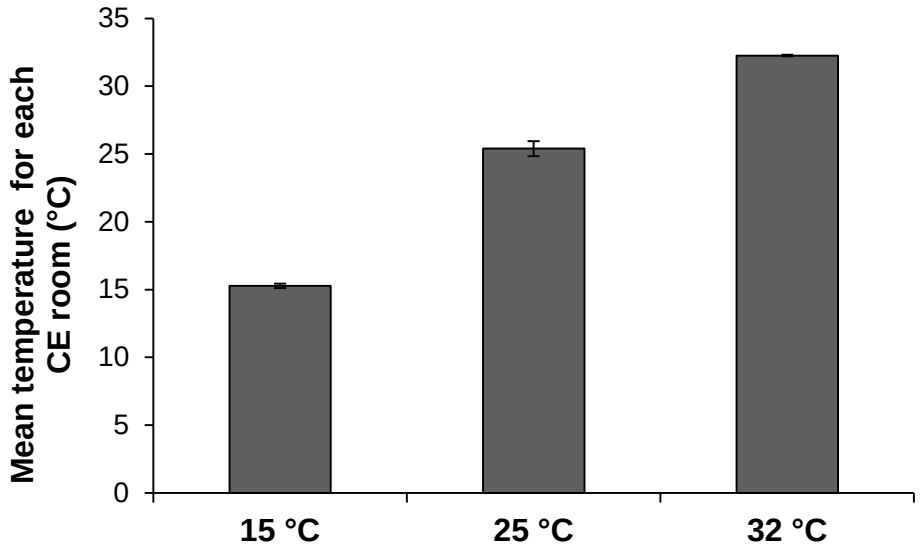

Figure 5.1 Mean air temperature (±SE) for each CE room temperature experiment (15 °C, 25 °C and 32 °C) throughout the duration of the pupation experiment.

The iButtons which were added to the soil during the development period prior to adult eclosion indicate that for the high temperature experiment, the soil surface temperature closely reflected the air surface temperature. In general, the air temperature showed more variation, with the occasional spike or drop in temperature occurring, most likely due to uncontrollable factors such as power failures (Fig. 5.2).

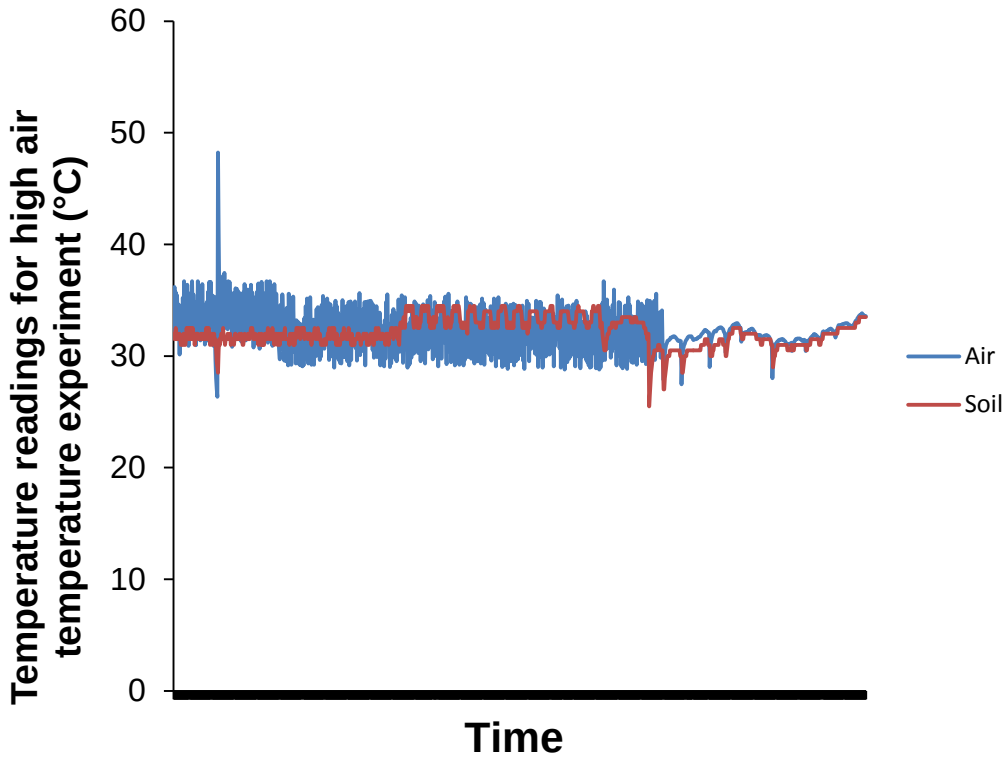

Figure 5.2 Temperature trends recorded at hourly intervals for air and soil for the high air temperature experiment for the duration of adult FCM development and eclosion.

The low temperature experiment showed a fairly consistent trend of the soil being approximately one degree cooler than the air temperature during adult FCM development and eclosion. The air temperature varied very little while the soil temperature had minor spikes in temperature fairly consistently. Over this time period the air temperature was approximately 16 °C, rather than the 15 °C at which the CE room had been set after pupation had taken place and the FCM pupae were developing in the soil (Fig. 5.3).

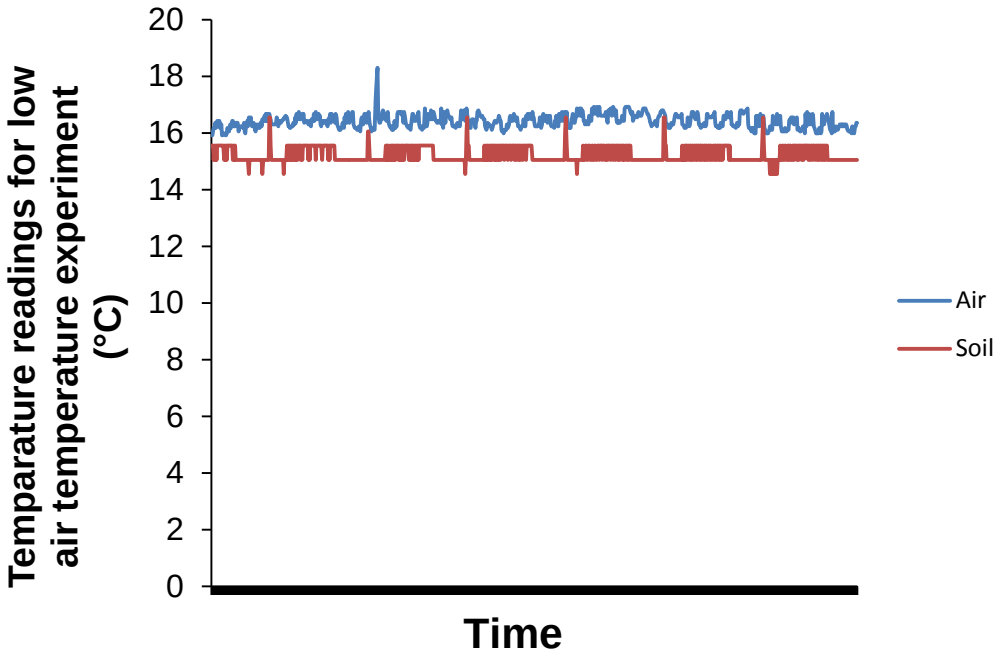

Figure 5.3 Temperature trends recorded at hourly intervals for air and soil for the low air temperature experiment for the duration of adult FCM development and eclosion.

5.3.2 General FCM Biology and High Air Temperature

High air temperature had no significant effect on the median amount of time that was spent by FCM larvae wandering on the soil surface between the three different soil texture classes ($H_{(2,90)} = 4.761$, $p = 0.093$). Variation in wandering time was high, with larvae generally having selected a pupation site within one to eight minutes after dropping onto the soil (Fig. 5.4).

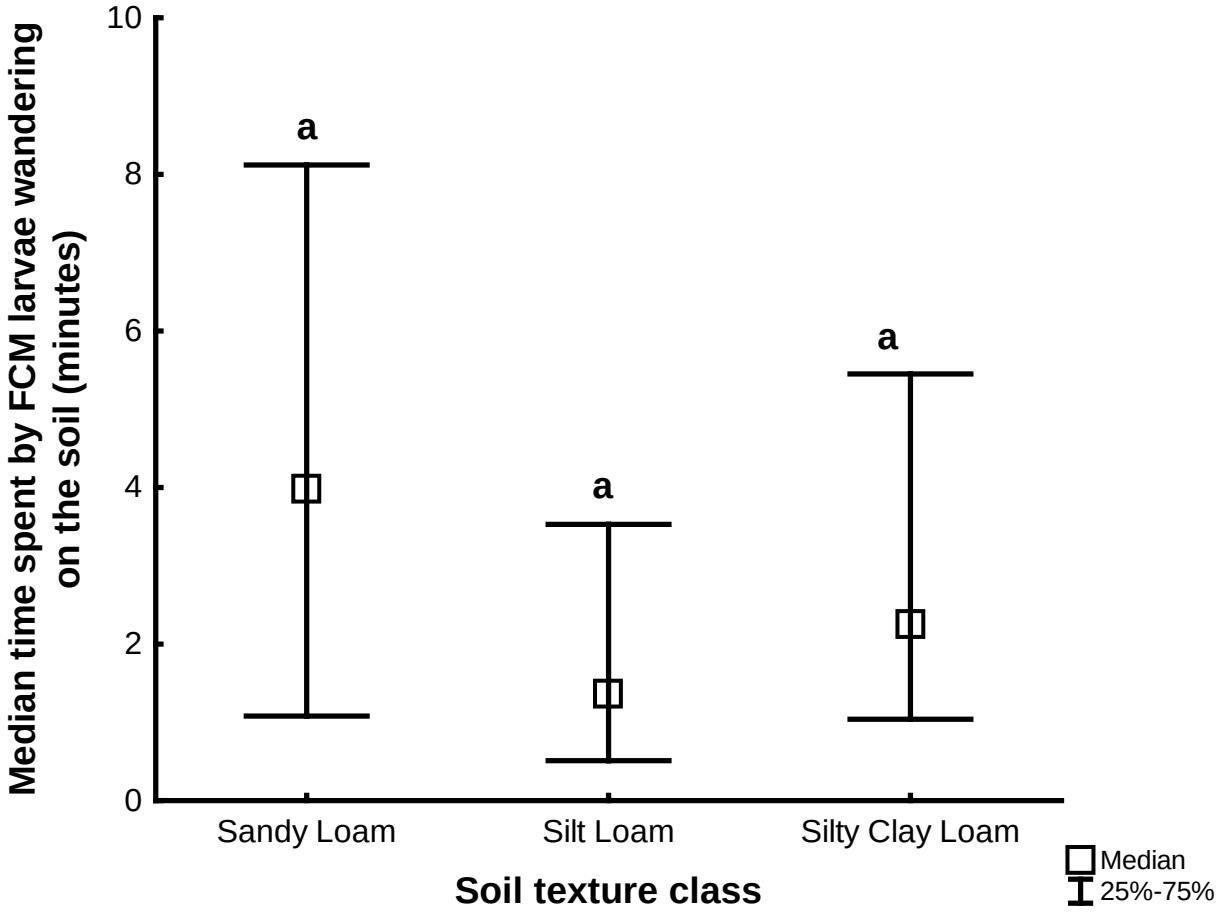

Figure 5.4 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection on soil of the three different soil texture classes with an average air temperature of 32 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant association was found between the median distance wandered by FCM larvae and a high constant air temperature for the three different soil texture classes ($H_{(2,90)}$ = 0.469, p = 0.791). For the sandy loam and silty clay loam soils, the variation in wandering distance was far higher than that of the silt loam (Fig. 5.5).

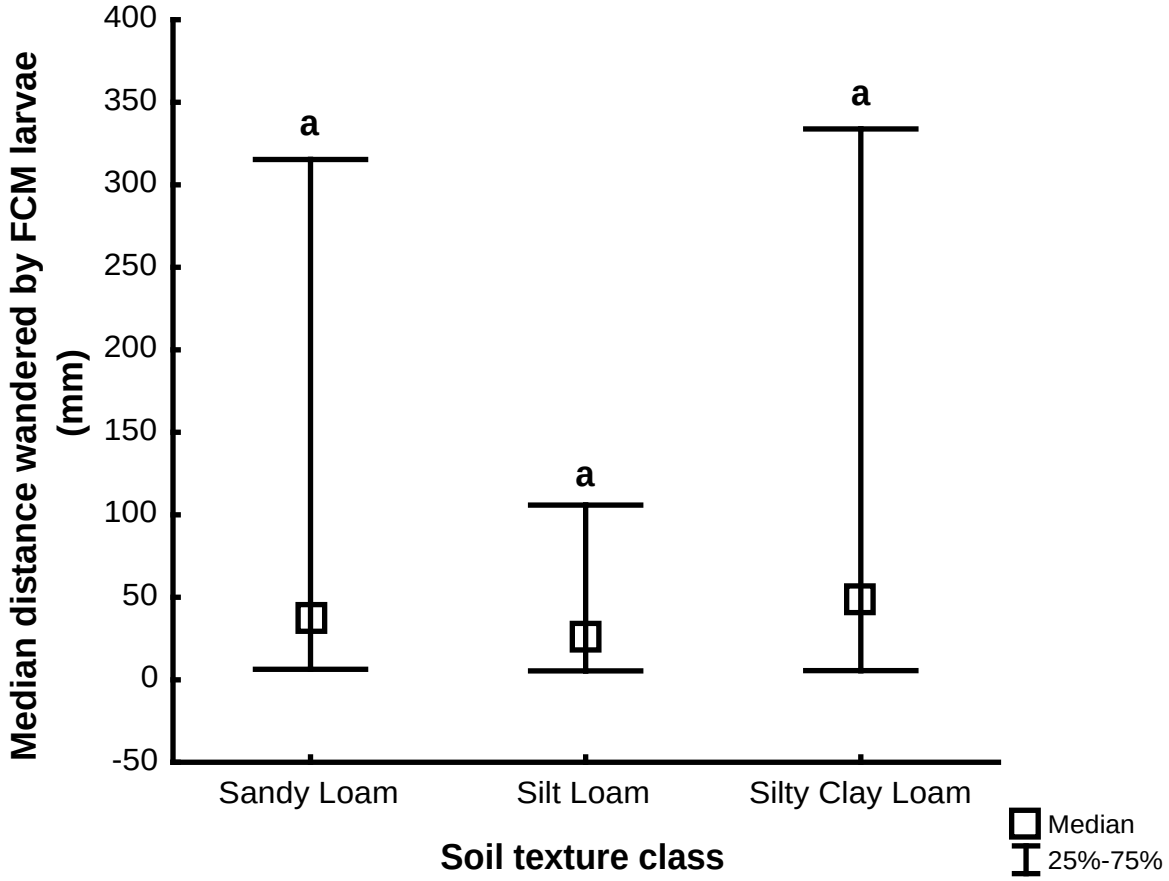

Figure 5.5 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection for the three different soil texture classes at an average air temperature of 32 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time taken to form the pupal cocoon was unaffected by the high air temperature no significant differences were found amongst the three different soil texture classes ($H_{(2,90)} = 2.191$, $p = 0.334$) (Fig. 5.6).

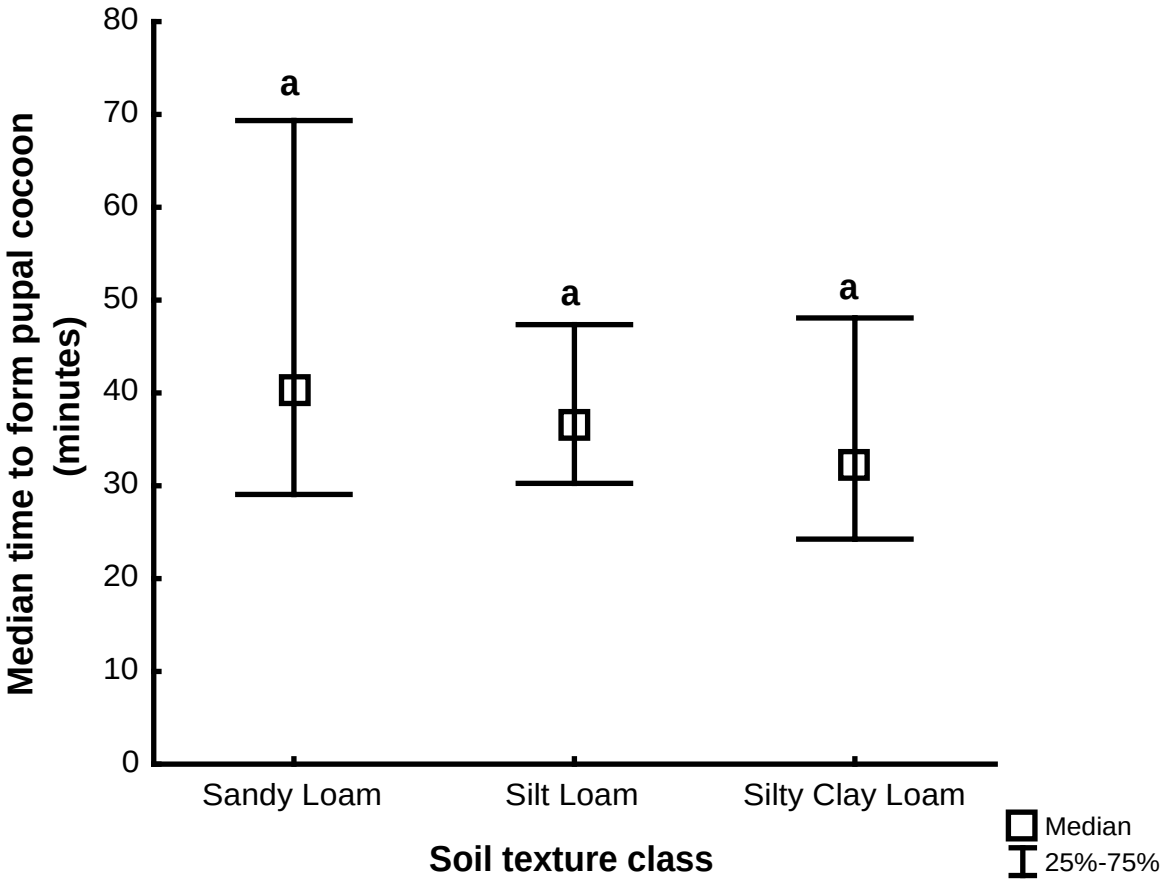

Figure 5.6 Median time taken for FCM larvae to form a pupal cocoon in soil of the three different soil texture classes with an average air temperature of 32 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median depth of pupation for FCM cocoons formed in the three different soil texture classes were almost identical, with all cocoons being formed on the surface layer of the soil. No significant relationship between pupation depth and high air temperature was found between the three soil texture classes ($H_{(2,90)} = 1.776$, $p = 0.411$) (Fig. 4.7). All 90 larvae pupated in a horizontal orientation in the soil.

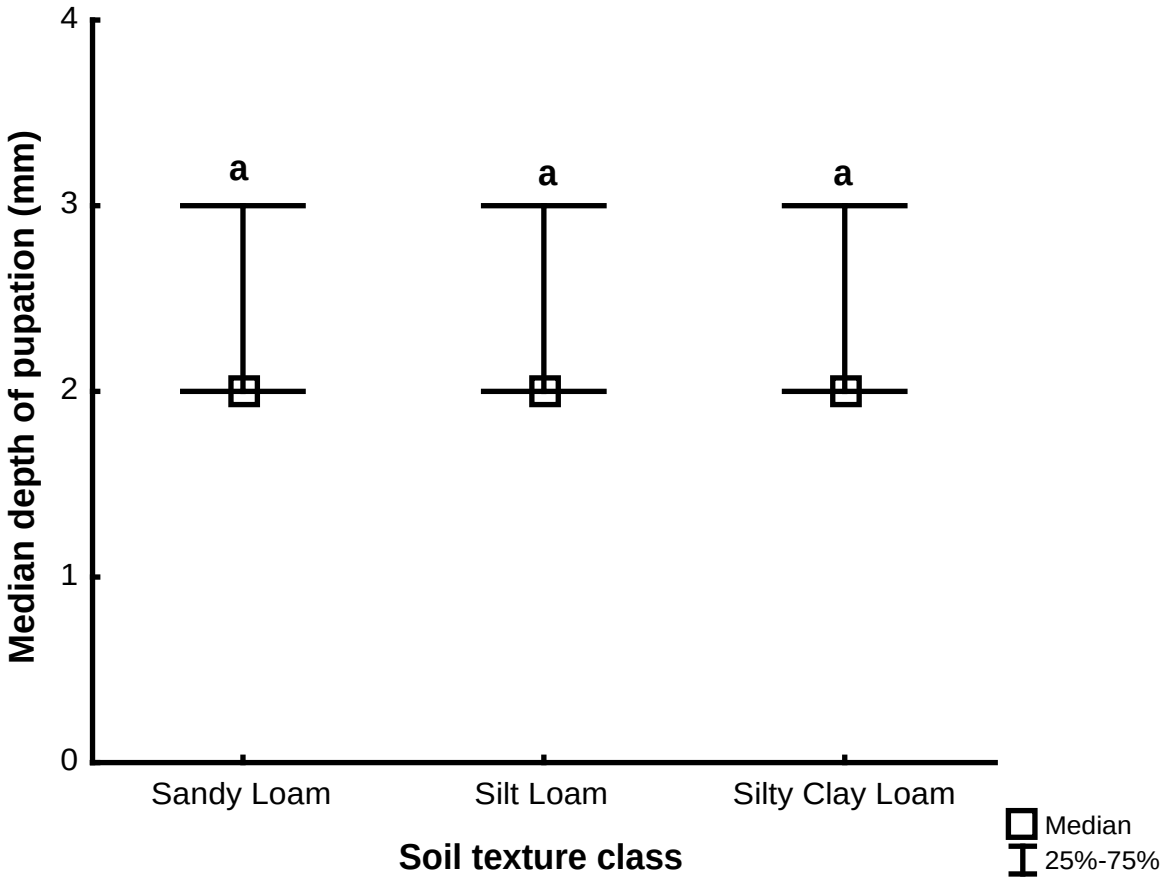

Figure 5.7 Median depth of FCM pupation in three different soil texture classes with an average air temperature of 32 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Soil texture class had no significant effect on the median amount of time that was taken for adult FCM to develop and eclose from the pupal cocoons formed at the high air temperature ($H_{(2,23)} = 1.707$, $p = 0.426$) (Fig. 5.8).

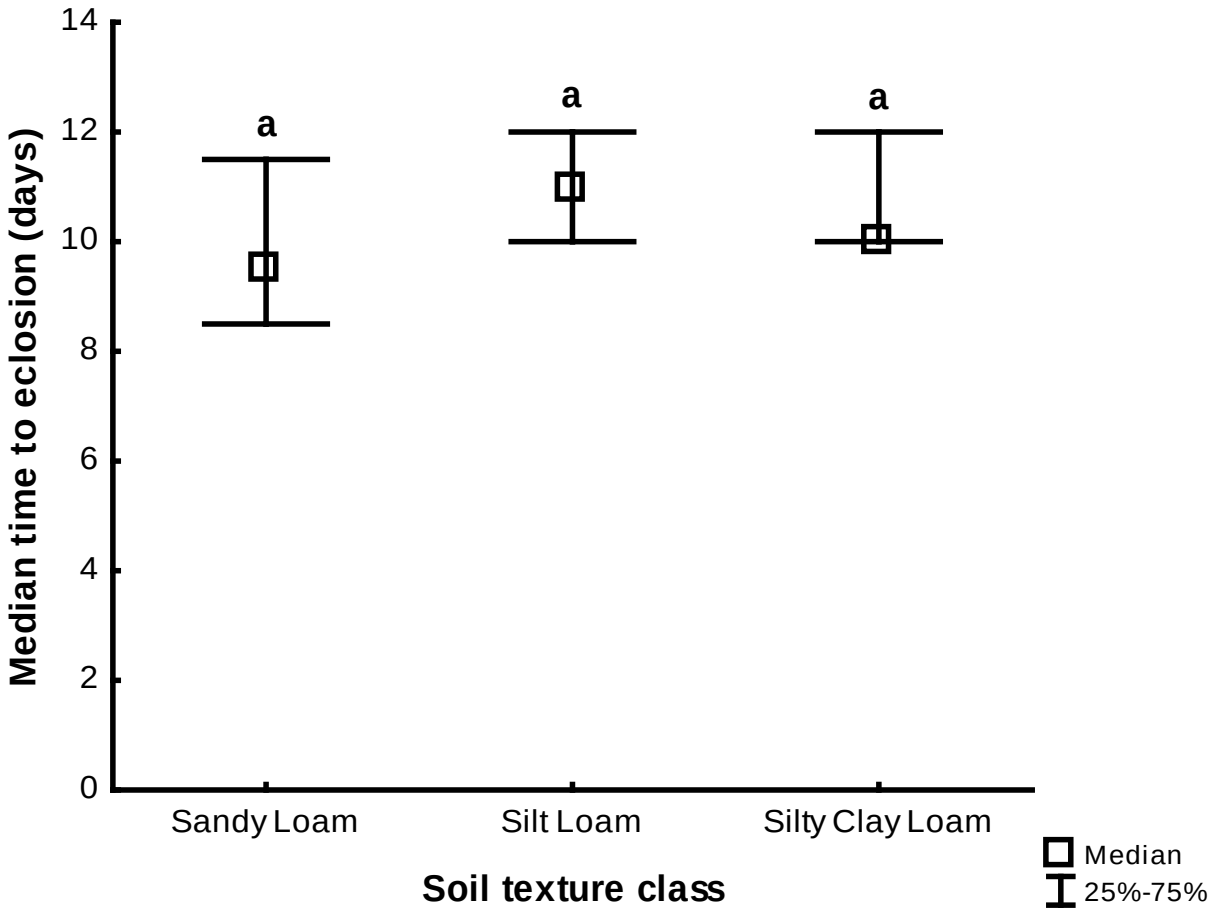

Figure 5.8 The median amount of time taken for adult FCM to eclose from the pupae formed in the soil of the three different soil texture classes with an air temperature of 32 °C (n = 23). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A significant association was found to exist between successful FCM adult eclosion and high air temperature between the different soil texture classes (Chi-square = 19.0, df = 2, $p < 0.000$). The percentage of eclosion was the highest for sandy loam at 53.3 %, while silt clay loam was 16.7 % and silt loam had just a 6.7 % eclosion success (Fig. 5.9).

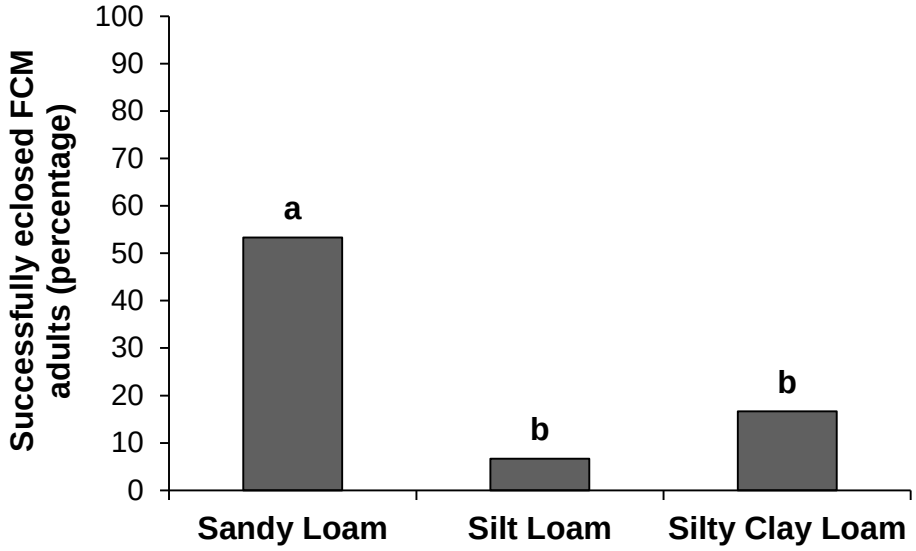

Figure 5.9 The percentage of successfully eclosed FCM adults from pupae formed in soil of the three different soil texture classes at an average air temperature of 32 °C (n = 23). Different letters denote significant differences (Chi-square test, $p < 0.05$).

5.3.3 General FCM Biology and Low Air Temperature

Larval wandering time was influenced the cold air temperature tested. FCM on the sandy loam soil wandered for a significantly longer time period than those on the silt loam soil, with no difference being found between silty clay loam and either of the other two soil texture classes ($H_{(2,90)} = 7.543$, $p = 0.023$) (Fig. 5.10).

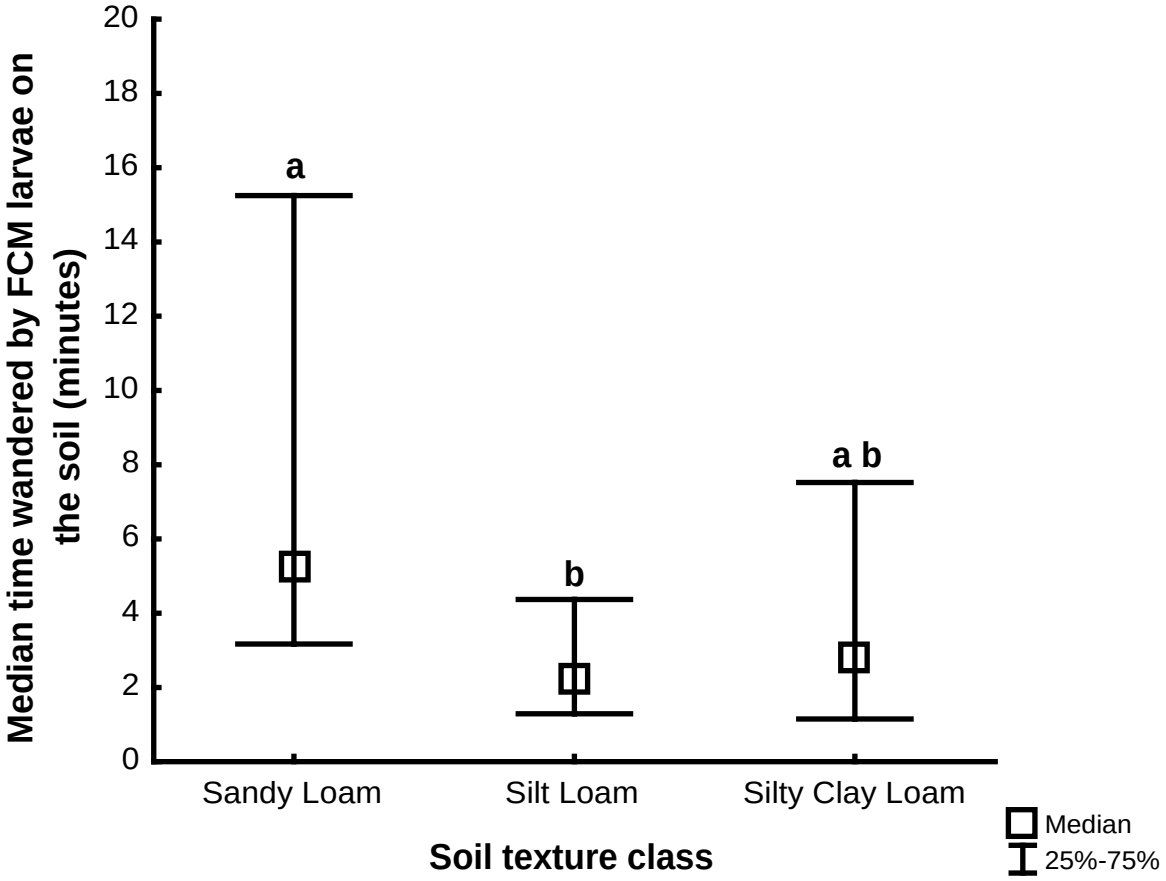

Figure 5.10 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection on soil of the three different soil texture classes with an average air temperature of 15 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

While the wandering time did show a significant difference between certain soil texture classes, this was not the case for the wandering distance. No significant differences were found between the three soil texture classes at the low air temperature ($H_{(2,90)} = 0.517$, $p = 0.772$). The variability in wandering distance was high once again (Fig. 5.11).

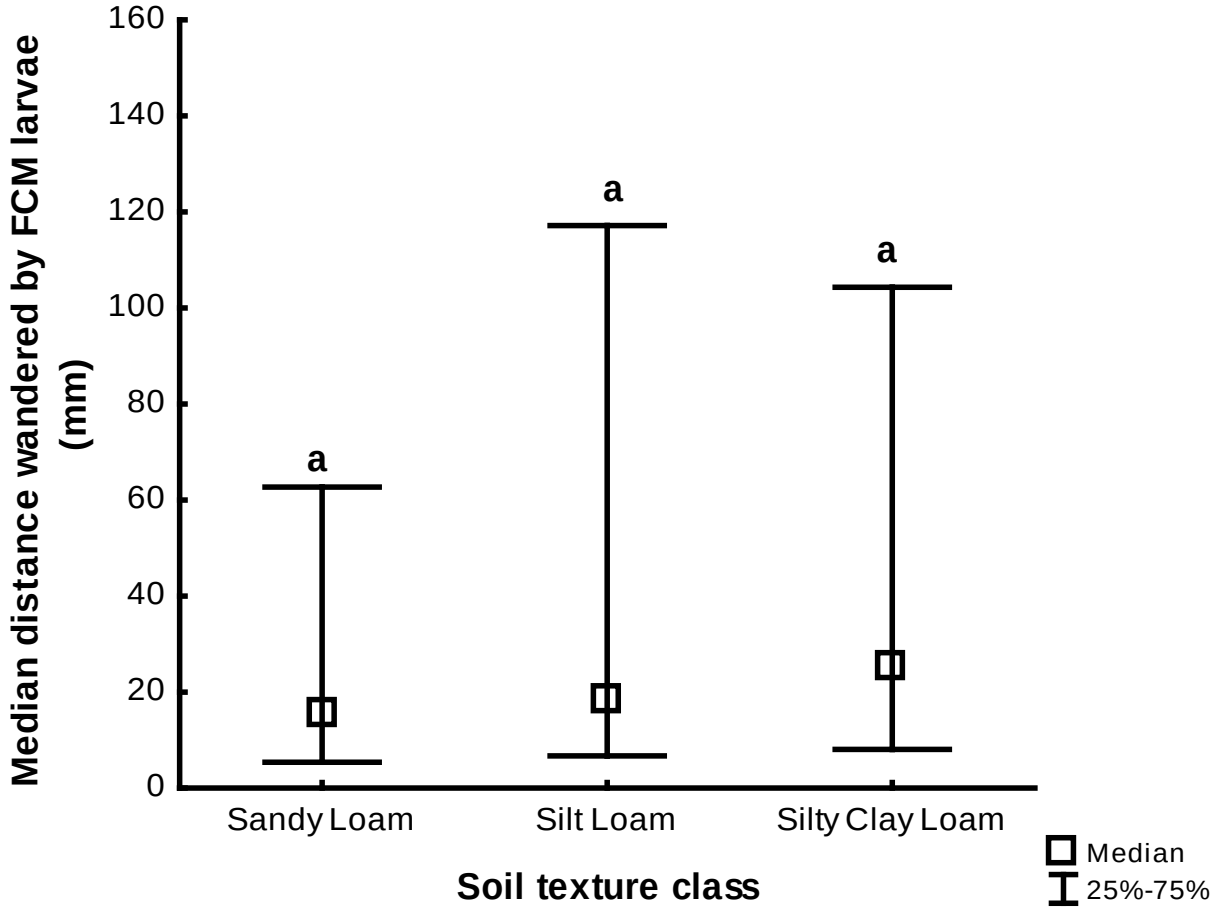

Figure 5.11 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection for the three different soil texture classes at an average air temperature of 15 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant difference was detected in the amount of time taken by FCM larvae to spin the pupal cocoon at the low air temperature between the three different soil texture ($H_{(2,78)}$ = 3.861, p = 0.145) (Fig. 5.12). In this case the sample size was reduced, as 13 % of the larvae did not spin a pupal cocoon immediately, rather remaining in the same position for an extended period of time and taking 24 hours or more to spin the pupal cocoon. This behaviour was not common and since it was impossible to determine exactly when the cocoon was spun and how long this took, these outliers were removed from the dataset.

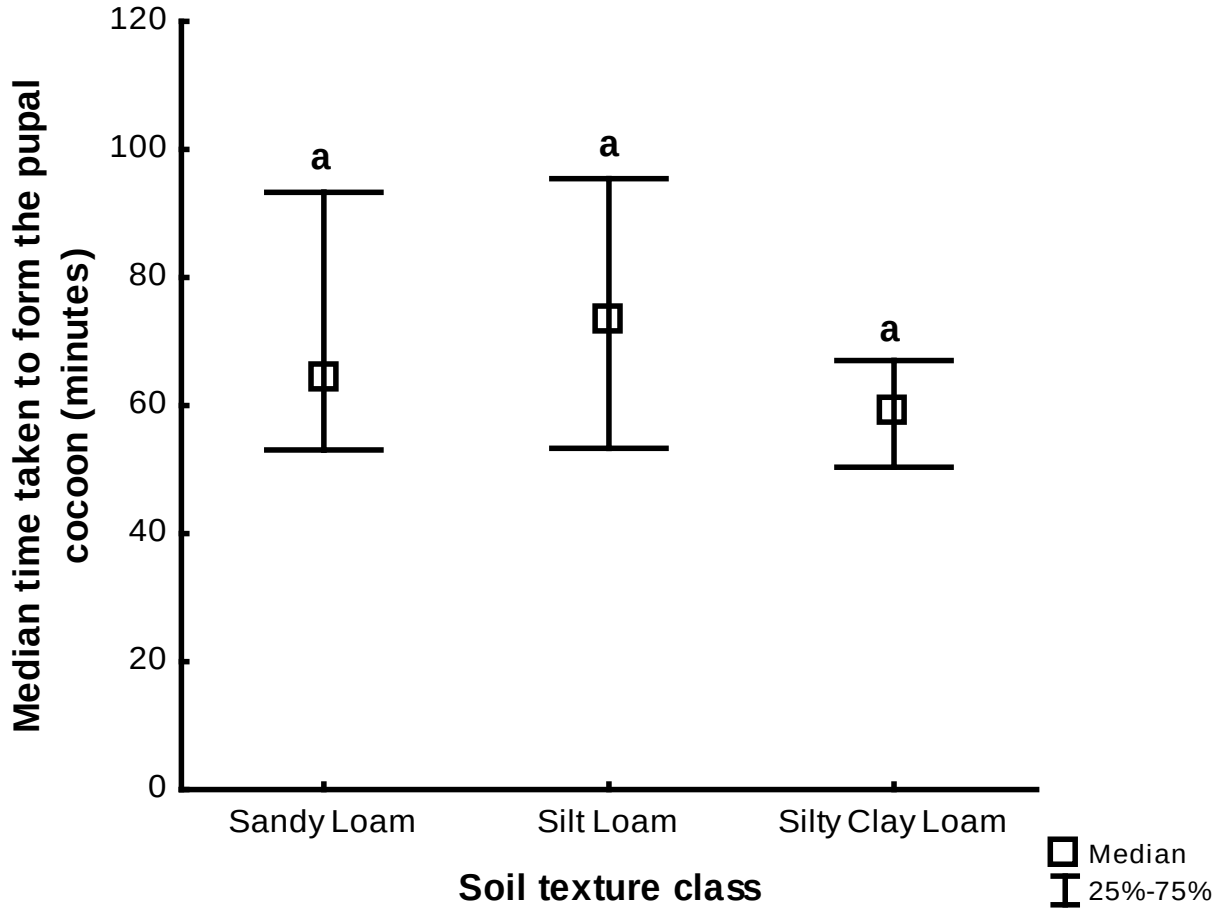

Figure 5.12 Median time taken for FCM larvae to form a pupal cocoon in soil of the three different soil texture classes with an average air temperature of 15 °C (n = 78). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

There was no significant differences in the median depth of pupation for FCM cocoons formed at low air temperature for the three different soil texture classes ($H_{(2,90)} = 1.776$, $p = 0.411$). All of the cocoons were spun in the upper 3 mm of the soil (Fig. 5.13). All of the pupae formed in the soil were in a horizontal orientation.

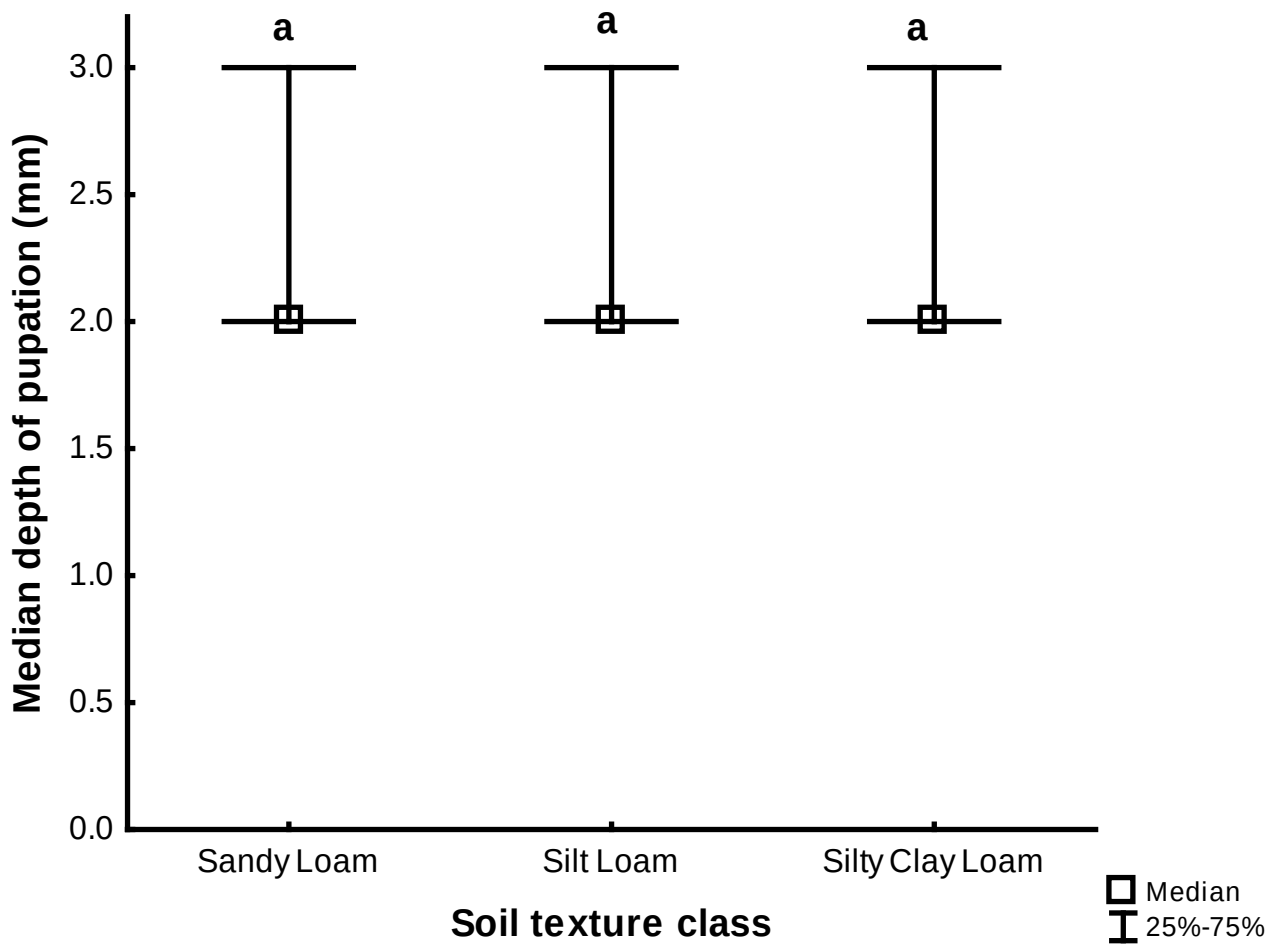

Figure 5.13 Median depth of FCM pupation in three different soil texture classes with an average air temperature of 15 °C (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time taken for development and adult eclosion was not significantly impacted by the cold air temperature when comparing between the three different soil texture classes ($H_{(2,24)} = 0.699$, $p = 0.705$) (Fig. 5.14).

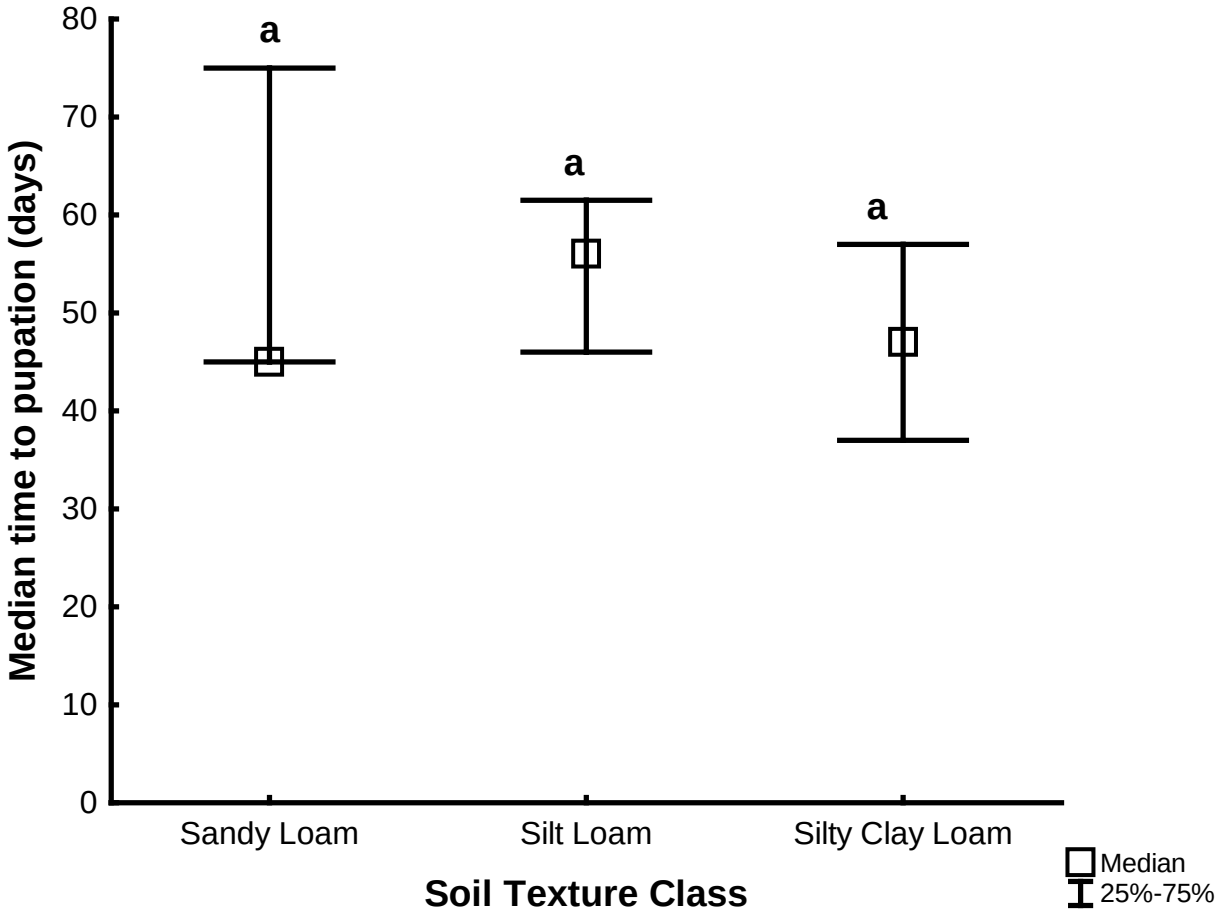

Figure 5.14 The median amount of time taken for adult FCM to eclose from the pupae formed in the soil of the three different soil texture classes with an air temperature of 15 °C (n = 24). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The impact of cold air temperature on the eclosion success of FCM was highly negative with only 26.7 % of the pupae actually producing viable adult moths. A significant association was found to exist between soil texture class and FCM eclosion at the cold air temperature where sandy loam had significantly higher eclosion success (43.3 %) than that of either silt loam (13.3 %) or silty clay loam (23.3 %) (Chi-square = 7.16, df = 2, p = 0.028) (Fig. 5.15).

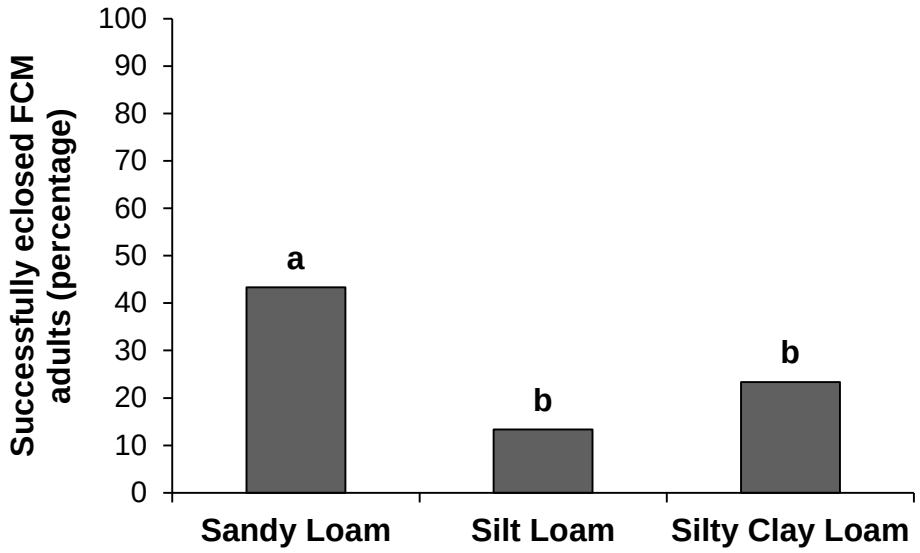

Figure 5.15 The percentage of successfully eclosed FCM adults from pupae formed in soil of the three different soil texture classes at an average air temperature of 15 °C (n = 24). Different letters denote significant differences (Chi-square test, $p < 0.05$).

5.3.4 Comparison of Temperature Effects on FCM Pupation

All three soil texture classes were combined in order to determine the overall impact of air temperature on FCM pupation. Larvae spent a significantly longer time wandering on the soil surface at 15 °C when compared to the 25 °C experiment ($H_{(2,270)} = 7.465$, $p = 0.002$). No difference was found between the 32 °C experiment and the other two temperatures (Fig. 5. 16).

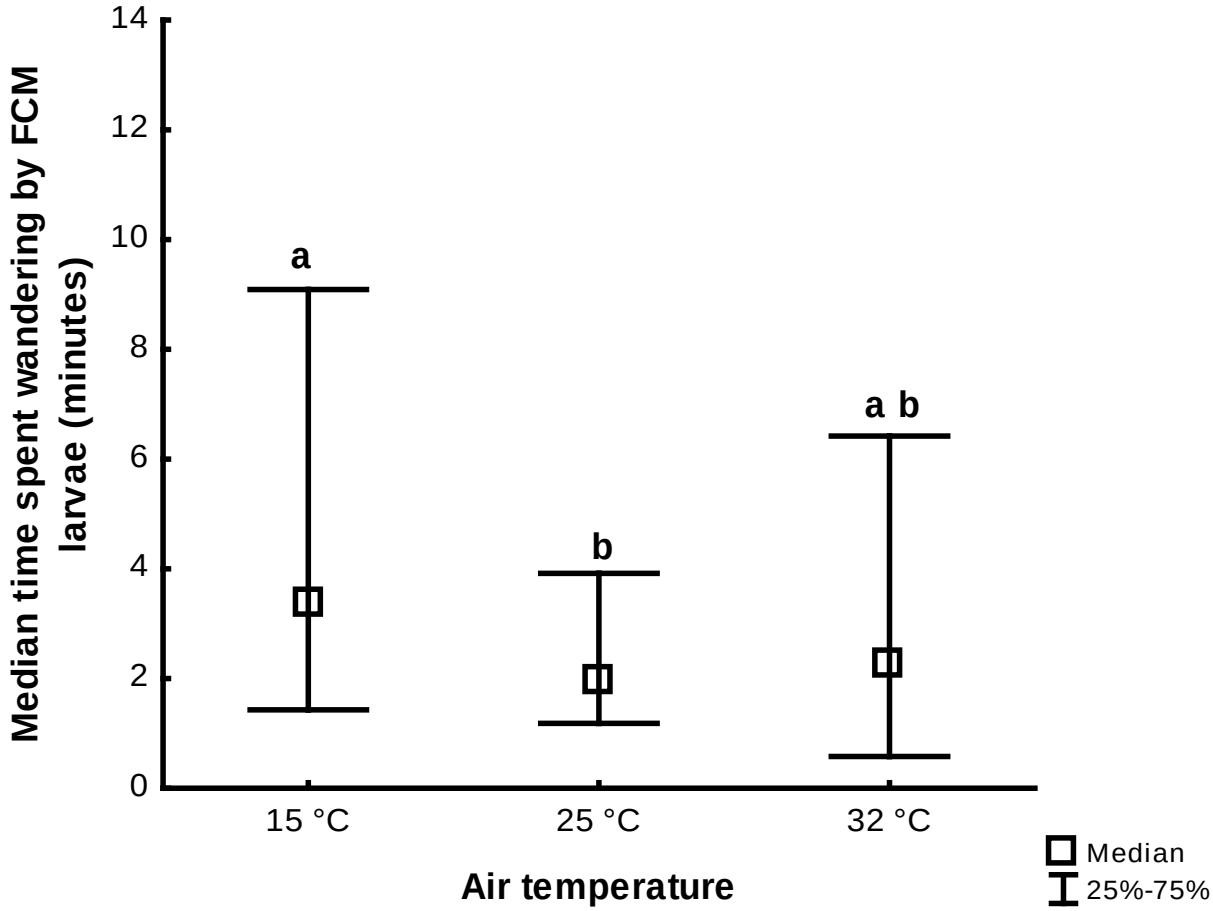

Figure 5.16 The median amount of time wandered by FCM larvae on the soil prior to pupation site selection on soil of all three different soil texture classes (combined) with an air temperature of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The differences in wandering time did not translate to the wandering distance as no significant differences were found between the three different air temperatures ($H_{(2,270)}$ = 5.143, p = 0.076). Wandering distance was variable for both the low and high temperatures, particularly for 32 °C (Fig. 5.17).

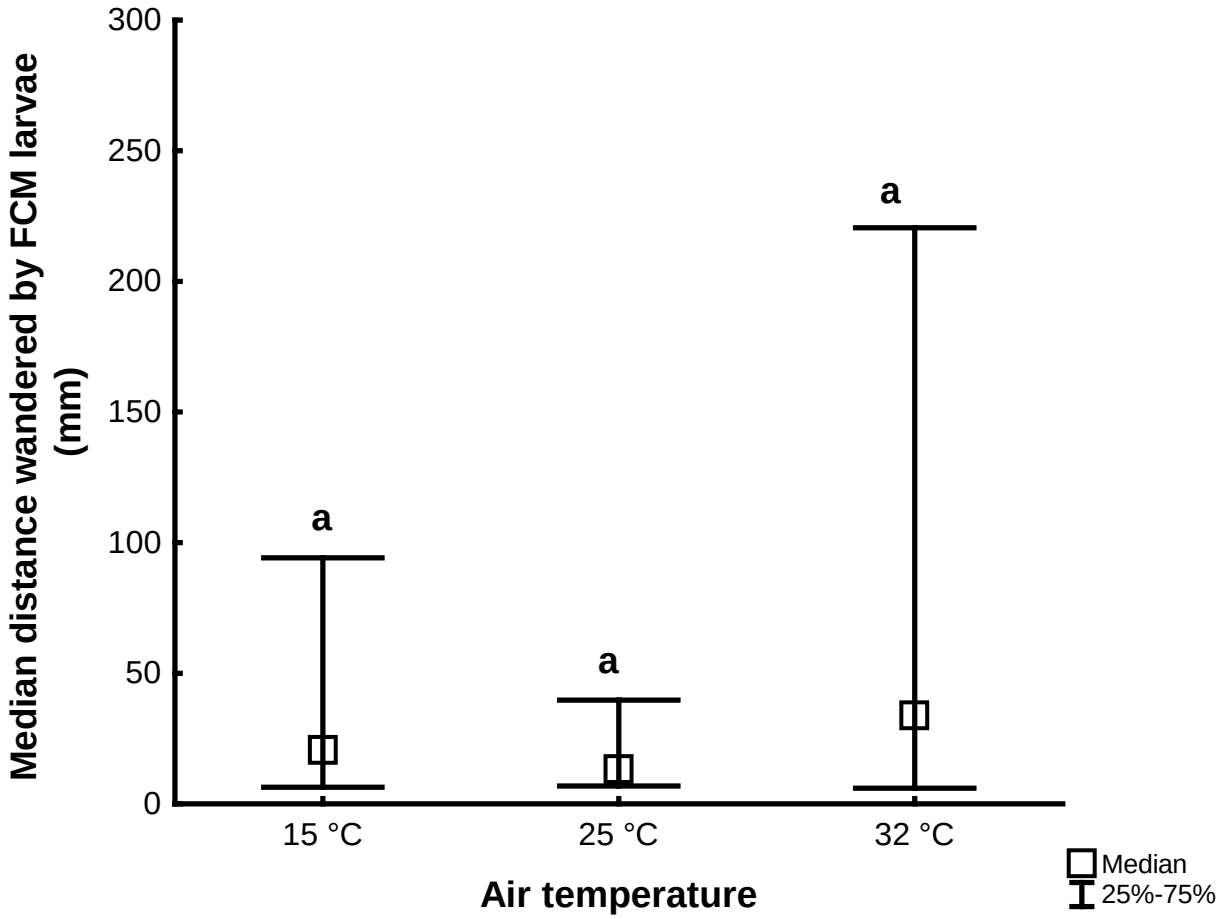

Figure 5.17 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection on soil of all three different soil texture classes (combined) with air temperatures of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, p < 0.05).

Air temperature did have an effect on time taken for pupal cocoon formation. Larvae spinning their cocoons at 15 °C took significantly longer to do so than those at either 25 °C or 32 °C ($H_{(2,251)} = 67.905$, $p < 0.0001$). No difference was found between the 25 °C and 32 °C experiments (Fig. 5.18).

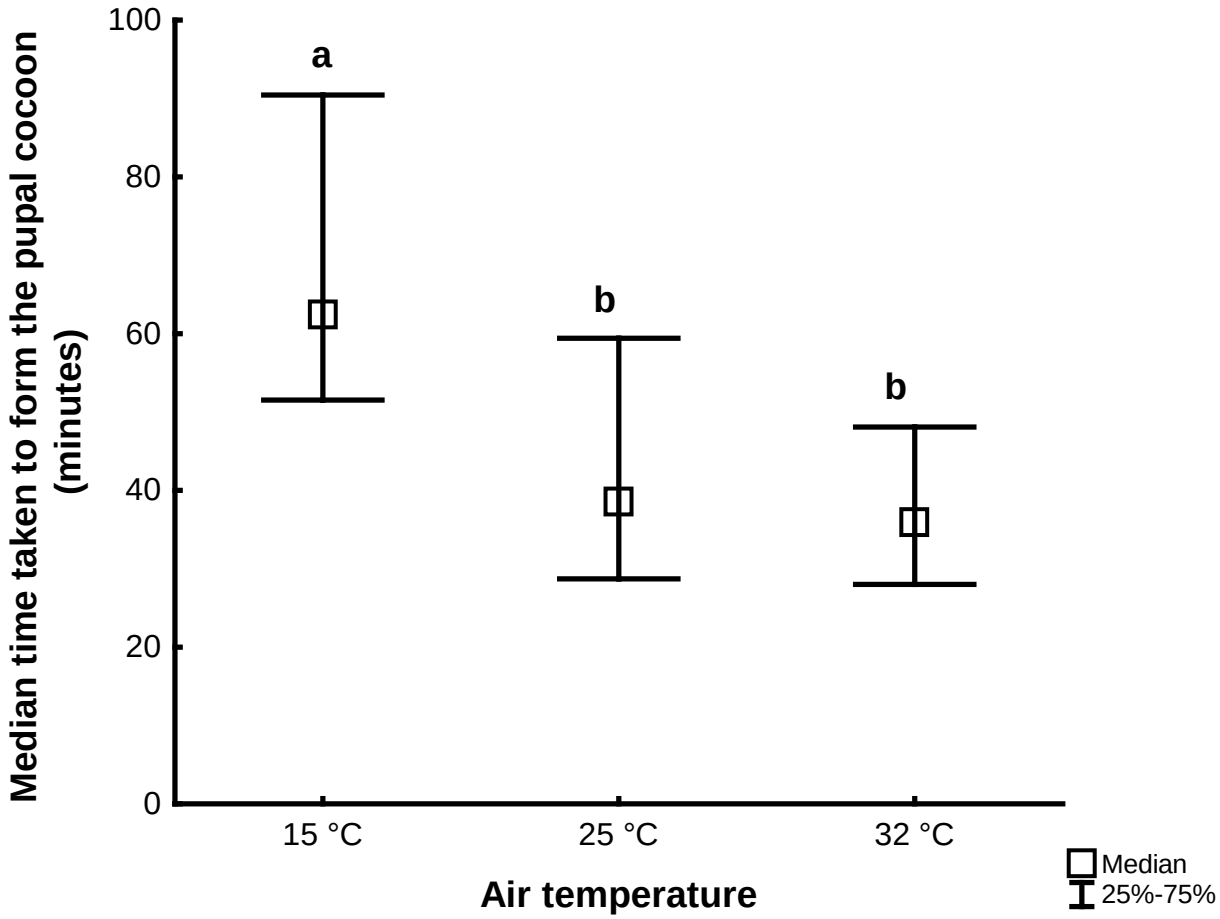

Figure 5.18 Median time taken for FCM larvae to form a pupal cocoon in soil of all three different soil texture classes (combined) with air temperatures of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 251). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

When comparing the pupation depths of FCM larvae at the three different temperatures, no significant difference was found in depth between the three air temperatures ($H_{(2,270)}$ = 0.597, p = 0.742.) All larvae for all air temperatures pupated on the soil surface in the within the top 3 mm (Fig. 5.19).

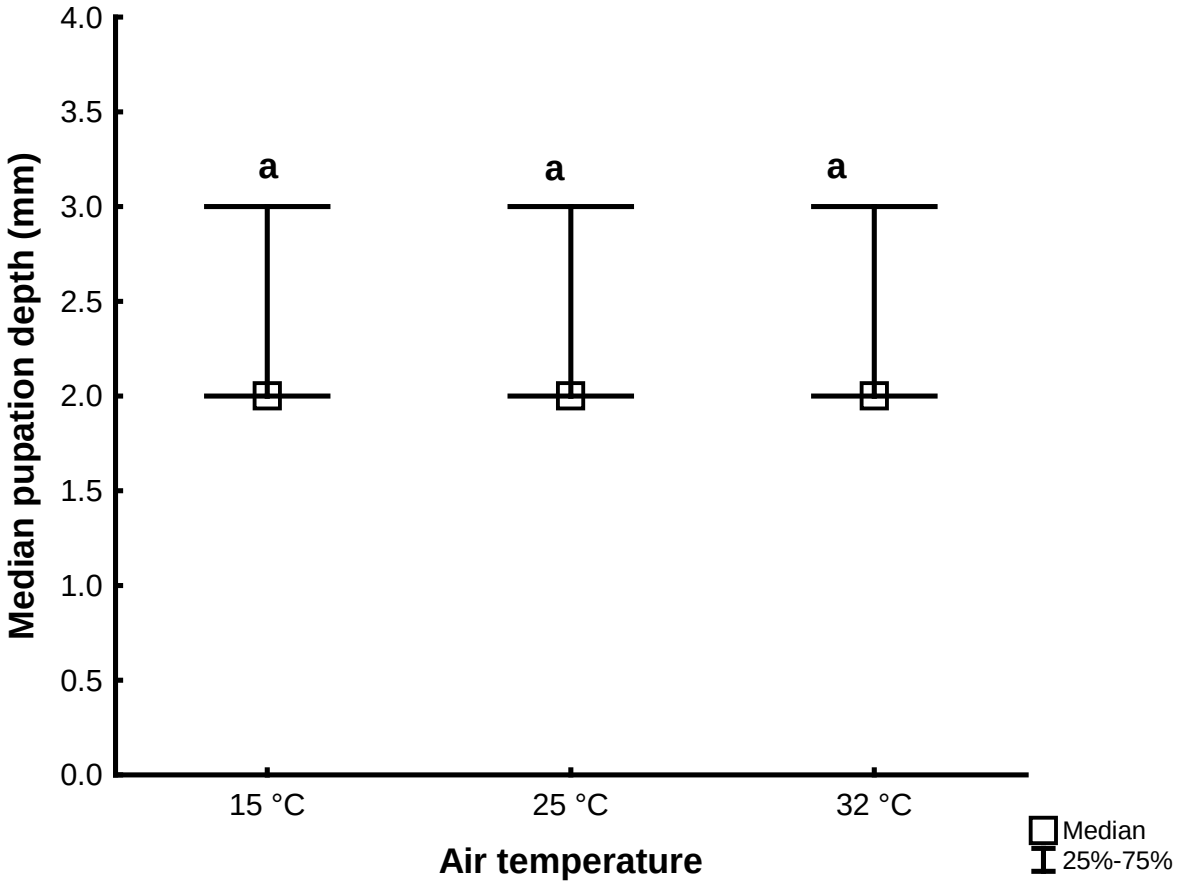

Figure 5.19 Median depth of FCM pupation in soil of all three different soil texture classes (combined) with air temperatures of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

There was a substantial difference in time to pupation between the different air temperatures. For the low air temperature treatment, FCM took significantly longer to develop and eclose than either the 25 °C or 32 °C experiments. Development was also significantly more rapid at 32 °C than 25 °C ($H_{(2,110)} = 70.484$, $p < 0.000$). Far more variation in adult eclosion time was found for the low temperature experiment than either of the other two temperatures (Fig. 5.20).

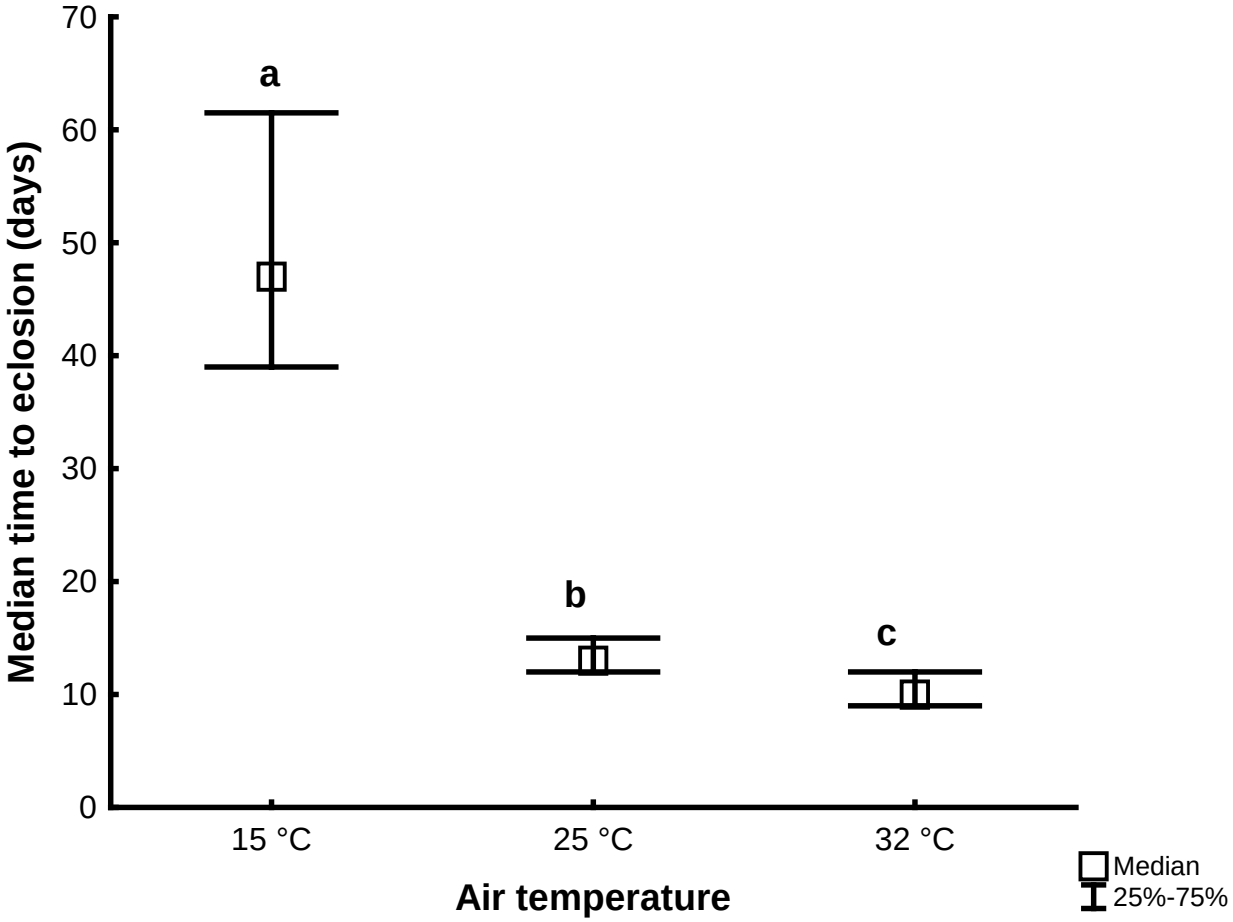

Figure 5.20 The median amount of time taken for adult FCM to eclose from the pupae formed in the soil of all three different soil texture classes (combined) with air temperature of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 110). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The effect of air temperature on the eclosion success of FCM was clearly demonstrated with a significant association being found between air temperature and FCM eclosion. The 25 °C air temperature had a significantly higher eclosion success rate (73.3 %) than either the 15 °C (26.7 %) or 32 °C (25.6 %) experiments (Chi-square = 55, df = 2, $p < 0.000$). Both high and low temperatures had a negative impact on FCM eclosion success (Fig. 5.21).

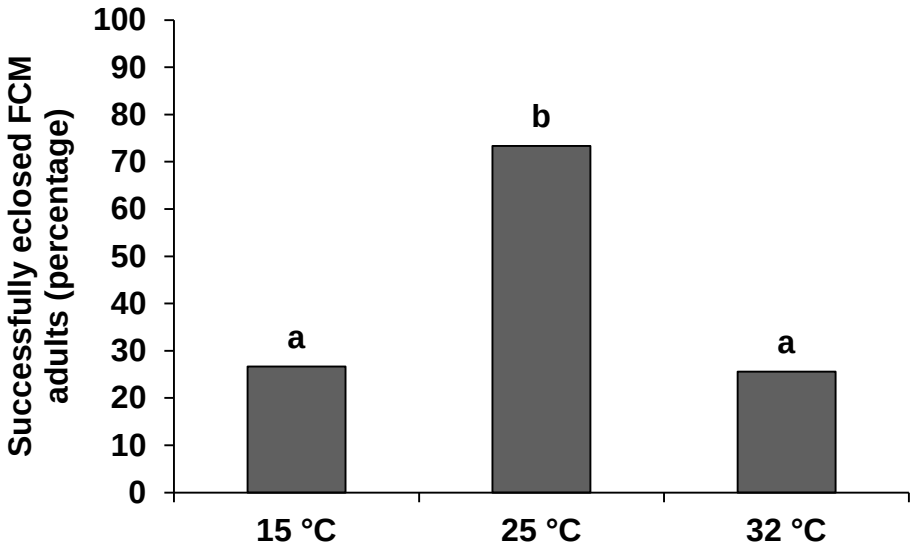

Figure 5.21 The percentage of successfully eclosed FCM adults from pupae formed in soil with the three different soil texture classes (combined) at temperatures of 15 °C (low temperature), 25 °C or 32 °C (high temperature) (n = 110). Different letters denote significant differences (Chi-square test, $p < 0.05$).

5.4 DISCUSSION

In an orchard under natural conditions, FCM pupae in the soil will be exposed to temperature fluctuations throughout the day-night cycles and also to seasonal temperature changes. This experiment makes use of constant temperatures to gain an improved understanding of the overall influence of temperature on FCM pupation habits and development. The average air temperature maintained by the CE rooms over the course of the experiments showed that the temperature was very close to the goal temperatures of 15 °C, 25 °C and 32 °C. When iButtons were added to the soil during the experiment, these clearly

reflected that the air temperature and soil surface temperature were very similar. Soil is known to heat up faster than the surrounding air and may be anywhere from 1 to 5 degrees higher in temperature (Osman 2013). In the 32 °C experiment, the soil did not reflect this and this is likely due to an artificial light source being used, rather than the soil being heated by the sun and receiving solar energy (Brady & Weil 2000; Osman 2013). The low air temperature experiment showed that the soil was cooler than the surrounding air temperature, indicating that once cooled, the ability of the soil to heat up again was compromised without the assistance of solar radiation.

The high temperature experiment had very little impact on the biology and behaviour of FCM between the three different soil texture classes when wandering and forming the pupal cocoon. The only influence that the high temperature did have was on the eclosion success of the FCM adults which was lower than had been previously recorded for all three soil texture classes. The overall pattern of eclosion success remained consistent with that of most of the previous experiments where sandy loam had the highest eclosion success rate, followed by silty clay loam and finally silt loam where only two adult moths eclosed. This shows that heat stress will reduce the survival of FCM, probably both through direct desiccation and increased vulnerability to pathogens as a result of stress (Vittum 2003). Pupal desiccation was common and evidence of pathogens was found in certain cases.

The lower air temperature experiment did result in an increase in wandering time of larvae on sandy soils rather than silt loam soils. No major changes in soil temperature were detected in the low temperature experiment when comparing air and soil temperatures. Although sieved, the soils had settled somewhat and it is possible that the sandy loam with more, larger sand particles would have been slightly better aerated with cold air (Brady & Weil 2000) which FCM may have found unfavourable and resulted in an increase in wandering time. This did not, however, result in an increase in wandering distance of the larvae. Other than this, there was no influence of low air temperature on the variables measured between the different soil classes. However, changes in FCM behaviour were noted using the camera footage which showed an increased number of larvae abandoning their initial pupation site and continuing wandering in order to find another site. Movement of FCM from their initial pupation site selection to a more favourable secondary site has been noted by Daiber (1979c) where larvae which were artificially buried underneath the soil would abandon their first pupal cocoon and move up to the more favourable soil surface. Furthermore, in this experiment, once again all of the larvae pupated on top of the soil surface or in the upper few

millimetres, which therefore appears to be their area of preference. This was the case almost exclusively, with no increase in pupation depth to attempt to escape either the high or low temperatures they were being exposed to.

When all three soil texture classes were combined, the importance of temperature and its effects became more apparent. Larval wandering time did increase at the lower temperature however, this did not result in an increase in wandering distance. This increase in wandering time is attributed to the colder temperatures reducing the ability of the larvae to move rapidly, as insects are ectothermic and need an external source of heat to raise their body temperature (Knapp & Casey 1986) and hence their activity levels. However they ultimately did not move any further than larvae at the other two temperatures. This reduction in general movement and speed was also shown for the pupal case formation where larvae at 15 °C took the longest time to spin the pupal cocoon. Prolonged lower temperatures would therefore leave the larvae more at risk to predators and pathogens, as their exposure time to these threats would be increased. Although it was expected that larval wandering at 32 °C might decrease and larvae would pupate more rapidly to attempt to reduce their chances of desiccation, this was not the case.

The lower temperature predictably resulted in a much longer pupal developmental period as pupal metabolic rate would be slowed. This extended time taken for pupal development was reported in the results of Daiber (1979c), although for this experiment it is known that the soil temperature was almost a full degree cooler than that of the air which would have increased the development time. The more rapid developmental time for the FCM that pupated at 32 °C was anticipated and has been found to decrease the length of the pupal period for other species as well, such as the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) (Bernier *et al*. 2014).

The high and low air temperatures did have a negative influence on FCM eclosion with less than 30 % of adults eclosing from pupae formed at these temperatures. The majority of the moths appeared incapable of adapting to the stress caused by the unfavourable temperatures, with many desiccating, not developing, or occasionally succumbing to disease. The inability of FCM to physiologically adapt to low temperatures through diapause or cold-hardening has been well-documented (Stotter & Terblanche 2009; Boardman *et al*. 2012; Terblanche *et al.* 2014). Survival rates of FCM at the low air temperature in this experiment indicate that FCM may be poorly suited for survival in colder areas or over periods of

prolonged reduced temperature. Prolonged exposure to high temperatures is also unfavourable for FCM eclosion. However, eclosion success remains highly dependent on soil texture class with sandy soils showing the highest percentages of FCM eclosion which may require additional control measures.

The implications of temperature on entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) vary according to the thermal limits of the particular species and the environment itself (Barbercheck 1992). Entomopathogenic fungi are able to survive better at much higher temperatures than FCM, as death of conidia only occurs at around 50 °C (Barbercheck 1992). When compared to the eclosion rates at 25 °C, FCM eclosion decreased by 47 % at 15 °C and 48 % at 32 °C. Even at 25 °C, which is close to the optimal temperature for FCM development, only 73 % were able to eclose successfully. Considering that peak germination of *Beauvaria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschinkoff) Sorokin occurs between 25 and 30 °C and that sporulation is still able to occur at 10 °C, temperature fluctuations are fairly unlikely to be problematic for EPFs, unless they are very extreme (Barbercheck 1992). Of much greater concern is the impact of UV radiation on EPF as was discussed in Chapter 3.

Entomopathogenic nematodes (EPNs) on the other hand are known to be very temperature-sensitive (Vittum 2003). Temperature tolerance of EPNs is highly variable, both amongst species and strains of nematodes (Barbercheck 1992; Stuart *et al.* 2006). The only commercially available nematode species in South Africa, *Heterorhabditis bacteriophora* Poinar, can survive cold temperatures of up to -19 °C, although only for short periods and is able to survive freezing (van Zyl & Malan 2014). While survival at these temperature extremes may be possible, both reproduction and establishment of *H. bacteriophora* becomes highly compromised when temperatures drop below 15 °C (Grewal *et al.* 1994). The upper temperature limit of this species is not specified however, since nematodes are so sensitive to temperature, van Zyl & Malan (2014), recommend the use of more temperature-tolerant strains and careful timing of the application of EPNs to the soil, both seasonally and at the most appropriate time of day. The infectivity of EPNs to FCM pupae is likely to be affected by temperature. Linked to this, is that EPNs are also negatively affected by UV radiation (van Zyl & Malan 2014) which would also be of concern in field applications.

6

The influence of soil texture class and soil moisture on FCM pupation

6.1 INTRODUCTION

In a citrus orchard, the amount of water required by the trees for good quality fruit production is high and natural rainfall alone is not sufficient (Wellington 1955; Mather 1999). Irrigation systems with water being applied directly to the soil are therefore the norm (Wellington 1955; Mather 1999). This soil moisture has a significant effect on the behaviour of soil particles and plays a large role in chemical reactions in the soil (Brady & Weil 2000). The effect on soil particle behaviour includes swelling or shrinking of particles, adherence to one another and the formation of aggregates (Brady & Weil 2000). Water movement in the soil occurs according to the soil water potential gradient where movement rate and direction are determined by differences in energy levels of water between sites or conditions such as soil dryness (Brady & Weil 2000).

In wet soils water molecules are able to move freely, as these are not held closely to the soil particle surface (Brady & Weil 2000). This is due to the retention of water in large pores between particles or thick water films around them. Molecule movement in dry soils is restricted as the water is held in much smaller pores and in a thin layer around the soil particles, resulting in much stronger adherence between the water molecules and the soil particle (Brady & Weil 2000). Soil texture has a very strong influence on moisture content as the ability of soils to retain moisture is partially dependent on soil texture. Soils with a higher sand content contain large sized soil particles of a coarse texture, resulting in larger macropores between particles and a lower specific surface area for water adsorption, both of which increase soil drainage and decrease the ability of sandier soils to retain water (Brady & Weil 2000; Schaetzl & Anderson 2005; Osman 2013). Soils with higher clay content behave

in the opposite manner, as the small size and fine texture of these particles results in small pores and a larger specific surface area for water adsorption (Brady & Weil 2000; Schaetzl & Anderson 2005; Osman 2013). Soil moisture also interacts closely with other abiotic factors such as soil temperature through evaporation as well as aeration when soil air pockets fill with water as the soil becomes more saturated reducing oxygen availability (Brady & Weil 2000).

Improving the understanding of the important role which soil moisture plays on invertertebrate seasonal ecology and insect behaviour was identified by Tauber *et al.* (1998). Changes in edaphic insect behaviour in response to soil moisture content have been noted, with moisture loss being of serious concern to these soil-dwelling insects (Villani & Wright 1990). The high surface to volume ratio of insects due to their small size makes them decidedly vulnerable to waterloss (Villani *et al.* 1999; Villani & Wright 1990). For mobile insect life stages, the risk of moisture loss can be mitigated through habitat modifications performed by the insect or through movement to a more suitable microclimate (Villani *et al.* 1999). For the immobile pupal stages of the insect life cycle however, this is not possible, leaving edaphic pupae decidedly vulnerable to changes in soil moisture content.

Numerous studies on the effect of soil moisture, either in isolation or in combination with other abiotic factors such as temperature and compaction, have been performed where the majority of these focus on the impact of varying soil moisture on insect pupation depth and eclosion success (Roach & Hopkins 1979; Eskafi & Fernandez 1990; Murray & Zalucki 1990; Rickelmann & Bach 1991; Hennessey 1994; Jackson *et al.* 1998; Alyokhin *et al.* 2001; Dimou *et al.* 2003; Ellis *et al.* 2004; Hou *et al.* 2006; Hulthen & Clarke 2006; Chen & Shelton 2007; Yee 2013). Preferences for particular soil moisture levels have been found for other agricultural species (Rickelmann & Bach 1991; Alyokhin *et al.* 2001; Hulthen & Clarke 2006). Improving the understanding of any biological, behavioural or survival changes in FCM pupation due to soil moisture may assist not only in improving the understanding of the species as a whole, but also any possible implications for the use of entomopathogenic fungi (EPFs) and entomopathogenic nematodes (EPNs) on FCM. The moisture content of soil is known to play an important role in the infectivity of both EPFs (Barbercheck 1992; Ignoffo 1992; Jaronski 2010; Gul *et al.* 2014) and EPNs (Kung *et al.* 1991; Barbercheck 1992; Koppenhöffer *et al.* 1995; Hazir *et al*. 2003; Campos-Herrera *et al.* 2010; Stuart *et al.* 2006).

The aims of this chapter were therefore to determine the influence of different soil texture classes and different moisture levels on 1) FCM larval wandering time, 2) the distance wandered by FCM larvae prior to pupation site selection 3) time taken by FCM to spin the protective pupal cocoon, 4) the depth of pupation, 5) orientation of pupation (horizontal or vertical in soil profile), 6) amount of time taken to eclose and 7) the eclosion success. This was done for two soil moisture levels, as well as a comparison between the three soil texture classes and each soil moisture level, including dry soil. The overall impact of soil moisture when combining the soil texture classes was examined. Furthermore, choice experiments were run to determine whether FCM larvae showed any preferences for particular soil textures with differing soil moisture content.

6.2 MATERIALS AND METHODS

The commercial citrus orchard is a highly managed environment and the addition of moisture to the soil through irrigation is no exception. The majority of research on soil moisture makes use of different moisture levels i.e. 0 %, 50 % and 100 %, based on the moisture content for saturated soil. For this study the moisture levels selected were to be those that would reflect field conditions, specifically regarding irrigation and the soil conditions. Information concerning the way in which growers go about determining when to irrigate the soil and how much moisture to add was provided by Vahrmeijer, J. T. (pers. comm.[4]). Citrus growers typically monitor soil moisture content using tensiometers which measure soil water tension. Once the soil has dried out to the refill point, where 20 – 50 % of the easily available water (EAW) in the soil has been used, the soil reservoir needs replenishing. This moisture level represents a reading of approximately -25 kPa on a tensiometer when growers begin irrigation. The soil is then irrigated until the soil reservoir has been replenished and reaches field capacity (FC). This occurs when water which is not tightly bound to the soil particles (free water) is drained by gravity and trees are able to make use of this water without expending large amounts of energy to obtain it. This state is reached at a tensiometer reading of approximately -10 kPa. Since these moisture levels would be the most typically found in the field, the refill point (RP) and field capacity (FC) were used for the experiments. The data from the soil texture experiment in Chapter 2 where the soil were dried in an oven 48 hours prior to use in the experiment was used as the dry experiment data

[4] Vahrmeijer, J.T. Department of Plant Production and Soil Science, University of Pretoria and Citrus Research International, South Africa.

for comparison with the two moist soil experiments. The soils used in the experiment (sandy loam, silt loam and silty clay loam) were sieved, autoclaved and dried before use, as was also performed for the other experiments as described in Chapter 2.

Since the necessary kPa and the saturation point of each soil texture (as previously calculated for the compaction experiment in Chapter 4) was known, it was possible to calculate the approximate amount of water which needed to be added to the soils in order to obtain the refill point and field capacity moisture levels. This was dependent on the weight of each of the soil texture classes, as each class differed slightly. Once dried, the approximate amount of distilled water (dH_2O) required was added to moisten, but not saturate, the soil and the kPa of soil was measured to determine whether the approximate moisture range had been achieved. These moisture readings were taken with a Bluemat digital tensiometer. This was repeated for all three soil texture classes, as the amount of water required and rates at which they dried out differed. The initial addition of dH_2O was done by measuring the water out in a graduated cylinder and pouring it evenly over the soil surface. The approximate soil moisture required in order to achieve the estimated kPa was calculated for both the refill point and field capacity soil moisture contents for each soil texture class.

During the experiments moisture readings for each soil texture were taken every 24 - 48 hours using a digital tensiometer. It was ensured that the entire ceramic tip was covered with soil in order to provide accurate readings. When the soil had dried out to below the desired level, the approximate amount of moisture needed was calculated and added to the soil surface using a standard plastic 250 ml spray bottle. The dH_2O was manually sprayed on the soil surface using a medium to fine spray setting. This was maintained throughout the experiment until eclosion, with non-eclosed pupae being checked following a three week period for evidence of mortality. All experiments were run under the same conditions as described in Chapter 2, with controlled environment (CE) rooms being used and temperature being set to 25 °C. Larval behaviour was filmed and analysed as described previously, with larvae being allowed to drop into the soil naturally from vials containing artificial diet, suspended above the plastic containers with soil. This was done using 30 FCM larvae per soil texture class for each experiment, with each larva being treated as an individual replicate.

Choice experiments to determine whether the FCM larvae would show any particular preferences for certain soil moistures when pupating were also performed. The experimental design was similar to that for the soil texture class choice experiments in Chapter 2, however, in this case the division that separated the experimental arena into two equal sections was plastic in order to avoid any moisture movement between the sections. The choice experiments were: 1) dry soil versus refill point moisture content (± -30 kPa), 2) dry soil versus field capacity moisture content (± -10 kPa) and 3) refill point moisture level versus field capacity moisture level. Ten larvae per replicate were allowed to naturally drop into the soil from a Petri dish suspended 20 cm above the choice arena and select a side for pupation. This was performed for each of the soil texture classes, with three replicates for each soil texture. The pupation site was marked and checked after 24 and 48 hours to ensure that the larva had not abandoned it and moved elsewhere. In addition to this, an experiment testing FCM preference for pupating in soil or an area with no soil was tested. In this case no barrier was used between the area of soil and no soil, with the soil layer only being 1.5 cm in depth. The distance between pupal cocoons in the moisture choice experiments were also measured in order to determine how close to one another larvae would pupate.

6.2.3 Statistical Analysis

The statistical analyses were run using the statistical software Statistica Version 10 (2011). The data sets were found to be non-parametric when tested for normality, thus Kruskal-Wallis tests were used when comparing multiple independent groups with a multiple comparison of mean ranks post-hoc test being used. The data sets with categorical variables were analysed using a Chi-square test. This included the choice experiments.

6.3 RESULTS

6.3.1 Soil Moisture

The provided soil moisture tension measurement for the refill point was given as approximately -25 kPa. The average soil moisture readings for the three soil texture classes were -23.96 kPa for sandy loam, -26.43 kPa for silt loam and -21.59 kPa for silty clay loam. All three of these fell into the approximate range of the refill point. The field capacity measurement was provided as approximately -10 kPa. For sandy loam the mean soil water tension measurement was -12.84 kPa, silt loam was -12.68 kPa and silty clay loam was -9.02 kPa. All three of these also fell into the approximate soil water tension measurement given for field capacity (Fig. 6.1).

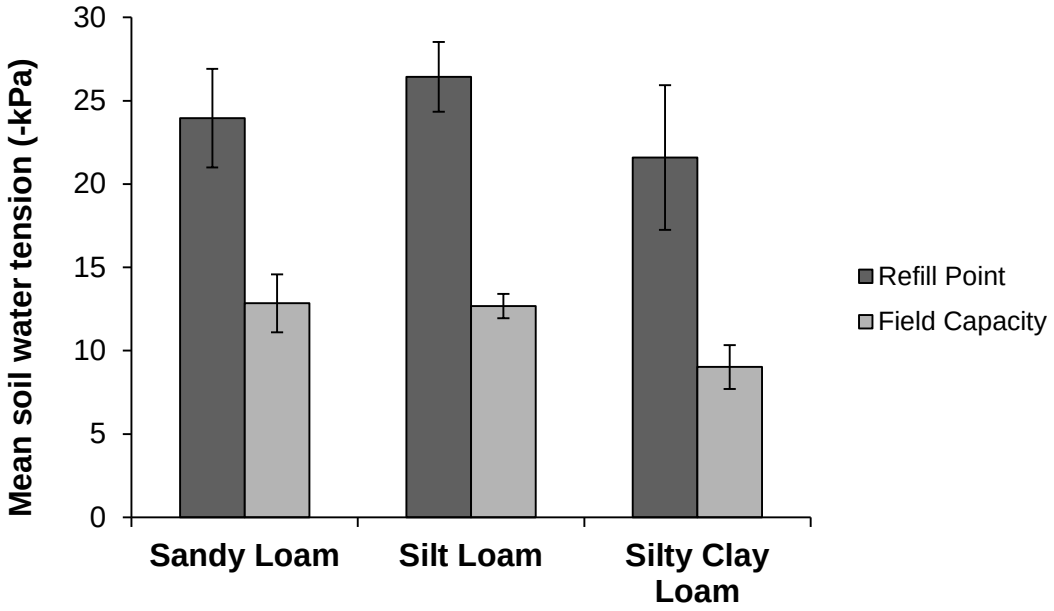

Figure 6.1 Mean soil water tension measurements (± SE) for the three different soil texture classes (sandy loam, silt loam and silty clay loam) at the refill point and field capacity soil moisture levels

6.3.2 General FCM Biology and Refill Point Soil Moisture

There was no significant relationship between the three different soil texture classes (sandy loam, silt loam and silt clay loam) and the amount of time wandered by FCM larvae on the soil surface prior to selection of a pupation site when the soil moisture was maintained at the refill point ($H_{(2,90)} = 0.45$, $p = 0.796$) (Fig. 6.2).

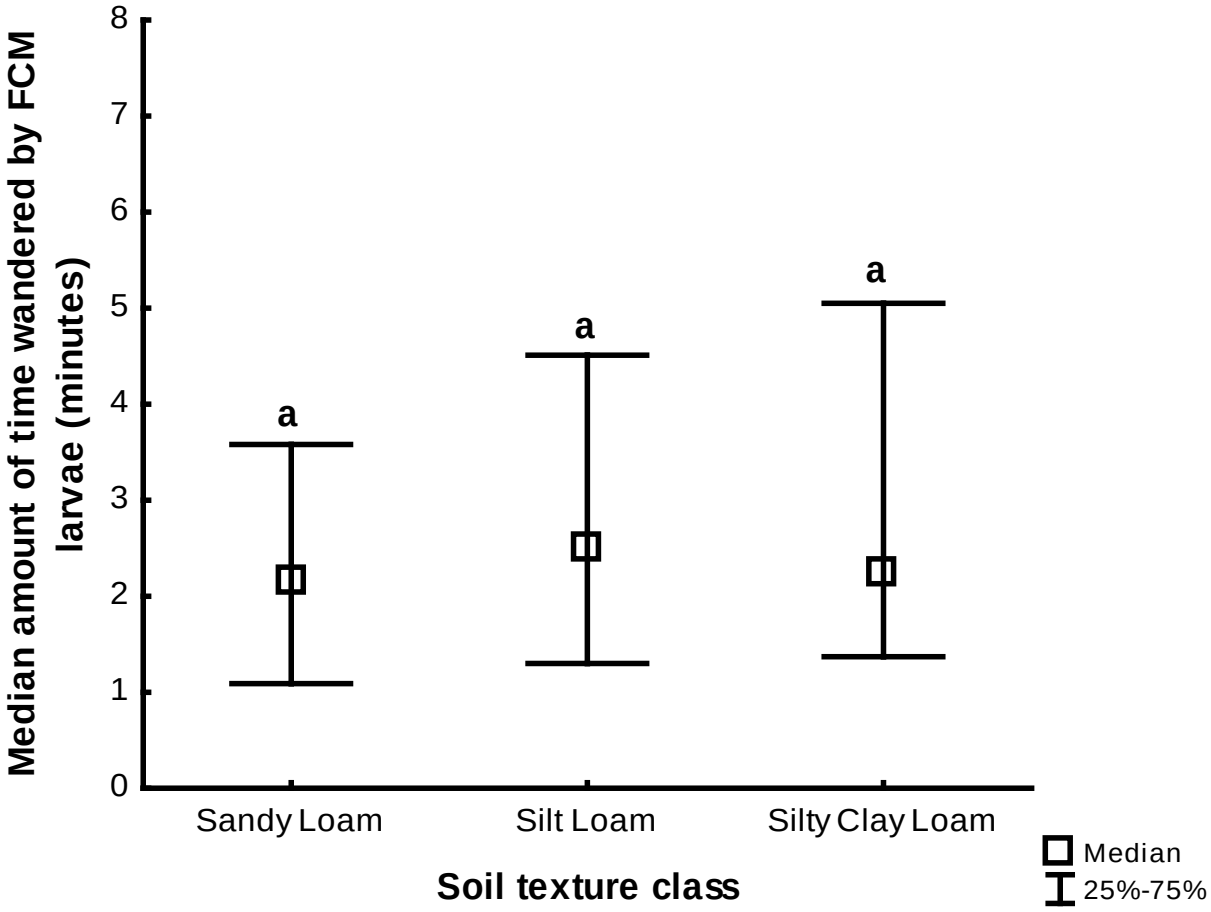

Figure 6.2 The median amount of time wandered by FCM larvae on the soil surface of the three different soil texture classes prior to pupation site selection with soils being maintained at the soil moisture refill point (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The comparison of the distance wandered by FCM larvae on the soil surface between the three soil texture classes at the soil moisture RP revealed no significant differences between any of the three soils ($H_{(2,90)} = 4.781$, $p = 0.092$). Wandering distance was variable, particularly for larvae pupating in the silt clay loam soil (Fig. 6.3).

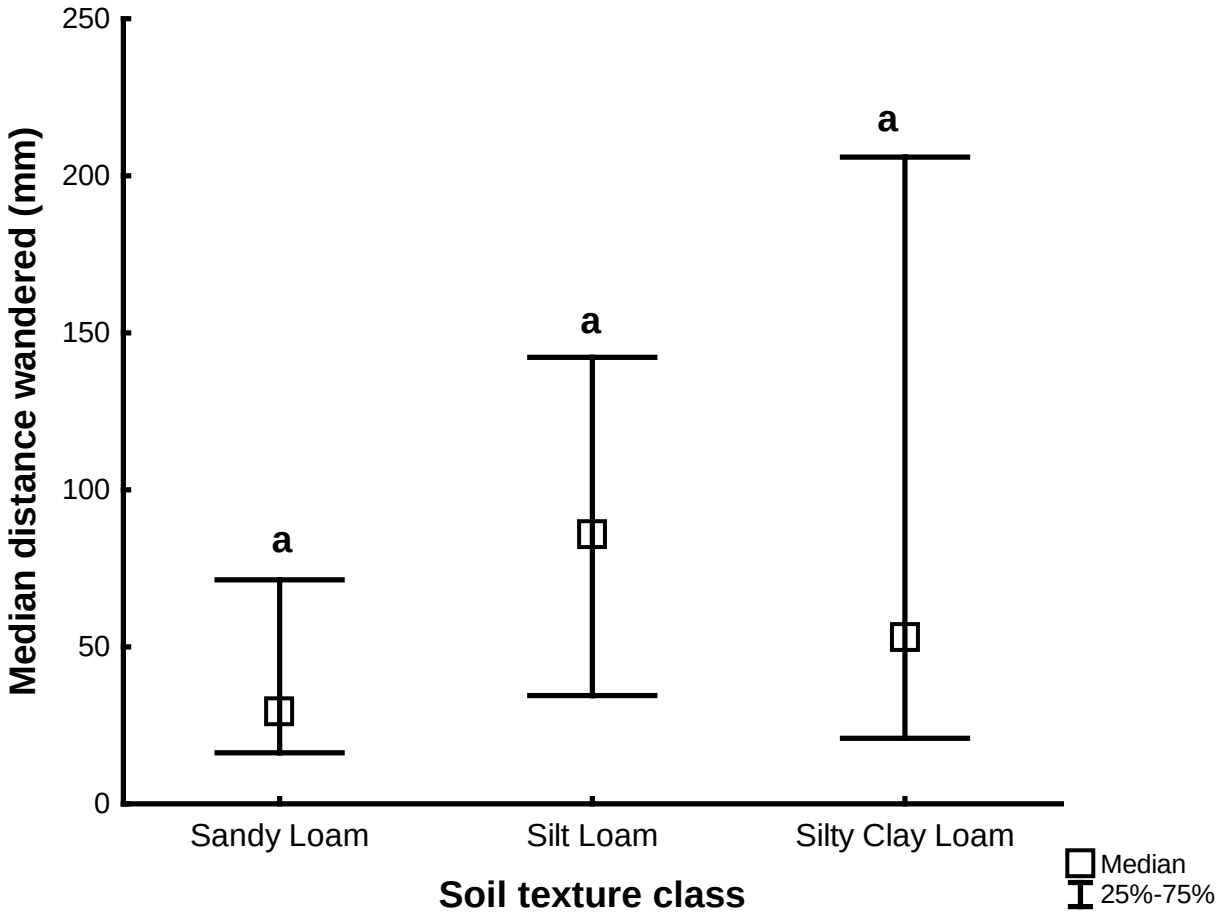

Figure 6.3 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection for the three different soil texture classes with soils being maintained at the soil moisture refill point (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

When the time taken by FCM larvae to form the pupal cocoon was analysed, it was found that larvae in the silty clay loam soil took significantly less time to form the pupal cocoon than larvae in either the sandy loam or silt loam soils ($H_{(2,90)} = 15.989$, $p = 0.003$). No difference in length of time was found between silt loam and sandy loam (Fig. 6.4).

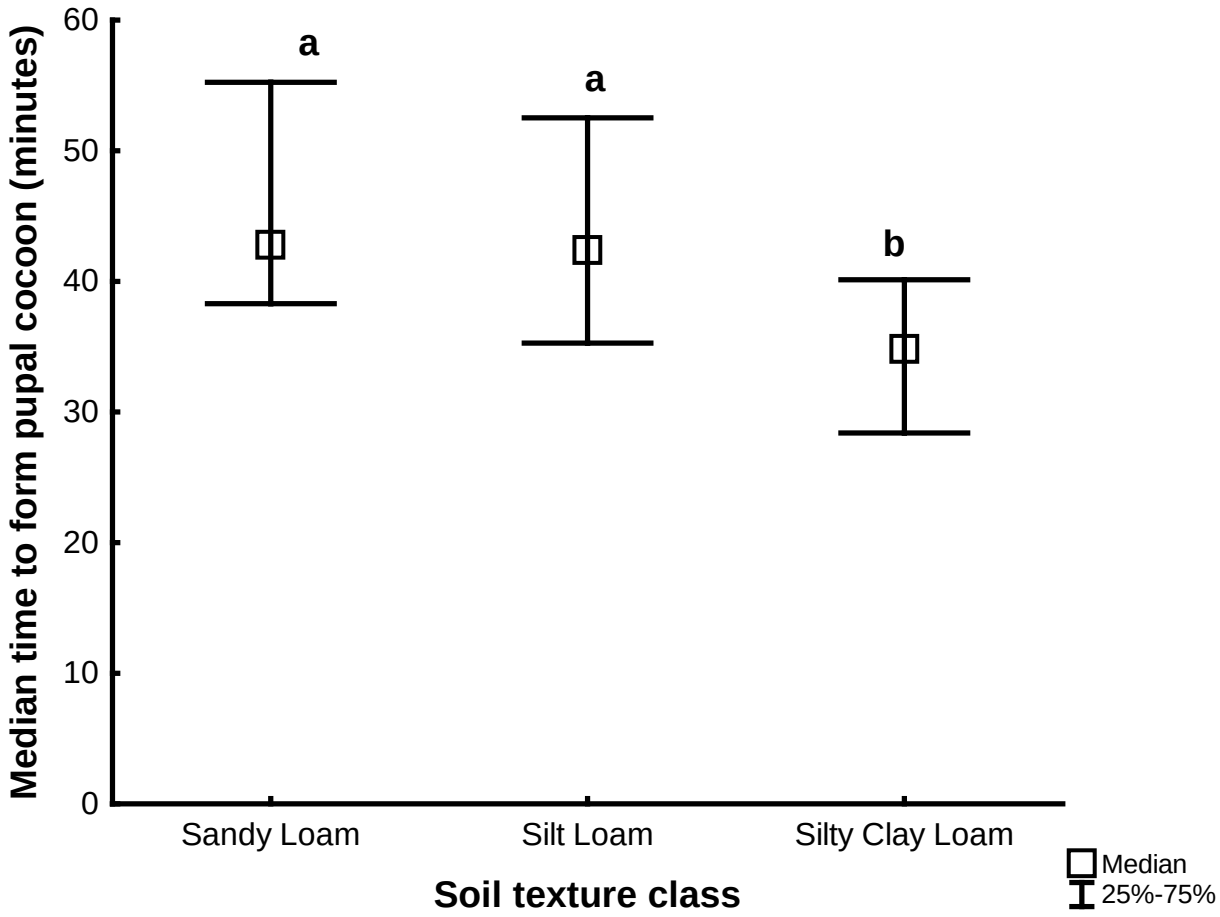

Figure 6.4 Median time taken for FCM larvae to form a pupal cocoon in soil of the three different soil texture classes with soils being maintained at the soil moisture refill point (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

There was no significant difference in pupation depth between the three different soil texture classes when the soils were kept at the soil moisture RP ($H_{(2,90)} = 4.091$, $p = 0.129$) (Fig. 6.5). All of the pupae formed in the RP moisture soil were horizontally orientated.

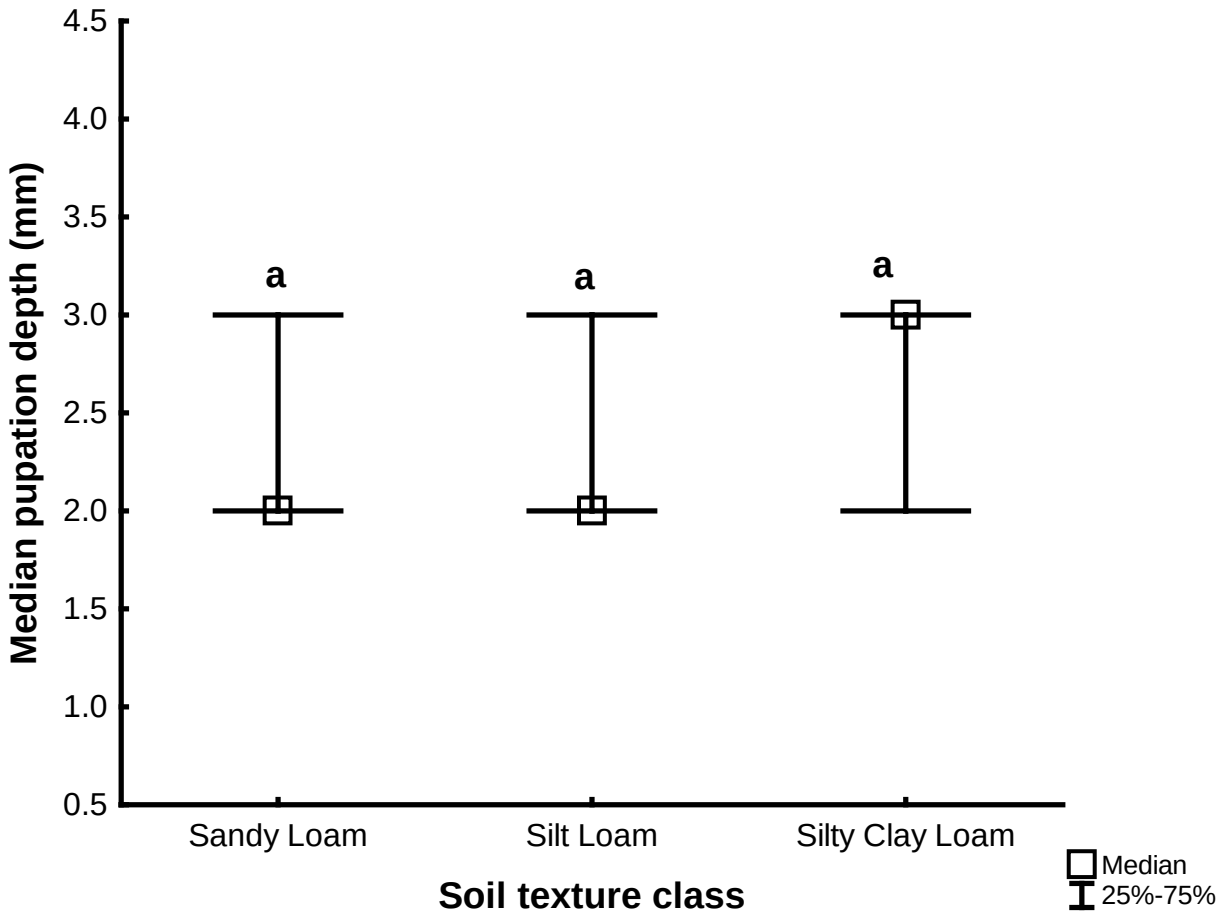

Figure 6.5 Median depth of FCM pupation in three different soil texture classes with soils being maintained at the soil moisture refill point (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant difference was found in the time taken for adult FCM to eclose from pupae formed in the three different soil texture classes with the soil moisture content being maintained at the refill point ($H_{(2,66)} = 3.4$, $p = 0.183$). Adult FCM development generally took between 13 and 20 days to complete at this moisture level (Fig. 6.6).

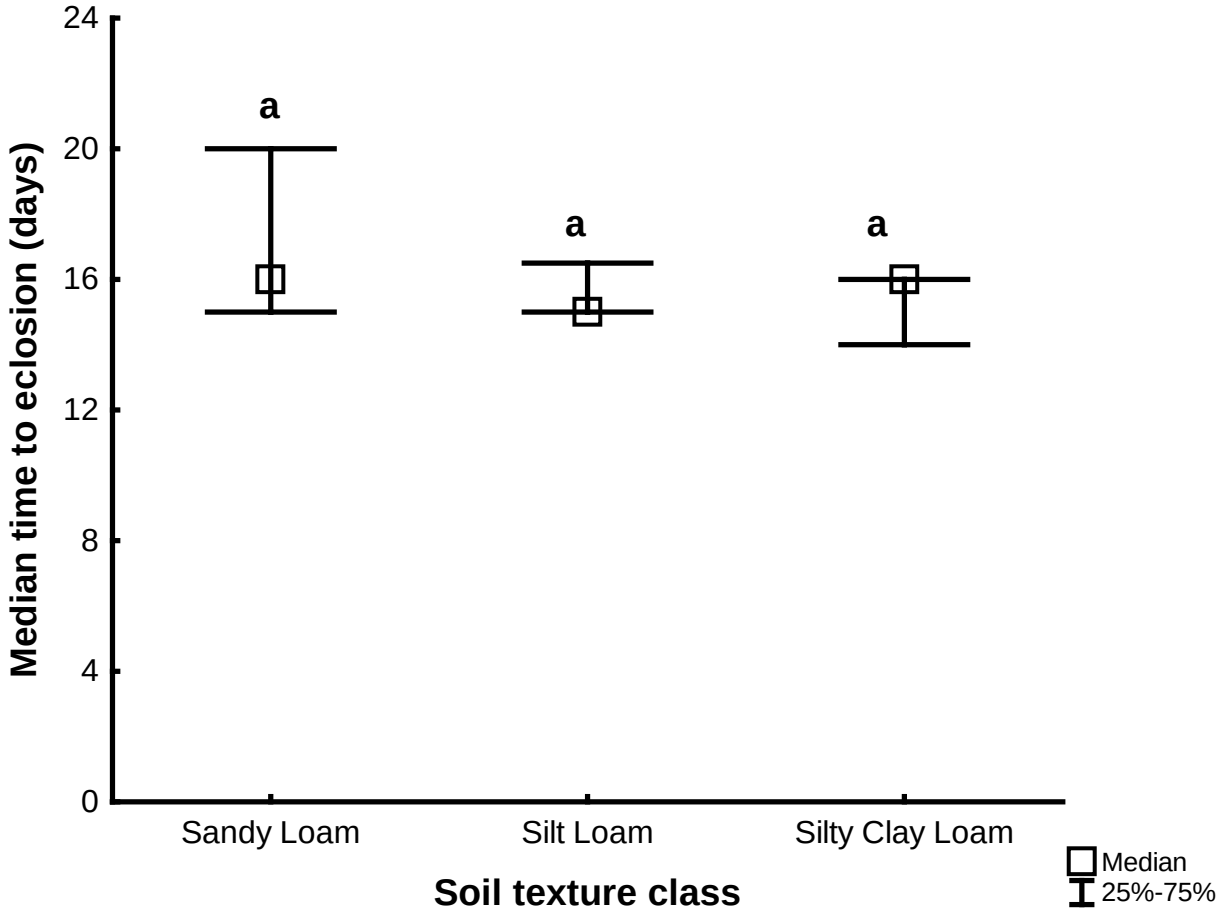

Figure 6.6 The median amount of time taken for adult FCM to eclose from the pupae formed in soil of the three different soil texture classes with soils being maintained at the soil moisture refill point (n = 66). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Significantly more adult FCM eclosed from silty clay loam (86.67 %) than silt loam (56.67 %), however no difference was found between sandy loam and the other two soil texture classes (76.67 %) (Chi-square = 7.159, df = 2, p = 0.028) (Fig. 6.7) at the RP for soil moisture.

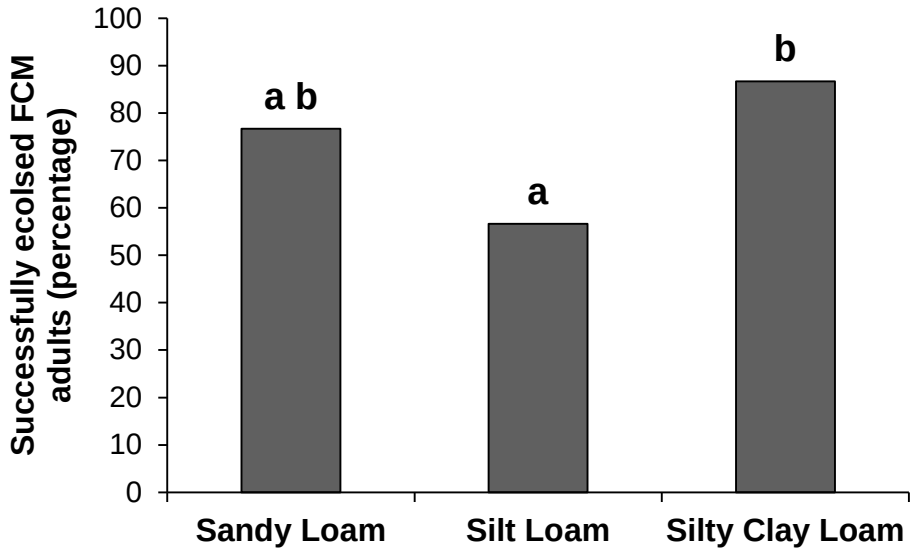

Figure 6.7 The percentage of successfully eclosed FCM adults from pupae formed in the three different soil texture classes with soils being maintained at the soil moisture refill point (n = 66). Different letters denote significant differences (Chi-square test, $p < 0.05$).

6.3.2 General FCM Biology and Field Capacity Soil Moisture

A comparison of the three soil texture classes showed no significant differences in the amount of time wandered prior to pupation site selection between the soil textures when the soils were kept at field capacity soil moisture ($H_{(2,90)} = 4.628$, $p = 0.99$) (Fig. 6.8).

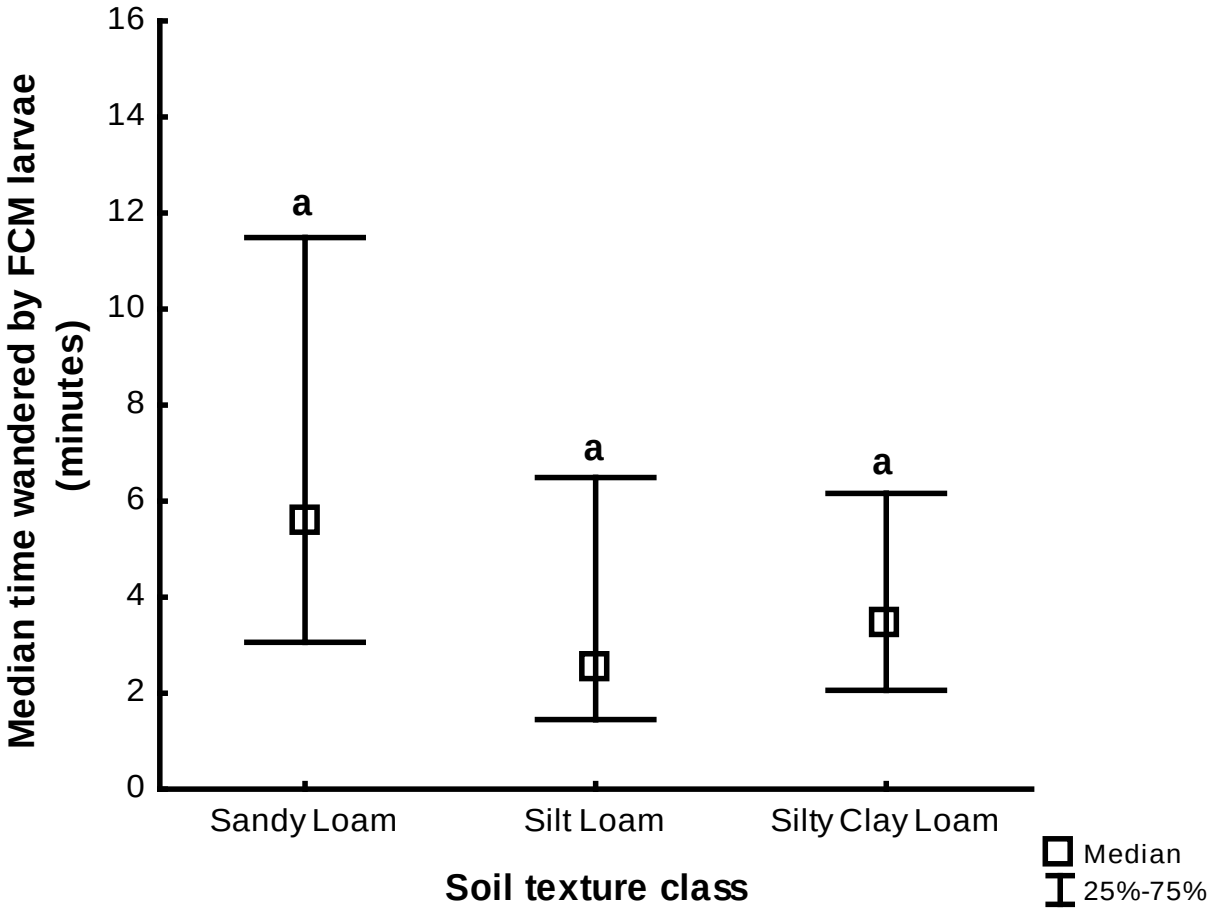

Figure 6.8 The median amount of time wandered by FCM larvae on the soil of the three different soil texture classes prior to pupation site selection with soils being maintained at the soil moisture field capacity (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant difference was found in the wandering distance between the three soil texture classes when the soil moisture content was maintained at FC ($H_{(2,90)} = 4.833$, $p = 0.082$). The variation in wandering distance was high, particularly for FCM larvae wandering on the silty clay loam soil (Fig. 6.9).

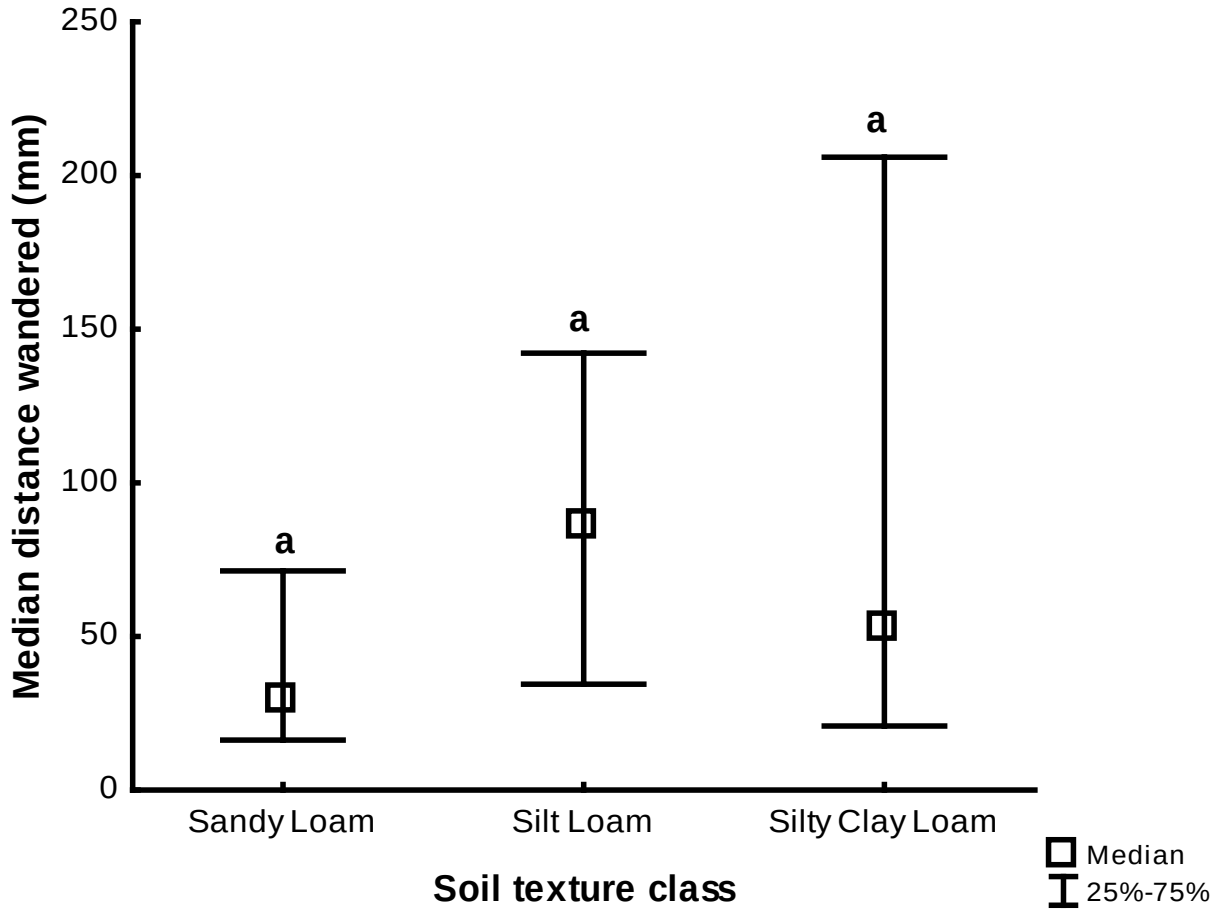

Figure 6.9 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection for the three different soil texture classes with soils being maintained at the soil moisture field capacity (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Soil texture class had no significant effect on the amount of time taken by FCM larvae to form the pupal cocoon with the soil moisture at FC ($H_{(2,90)} = 0.528$, $p = 0.768$) (Fig. 6.10).

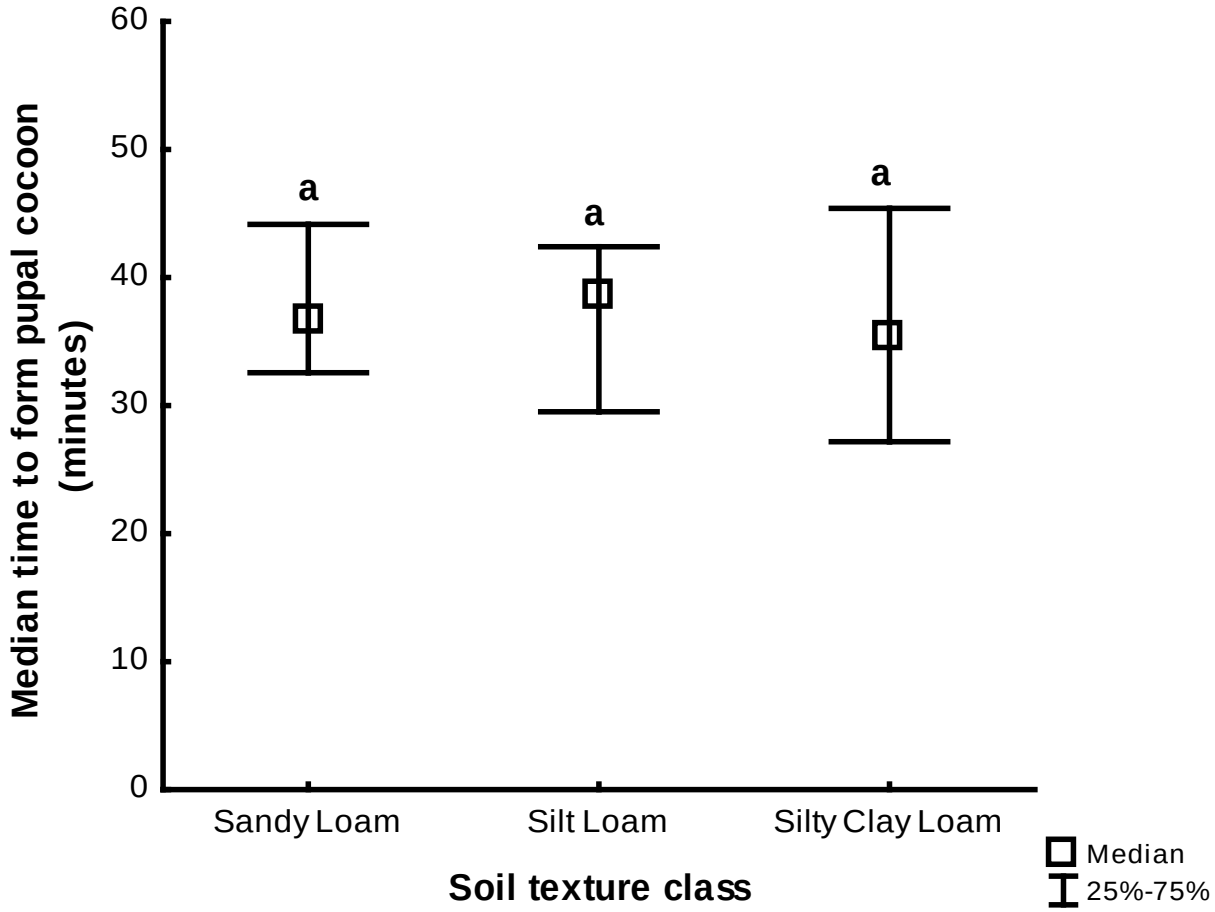

Figure 6.10 Median time taken for FCM larvae to form a pupal cocoon in soil of the three different soil texture classes with soils being maintained at the soil moisture field capacity (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The depth of FCM pupation in the soil was affected by soil texture class when the soil moisture was maintained at FC. Pupation was significantly deeper in silt soil than in silty clay loam soil at this moisture level ($H_{(2,90)}$ = 12.102, p = 0.002). No difference was found between the sandy loam and the other two soil texture classes (Fig. 6.11). In this case, 21 out of the 90 (23 %) pupal cases formed were in a vertical orientation, while the remainder were all horizontally orientated in the soil.

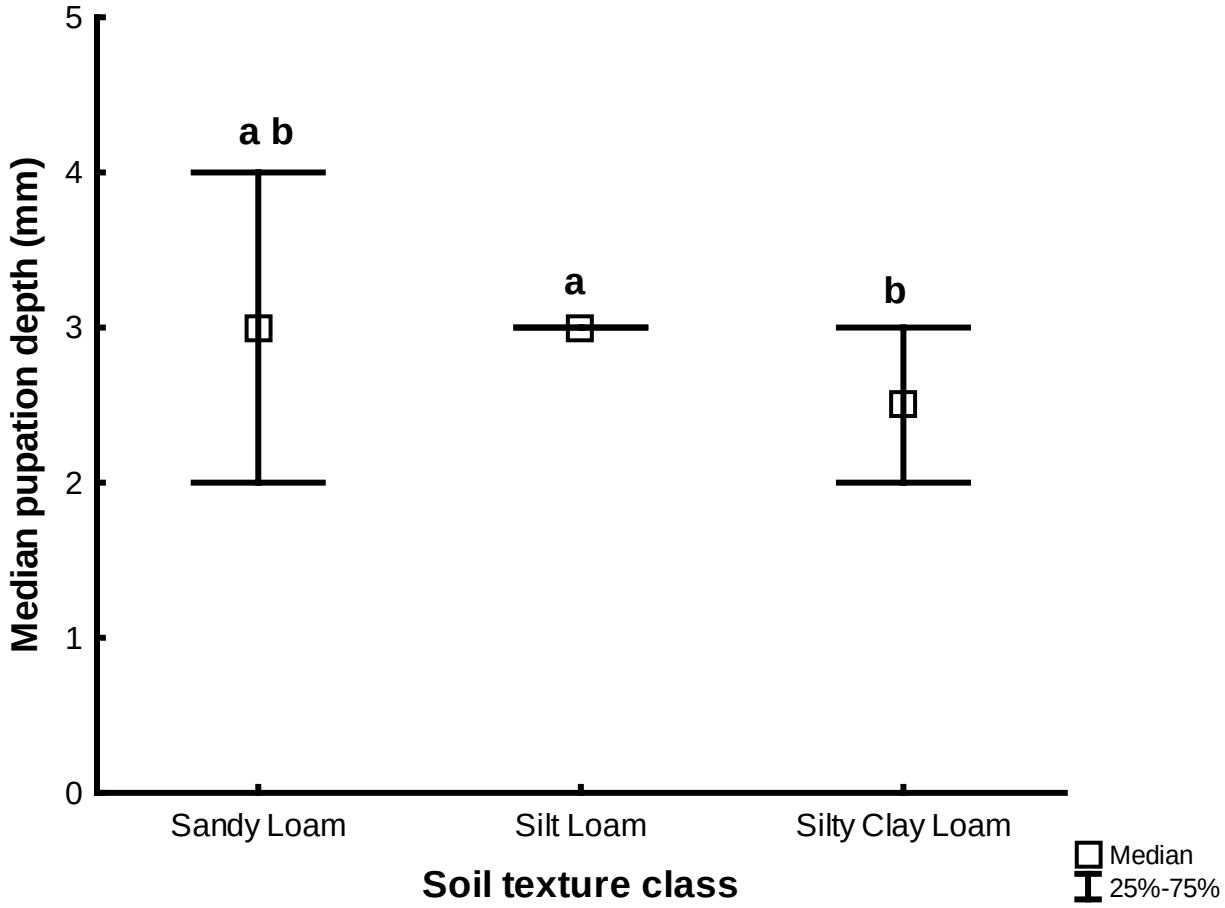

Figure 6.11 Median depth of FCM pupation in three different soil texture classes with soils being maintained at the soil moisture field capacity (n = 90). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Soil texture class had no significant effect on the amount of time taken for FCM adults to eclose when the soil moisture was kept at field capacity ($H_{(2,68)} = 2.648$, $p = 0.266$). Variation in eclosion time was highest for pupae formed in the silt loam soil (Fig. 6.12).

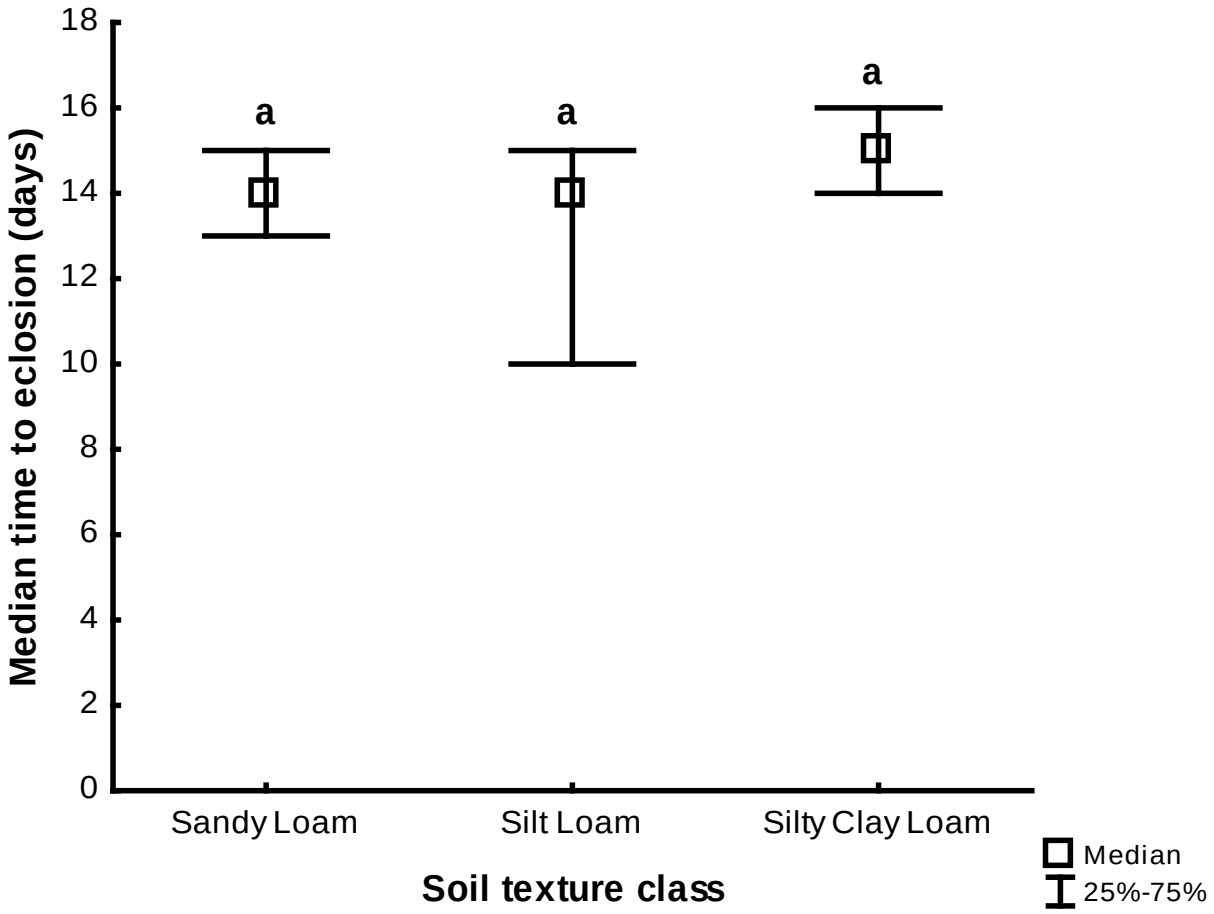

Figure 6.12 The median amount of time taken for adult FCM to eclose from the pupae formed in compacted soil of the three different soil texture classes with soils being maintained at the soil moisture field capacity (n = 68). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The percentage of successfully eclosed adult FCM was high, with a significant association being found between soil texture class and FCM eclosion at field capacity. Adult eclosion was highest (100 %) in sandy loam, which was significantly higher than that of silty clay loam (70 %), but not that of silt loam (90 %) (Chi-square = 9.447, df = 1, p = 0.009) (Fig. 6.13).

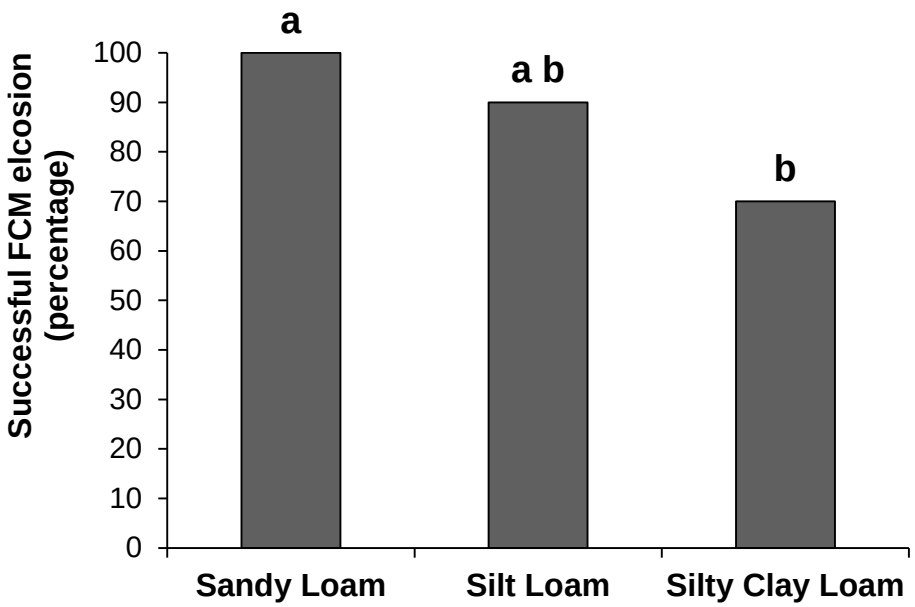

Figure 6.13 The percentage of successfully eclosed FCM adults from pupae formed in moist soil at field capacity for the three different soil texture classes (n = 68). Different letters denote significant differences (Chi-square test, $p < 0.05$).

6.3.3 Comparison of Soil Texture Class and Soil Moisture Content

When comparing each soil texture and each moisture content used, the amount of time wandered by FCM larvae on sandy loam soil at RP moisture was significantly lower than that of sandy loam dry soil or sandy loam FC soil ($H_{(8,270)} = 22.77$, $p = 0.037$). No other significant differences were found between the soil textures and moistures (Fig. 6.14).

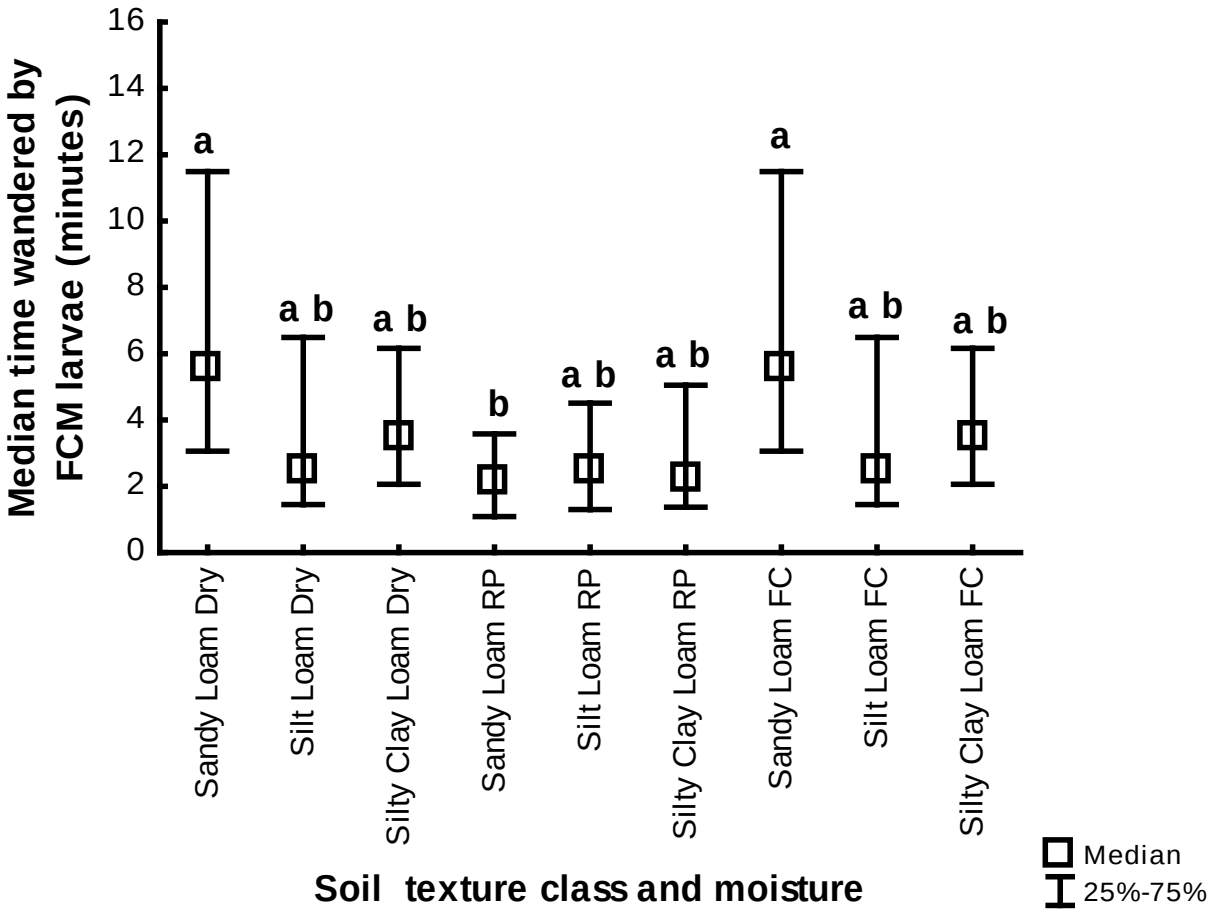

Figure 6.14 The median amount of time wandered by FCM larvae on the soil surface for each soil texture class and soil moisture content prior to pupation site selection (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A comparison of each soil texture and each moisture content showed a significantly higher wandering distance for all of the soil texture classes where moisture was added to the soil (refill point and field capacity) than the sandy loam dry, silt loam dry and silty clay loam dry treatments ($H_{(8,270)} = 166.178$, $p < 0.0001$). No significant differences were found between each of the dry treatments or between the three moisture treatments at either RP or FC (Fig. 6.15).

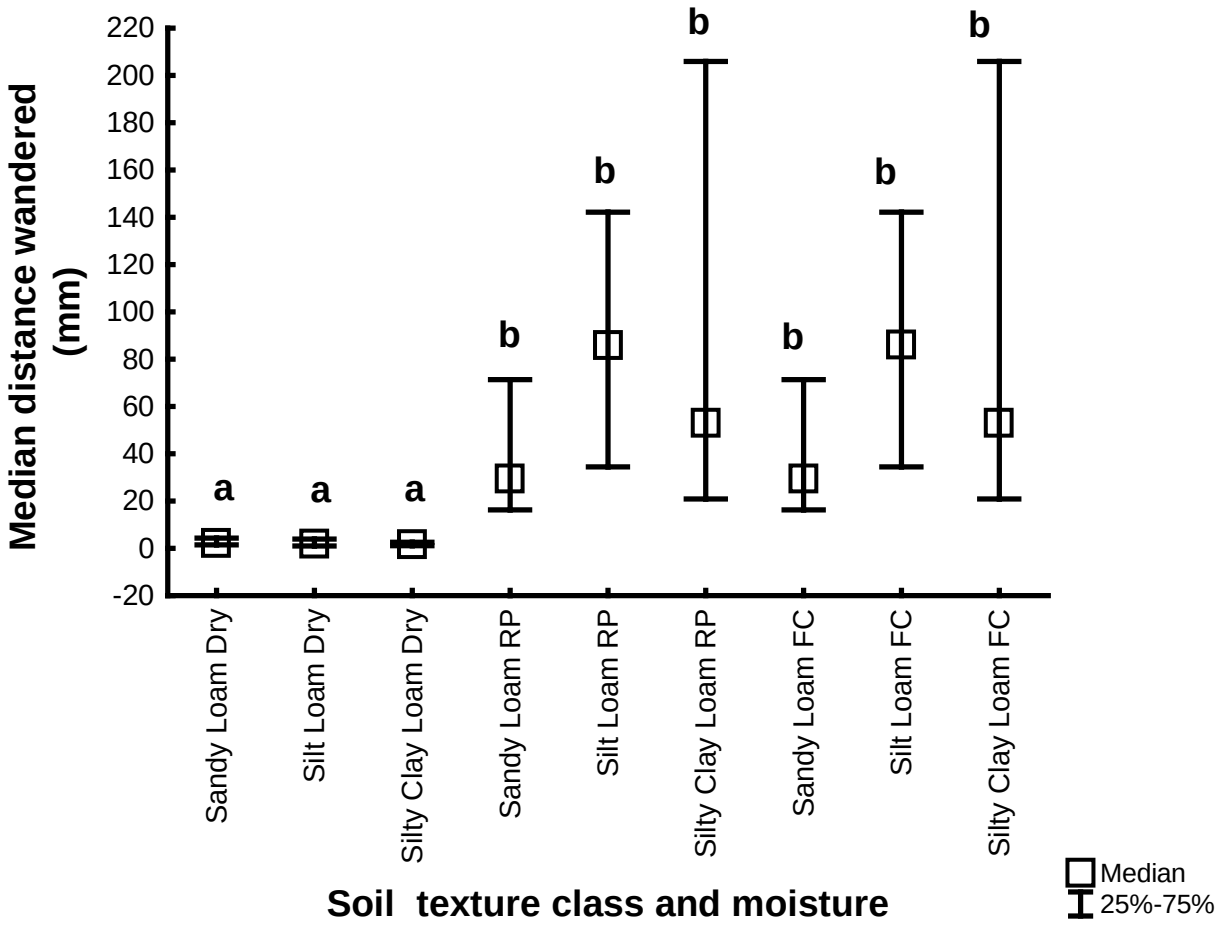

Figure 6.15 The median distance wandered by FCM larvae on the soil surface for each soil texture class and soil moisture content prior to pupation site selection (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

No significant difference in pupal cocoon formation time was found when comparing each soil texture and each moisture content used ($H_{(8,263)} = 15.35$, $p = 0.052$). The variation in pupal cocoon formation time was larger for the dry soils, in particular sandy loam and silt loam in comparison to the soils where moisture had been added (Fig. 6.16).

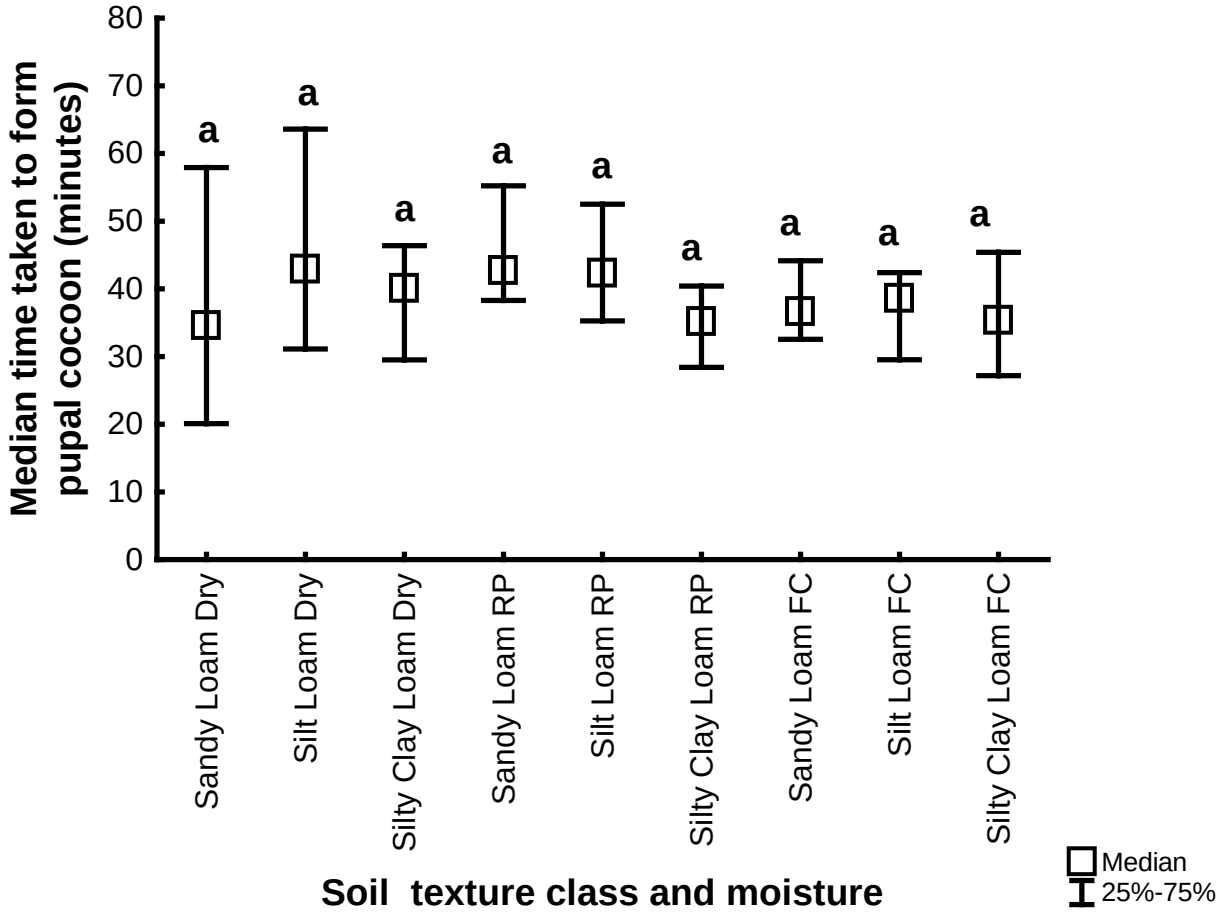

Figure 6.16 The median amount of time taken by FCM larvae to spin the pupal cocoon for each soil texture class and soil moisture content prior to pupation site selection (n = 263). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Pupation depth differed significantly amongst treatments of different soil texture and moisture ($H_{(8,270)} = 44.947$, $p < 0.0001$). Silt loam soil at field capacity showed significantly deeper pupation in the soil than silt loam dry, silty clay loam dry and sandy loam RP treatments, while pupae in sandy loam soil at FC were significantly deeper in the soil than silty clay loam dry soil or sandy loam RP soil. No other significant differences were found between the soil textures and moistures. Larval pupation orientation within the soil at the refill point soil moisture and field capacity moisture varied. At the RP, all larvae pupated horizontally whilst at the FC, 23 % of larvae were found to have pupated vertically in the soil arena. The majority of these vertically orientated pupae were observed in the sandy loam or silt loam soil which is reflected by the differences in pupation depth (Fig. 6.17).

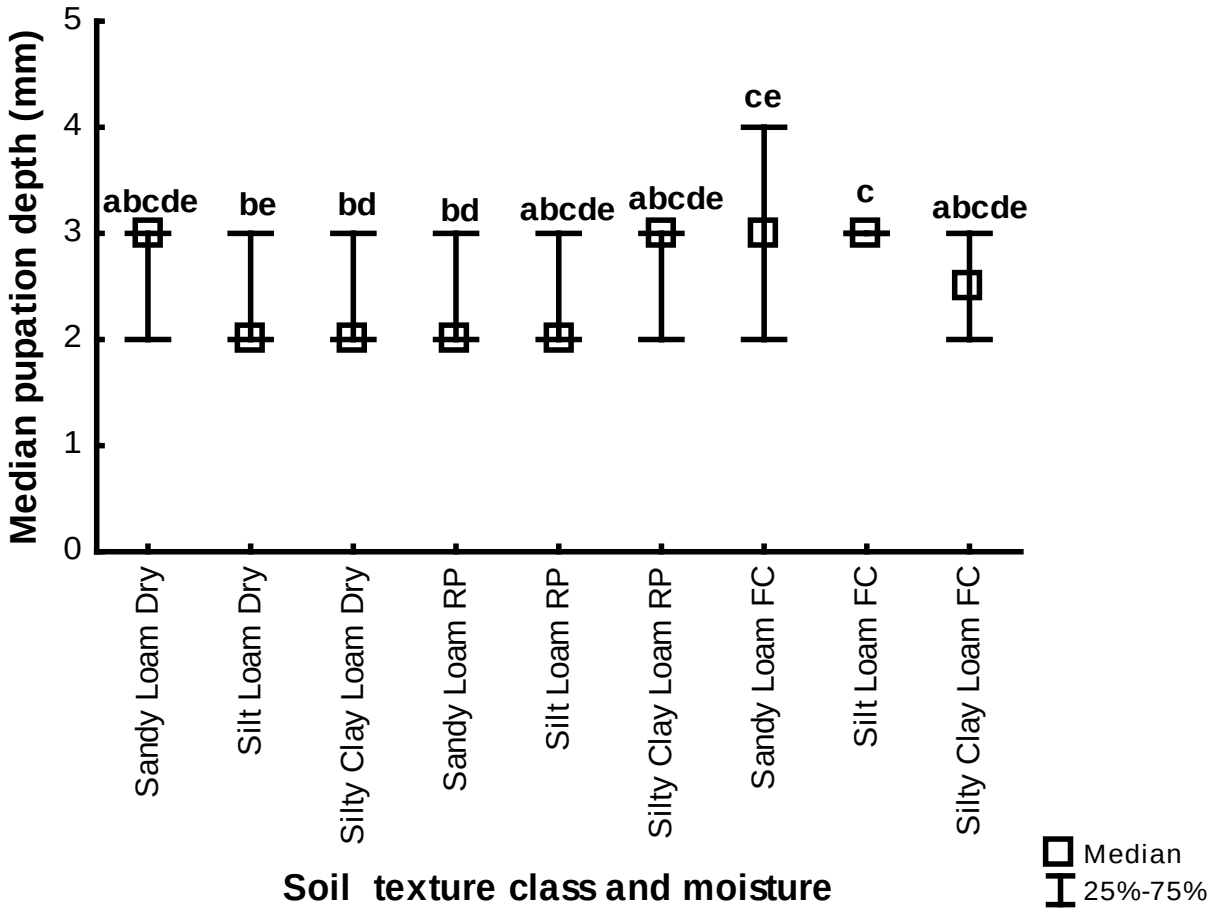

Figure 6.17 The median pupation depth for FCM pupae formed in each of the soil texture classes at each soil moisture content (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The median time to eclosion showed some significant differences between the treatments when comparing each soil texture and each moisture content used ($H_{(8,197)} = 56.786$, $p < 0.0001$). The eclosion time of pupae formed in silty clay loam dry soil was significantly lower than that of silt loam dry, all of the soil texture classes at the refill moisture point and silty clay loam at FC. The sandy loam refill point soil adult eclosion time was significantly longer than sandy loam dry, silty clay loam dry, sandy loam at FC and silt loam at FC (Fig. 6.18).

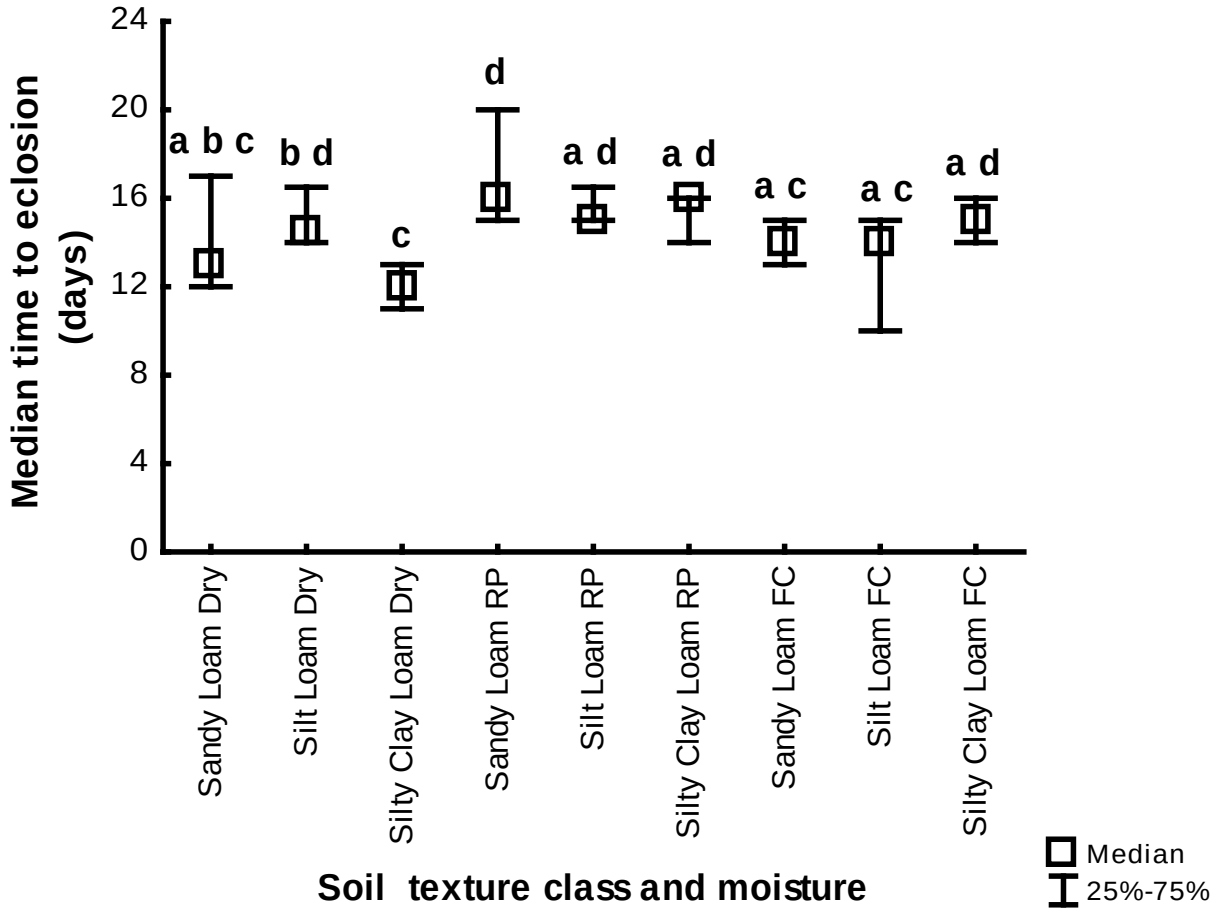

Figure 6.18 The median time taken for FCM adults to eclose from pupae formed in each of the soil texture classes at each soil moisture content (n = 197). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Significant differences between different soil textures of the same moisture content were also provided in the previous refill point and field capacity moisture content sections. When comparing all nine soil texture and moisture treatments, silt loam dry had lower FCM eclosion success than sandy loam dry, silty clay loam RP, sandy loam FC and silt loam FC. Eclosion success of silty clay loam dry was significantly lower than that of FCM in sandy loam FC, while eclosion success of pupae formed in Silt Loam RP soil was significantly lower than that of sandy loam dry, silty clay loam RP, sandy loam FC and silt loam FC (Chi-square = 34.224; df = 8; $p < 0.0001$). For sandy loam and silt loam, FC moisture levels increased eclosion success, but not always significantly (Fig. 6.19).

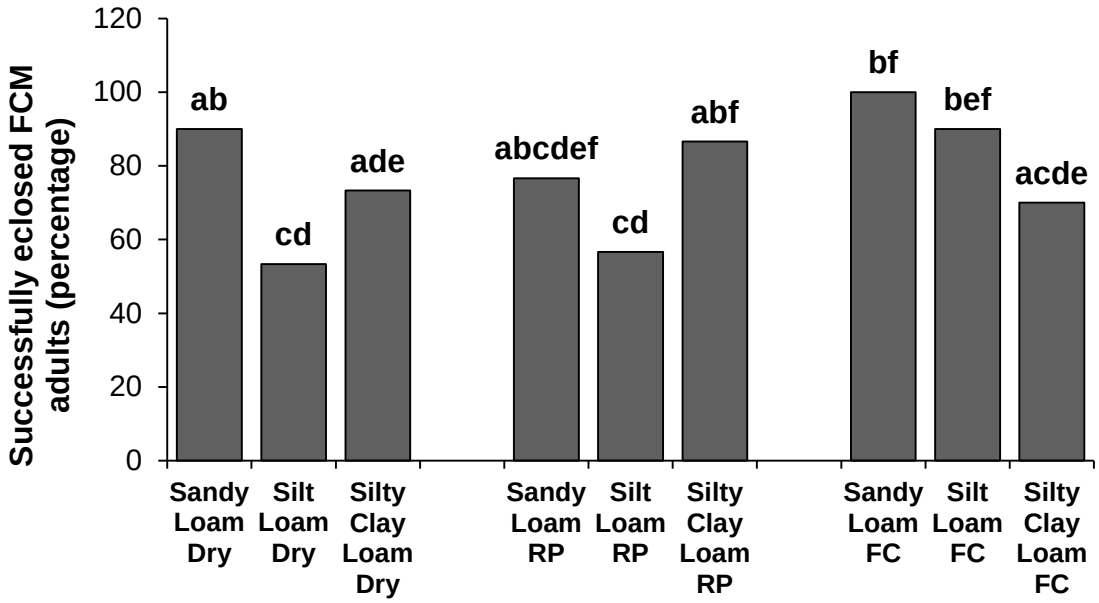

Figure 6.19 The percentage of successfully eclosed FCM adults from pupae formed in each of the soil texture classes at each soil moisture content (n = 197). Different letters denote significant differences (Chi-square test, $p < 0.05$).

6.3.4 Comparison of Different Soil Moisture Contents

For a comparison of the overall effect of soil moisture, irrespective of soil texture, all three textures were combined. The amount of time spent by FCM larvae wandering on the soil surface was significantly higher for soils when at FC moisture than at the RP or when dry ($H_{(2,270)} = 17.849$, $p = 0.0001$) (Fig. 6.20).

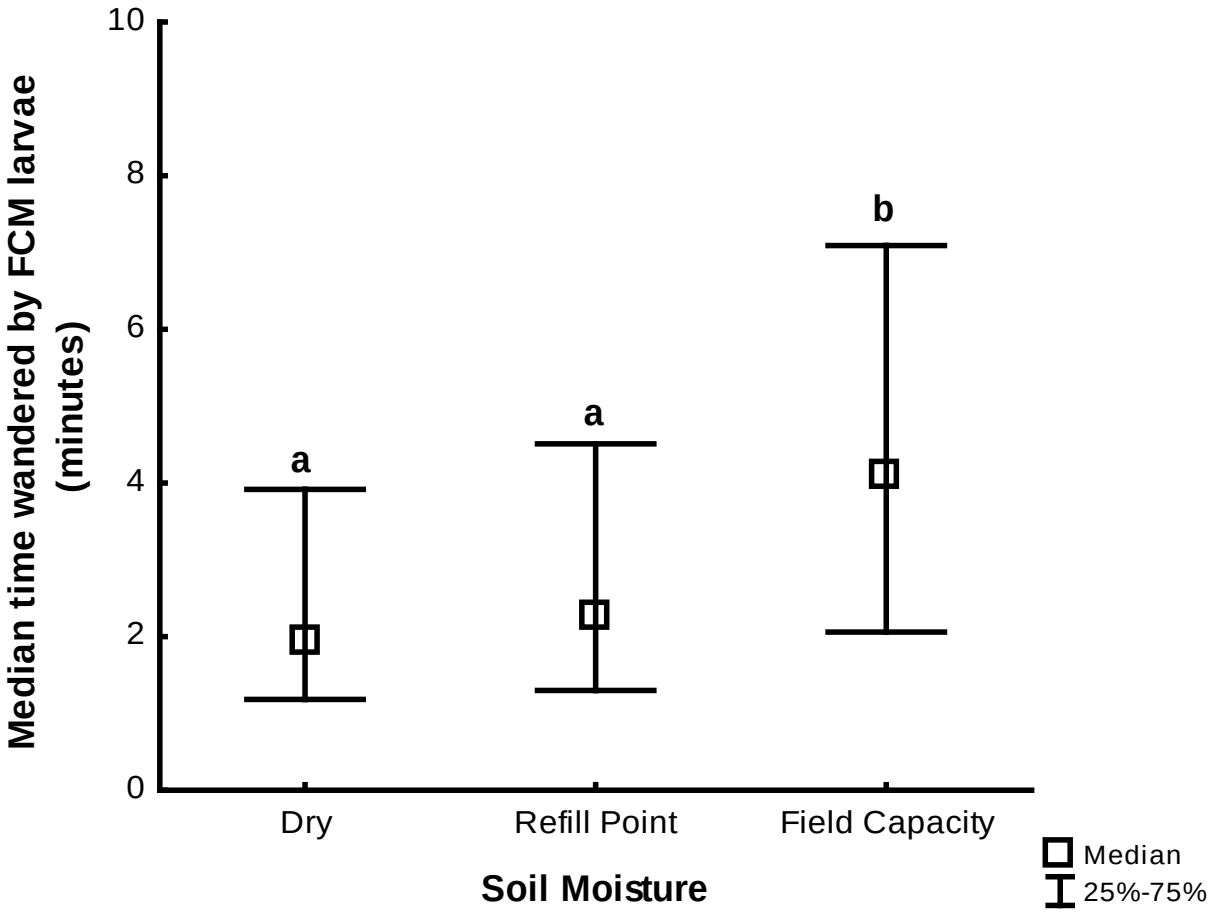

Figure 6.20 The median amount of time wandered by FCM larvae on the soil surface of the three different soil texture classes prior to pupation site selection with soils of varying moisture content (dry, refill point or field capacity) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

A significant increase in distance wandered by FCM larvae prior to pupation site selection was found for both the RP and FC soil moisture content in comparison to the dry soil when all three soil texture classes were combined ($H_{(2,270)} = 33.909$, $p < 0.0001$). No difference was found between the RP and FC soil moisture. Wandering distance was highly variable for both the refill point and field capacity moisture content, far more so than the in the dry soil (Fig. 6.21).

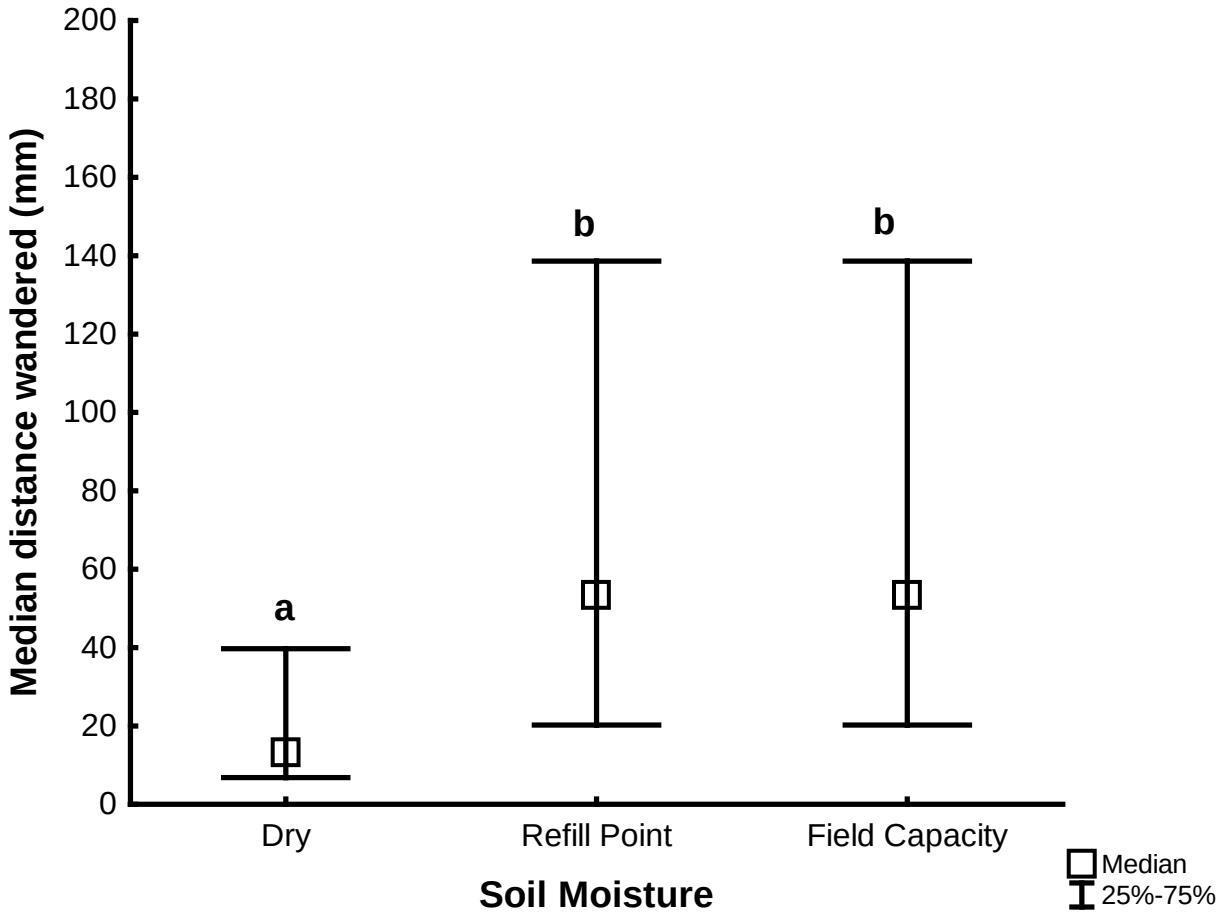

Figure 6.21 The median distance wandered by FCM larvae on the soil surface, prior to pupation site selection for all three different soil texture classes combined with soils of varying moisture content (dry, refill point or field capacity) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

Soil moisture had no significant effect on the amount of time it took for FCM larvae to spin the pupal cocoon when comparing the dry, refill point and field capacity soil moistures ($H_{(2,263)} = 3.514$, $p = 0.173$) (Fig. 6.22).

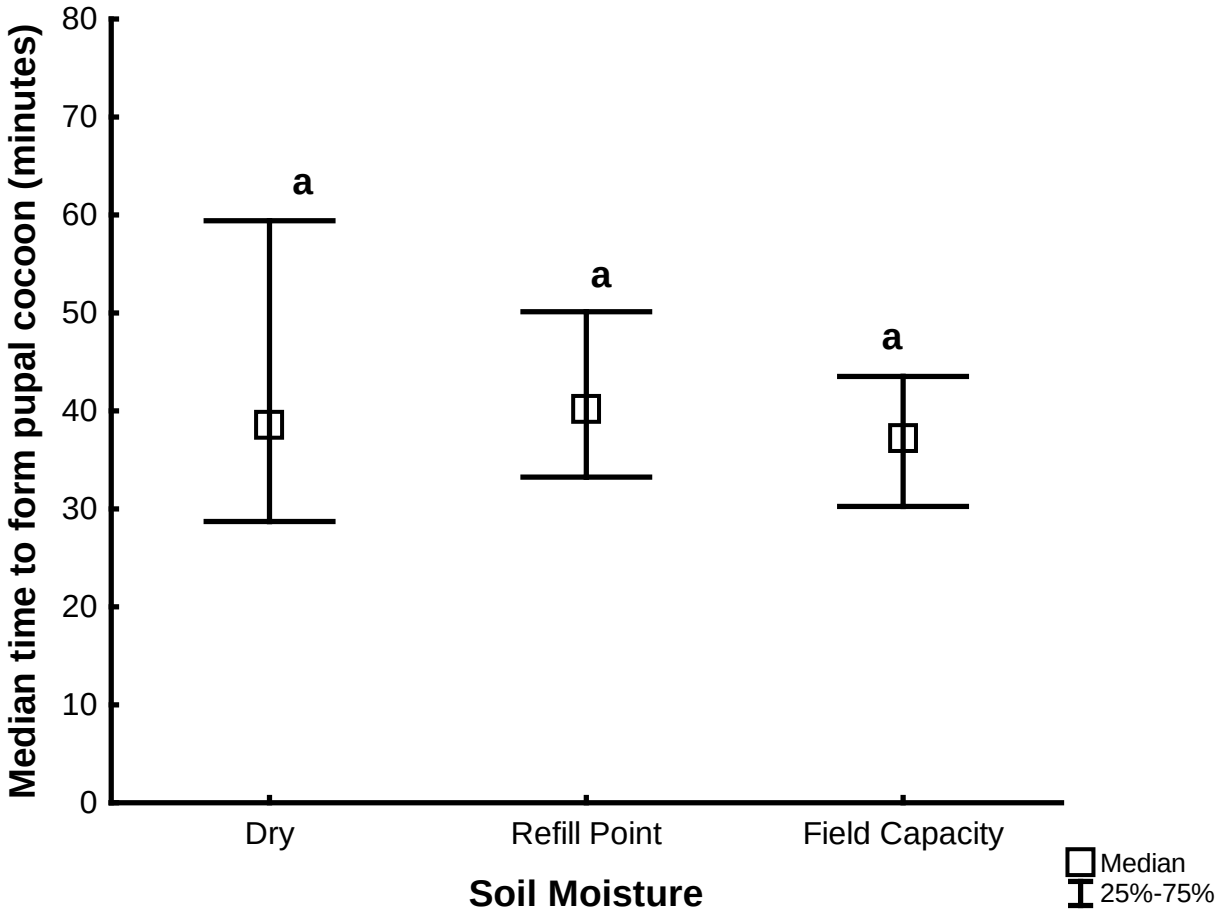

Figure 6.22 Median time taken for FCM larvae to form a pupal cocoon in soil for all three soil texture classes combined with soils of varying moisture (dry, refill point or field capacity) (n = 263). Different letters denote significant differences ($p < 0.05$).

Soil moisture content had a significant effect on FCM median pupation depth when the three soil textures were combined. Pupae formed in soil maintained at the field capacity were significantly deeper than those formed in dry soil or soil maintained at the refill point ($H_{(2,270)} = 20.851$, $p < 0.001$) (Fig. 6.23).

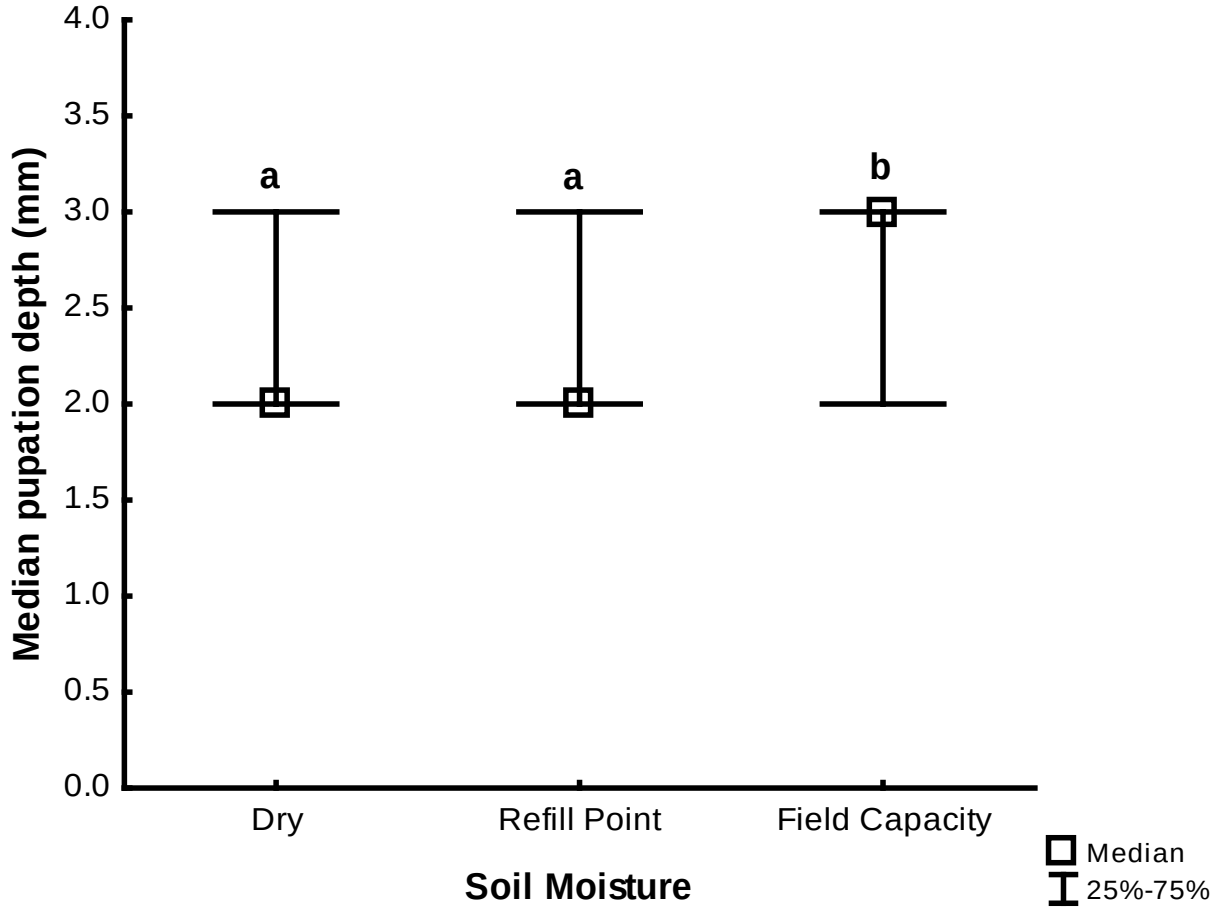

Figure 6.23 The overall effect of soil moisture on median depth of FCM pupation in soils of varying moisture (dry, refill point or field capacity) (n = 270). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

The overall impact of soil moisture on FCM adult eclosion showed that the amount of time taken for the pupae formed in the soil at the refill moisture point to eclose was significantly longer than those in either the dry soil or soil kept at field capacity ($H_{(2,197)} = 37.925$, $p < 0.0001$). No significant difference in eclosion time was found between pupae formed in the dry soil or soil kept at field capacity (Fig. 6.24).

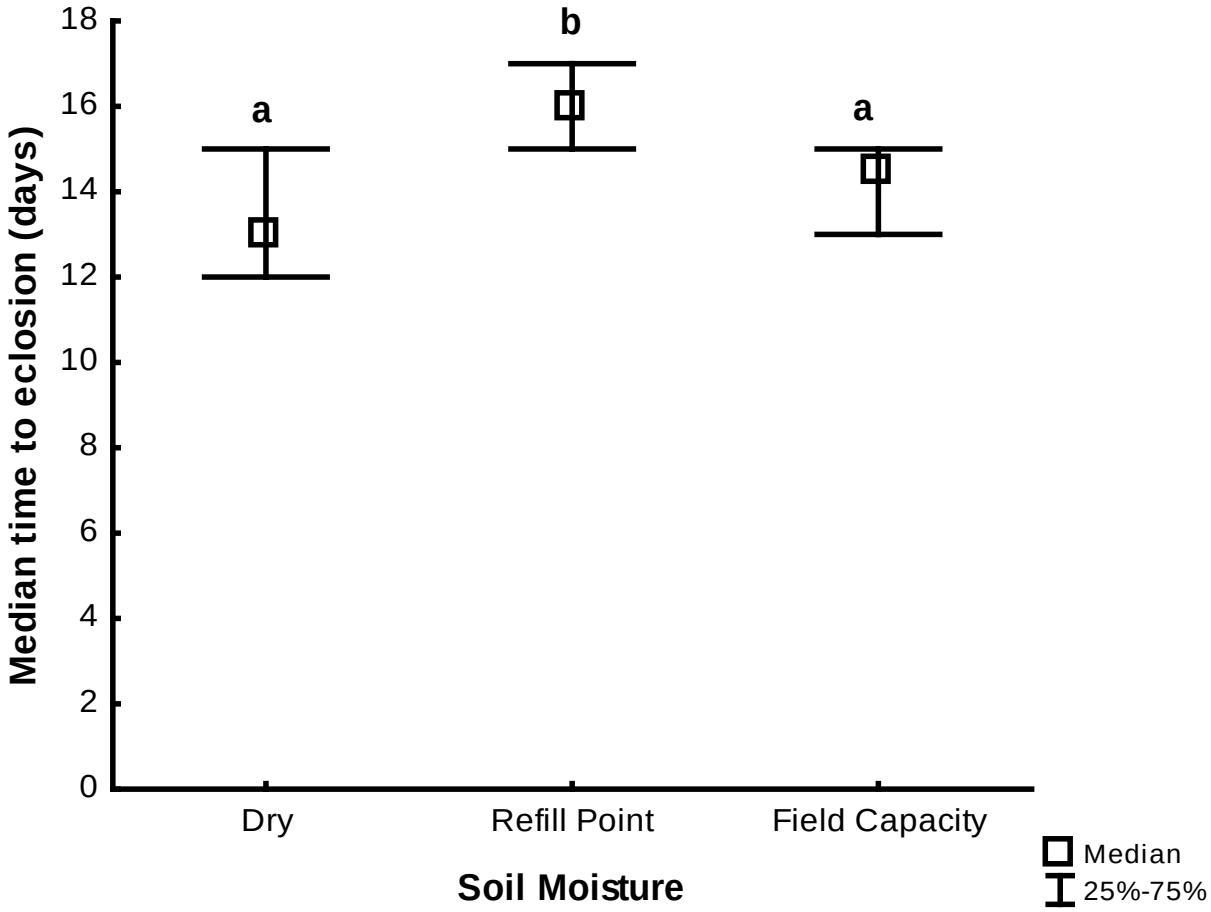

Figure 6.24 The median amount of time taken for adult FCM to eclose from the pupae formed in soils of varying moisture (dry, refill point or field capacity) (n = 197). Different letters denote significant differences (multiple comparisons of mean ranks, $p < 0.05$).

When comparing the effect of overall soil moisture on the percentage of successfully eclosed adult FCM it was found that there was no significant association between soil moisture and adult eclosion (Chi-square = 5.755; df = 1; p = 0.056) While not significant, eclosion success did increase as the soil moisture content increased (Fig. 6.25).

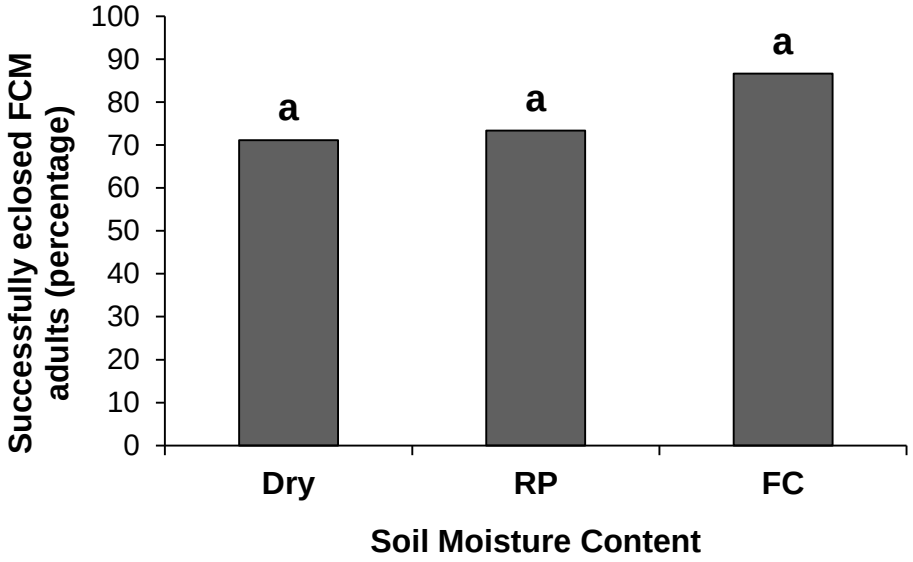

Figure 6.25 The percentage of successfully eclosed FCM adults from pupae formed in soil at each of the soil moisture contents (dry, refill point and field capacity) (n = 197). Different letters denote significant differences (Chi-square test, $p < 0.05$).

6.3.5 Soil Moisture Choice Experiments

FCM larvae were allowed a choice between dry soil and soil moisture content kept at the refill point for each of the three soil texture classes. FCM did not show a significant preference for moist soil at the refill point over dry for sandy loam (Chi-square= 3.33, df = 1, p = 0.068), silt loam (Chi-square = 3.33, df = 1, p = 0.068) or silty clay loam (Chi-square = 0.133, df = 1, p = 0.715) (Fig. 6.26).

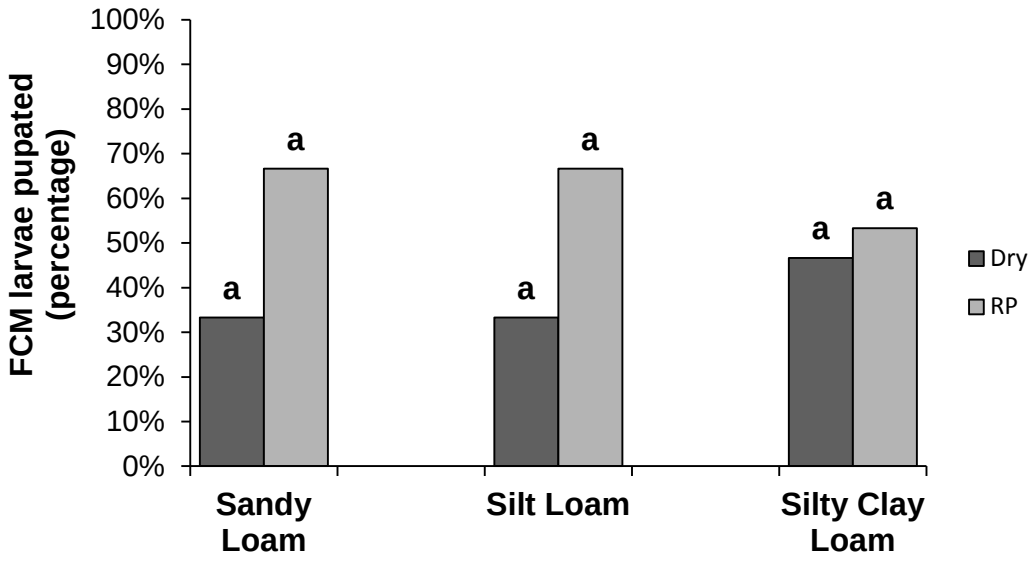

Figure 6.26 The percentage of FCM larvae pupating in either dry soil or refill point moisture soil of the three different soil texture classes in choice experiments (n = 30 per choice test). Different letters denote significant differences (Chi-square test, $p < 0.05$).

In the choice experiments between dry soil and soil at field capacity moisture, no preference was found between the two soil treatments for sandy loam soils (Chi-square = 15, df = 1, p = 1). However, larvae did show a significant preference for soil at field capacity over dry soil for the silt loam (Chi-square = 6.533, df = 1, p = 0.011) and the reverse was true for silty clay loam with a preference for dry soil (Chi-square = 4.8, df = 1, p = 0.028) (Fig. 6.27).

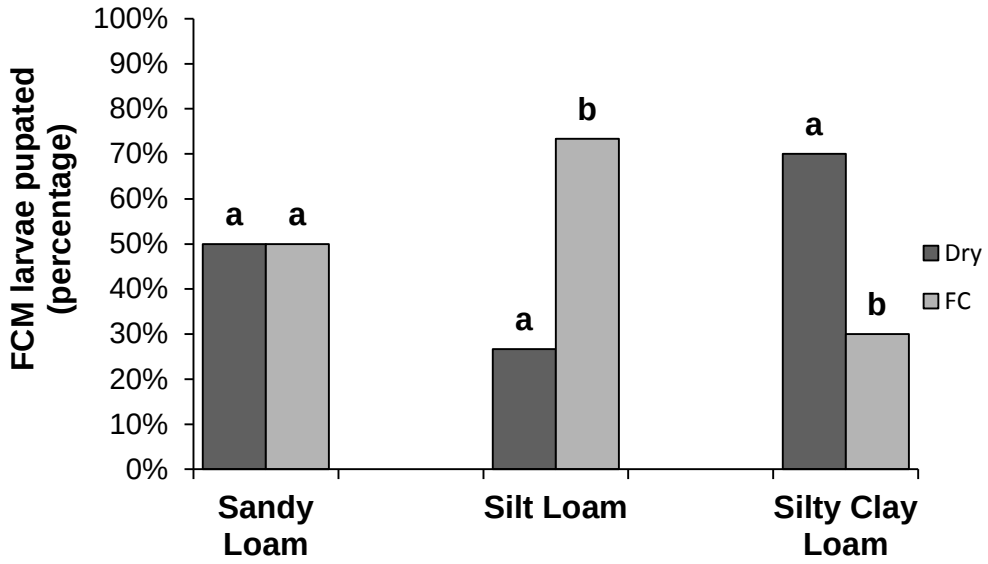

Figure 6.27 The percentage of FCM larvae pupating in either dry soil or field capacity moisture soil of the three different soil texture classes in choice experiments (n = 30 per choice test). Different letters denote significant differences (Chi-square test, $p < 0.05$).

No significant preference was found when comparing the two soil moisture contents for any of the three soil texture classes with the same percentages of FCM pupating in each moisture level (60 % at FC and 40 % at RP) for the three soil textures (Chi-square = 1.2, df = 1, p = 0.273) (Fig. 6.28).

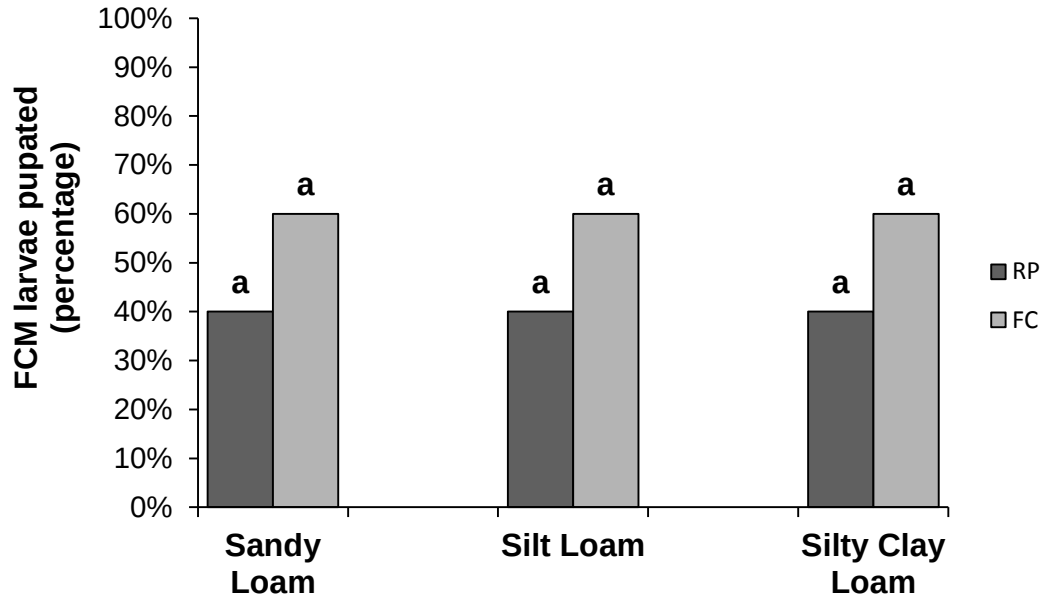

Figure 6.28 The percentage of FCM larvae pupating in either soil at refill point or field capacity moisture soil for the three different soil texture classes in choice experiments (n = 30 per choice test). Different letters denote significant differences (Chi-square test, $p < 0.05$).

An overall comparison was done to determine whether FCM would show a preference for any particular soil moisture content for pupation, when not taking soil texture class into account, therefore all three soil textures were combined. Although FCM showed a preference for refill point moisture soil over dry soils (Chi-square = 5.378, df = 1, p = 0.02) (Fig. 6.29), no difference in preference was found for either dry versus field capacity soils (Chi-square = 3.6, df = 1, p = 0.058) or refill point versus field capacity soil moistures (Chi-square = 0.4, df = 1, p = 0.527). An experiment using soil versus no soil with the three soil texture classes combined showed that FCM has a significant preference for pupating in soil (Chi-square = 82.178, df = 1, p = 0) should it be available.

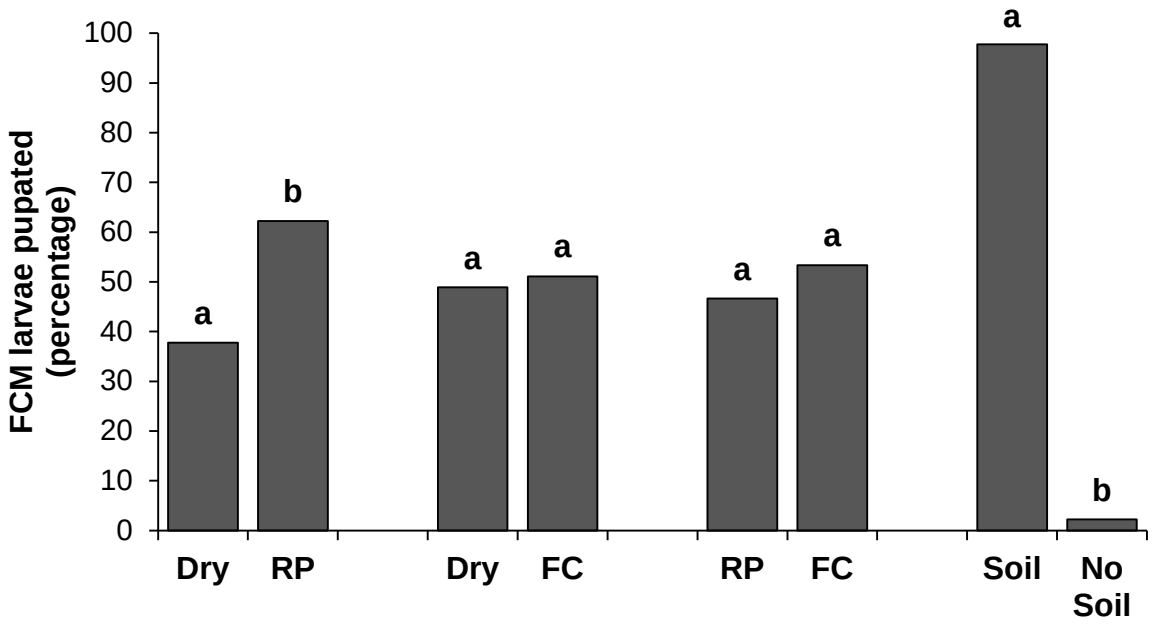

Figure 6.29 Soil moisture choice tests showing the percentage of FCM larvae pupating in dry versus refill point moisture (n = 90), dry versus field capacity moisture (n = 90), refill point versus field capacity moisture (n = 90) and pupation preference for soil versus no soil, with all soil texture classes combined (n = 30). Different letters denote significant differences (Chi-square test, $p < 0.05$).

6.3.6 Pupal Cocoon Distance Measurements

The average distance between each of the pupal cocoons formed was measured for the choice experiment with dry soil and soil kept at the refill point, as it was noted that the pupal cocoons were in close proximity to each other, both in this and the previous experiments. The mean distance between pupae in sandy loam was 28.73 mm, in silt loam, 19.23 mm and in silty clay loam soil, 31.26 mm (Fig. 6.29).

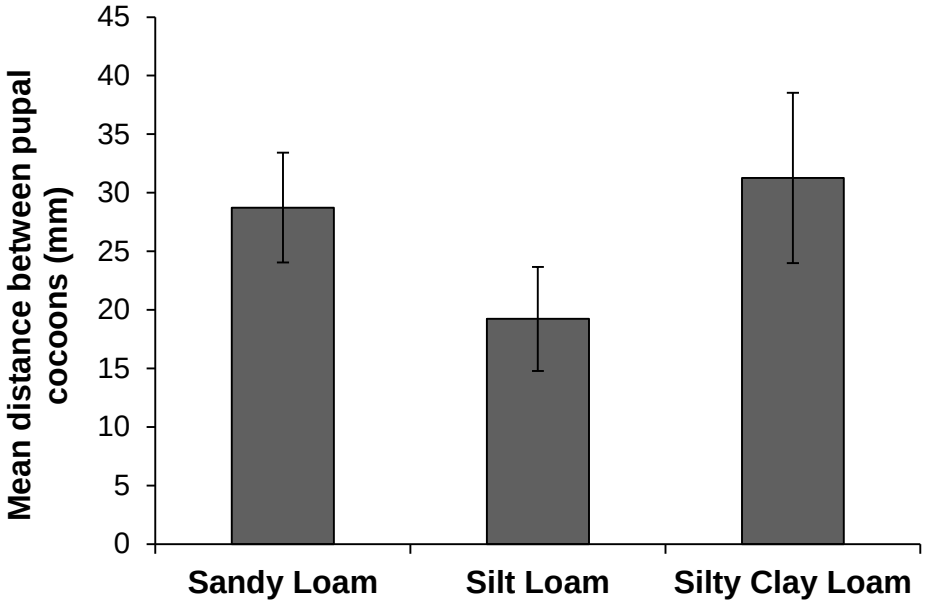

Figure 6.30 The mean distance (± SE) between FCM pupal cocoons formed in soils from the three different soil texture classes (n = 90) during the dry versus refill point choice experiment.

6.4 DISCUSSION

The importance of soil moisture on insect pupation has been well-established through numerous previous studies (e.g. Roach & Hopkins 1979; Murray & Zalucki 1990; Rickelmann & Bach 1991; Jackson *et al.* 1998; Alyokhin *et al.* 2001; Dimou *et al.* 2003; Hou *et al.* 2006; Chen & Shelton 2007; Yee 2013). In this study, the effect of dry soil, moist soil maintained at the refill point (approximately -30 kPa) and moist soil maintained at field capacity (approximately -10 kPa) were used. All three of the soil texture classes for both the refill moisture point and field capacity were able to be maintained at the approximate target kPa. The silty clay loam soil had a lower kPa for both the RP and FC measurements which is thought to be due to the higher amount of clay particles in this soil. Clay soil particles are able to retain water far more effectively than either sand or silt particles as a result of their high surface to volume ratio (Brady & Weil 2000). When the three different soil texture classes (sandy loam, silt loam and silty clay loam) were maintained at the refill moisture point or at field capacity, there was no influence on the wandering time or distance of the FCM larvae prior to selection of a suitable pupation site. Formation of the pupal cocoon in soils kept at the refill moisture point did result in larvae spinning the pupal cocoon more rapidly in silty clay loam soils than silt loam. The particularly adhesive nature of clay particles when moist (Brady & Weil 2000) may have allowed for more rapid incorporation of these into the pupal cocoon resulting in the pupal cocoon forming more rapidly than silt loam. Surprisingly, no difference was found between sandy loam and silty clay loam or between any of the soil texture classes when the soils were at FC, as the moisture retaining effect of increased clay particles would have been expected for these.

Pupation depth increased for cocoons formed in silt loam soil at field FC over cocoons formed in silty clay loam soil. Based on observation during the experiment, this was largely due to the soils cracking and forming aggregates. The formation of the silt loam soil resulted in slightly deeper soil cracks allowed FCM larvae to move more deeply into the soil without having to physically burrow into it. The silty clay loam soil retained more water for longer, resulting in slightly less cracking of the soil. The length of time taken until adult eclosion for FCM did not differ amongst the three different soil texture classes at either the refill point or at field capacity. Soil moisture has been known to effect the development time prior to adult emergence of Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), with

development being fastest at 70 % soil moisture and slowest at 30 % (Hou *et al.* 2006), however for FCM no difference in eclosion time was found.

The eclosion success results showed that the trend of low eclosion success rate of FCM in silt loam soil remained, despite the addition of moisture in the RP experiment. This changed for the FC experiment where the increase in soil moisture had a positive effect on FCM eclosion in silt loam. The dusty nature of the silt loam soil was eliminated when moisture was added to soil and this may well have reduced FCM pupal desiccation. The positive effect on FCM eclosion success was particularly noticeable for the FC experiment. In experiments testing the effects of moisture on insect pupation in other species, high soil moisture content results in an increase in pupal mortality (Roach & Hopkins 1979; Eskafi & Fernandez 1990; Lapointe & Shapiro 1999; Hulthen & Clarke 2006; Hou *et al*. 2006; Chen & Shelton 2007). In the majority of these experiments however, the soils were at 100 % moisture content and completely saturated which left little air in the soil for the pupae. In this study, the soils were not saturated, but rather maintained at field capacity which would have resulted in better soil aeration. Furthermore, FCM pupated on the soil surface and the very upper layer of the soil. This contact with the surrounding air would have prevented oxygen availability from becoming a limiting factor in moist or wet soils, unlike in the majority of previous studies with other species where the larvae burrowed more deeply into the soil.

When comparing each of the soil texture classes at each moisture level, a clear trend in wandering time was not seen, but for the wandering distance it was evident that the addition of soil moisture resulted in an increase in FCM wandering distance prior to pupation site selection. The addition of moisture to the soils may have allowed for larvae to wander more easily as the soils were now more compact than the loose, dry soil. The moist soils had a less uniform environment than the loose, dry soil due to the formation of small cracks and soil aggregates. This allowed the larvae to search for the most protected and suitable pupation sites. Wandering time was far more variable in the refill point and field capacity moisture experiments than the dry soil experiment. Pupation depth did differ in certain cases, primarily between particular dry and field capacity soil textures. This was mainly due to the formation of the occasional air pocket and shallow cracks in the soil for the field capacity moisture experiment, while the dry soil provided a far more uniform environment. The change in the soil structure also allowed FCM larvae to pupate vertically in the soil cracks or between larger aggregates which were most prevalent in the silt loam soil. The eclosion time, when

comparing all soil textures and moistures was variable, but the pattern of decreased eclosion success of pupae in silt loam soil in comparison to sandy loam soil was apparent.

In order to determine the overall impact of moisture addition to the soil on FCM pupation, the results for the three soil texture classes were grouped together and compared with the previous dry soil experiment results. In this case, the wandering time was significantly higher for larvae which pupated in the soils kept at field capacity than either the dry or refill point soils. The larval wandering distance increased for both moisture levels when compared to the dry soil as has been previously discussed. An increase in pupation depth was found for the larvae pupating at FC. This is in contrast to research on other insect species where high levels of moisture resulted in more of the insects pupating at the soil surface, although these were at or just below soil moisture saturation levels (Ellis *et al.* 2004; Hou *et al.* 2006). Dimou *et al.* (2003) reported an increase in pupation depth for the olive fruit fly, *Bactrocera (Dacus) oleae (Gmel.)* (Diptera: Tephritidae) at 50 % field capacity moisture. This soil moisture content was thought to allow a favourable balance between soil moisture and oxygen for the larvae without surface soil pupation being required. Oxygen was not a limiting factor for FCM due to the overall shallowness of the pupation depth. Combining all three soil texture classes, there was no significant difference in eclosion success, although eclosion did increase with increasing moisture content for the FC experiment, with 100 % eclosion success reported in sandy loam soils at FC. However, while this indicates an overall trend, it is important to keep in mind the strong interaction between soil texture class and eclosion success as had been noted in the previous moisture experiments.

Insects such as Oriental fruit fly (Alyokhin *et al.* 2001), Queensland fruit fly, *Bactrocera tryoni* Froggatt (Diptera: Tephritidae) (Hulthen & Clarke 2006) and willow flea beetle, *Altica subplicata* LeC. (Coleoptera: Chrysomelidae) (Rickelmann & Bach 1991) have shown to be able to distinguish between, and show preference for, different soil moistures. In this study, FCM only showed a preference for FC soil over dry soil, particularly in silt loam soil. FCM mortality in dry silt loam soil was found to be high and thought to largely be due to desiccation therefore, by choosing more moist soil over dry soil, mortality as a result of desiccation is reduced. The reverse was found in silty clay loam where more FCM larvae chose to pupate in the dry soil. The high water retention capacity and potential waterlogging of silty clay loam made this a less favourable environment than the dry soil, prompting the larvae to pupate in the dry soil. When grouping the soils together this difference was lost and a preference for moist, RP soil over dry soil was recorded. Therefore the interaction between

different soil textures and moistures is very important and may alter FCM behaviour. The soil availability experiment showed that FCM larvae do prefer to pupate in soil if it is available. However, general observations of FCM behaviour during the course of the study revealed that if soil is not available, the larvae will spin a cocoon on another surface that is available and pupate there. This is unlike the small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) for whom the appropriate substrate is vital in order for pupation to occur (Meikle & Diaz 2012). FCM is able to adapt easily to change in its substrate environment.

In the choice experiments, measurement of the distance between the closest pupae revealed that FCM tended to pupate within close distance proximity to one another and in some cases, larvae would pupate directly adjacent to each other attaching themselves to another cocoon. This behaviour was also noted in previous experiments during the study. By attaching itself to another pupal cocoon, the larva expended less energy in formation of the cocoon. It is also speculated by the author that male and female larvae may have been pupating next to each other for ease of mating after eclosion. Sex pheromones have been isolated from adult female FCM (Henderson & Warren 1970; Read *et al.* 1974), but it is currently unknown whether these are emitted during the larval life stage as well. Unfortunately, this is difficult to confirm as sex determination at the larval stage cannot be done and in almost all cases where larvae pupated right next to one another, one of the two did not survive. The attachment of one pupal cocoon to another only occurred in 7.8 % of the pupal cocoons formed during this experiment, therefore this particular behaviour was not common, but overall FCM larvae tended to pupate within a close proximity of one another. Another possibility is that this behaviour was due to FCM larvae releasing an aggregation pheromone. Evidence of aggregation pheromone being produced by both male and female cocoon-spinning codling moth, *Cydia pomonella L.* (Lepidoptera: Olethreutidae) has been found (Jumean *et al.* 2004). While FCM may not show strong preferences for particular soil moisture content, a clumped distribution may be occurring in the field when larvae pupate in the soil as there was ample space for larvae to pupate further away from each other in the experimental arena. However, since this was a laboratory study, the larvae all dropped into the soil within a few hours of each other and their movement was restricted by the size of the experimental arena. Therefore it would be necessary to find evidence of this behaviour occurring in the field as well.

Both EPF and EPNs will be strongly influenced by the soil moisture content. In the case of EPF, water is required for most fungal species to germinate and subsequently infect their host (Barbercheck 1992; Ignoffo 1992). Sporulation may also only be possible if there is free water available in the soil (Ignoffo 1992) but large amounts may have a negative impact on the conidia of certain fungi (Barbercheck 1992). EPF application to the soil through soil drenches is often used, but the movement of conidia into the lower soil layers may be poor (Jaronski 2010), which is particularly influenced by soil texture (Garrido-Jurado *et al.* 2011). This is not anticipated to be as problematic for FCM as it may be for other species, as FCM appears to pupate at the surface and very upper layer of the soil, although this behaviour does still require confirmation in the field. Soil texture, structure and fungal spore size will play a pivotal role in conidia retention in the soil surface as some spores may disperse beneath the surface layers of the soil. Should the field results reflect a similar pupation depth to what has been found in this study, then fungal applications to the soil surface should allow for very good exposure of FCM to EPF. Research is currently being conducted to determine the effectiveness of applying EPF to citrus orchard soils via the irrigation system (drip or microjet irrigation) (Coombes, C. pers. comm.[1]).

For EPNs, soil moisture is essential for survival and dispersal. In order to move through the soil, EPNs require a film of soil water to be present around and between the soil particles and for dispersal to be most effective this water layer should be continuous and not too thin (Barbercheck 1992; Hazir *et al.* 2003; Stuart *et al.* 2006). Nematodes such as *Heterorhabditis bacteriophora* Poinar, which make use of cruiser behaviour and actively search for their host (van Zyl & Malan 2014), are particularly affected by this (Hazir *et al.* 2003). Moisture extremes where the soil becomes too dry and the water film too thin for effective nematode movement or when the soil becomes completely saturated with water and all the air pores between soil particles become completely waterlogged also restrict EPN movement (Koppenhöffer *et al.* 1995). The ability of EPNs to survive these conditions varies from species to species (Koppenhöffer *et al.* 1995).

Heterrorhabditis bacteriophora, the only commercially available EPN currently used in South Africa, appears to be able to survive in a range of soil moisture conditions from humid, subtropical areas of South Africa to more semi-arid regions (Hatting *et al.* 2009). This range was noted by Hatting *et al.* (2009) when sampling for naturally-occurring EPN species across South Africa. *Steinernema* species were also recovered from semi-arid regions. The reason for this may be that certain EPNs are able to survive dry soil conditions in a number of ways,

such as by lowering their metabolic rate (Hazir *et al.* 2003), adapting to drier conditions as soil water content gradually decreases (Patel *et al.* 1997) or even by making use of their insect host and remaining inside the cadaver until conditions become more favourable (Koppenhöfer *et al.* 1997). Soil texture will also have an impact on EPN distribution as nematode movement is known to be restricted in high clay soils and silty clay loam (Georgis & Poinar 1983). When sampling for nematodes throughout South Africa, Hatting *et al.* (2009) only recovered nematodes in soils which contained 20 % clay particles or less, indicating that high clay content soils may not be the most suitable environment for most nematode species. The interaction of high soil moisture and high clay content, with its high water retention capability and small soil pore sizes, may result in waterlogged soils reducing aerobic respiration for EPNs (Barbercheck 1992). A reduction in surface tension in high moisture soils can also inhibit nematode dispersal (Wallace 1971, cited in Babercheck 1992); therefore both soil texture class and the amount of soil moisture will need to be carefully taken into account prior to EPN application to the soil. Taking abiotic factors into account prior to EPN application to the soil has been recommended by van Zyl & Malan (2014).

The biology and behaviour of FCM under moist soil conditions is likely to be favourable for both EPF and EPNs. The increase in FCM larval wandering time at field capacity and in wandering distance of FCM at both the refill point and field capacity will increase the potential exposure of FCM to these biological control agents. The lack of larval burrowing into the soil may be a negative factor for EPF and EPN efficacy, as the surface of the soil will dry out more rapidly than the deeper soil layers post-irrigation and will also initially become more saturated with moisture after irrigation, leaving the biological control agents vulnerable to these moisture extremes. This is where factors such as ground cover and shading will have an important role to play in assisting in survival of these agents. A potential positive side to this surface and upper layer pupation would be the increased likelihood of contact between the biological control agents and FCM as they are applied to soil. Persistence in the soil and virulence against FCM will be crucial for both EPF and EPNs, particularly as FCM eclosion success improves in moist soils. Moisture will therefore play a key role in the effectiveness of both biological control agents for FCM control.

7

General Discussion

7.1 INTRODUCTION

The control of the soil-dwelling life stages of false codling moth (FCM) in citrus orchards is key to reducing the phytosanitary risk and financial losses resulting from this pest. Interest in controlling FCM at this stage of the life cycle has increased in recent years, particularly due to the potential of entomopathogenic fungi (EPF) and entomopathogenic nematodes (EPNs) for control of FCM being recognized (Goble *et al.* 2011; Malan *et al*. 2011; Coombes *et al.* 2013). There is great potential for these biological control agents to be incorporated into an IPM programme in citrus orchards, which would assist in the reduction of pesticide usage. However, a knowledge gap regarding FCM pupation biology, behaviour and survival was identified and this needed to be addressed.

The biology, behaviour and survival of FCM, including wandering time, wandering distance, time taken to form the pupal cocoon, pupation depth, pupal orientation, time to eclosion and eclosion success, were determined in three different soil texture classes (sandy loam, silt loam or silty clay loam) under varying abiotic conditions. The amount of time wandered on the soil surface was unaffected by soil texture class in isolation (Chapter 2) and did not vary between the soil textures when manipulating the soil ground cover (Chapter 3), compaction (Chapter 4), air temperature (Chapter 5) or soil moisture (Chapter 6). The wandering time was generally very short, with FCM larvae rapidly selecting a pupation site within minutes of dropping onto the soil surface. The only influence on wandering time was low air temperature where a decrease in metabolic rate slowed the wandering time, but without increasing the wandering distance. Low air temperature also resulted in more FCM larvae abandoning their initial choice of pupation site in favour of a secondary site, possibly in an attempt to escape the cold conditions.

The wandering distance of FCM larvae was highly variable for all abiotic factors tested. The only factor to result in a change in wandering distance was moisture, where an increase in wandering distance was found for moist, as opposed to dry soils. Wandering over more compact, moist soils with a less uniform environment in search of the most suitable site was thought to be the reason for this. Pupal cocoon formation was rapid once a suitable site had been selected and these typically included soil particles which provided an added element of camouflage for protection from predators (Danks 2002). The importance of the pupal cocoon against the natural elements was shown by the 100 % mortality rate of pupae formed without a cocoon due to desiccation.

Based on previous pupation studies on other species (Dimou *et al.* 2003; Ellis *et al.* 2004; Hou *et al.* 2006; Chen & Shelton 2007), FCM had an unusually shallow depth of pupation with pupation occurring on the soil surface or in the upper few millimetres of the soil, not more than 1 cm in depth. An increase in pupation depth was found for the compaction experiment and the field capacity moisture experiment. This was not as a result of larvae physically burrowing into the soil, but rather due to compaction and moisture, resulting in a more heterogeneous environment with soil cracks and aggregations which allowed for deeper pupation depth without the physical effort of burrowing more deeply into the soil. There is a possibility of increased vulnerability to predators by pupating on the soil surface, however, in the compact soils it was noted that pupal cocoons were often spun onto the soil surface, thus firmly attaching them to the soil, with the protective pupal cocoon being harder to remove and expose the immobile pupa underneath. This may provide an element of protection from predators, but the high risk of exposure to the natural elements and temperature fluctuations would remain, unless pupation occurred in a more shaded environment or underneath ground cover.

The length of time taken to develop between larval pupation and adult eclosion was unaffected by soil texture class, ground cover or compaction. The cold air temperature increased the length of time to adult eclosion, while the warmer air temperature decreased it. The eclosion success of FCM was very clearly influenced by both soil texture class alone and in combination with most other abiotic factors. A general trend of higher eclosion success for FCM pupae in the sandy loam soil compared to the silt loam was determined for the soil texture class, ground cover and temperature experiments. The loose nature of the silt loam soil in these experiments appeared to negatively impact FCM pupal survival. When the soil was compacted, or when moisture was added (for both the refill point and field capacity

moistures) eclosion success for silt loam was still less than the sandy loam but not significantly so. High (32 °C) and low (15 °C) air temperature had a very negative effect on FCM eclosion with both increasing mortality substantially compared to 25 °C. The addition of moisture had a positive effect on FCM eclosion, which is not unexpected as mortality due to desiccation in the dry soils was a leading cause of pupal death. Eclosion success in sandy soil with a field capacity moisture level was 100% and was also high for the other two soil textures at this moisture level.

The choice experiment revealed that FCM does not show distinct preferences for pupating in any particular soil texture class, despite the high mortality of pupae in silt loam soil. Although there was not a significant difference, more larvae did pupate in shaded soil over open soil. It is possible that a clearer preference for shaded areas might be found in the field where soil moisture content and temperature through solar energy from the sun, and not an artificial light source, would also play a role in the choice. Soil moisture and texture class did affect FCM larval choice of pupation site in the case of silt loam soil and silty clay loam and this is thought to be due to the water retention properties of these soil textures. FCM larvae do show a clear preference for pupating in soil over areas with no soil, but behavioural observations throughout the study show that soil is not essential for pupation to take place. When multiple FCM larvae pupated in the soil there was a tendency for larvae to pupate within a close distance of one another or at times right next to each other.

7.2 IMPLICATIONS OF FCM PUPATION BIOLOGY, BEHAVIOUR AND SURVIVAL FOR EPF AND EPN CONTROL PROGRAMMES AND DIRECT HABITAT MANIPUALTION

A better understanding of FCM pupation biology allows for this knowledge to be applied to EPF and EPN control programmes for FCM. In general, the amount of time FCM spent wandering on the soil surface in search of a suitable pupation site was just a few minutes. This short time period will result in a need for excellent coverage of EPFs and EPNs being applied to the soil. Improving the likelihood of rapid contact with EPFs or EPNs will increase FCM mortality. The wandering larval stage of FCM is known to be particularly vulnerable to EPN infection, although the infective juveniles (IJs) are also able to actively seek out their host (Riga 2004; Malan *et al.* 2011). The application of *H. bacteriophora* is 10 IJs/cm^2 when applying these EPNs on citrus (Danckwerts, K. pers. comm.[5]) and this application rate

[5] Danckwerts, K. River Bioscience, South Africa.

coupled with their active searching behaviour suggests that the probability of these EPNs finding and infecting FCM is high in spite of the rapid pupal cocoon formation of the moth. Accurately determining wandering distance under laboratory conditions can be difficult as the experimental arena size is likely to be a limiting factor and the environment is fairly homogenous, whereas under field conditions, the area available for pupation would be far greater and the environment much more variable. This high variability in the wandering distance is anticipated to be similar in the field, although the specific distance may be increased for certain individuals. To add to this, pupal cocoons were rapidly spun once a pupation site had been selected by the FCM larva, reducing the chances of direct larval contact between itself and the biological control agents. Studies have found that both EPF (Goble *et al.* 2011) and EPNs (Malan *et al.* 2011) are able to provide effective control against FCM pupae, with control of the larval life stage being even higher. A positive factor in the case of the EPF was that the vast majority of cocoons included soil particles and if EPF were already present in these, the probability of infection would be high, therefore application of EPF to the soil prior to known peak adult emergence times could potentially improve control. The shallow pupation depth of FCM would have both positive and negative implications for biological control of FCM. There is the possibility of increased contact of EPF and EPNs with pupating FCM when the biological control agents are first applied to the soil due to this shallow pupation. In the case of EPF, previous research has shown that the majority of the conidia remain in the surface layers of packed soil with only a limited number passively dispersing further into the soil profile (Garrido-Jurado *et al.* 2011). This could result in increased probability of contact between EPF and FCM post-application as well. It should be noted though that this retention and dispersal does differ with soil texture class and increased movement of EPFs into the soil has been noted for soils with a higher sand content (Garrido-Jurado *et al.* 2011).

A negative consequence of shallow pupation for biological control of FCM is the exposure of EPF and EPNs to the often harsh and variable environment there with substantial temperature and moisture fluctuations, particularly for largely bare soil (Brady & Weil 2000). For soils that are within the shade of the citrus tree canopy and have ground cover in the form of organic debris on the top of the soil, these effects may be somewhat reduced. Shaded areas will have less moisture loss and lower temperatures than unshaded areas, while ground cover also assists in moisture retention and UV protection (Brady & Weil 2000). Both of these are critical factors for EPF and EPN survival as both require moist soil conditions and UV

radiation exposure results in high mortality rates (Gaugler 1988; Fargues & Luz 2000; Hazir *et al.* 2003; Gul *et al.* 2014). Nematodes in particular are also sensitive to temperature extremes although their tolerance varies greatly between species (Barbercheck 1992; Gaugler 1988).

While not statistically significant in this experiment, a higher number of FCM did pupate in shaded soil rather than unshaded and it is possible that this number would be higher under field conditions. This is promising for biological control, as these cool, moist and UV protected areas are ideal for EPF and EPN survival and pathogenicity (Barbercheck 1992; Gaugler 1988). However, since up to 40 % of larvae would still be likely to pupate in unshaded areas, application of biological control agents would be needed in these areas, most likely at the edge of the tree canopy. It is possible that very thick ground cover may present an obstacle to EPN penetration into the soil if they are applied above the ground cover layer (Grewal *et al.* 2005). Up to 40 % of FCM larvae incorporated organic ground cover into their pupal cocoons, thus it is expected that EPFs and EPNs applied directly to the ground cover would come into contact with the pupae as the biological control agents moved down into the soil and IJs which may not be able to actively seek out FCM pupae as effectively if moisture is limited in the ground cover layer post-application to the soil. Ground cover and shading will differ between orchards of differing ages as the canopies of younger trees are less well-developed than older trees. This increase in bare soil of younger orchards could also influence the effectiveness of FCM control by EPFs and EPNs applied here.

Soil compaction at some level is very common in the citrus orchard, although the amount of compaction will differ according to soil texture class. FCM larvae did pupate at deeper depths in compacted soil, however since this was due to changes in the soil features, such as cracks and soil aggregates, EPFs and EPNs should still be able to reach these pupae when applied to the soil surface. EPN movement in the soil may be restricted by the level of soil compaction of each soil texture class, but this also varies between species (Portillo-Aguilar *et al.* 1999; Gruner *et al.* 2007). Sandy loam soil which had the highest sand particle content of the three soil texture classes used in this study had the highest eclosion success percentage for FCM. This eclosion success trend was seen for the majority of the experiments. This suggests that increased control measures may be required for citrus trees with sandier soils. The effectiveness of EPNs in sandy soils is anticipated as being higher due to easier movement in these soils with larger particle sizes, thereby increasing dispersal and infection of FCM pupae (Portillo-Aguilar *et al.* 1999; Gruner *et al.* 2007). The movement of fungal conidia into the

soil increases with sandier soil content (Quesada-Moraga *et al.* 2007; Garrido-Jurado *et al.* 2011) which may result in more frequent applications of EPFs being required for sandier soils.

Direct habitat manipulation would be difficult, based on the biology of FCM. While flooding of the agricultural habitat has been suggested for species which do not survive well at soil saturation (Eskafi & Fernandez 1990), the size of commercial citrus orchards and water scarcity in South Africa make this an impractical strategy. Habitat manipulation through upper soil layer removal, since FCM pupation appears to be so shallow, is not recommended due to the high cost, time that would be required and damage to the environment. The continued burial or pulping of fallen citrus fruit collected during orchard sanitation under the guidelines provided by Moore & Kirkman (2008) and Moore (2012) is strongly encouraged as this will prevent any FCM larvae inside the fruit having the opportunity to pupate in the soil.

7.3 FUTURE RESEARCH

The vast majority of previous studies conducted on insect pupation have been performed in the laboratory (e.g. Eskafi & Fernandez 1990; Murray & Zalucki 1990; Rieckelmann & Bach 1991; Ande 2004; Ellis *et al*. 2004; Hulthen & Clarke 2006; Chen & Shelton 2007; Zheng *et al*. 2011). This is largely due to the difficulty in observing and recording insect pupation habits under field conditions and being unable to control for variables other than the ones being investigated in the study. This work was also performed in the laboratory in order to improve knowledge of FCM pupation which could then be expanded on at a later stage. Confirmation of these results under field conditions would be very useful, particularly for the pupation depth and eclosion success. The use of video footage to capture insect pupation behaviour was a helpful data capturing technique. Observing FCM pupation behaviour in person would be impractical due to the length of time required for this and certain behaviours may be missed unless observation is constant. Furthermore, this footage can then be used to determine other behaviours such as wandering distance, which are otherwise difficult to measure by human observation without disturbing the insect and these techniques could be applied to future insect pupation studies.

In this study, the influence of each individual abiotic variable was examined to determine the individual effects of these on FCM pupation. In the field it would be a combination of these factors that FCM would be exposed to when pupating in the soil, therefore a study establishing the combined effect of abiotic factors on FCM pupation biology, behaviour and survival would be useful. The commercial orchard is a highly productive and therefore carefully managed system with a number of inputs such as herbicides, fertilizers and mulches being added to the soil (Kibblewhite *et al.* 2008). These must be carefully managed in the complex soil environment, as high productivity must not come at the cost of ecosystem health, in order for agriculture to remain sustainable (Kibblewhite *et al.* 2008). Inputs such as these may well have an impact on the target pest (FCM), the biological control agents (EPF and EPNs) and the interactions between them. Determining such potential impacts would be useful for biological control.

It would be useful to test the physiological tolerance of EPF isolates to the abiotic factors mentioned above, as well as any nematode species or isolates currently being identified for commercialization to determine how their specific biology might overlap with FCM. This knowledge would be useful when making decisions about biological control agent application to the soil and including these on the instructions for usage. The importance of correct usage must be stressed to growers as deviations from this would result in the effectiveness of the product being compromised. This is essential as one of the main factors for grower acceptance of control strategies such as EPNs is reliability (Dolinski *et al.* 2012) which would be affected by incorrect usage.

The value of pest biology studies is highlighted by this work and should be considered an important part of any biological control programme. Understanding the pest biology improves the chance of the biological control agent performing at an optimal level by selecting the most appropriate species of biological control agent for pest control, improving the application recommendations to growers for the most effective control and providing likely explanations if the biological control agents do not perform as well as expected. If performance is lower than expected, the knowledge of both pest and biological control agent biology can be applied in order to improve it. Control of pests such as FCM using biological control would be an excellent addition to the other control options available to growers as part of an IPM programme. Reduction of pesticide usage is essential for growers as the export markets demand increasingly lower maximum residue levels (MRLs) of chemicals on fruit (CGA Annual Report 2011). The need for alternative, IPM-compatible control strategies for

FCM in citrus is therefore high, with the use of EPF and EPNs showing good potential as such alternatives.

The importance of understanding pest biology, behaviour and survival cannot be underestimated when control is being attempted. While direct habitat manipulation for control of the soil-dwelling life stage of FCM would be challenging in large orchards, the use of EPF and EPN products is very promising. Overlap between the biology of the moth and the biological control agents does exist and will be useful for future product development and products already on the market. This information regarding FCM pupation habits has assisted in closing the knowledge gap surrounding the pupal life stage and can also be built on through further research. In addition, this work may also be potentially useful for any new control strategies for the soil-dwelling life stage of FCM that are identified for development in the future.

8

References

ALBRIGO, L.G., WANG, N., SPANN, T., DENG, X. & CARLOS, E.F. 2009. Critical pests and diseases imperil world citrus. *Chronica Horticulturae* **49**: 11-13.

ALTIERI, M.A. & LETOURNEAU, D.K. 1982. Vegetation management and biological control in agroecosystems. *Crop Protection* **1**: 405-430.

ALTIERI, M.A., PONTI, L. & NICHOLLS, C.I. 2005. Manipulating vineyard biodiversity for improved insect pest management: case studies from northern California. *International Journal of Biodiversity Science & Management* **1**: 191-203.

ALYOKHIN, A.V., MILLE, C., MESSING, R.H. & DUAN, J.J. 2001. Selection of pupation habitats by Oriental fruit fly larvae in the laboratory. *Journal of Insect Behaviour* **14**: 57-67.

ANDE, AT. 2004. The pupal habits of *Cirina forda* Westwood (Lepidoptera: Saturniidae), a leading food insect in Kwara State, Nigeria. *Journal of Sustainable Tropical Agricultural Research* **9**: 97-100.

BARBERCHECK, M.E. & KAYA, H.K. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology* **20**: 582-589.

BARBERCHECK, M.E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. *The Florida Entomologist* **75**: 539-548.

BARNARD, H.O. & DU PREEZ, C.C. 2004. Soil fertility in South Africa: the last twenty five years. *South African Journal of Plant and Soil* **21**: 301-315.

BARRTT, H.C. & RHODES, A.M. 1976. A numerical taxonomic study of affinity relationships in cultivated citrus and its close relatives. *Systematic Botany* **1**: 105-136.

BATEY, T. 2009. Soil compaction and soil management – a review. *Soil Use and Management* **25**: 335-345.

BEDFORD, E.C. 1998. Introduction (Part I), Methods of controlling citrus pests (Part II) and Pesticides (Part III). In: *Citrus Pests in the Republic of South Africa*. E.C.G Bedford, M.A. Van den Berg & E.A. de Villiers (Eds.). Dynamic Ad: Nelspruit, South Africa. 288 pp.

BERNIER, M., FOURNIER, V. & GIOVENAZZO, P. 2014. Pupal Development of *Aethina tumida* (Coleoptera: Nitidulidae) in thermo-hygrometric soil conditions encountered in temperate climates. *Journal of Economic Entomology* **107**: 531-537.

BOARDMAN, L., GROUT, T.G. & TERBLANCHE, T.S. 2012. False codling moth *Thaumatotibia leucotreta* (Lepidoptera, Tortricidae) larvae are chill-susceptible. *Insect Science* **19**: 315-328.

BOVÉ, J.M. 2014. Huanglongbing or yellow shoot, a disease of Gondwanan origin: Will it destroy citrus worldwide? *Phytoparasitica* **42:** 579-583.

BRADY, N.C. & WEIL, R.R. 2000. *Elements of the nature and properties of soils*. Prentice-Hall Inc., New Jersey, USA. 559 pp.

BUGG, R.L. & WADDINGTON, C. 1994. Using cover crops to manage arthropod pests of orchards: a review. *Agriculture, Ecosystems and Environment* **50**: 11-28.

CAMMACK, J.A., ADLER, P.H., TOMBERLIN, J.K., ARAI, Y. & BRIDGES JR., W.C. 2010. Influence of parasitism and soil compaction on pupation of the green bottle fly, *Lucilia sericata*. *Entomologia Experimentalis et Applicata* **136**: 134–141.

CAMPOS-HERRERA, R., STUART, R.J., EL-BORAI, F., GUTIERREZ, C. & DUNCAN, L. 2010. Entomopathogenic nematode ecology and biological control in Florida citrus orchards. In: *Integrated management of arthropod pests and insect borne diseases*. Springer, Netherlands. pp 101-130.

CARPENTER, J.E., BLOEM, S. & HOFMEYR, J.H. 2004. Acceptability and suitability of eggs of false codling moth (Lepidoptera: Tortricidae) from irradiated parents to parasitism by *Trichogrammatoidea cryptophlebia* (Hymenoptera: Trichogrammatidae). *Biological Control* **30**: 351-359.

CATLING, H.D., ASCHENBORN, H., 1974. Population studies of the false codling moth, *Cryptophlebia leucotreta* Meyr., on citrus in the transvaal. *Phytophylactica* **6**: 31–38.

CHAPMAN, R.F. 2013. The insects: structure and function, 5th edition. S.J. Simpson & A.E. Douglas (Eds.). Cambridge University Press, Cambridge, UK. 929 pp.

CHARLESTON, D.S., KFIR, R., VAN RENSBURG, N.J., BARNES, B.N., HATTINGH, V., CONLONG, D.E., VISSER, D. & PRINSLOO, G.J. 2003. Integrated pest management in South Africa. In: *Integrated Pest Management in the Global Arena*. K.M. Maredia, D. Dakouo & D. Mota-Sanchez (Eds.). CABI Publishing, Oxon, U.K. pp. 167-194.

CHEN, M. & SHELTON, A.M. 2007. Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. *Environmental Entomology* **36**: 1349-1355.

CHEN, F., PORTER, D. & VOLLRATH, F. 2012. Morphology and structure of silkworm cocoons. *Materials Science and Engineering C* **32**: 772-778.

CGA (CITRUS GROWERS ASSOCIATION OF SOUTHERN AFRICA): ANNUAL REPORT. 2007. http://cdn.webhouse.co.za/site/files/5438/CGA%20Annual%20Report%20-%202007%20web.pdf. Accessed: 28/03/2013.

CGA (CITRUS GROWERS ASSOCIATION OF SOUTHERN AFRICA): ANNUAL REPORT. 2011. http://cdn.webhouse.co.za/site/files/5438/CGA%20Annual%20Report%202011%2es(1).pdf. Accessed: 25/01/2013.

CGA (CITRUS GROWERS ASSOCIATION OF SOUTHERN AFRICA): ANNUAL REPORT. 2014. http://cdn.webhouse.co.za/site/files/5438/CGA%20Annual%20Report%202014% 20e12.pdf. Accessed: 25/01/2013.

CGA (CITRUS GROWERS ASSOCIATION OF SOUTHERN AFRICA): KEY INDUSTRY STATISTICS. 2012. http://www.cga.co.za/site/files/5438/CGA%20Stats%20Book%202012%20e(1).pdf. Accessed: 25/01/2013.

COOMBES, C.A. 2012. Entomopathogenic fungi for control of soil-borne life stages of false codling moth, *Thaumatotibia leucotreta* (Meyrick) (1912) (Lepidoptera: Tortricidae). MSc Thesis, Rhodes University.

COOMBES, C.A., HILL, M.P., MOORE, S.D., DAMES, J.F. & FULLARD, T. 2013. Persistence and virulence of promising entomopathogenic fungal isolates for use in citrus orchards in South Africa. *Biocontrol Science and Technology* **23**: 1053-1066.

CORNELL SOIL HEALTH TRAINING MANUAL. 2009. What is soil health? *South African Fruit Journa*l June/July **8**: 72-73.

DAFF (Department of Agriculture, Forestry and Fisheries). August 2010. Step-by-step Export Manual for the South African Fruit Industry. http://www.nda.agric.za/doaDev/sideMenu/plantHealth/docs/ExportManual_SAfruitIndustryAug2010.pdf. Accessed: 25/01/2013.

DAFF (Department of Agriculture, Forestry and Fisheries). 2011. A profile of the South African citrus market value chain. http://www.daff.gov.za/docs/AMCP/CitrusMVCP2010-11.pdf. Accessed: 25/01/2013.

DAIBER, C.C. 1979a. A study of the biology of the false codling moth [*Cryptophlebia leucotreta* (Meyr.)]: the egg. *Phytophylactica* **11**: 129-132.

DAIBER, C.C. 1979b. A study of the biology of the false codling moth [*Cryptophlebia leucotreta* (Meyr.)]: the larva. *Phytophylactica* **11**: 141-144.

DAIBER, C.C. 1979c. A study of the biology of the false codling moth [*Cryptophlebia leucotreta* (Meyr.)]: the cocoon. *Phytophylatica* **11**: 151-157.

DANKS, H.V. 2002. Modification of adverse conditions by insects. *Oikos* **99**: 10-24.

DEWITTE, O., JONES, A., SPARGAAREN, O., BREUNING-MADSEN, H., BROSSARD, M., DAMPHA, A., DECKERS, J., GALLALI, T., HALLETT, S., JONES, R., KILSARA, M., LE ROUX, P., MICHÉLI, E., MONTANARELLA, L., THIOMBANO, L., VAN RANST, E., YEMEFACK, E. & ZOUGMORE, R. 2013. Harmonisation of the soil map of Africa at the continental scale. *Georderma* **211-212**: 138-153.

DIMOU, I., KOUTSIKOPOULOS, C., ECONOMOPOULOS, A.P. & LYKAKIS, J. 2003. Depth of pupation of the wild olive fruit fly, *Bactrocera* (*Dacus*) *oleae* (Gmel.) (Dipt., Tephritidae), as affected by soil abiotic factors. *Journal of Applied Entomology* **127**: 12-17.

DIXIE, G. 1999. Summer citrus: The role and prospects for Southern Africa. In: *Southern African Agribusiness: Gaining through Regional Collaboration*, Volume 23-424. S Jaffe (Ed.). The World Bank Technical Series, Washington, D.C.

DOLINSKI, C., CHOO, H.Y. & DUNCAN, L.W. 2012. Grower acceptance of entomopathogenic nematodes: case studies on three continents. *Journal of Nematology* **44**(2): 226-235.

DOMINICK, O.S. & TRUMAN, J.W. 1984. The physiology of wandering behaviour in *Manduca sexta* I. temporal organization and the influence of internal and external environments. *Journal of Experimental Biology* **110**: 35-51.

DRYDEN, M.W. & SMITH, V. 1994. Cat flea (Siphonaptera: Pulicidae) cocoon formation and development of naked flea pupae. *Journal of Medical Entomology* **31**: 272-277.

ELLIS, J.D., HEPBURN, R., LUCKMAN, B. & ELZAN, P.J.. 2004. Effects of soil type, moisture, and density on pupation success of *Aethina tumida* (Coleoptera: Nitidulidae). *Environmental Entomology* **33**: 794-798.

ERICHSEN, C. & SCHOEMAN, A. 1992. Economic losses due to insect pests on avocado fruit in the Nelspruit/Hazyview region of South Africa during 1991. South African Avocado Growers' Association Yearbook **15**: 49-54.

ESKAFI, F.M. & FERNANDEZ, A. 1990. Larval-pupal mortality of Mediterranean fruit fly (Diptera: Tephritidae) from interaction of soil, moisture, and temperature. *Environmental Entomology* **19**: 1666-1670.

ESWARAN, H., ALMARAZ, R., VAN DEN BERG, E., & REICH, P. 1997. An assessment of the soil resources of Africa in relation to productivity. *Geoderma* **77**: 1-18.

FARGUES, J. & LUZ, C. 2000. Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauvaria bassiana* for *Rhodnius prolixus*. *Journal of Invertebrate Pathology* **75**: 202-211.

FEY, M.V. 2010. Soils of South Africa. Cambridge University Press, Cambridge, UK. pp 287.

FULLARD, T. & HILL, M.P. 2013. Impact of insecticides on the reproductive potential of false codling moth, *Thaumatotibia leucotreta* (Meyrick, 1913) (Lepidoptera: Tortricidae). *African Entomology* **21**: 310-315.

GARRIDO-JURADO, I., TORRENT, J., BARRÓN, V., CORPAS, A., & QUESADA-MORAGA, E. 2011. Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera: Tephritidae). *Biological Control* **58**: 277-285.

GAUGLER, R. 1988. Ecological considerations in the biological control of soil-inhabiting insects with entomopathogenic nematodes. *Agriculture, Ecosystems and Environment* **24**: 351-360.

GENDALL, K.L. 2007. *Agathis bishopi* (Nixon) (Hymenoptera: Braconidae): its biology and usefulness as a biological control agent for false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), on citrus. MSc Thesis, Rhodes University.

GEORGALA, M.B. 1969. Control of false codling moth and fruit flies in citrus orchards. *The South African Citrus Journal* January 1-7.

GEORGIS, R. & POINAR JR, G.O. 1983a. Effect of soil texture on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). *Journal of Nematology* **15**: 308-311.

GEORGIS, R., & POINAR JR, G.O. 1983b. Effect of soil texture on the distribution and infectivity of *Neoaplectana glaseri* (Nematoda: Steinernematidae). *Journal of nematology* **15**: 329-332.

GEORGIS, R., & POINAR JR, G.O. 1983c. Vertical migration of *Heterorhabditis bacteriophora* and *H. heliothidis* (Nematoda: Heterorhabditidae) in sandy loam soil. *Journal of nematology* **15**: 652-654.

GEORGIS, R., KOPPENHÖFER, A.M., LACEY, L.A., BÉLAIR, G. DUNCAN, L.W. GREWAL, P.S., SAMISH, M. TAN, TORR, L.P. & VAN TOL, R.W.H.M. 2006. Successes and failures in the use of parasitic nematodes for pest control. *Biological Control* **38**: 103-123.

GOBLE, T.A. 2009. Investigation of entomopathogenic fungi for control of false codling moth, *Thaumatotibia leucotreta*, Mediterranean fruit fly, *Ceratitis capitata* and Natal fruit fly, *C. rosa* in South African Citrus. MSc Thesis, Rhodes University.

GOBLE, T.A., DAMES, J.F., HILL, M.P. & MOORE, S.D. 2011. Investigation of native isolates of entomopathogenic fungi for the biological control of three citrus pests. from citrus soils in the Eastern Cape Province, South Africa. *BioControl Science and Technology* **21**: 1193-1211.

GRANT, J.A. & VILLANI, M.G. 2003. Soil moisture effects on entomopathogenic nematodes. *Environmental Entomology* **32** (1): 80-87.

GREWAL, P.S., SELVAN, S. & GAUGLER, R. 1994. Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. *Journal of Thermal Biology* **19**: 245-253.

GREWAL, P.S., KOPPENHÖFER, A.M. & CHOO, H.V. 2005. Chapter 7: Lawn, turfgrass and pasture application. In: *Nematodes as Biocontrol Agents*. P.S. Grewal, R-U Ehlers, D.I. Shapiro-Ilan (Eds.). CAB International, Oxfordshire, UK. Pp 115-146.

GRUNER, D.S., RAM, K. & STRONG, D.R. 2007. Soil mediates the interaction of coexisting entomopathogenic nematodes with an insect host. *Journal of Invertebrate Pathology* **94**: 12-19.

GUL, H.T., SAEED, S. & KHAN, F.Z.A. 2014. Entomopathogenic fungi as effective insect pest management tactic: A review. *Applied Sciences and Business Economics* **1**: 10-18.

GULLAN, P.J. & CRANSTON, P.S. 2000. *The insects: An outline of entomology*, 2nd edition. Blackwell Science Ltd., Oxford, UK. 470 pp.

GURR, G.M., WRATTEN, S.D. & LUNA, J.M. 2003. Multi-function agricultural biodiversity: pest management and other benefits. *Basic and Applied Ecology* **4**: 107-116.

HÅKANSSON, I. & VOORHEES, W.B. 1998. Soil Compaction. In: *Methods for the Assessment of Soil Degradation*. R. Lal, W.H. Blum, C. Valentine & B.A. Stewart (Eds.). CRC Press LLC, Florida, USA. pp 161-180.

HAMZA, M.K. & ANDERSON, W.K. 2005. Soil compaction in cropping systems : A review of the nature, causes and possible solution. *Soil & Tillage Research* **82**: 121-145.

HATTING, J., STOCK, S. P. & HAZIR, S. 2009. Diversity and distribution of entomopathogenic nematodes (Steinernematidae, Heterorhabditidae) in South Africa. *Journal of Invertebrate Pathology* **102**: 120-128.

HAZIR, S., KAYA, H.K., STOCK, S.P. & KESKIN, N. 2003. Entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) for biological control of soil pests. *Turkish Journal of Biology* **27**: 181-202.

HENDERSON, H.E., & WARREN, F.L. 1970. Sex-pheromone of the false codling moth *Cryptophlebia leucotreta* Meyr.,(formerly *Argyroploce leucotreta* Meyr.), synthesis and bioassay of trans-dodec-7-en-1-yl acetate and related compounds. *S Afr Chem Inst J*.

HENNESSEY, M.K. 1994. Depth of pupation of Caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. *Environmental Entomology* **23**: 1119-1123.

HOFMEYR, J.H. & BURGER, B.V. 1994. Controlled-release pheromone dispenser for use in traps to monitor flight activity of false codling moth. *Journal of Chemical Ecology* **21**: 355 – 364.

HORROCKS, N.P.C., VOLLRATH, F. & DICKO, C. 2013. The silkmoth cocoon as a humidity trap and waterproof barrier. *Comparative Biochemistry and Physiology, Part A* **164**: 645-652.

HOU, B., XIE, Q. & ZHANG, R. 2006. Depth of pupation and survival of the Oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae) pupae at selected soil moistures. *Applied Entomology and Zoology* **41**: 515-520.

HULTHEN, A.D. & CLARKE, A.R. 2006. The influence of soil type and moisture on pupal survival of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae). *Australian Journal of Entomology* **45**: 16-19.

IGNOFFO, C.M. 1992. Environmental factors affecting persistence of entomopathogens. *Florida Entomologist* **75**: 516-525.

INGLIS, G.D., GOETTEL, M.S., BUTT, T.M. & STRASSER, H. 2001. Chapter 3: Use of Hyphomycetous fungi for managing insect pests. In: *Fungi as biocontrol agents: progress, problems and potential*. T.M. Butt, C.W. Jackson & N. Magan (Eds.). CABI Publishing, Oxon, UK. pp 23 – 69.

JACKSON, C.G., LONG, J.P. & KLUNGNESS, L.M. 1998. Depth of pupation in four species of fruit flies (Diptera: Tephritidae) in sand with and without moisture. *Journal of Economic Entomology* **91**: 138-142.

JARONSKI, S.T. 2010. Ecological factors in the inundative use of fungal entomopathogens. *Biocontrol* **55**: 159-185.

JUMEAN, Z., ROWLAND, E., JUDD, G.J. & GRIES, G. 2004. Male and female *Cydia pomonella* (Lepidoptera: Olethreutidae) larvae produce and respond to aggregation pheromone. *The Canadian Entomologist* **136**: 871-873.

KARLEN, D.L., MAUSBACH, M.J., DORAN, J.W., CLINE, R.G., HARRIS, R.F. & SCHUMAN, G.E. 1997. Soil quality: a concept, definition, and framework for evaluation (a guest editorial). *Soil Science Society of America Journal* **61**: 4-10.

KAYA, H.K., & GAUGLER, R. 1993. Entomopathogenic nematodes. *Annual Review of Entomology* **38**: 181-206.

KEVAN, P.G., JENSEN, T.S. & SHORTHOUSE, J.D. 1982. Body temperatures and behavioral thermoregulation of high Arctic woolly-bear caterpillars and pupae (*Gynaephora rossii*, Lymantriidae: Lepidoptera) and the importance of sunshine. *Arctic and Alpine Research* **14**: 125-136.

KIBBLEWHITE, M.G., RITZ, K. & SWIFT, M.J. 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1492), 685-701.

KIRKMAN, W. 2007. Understanding and improving the residual efficacy of the *Cryptophlebia leucotreta* granulovirus (CRYPTOGRAN). MSc Thesis, Rhodes University.

KIRKMAN, W. & MOORE, S.D. 2007. A study of alternative hosts for the false codling moth, *Thaumatotibia (=Cryptophlebia) leucotreta* in the Eastern Cape. *South African Fruit Journal* **6**: 33-38.

KIRKMAN, W., MOORE, S., DE BEER, F., HOFFMANN, K., BAM, L. & NSHIMIRIMANA, R. 2014. Seeing the invisible: X-ray for post-harvest detection of FCM. *Proceedings of the 8th Citrus Research Symposium* pp 33. Central Drakensberg, South Africa.

KNAPP, R., & CASEY, T. M. 1986. Thermal ecology, behavior, and growth of gypsy moth and eastern tent caterpillars. *Ecology* **67**: 598-608.

KOPPENHÖFER, A.M., KAYA, H.K. & TAORMINO, S.P. 1995. Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. *Journal of Invertebrate Pathology* **65**:193-199.

KOPPENHÖFER, A.M., BAUR, M.E., STOCK, S.P., CHOO, H.Y., CHINNASRI, B. & KAYA, H.K. 1997. Survival of entomopathogenic nematodes within host cadavers in dry soil. *Applied Soil Ecology* **6**: 231-240.

KOZLOWSKI, T.T. 1999. Soil compaction and growth of woody plants. *Scandinavian Journal of Forest Research* **14**: 596-619.

KUNG, S-P, GAUGLER, R. & KAYA, H.K. 1990a. Soil type and entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* **55**: 401-406.

KUNG, S-P., GAUGLER, R., & KAYA, H.K. 1990b. Influence of soil pH and oxygen on persistence of *Steinernema* spp. *Journal of Nematology* **22**: 440-445.

KUNG, S-P., GAUGLER, R., & KAYA, H.K. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. *Journal of Invertebrate Pathology* **57**: 242-249.

LACEY, L.A. & GEORGIS, R. 2012. Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology* **44**: 218-225.

LAPOINTE, S.L., & SHAPIRO, J.P. 1999. Effect of soil moisture on development of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Florida Entomologist* **82**: 291-299.

LEWIS, E.E. 2002. Chapter 10: Behavioural Ecology. In:*Entomopathogenic nematology*, R. Gaugler (Ed). CABI International. pp 205–223.

LIANG, W. & HUANG, M. 1994. Influence of citrus ground cover plants on arthropod communities in China: a review. *Agriculture, Ecosystems and Environment* **50**: 29-37.

LOVE, C.N., HILL, M.P. & MOORE, S.D. 2014. *Thaumatotibia leucotreta* and the Navel orange: ovipositional preferences and host susceptibility. *Journal of Applied Entomology* **138**: 600-611.

MAHROOF, R., SUBRAMANYAM, B., THRONE, J.E. & MENON, A. 2003. Time-mortality relationships for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. *Journal of Economic Entomology* **96**: 1345-1351.

MALAN, A.P., KNOETZE, R. & MOORE, S.D. 2011. Isolation and identification of entomopathogenic nematodes from citrus orchards in South Africa and their biocontrol potential against false codling moth. *Journal of Invertebrate Pathology* **108**: 115-125.

MANRAKHAN, A., DANEEL, J-H. & MOORE, S.D. 2014. The impact of naturally occurring entomopathogenic nematodes on false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae), in citrus orchards. *Biocontrol Science and Technology* **24**: 241-245.

MATHER, C. 1999. Agro-commodity chains, market power and territory: re-regulating South African citrus exports in the 1990s. *Geoforum* **30**: 61-70.

MATHER, C. & GREENBERG, S. 2003. Market liberalisation in post-Apartheid South Africa: the reconstruction of citrus exports after 'deregulation'. *Journal of Southern African Studies* **29**: 393-412.

MATHER, C. & ROWCROFT, P. 2004. Citrus, apartheid and the struggle to (re)present Outspan oranges. In: *Geographies of Commodity Chains*. A. Hughes & S. Reimer (Eds.). Routledge, London, U.K. pp 156-172.

MEIKLE, W.G. & DIAZ, R. 2012. Factors affecting pupation success of the small hive beetle, *Aethina tumida*. *Journal of Insect Science* **12**: 1-9.

MERMUT, A.R. & ESWARAN, H. 2001. Some major developments in soil science since the mid-1960s. *Geoderma* **100**: 403-426.

MITCHELL, E.R. 1975. Disruption of pheramonal communication among coexistent pest insects with multichemical formulations. *BioScience* **25**: 493-499.

MÖLLER, M. & ASSOULINE, S. 2007. Effects of a shading screen on microclimate and crop water requirements. *Irrigation Science* **25**: 171-181.

MOORE, G.A. 2001. Oranges and lemons: clues to the taxonomy of Citrus from molecular markers. *TRENDS in Genetics* **17**: 536-540.

MOORE, S.D. 2002. The development and evaluation of *Cryptophlebia leucotreta* granulovirus (CrleGV) as a biological control agent for the management of the false codling moth, *Cryptophlebia leucotreta*, on citrus. PhD Thesis, Rhodes University.

MOORE, S.D. 2012. Moths and butterflies: false codling moth. In: *Citrus Research International IPM Production Guidelines, Volume 3*, Part 9.4. pp 1-9.

MOORE, S.D. & KIRKMAN, W. 2008. Citrus orchard sanitation with emphasis on false codling moth control. *South African Fruit Journal* **09**: 57-60.

MOORE, S.D. & HATTINGH, V. 2012. A review of current pre-harvest control options for false codling moth in citrus in southern Africa. *South African Fruit Journal* **11**: 82-85.

MOORE, S.D., HENDRY, D.A. & RICHARDS, G.I. 2011. Virulence of a South African isolate of the *Cryptophlebia leucotreta* granulovirus to *Thaumatotibia leucotreta* neonate larvae. *Biocontrol* **56**: 341-352.

MOORE, S.D., RICHARDS, G.I., CHAMBERS, C. & HENDRY, D. 2014. An improved larval diet for commercial mass rearing of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae): short communication. *African Entomology* **22**: 216-219.

MOREY, A.C., HUTCHISON, W.D., VENETTE, R.C. & BURKNESS, E.C. 2012. Cold hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) pupae. *Environmental Entomology* **41**: 172-179.

MURRAY, D.A.H. & ZALUCKI, M.P. 1990. Effect of soil moisture and simulated rainfall on pupal survival and moth emergence on *Helicoverpa punctigera* (Wallengren) and *H. armigera* (Hübner) (Lepidoptera: Noctuidae). *J. Aust. Ent. Soc*. **29**: 193-197.

NDOU, P. 2012. The competitiveness of the South African citrus industry in the face of the changing global health and environmental standards. PhD Thesis, University of Fort Hare.

NDOU, P. & OBI, A. 2011. The business environment and competitiveness of the South African Citrus Industry. *The International Food and Agribusiness Management Association (IFAMA) 21st Annual World Symposium, Frankfurt, Germany*. 20-23 June.

NEWTON, P.J. 1988. Inversely density-dependent egg parasitism in patchy distributions of the citrus pest *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae) and its agricultural efficiency. *Journal of Applied Ecology* **25**: 145-162.

NEWTON, P.J. 1989. The influence of citrus fruit condition on egg laying by the false codling moth, *Cryptophlebia leucotreta*. *Entomologia Experimentalis et Appl*icata **52**: 113-117.

NEWTON, P.J. 1990. Ovipositional preferences amongst navel sweet orange types by the false codling moth, *Cryptophlebia leucotreta*. *Annals of Applied Biology* **116**: 143-150.

NEWTON, P.J. 1998 False codling moth *Cryptophlebia leucotreta* (Meyrick). In: *Citrus pests in the Republic of South Africa*. E.C.G. Bedford, M.A. van den Berg, E.A. de Villiers (Eds.). Dynamic Ad, Nelspruit. pp 192-199.

NEWTON, P.J. & ODENDAAL, W.J. 1990. Commercial inundative releases of *Trichogrammatoidea cryptophlebiae* [Hym.: Trichogrammatidae] against *Cryptophlebia leucotreta* [Lep.: Tortricidae] in citrus. *Entomophaga* **35**: 545-556.

NICOLOSI, E., DENG, Z.N., GENTILE, A., LA MALFA, S., CONTINELLA, G. & TRIBULATO, E. 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics*. **100**: 1155-1166.

NIEUWOUDT, H.C. 1987. *U.S. Patent No. PP6,047*. Washington, DC: U.S. Patent and Trademark Office.

NOVELLI, V.M., CRISTOFANI, M., SOUZA, A.A & MACHADO, M.A. 2006. Development and characterisation of polymorphic microsatellite markers for the sweet orange (*Citrus sinensis* L. Osbeck). *Genetics and Molecular Biology* **29**: 90-96.

OPOKU-DEBRAH, J.K., HILL, M.P., KNOX, C. & MOORE, S.D. 2013. Overcrowding of false codling moth, *Thaumatotibia leucotreta* (Meyrick) leads to the isolation of five new *Cryptophlebia leucotreta* granulovirus (CrleGV-SA) isolates. *Journal of Invertebrate Pathology* **112**: 219-228.

OSMAN, K.T. 2013. Chapter 5: Physical properties of soil. In: *Soils: Principles, Properties and Management*. Springer Science + Business Media, Dordrecht, The Netherlands. pp. 49-66.

O'SULLIVAN, M.F., & SIMOTA, C. 1995. Modelling the environmental impacts of soil compaction: a review. *Soil and Tillage Research* **35**:: 69-84.

PATEL, M.N., PERRY, R.N. & WRIGHT, D.J. 1997. Desiccation survival and water contents of entomopathogenic nematodes, *Steinernema* spp. (Rhaditida: Steinernematidae). *International Journal for Parasitology* **27** (1): 61-70.

PIMENTAL, D., TERHUNE, E.C., DRITSCHILO, W., GALLAHAN, D., KINNER, N., NAFUS, D., PETERSON, R., ZAREH, N., MISITI, J. & HABER-SCHAIM, O. 1977. Pesticides, insects in foods, and cosmetic standards. *Bioscience* **27**: 178-185.

PIMENTAL, D., KIRBY, C. & SHROOF, A. 1993. Chapter 4: The relationship between "cosmetic standards" for foods and pesticide use. In: *The pesticide question*: *environment, economics, and ethics*. D. Pimental & H. Lehman (Eds.). Chapman & Hall, London, UK. pp. 85-105.

POLLACK, S.L. 2001. Consumer demand for fruit and vegetables: the U.S. example. In: *Changing Structure of Global Food Consumption and Trade.* A. Regmi (Ed.). Market and Trade Economics Division, Economic Research Service, U.S. Department of Agriculture, Agriculture and Trade Report.

POOLE, N.D., MARTÍNEZ, L.C-M. & GIMÉNEZ, F.V. 2007. Quality perceptions under evolving information conditions: implications for diet, health and consumer satisfaction. *Food Policy* **32**: 175-188.

PORTILLO-AGUILAR, C., VILLANI, M.G., TAUBER, M.J., TAUBER, C.A. & NYROP, J.P. 1999. Entomopathogenic nematode (Rhabditida: Heterorhabditidae and Steinernematidae) response to soil texture and bulk density. *Environmental Entomology* **28**: 1021-1035.

PROKOPY, R.J. 1994. Integration in orchard pest and habitat management: a review. *Agriculture, Ecosystems and Environment* **50:** 1-10.

QUESADA-MORAGA, E., NAVAS-CORTÉS, J.A, MARANHAO, E.A.A, ORTIZ-URQUIZA, A. & SANTIAGO-ÁLVAREZ, C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. *Mycological Research* **111**: 947-966.

READ, J.S., HEWITT, P.H., WARREN, F.L. & MYBERG, A.C. 1974. Isolation of the sex pheromone of the moth *Argyroploce leucotreta*. *Journal of Insect Physiology* **20**(3): 441-450.

REINECKE, J.P., BUCKNER, J.S. & GRUGEL, S.R. 1980. Life cycle of laboratory-reared tobacco hornworms, *Manduca sexta*, a study of development and behaviour, using time-lapse cinematography. *Biological Bulletin* **158**: 129-140.

RICKELMANN, K.M. & BACH C.E. 1991. Effects of soil moisture on the pupation behavior of *Altica subplicata* (Coleoptera: Chrysomelidae). *The Great Lakes Entomologist* **24**: 231-237.

RIGA, E. 2004. Chapter 3: Orientation Behaviour. In: *Nematode behaviour*. R. Gaugler & A.L. Bilgrami (Eds.). CABI Publishing, Oxfordshire, UK. pp 63-90.

ROACH, S.H. & HOPKINS, A.R. 1979. *Heliothis* spp.: Behavior of prepupae and emergence of adults from different soils at different moisture levels. *Environmental Entomology* **8**: 388-391.

ROACH, S.H. & CAMPBELL, R.B. 1983. Effects of soil compaction on bollworm (Lepidoptera: Noctuidae) moth emergence. *Environmental Entomology* **12**: 1883-1886.

SAPONARI, M., DE BAC, G., BREITHAUPT, J., LOCONSOLE, G., YOKOMI, R.K. & CATALANO, L. 2010. First Report of '*Candidatus* Liberibacter asiaticus' associated with Huanglongbing in sweet orange in Ethiopia. *Plant Disease* **94**: 482-482.

SCHAETZL, R. & ANDERSON, S. 2005. Chapter 5: Basic concepts: Soil physics. In: *Soils: Genesis and Geomorphology*. Cambridge University Press, Cambridge, U.K. pp. 82-92.

SCORA, R.W. 1975. On the history and origin of citrus. *Bulletin of the Torrey Botanical Club* **102**: 369-375.

SHAH, P.A. & PELL, J.K. 2003. Entomopathogenic fungi as biological control agents. *Applied Microbiology and Biotechnology* **61**: 413-423.

SILALAHI, J. 2002. Anticancer and health protective properties of citrus fruit components. *Asia Pacific Journal of Clinical Nutition.* **11**: 79-84.

SILVERMAN, J. & RUST, M.K. 1985. Extended longevity of the pre-emerged adult cat flea (Siphonaptera: Pulicidae) and factors stimulating emergence from the pupal cocoon. *Annals of the Entomological Society of America* **78**: 763-768.

SIMMONS, A.M. 1993. Effects of constant and fluctuating temperatures and humidities on the survival of *Spodoptera frugiperda* pupae (Lepidoptera: Noctuidae). *Florida Entomologist* **76**: 333-340.

SMITH, D. & PEÑA, J.E. 2002. Tropical citrus pests. In: *Tropical Fruit Pests and Pollinators: Biology, Economic Importance, Natural Enemies and Control*. J.E. Peña, J.L. Sharp & M. Wysoki (Eds.). CABI Publishing, Oxon, U.K. pp 57-101.

SPIEGEL-ROY, P. & GOLDSCHMIDT, E.E. 1996. The biology of citrus. Cambridge University Press, Cambridge, UK. 230 pp.

STIBICK, J. 2010. New pest response guidelines: false codling moth *Thaumatotibia leucotreta*. USDA–APHIS–PPQ–Emergency and Domestic Programs, Riverdale, Maryland.[Available:http://www.aphis.usda.gov/import_export/plants/manuals/emergency/down loads/nprg-fcm.pdf]. Accessed: 31/03/2013.

STOTTER, R.L. 2011. Monitoring the efficacy of Sterile Insect Technique to control false codling moth in the Citrusdal area (Western Cape Province, South Africa). Citrus Research International Annual Research Report 2010-11. pp 39-56.

STOTTER, R. L. & TERBLANCHE, J.S. 2009. Low-temperature tolerance of false codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) in South Africa. *Journal of Thermal Biology* **34**: 320-325.

STUART, R.J., BARBERCHECK, M.E., GREWAL, P.S., TAYLOR, R.A. & HOY, C.W. 2006. Population biology of entomopathogenic nematodes: Concepts, issues, and models. *Biological Control* **38**: 80-102.

TAUBER, M.J., TAUBER, C.A., NYROP, J.P. & VILLANI, M.G. 1998. Moisture, a vital but neglected factor in the seasonal ecology of insects: hypotheses and tests of mechanisms. *Environmental Entomology* **27**(3): 523-530.

TERBLANCHE, J.S., JAGER, Z., BOARDMAN, L. & ADDISON, P. 2014. Physiological traits suggest limited diapause response in false codling moth, *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Journal of Applied Entomology* **138**: 683-691.

TIMM, A.E., GEERTSEMA, H. & WARNICH, L. 2010. Population genetic structure of economically important Tortricidae (Lepidoptera) in South Africa: a comparative analysis. *Bulletin of Entomological Research* **100**: 421-431.

TREVORS, J.T. 1996. Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods* **26**: 53-59.

TURLINGS, T.C.J., HILTPOLT, I., RASMANN, S., 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant and Soil* **358**: 51-60.

URQUHART, P. 1999. IPM and the citrus industry in South Africa. Gatekeeper series No. SA86, Sustainable Agriculture and Rural Livelihoods Programme of the International Institute for Environment and Development.

VAN DEN BERG, M.A. 2001. *Cryptophlebia leucotreta* (Meyrick) False codling moth. In: *Pests and Beneficial Arthropods of Tropical and Non-citrus Subtropical Crops in South Africa*. M.A. van den Berg, E.A. de Villiers & P.H. Joubert (Eds).ARC-Institute for Tropical and Sub-Tropical Pests, Nelspruit, South Africa. pp 320-325.

VAN ZYL, C. & MALAN, A.P. 2014. The role of entomopathogenic nematodes as biological control agents of insect pests, with emphasis on the history of their mass culturing and *in vivo* production: review article. *African Entomology* **22**: 235-249.

VENETTE, R.C., DAVIS, E.E., DA COSTA, M., HEISLER, H. & LARSON, M. 2003. Mini Risk Assessment: false codling moth, *Thaumatotibia* (=*Cryptophlebia*) *leucotreta* (Meyrick) [Lepidoptera: Tortricidae]. University of Minnesota, Department of Entomology, CAPS PRA. 1-30. http://www.aphis.usda.gov/ppq/ep/pest detection / pra /tleucotretapra.pdf. Accessed: 02/03/2012.

VILLANI, M.G. & WRIGHT, R.J. 1990. Environmental influences on soil macroarthropod behaviour in agricultural systems. *Annual Review of Entomology* **35**: 249-269.

VILLANI, M.G., ALLEE, L.L., DIAZ, A., & ROBBINS, P.S. 1999. Adaptive strategies of edaphic arthropods. *Annual Review of Entomology* **44**: 233-256.

VITTUM, P.J. 2003. Soil habitats. In: *Encyclopedia of Insects*. V.H. Resh & R.T. Cardé (Eds.). Academic Press, California, USA. pp 1053-1057.

WEBBER, H. J. 1967 History and development of the citrus industry. In: *The Citrus Industry, Vol. 1*. W. Reuther, H.J. Webber, L.D. Batchelor (Eds.) University of California Press, Berkeley. pp 1–39.

WELLINGTON, J.H. 1955. Southern Africa: A Geographical Study, Vol. 2: Economic and Human Geography. Cambridge University Press, Cambridge, U.K. 320 pp.

WILSON, M.J., EHLERS, R.U., GLAZER, I. 2012 Entomopathogenic nematode foraging strategies – is *Steinernema carpocapsae* really an ambush forager? *Nematology* **14**: 389-394.

YEE, W. L. 2013. Influence of media type and moisture on adult development and pupal mortality in *Rhagoletis indifferens* (Diptera: Tephritidae). *Environmental Entomology* **42**: 595-604.

ZHENG, X-L, CONG, X-P, WANG, X-P, LEI, C-L. 2011. Pupation behaviour, depth, and site of *Spodoptera exigua*. *Bulletin of Insectology* **64**: 209-214.