

**The morphology and ecology of the Carob
moth (*Ectomyelois ceratoniae*) (Zeller) in
citrus orchards of the Western Cape, South
Africa**

by
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Abstract

The Carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae: Phyticitinae) became known initially as a Mediterranean pest of stored commodities such as pods of the Carob tree (*Ceratonia siliqua*) and dates, but became a pest of phytosanitary concern in South Africa when recorded in 1974 as a pest of citrus in the Citrusdal area in the Western Cape.

Since then it has been a pest of questionable concern to the citrus industry. In its larval stage the Carob moth is often confused with that of the False Codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), presenting a problem when contaminated fruit exports are intercepted at ports of entry. The aim of this study was thus to establish some guidelines for the development of an integrated pest management programme, which will enable growers to more effectively manage Carob moth infestations as well as to present morphological detail to facilitate definite identification of the Carob moth in all of its life stages.

This was achieved by collating and screening all available literature, ranging from obscure historical to modern texts, to arrive at a clear understanding of key morphological features of use to classify the Carob moth from ordinal to the species level. These features were then used and supplemented to produce a detailed morphological study of the Carob moth's life cycle. Morphological detail was then condensed into a user-friendly key based on and restricted to the most distinguishing characteristics to aid the identification of the Carob moth and the False Codling moth and to point out morphological characteristics separating the two species.

A field study was also carried out in the Western Cape to determine the Carob moth's seasonal cycle within local citrus orchards. This was determined by using a pheromone based trapping system and a set protocol for damage assessment by actively monitoring for two growing

seasons. A pheromone lure preference trial was conducted in all areas of study to assess two commercially available lures.

The outcomes of this study aim towards a better understanding of the nomenclatorial and morphological history of the Carob moth, as well as serving as a user friendly morphological identification key. The field results showed a clear seasonal cycle history of the Carob moth within citrus orchards of the Western Cape, closely following the phenology of the citrus tree. A lure preference was recorded for only one of the study areas. The Carob moth was found to be a minor pest, compared to False Codling moth, and presented more of an economic threat in certain areas with suitable hosts. A longer study should be undertaken to ascertain factors affecting the sporadic nature of the pest.

Opsomming

Die Karobmot, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae: Phycitinae), was aanvanklik bekend as 'n Mediterreense plaag van gestoorde produkte, soos, byvoorbeeld, die peule van die Johannesbroodboom (*Ceratonia siliqua*) en dadels, maar het 'n plaag van fitosanitêre belang in Suid-Afrika geword toe dit in 1974 as 'n plaag van sitrus in die Citrusdal gebied in die Wes-Kaap bekend geword het.

Sedertdien is dit 'n plaag van groot belang vir die sitrusbedryf. In sy larwale stadium word die Karobmot dikwels verwar met dié van die Valskodlingmot, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), wat op sy beurt 'n probleem veroorsaak wanneer besmette uitvoervrugte by invoerhawens onderskep word. Die doel van hierdie studie was dus gemik om sekere gidslyne daar te stel vir die ontwikkeling van 'n geïntegreerde plaagbestuurprogram wat produsente in staat sal stel om besmettings van die Karobmot beter te bestuur, asook om morfologiese besonderhede wat die juiste identifikasie van die Karobmot in al sy stadia bevorder beskikbaar te stel.

Dit is bereik deur die samestelling en nagaan van alle beskikbare literatuur, wisselend van skaars histories tot modern, om sodoende 'n duidelike begrip van sleutel morfologiese kenmerke van nut om die Karobmot van ordinale tot spesiesvlak te klassifiseer, te verkry. Hierdie kenmerke is dan gebruik en aangevul om 'n gedetailleerde morfologiese van die lewenssiklus van die Karobmot daar te stel. Morfologiese besonderhede is dan gekondenseer tot 'n gebruikersvriendelike sleutel gebaseer op, en beperk tot, die mees onderskeidende kenmerke as hulp by die identifikasie van die Karobmot en die Valskodlingmot om morfologiese kenmerke wat die twee spesies skei, uit te wys.

'n Veldstudie is in die Wes-Kaap bykomend uitgevoer om die Karobmot se seisoenale siklus in sitrusboorde te bepaal. Dit is bereik deur die gebruik van 'n feromoon-gebaseerde

vangsisteem and 'n gestelde protokol vir skadebepaling deur aktiewe monitoring gedurende twee groeiseisoene. 'n Feromoon voorkeurproef is ook in alle studiegebiede uitgevoer.

Die uitkomste van hierdie studie poog om 'n beter begrip van die nomenklatoriese en morfologiese geskiedenis van die Karobmot daar te stel, maar ook om as 'n gebruikersvriendelike morfologiese identifikasie sleutel te dien. Die veldresultate toon 'n duidelike seisoenale siklus geskiedenis van die Karobmot, in ooreenstemming met die fenologie van sitrus in sitrusboorde van die Wes-Kaap. 'n Lokvoorkeur is vir net een van die studiegebiede aangeteken. Die Karob mot was gevind as 'n plaag van minder belang, in vergelyking met die False Codling mot. Dit was ook gevind dat die Karob mot meer ekonomies skadelik is in areas waar daar meer toepaslike gashere is. 'n Langer studie is nodig om al die faktore in te reken.

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Chapter 1

General Introduction

1.1 Introduction

This study concerns a phytosanitary, lepidopteran pest from the Pyralidae family. The Carob moth (*Ectomyelois ceratoniae*) (Zeller), (Lepidoptera: Pyralidae) is a widely distributed moth that causes considerable damage in the larval part of its life cycle. This damage affects a variety of economically important host plants as well as stored products. The larvae hatch and bore into fruits causing unsightly damage to both the rind and flesh of the fruit (Neunzig 1979). There is also great concern for detection at ports of entry, which may lead to consignment rejection and more seriously the halt of all fruit exports due to infestation problems.

The Carob moth was first recorded in South Africa in 1974, emerging from damaged fruit in the Citrusdal area of the Western Cape Province (Honiball & Catling 1998). Since then reports have been received of large infestations of larvae detected from cultivated citrus fruits. The pomegranate industry also has a major concern over Carob moth infestations, as it has been recorded as a primary pest on pomegranates in countries such as Israel and Iran (Avidov 1961; Gothilf 1984). Primarily the Carob moth is a stored product pest, its first description was based on moths that emerged from stored fruits and nuts in the basement of a shop (Zeller 1839). This is of concern to the nut industry in South Africa.

Carob moth larvae and False Codling moth (FCM) larvae *Thaumatotibia (Cryptophlebia) leucotreta* (Meyrick) (Lepidoptera: Tortricidae) are very similar in appearance and host plant preference and therefore are commonly confused by producers (Honibal & Catling 1998). They are both pink in colour and often found within the same type of fruit, but have distinct morphological differences that can be used to distinguish between them (Honiball & Catling 1998).

Both Carob moth and FCM larvae are concealed feeders and it is therefore of no consequence if chemical sprays are used (Scoble 1992; Mediouni & Dhouibi 2007). Therefore a multidisciplinary approach is the best way to achieve control of larval infestations within orchards and across a landscape. This approach must include a sound identification of the specific pest based on morphological characters and molecular studies, an accurate knowledge of host preferences and population dynamics of the pest, and an accurate monitoring system.

This study was therefore undertaken to collate all historical and modern literature with the aim of highlighting the morphological identity of the Carob moth. This was then used as a basis for a more in-depth morphological study which also aided in producing a diagnostic key to determine the differences between the Carob moth and FCM in all life stages, to aid producers and stakeholders in decision making. An investigation was also undertaken to determine the basic biology and seasonal life cycle of the Carob moth on citrus, with the aim of the further development of an integrated pest management programme.

1.2 Global distribution of the Carob moth

The Carob moth was first described by Zeller (1839) from a specimen he was given by a shopkeeper by the name of Mr Schmidt. Thereafter it was found in numerous localities around the globe (Table 1; Figure 1). In 1974, Honiball and Catling reared the Carob moth from damaged fruits in the Citrusdal area, Western Cape, and this was the first recording of Carob moth in South Africa (Honiball & Catling 1998).

Table 1: A summary of all localities of where *Ectomyelois ceratoniae* was observed, as compiled from literature sources.

	Zeller (1839)	Fischer von Röslerstamm (1838)	Hampson (1903)	Warren and Rothschild (1905)	Dyer (1911)	Durrant (1915)	Blunck (1953)	Heinrich (1956)	Zimmerman (1958)	Meyrick (1895)	Calderon <i>et al.</i> (1969)	Cox (1979)	Avidov (1961)	Aitken (1963)	Gothlif (1984)	Al-Izzi <i>et al.</i> (1985)	Honiball & Catling (1998)
Laibach (Austria)	x	x															
England						x				x							
Ceylon (Sri-Lanka)			x														
Sudan				x													
Algeria						x											
Europe							x			x							
Syria							x			x							
Africa							x			x							
USA							x	x							x		
Jamaica								x									
Argentina								x									
Mediterranean								x			x			x	x		
Hawaii																	
West Indies										x							
South America										x							
Southern Russia												x				x	

Israel																		X					
Cyprus																			X				
Iran																					X		
Australia																					X	X	
South Africa																					X		X
Zimbabwe																							X



Figure 1 : The global distribution of the Carob moth based on published literature. (Acquired from <http://geology.com/world/world-map.shtml>)

1.3 Hosts of the Carob moth

The larvae of the Carob moth confine their attack not only to a variety of stored food products, but will also attack hosts in the wild. The very first record of larval attack has been reported by Zeller (1839) and Fischer von Röslerstamm (1839), both reporting attack on stored pods of *Ceratonia siliqua*, the Carob Tree. Sorhagen (1881a, 1881b) reported the larva as feeding on dried raisins and dried figs in storage; he also refers to larvae retrieved from dates (Sorhagen 1881b). Dyer (1911) bred individuals from dried loquat fruits; larvae were found on dates by Durrant (1915). In Table 2, a summary is given of reports of the larvae attacking a variety of commodities.

Table 2: Summary of recorded host plants affected by the Carob moth *Ectomyelois ceratoniae* based on available literature sources.

	Heinrich, (1956)	(Blunck, 1953)	Zimmerman, (1958)	(Calderon et al. 1969)	(Gothilf, 1969)	(Catling, 1979)	(Neunzig, 1979)	(Gothilf, 1984)	(Al-Izzi et al. 1985)	(Solis, 1986)	(Mehrnejad, 1995)	(Nay & Perring, 2005)	(de Villiers, 2001)
<i>Acacia sp.</i>											x		
<i>Acacia farnesiana</i>			X		x		x						
<i>Annona sp.</i>										x			
<i>Arachis hypogaea</i> (Peanut)											x		
<i>Bequaertiodendron magalismontanum</i>						x							
<i>Caesalpinia sappou</i>			X										
<i>Capsicum sp.</i>										x			
<i>Carissa grandiflora</i>	x												
<i>Cassia sp.</i>	x		X							x			
<i>Castanea sativa</i>							x						
<i>Ceratonia siliqua</i>	x	x	X	x	x	x		x					
<i>Cereus sp.</i>										x			
<i>Chimonanthus sp.</i>										x			

<i>Citrus paradise</i> (Grape fruit)												x		
<i>Citrus sp.</i>				x		x	x	x						
<i>Cydonia oblonga</i> (Quince)		x					x					x		
<i>Cucubita sp.</i>											x			
<i>Cydonia sp.</i>											x			
<i>Dioscorea sp.</i>											x			
<i>Diospyros sp.</i>											x			
<i>Englerophytum</i> <i>magalismontanum</i>						x								X
<i>Erisbotyra japonica</i>	x						x							
<i>Erythrina</i> <i>monosperma</i>			X											
<i>Ficus carica</i>											x			
<i>Ficus sp.</i> (Figs)	x	x					x	x						
<i>Haematoxylon</i> <i>campechianum</i>			X											
<i>Juglans sp.</i>						x					x			
<i>Livitona chinensis</i>	x													
<i>Macadamia sp.</i> (Macadamia)						x								X

<i>Malus domestica</i> (Apples)						x	x				x		
<i>Malus sp.</i>										x			
<i>Phaseolus sp.</i>										x			
<i>Phoenix dactylifera</i> (dates)	x	x					x	x			x	x	
<i>Phoenix sp.</i>										x			
<i>Pistacia vera</i> (Pistachio)											x		
<i>Prosopis juliflora</i>			x										
<i>Prunus dulcis</i> (Almond)				x			x	x					
<i>Prunus sp.</i> (Stone fruit)											x		
<i>Punica granatum</i> (Pomegranate)							x		x		x		
<i>Punica sp.</i>										x			
<i>Pyrus sp.</i> (Pear)											x		
<i>Quercus sp.</i> (Acorns)						x							
<i>Robinia sp.</i>	x												
<i>Samanea saman</i>													
<i>Sesbania sp.</i>										x			

<i>Tamarindus indica</i>							x						
<i>Tamarindus sp.</i>	x	x								x			
<i>Vachellia insularis</i>	x												
<i>Vigna sp.</i>										x			
<i>Ximenia caffra</i>						x							x
<i>Ziziphus spina- christi</i>							x						

1.4 Pest status and economic importance

The Carob moth is a field pest that occurs on growing fruits and carobs. Split fruit are more prone to infestation by larva as penetration into the fruit occurs more readily. There is also a preference towards fruit covered in sooty mould (Honiball & Catling 1998). The order Lepidoptera has 107 pest species and an average pest status of 22.0%. This calculation is based on the formula below, drawn from Moran (1983), who specifically worked on South African insect and mite pests on cultivated crops:

$$\text{Pest status} = [a/5 + b + c + (d/107 \times e)]$$

- (a) A measure of research effort,
- (b) The number of crops on which a particular pest was recorded,
- (c) Pesticide usage,
- (d) The value of the crop, and
- (e) The importance of each pest on each crop

The family Pyralidae into which the Carob moth belongs, has a pest status of 2.7% (Moran 1983). The Carob moth is a phytophagous insect that is termed a minor pest on fruit crops (Mehrnejad 2001). It can however become a bigger problem if local conditions are adequate (dry and hot) for fast growth and reproduction (Mehrnejad 2001). The Carob moth is the main pest of carob trees in Mediterranean regions, but also attacks a variety of agricultural crops in many different parts of the world (Mehrnejad 1995; Gothilf 1968; Heinrich 1956; Mediouni & Dhouibi 2007). Carob moth accounts for 10-40% of date crop damage in the US annually (Park 2010; Norouzi *et al.* 2008; Nay *et al.* 2006). In Tunisia, the annual infestation rates can be anything from 20% in dates to 80% in pomegranates (Mediouni & Dhouibi 2007). The Carob moth accounts for an average damage of 25-30% for pomegranate yields in Iran, where second and third instar larvae feed on the flesh of the fruit and also allow the entry to pathogenic fungi (Peyrovi *et al.* 2011). Since the commercial production of pomegranates in the Western Cape, the Carob moth has been reported as infesting the fruit in various

localities in this region. Total damage on South African citrus plantations is difficult to gauge, because of frequent confusion with the False Codling moth *T. leucotreta* (Catling 1979; de Villiers 2001).

The Carob moth appears to be extending its distribution in southern Africa, with Carob moth incidence increasing, with many reports of damage coming from the Lowveld areas of Mpumalanga and the Northern Province (Honibal & Catling 1998). It has become a major pest of pecan nuts in the Vaalharts district of the Northern Cape and more recently (2006 onwards) reported on citrus in the Eastern and Western Cape, South Africa (personal communication with Sean Moore).

The increase in incidence of the Carob moth infestations can be due to the increased use of synthetic pyrethroids and Insect Growth Regulators (IGRs) applied to citrus crops to control thrips, *Scirtothrips aurantii* and armoured scale insects (Honiball & Catling 1998). The use of these chemicals creates an upset in the natural balance of an ecosystem, resulting in outbreaks of normally controllable insects. Mealybugs, *Planococcus citri*, Australian bug, *Icerya purchasi* Maskell, and citrus wax scale, *Ceroplastes brevicauda*, create a sooty mould on the fruit which in turn attracts and indirectly causes the proliferation of the Carob moth (Honiball & Catling 1998).

1.5 Management

1.5.1 Monitoring

Pheromone trapping systems have been developed for the adequate monitoring of the Carob moth. The dispensers used for the trapping of Carob moth contain a female pheromone lure 7, 9, 11-dodecatrien-1-ol, formate, (7Z, 9E) which aids in attraction of male moths. In South Africa, there are two registered pheromone dispensers available for the detection of Carob moth. Two traps are available to capture adults, these being a beige PVC pipe trap and the yellow delta (wing) trap (Moore 2012) (Fig. 2).

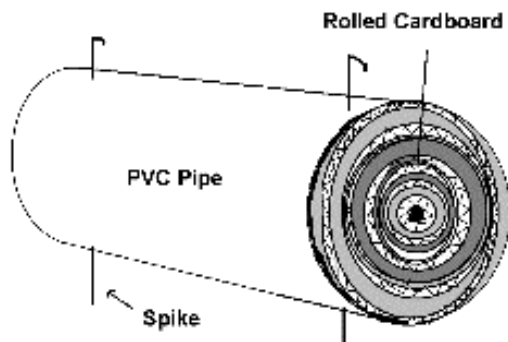


Figure 2: (Left) PVC pipe trap design used to trap insects within the trap when baited with a pheromone lure. (Right) Yellow delta trap used in conjunction with a sticky pad to trap insects inside the trap also when baited with a pheromone lure.

No treatment thresholds are available for this pest (Moore 2012)

1.5.2 Chemical Control

There are no registered chemical sprays to combat Carob moth in South Africa, while no previous literature mentions the use of a specific pesticide to combat Carob moth infestation in any crops. False Codling moth is however controlled using an array of chemical pesticides: cypermethrin, E-8 dodecenyl acetate/Z-8 dodecenyl acetate/ E/Z-8 dodecenol, fenpropathrin, parathion-methyl, pheromone/permethrin, teflubenzuron and triflumuron, which could also inadvertently effect Carob moth populations (Directorate: Food Safety and Quality Assurance 2007)

1.5.3 Biological Control

The efficacy of a bio-control agent has been explored by Harpaz & Wysoki (1984) who tested *Bacillus thuringiensis* Berliner in laboratory trials with positive results.

Sixteen natural enemies of the Carob moth have been recorded in Israel (Gothilf 1964). A list of parasitoids reported to parasitise *E. ceratonia* was published by Gothilf (1968). The larval parasitoids included: *Goniozus gallicola* Fouts (Bethylidae), *Persierola emigrata* Rohwer (Bethylidae) and *Microbracon pembertoni* (Zimmerman 1958), *Apanteles lacteus* Nees (Braconidae), *Apanteles myeloenta* Wilkinson (Braconidae), *Bracon brevicornis* Wesmael

(Braconidae), *Bracon mellitor* Say (Braconidae), *Rogas testaceus* Fabricius (Braconidae), *Acrocephalus mitys* Walker (Chalcididae), *Brachymeria aegyptiaca* Masi (Chalcididae) and *Anisopteromalus calandrae* Howard (Pteromalidae). The egg parasitoids include: *Phanerotoma dentata* Panzer (Braconidae), *Phanerotoma flavitestacea* Fischer (Braconidae), *Gelis* sp. (Ichneumonidae), *Herpestomus arridens* Grav (Ichneumonidae) and *Horogenes* sp. Diadegma (Ichneumonidae). The pupal parasitoids included: *Pristomerus vulnerator* Panzer (Ichneumonidae) and the hyperparasitoids included: *Perilampus tristis* Mayr (Perilampidae).

In South Africa only one species of parasitoid has been recorded: *Phanerotoma ornatulopsis* De Seager (Braconidae), which was reared from infested acorns in the Citrusdal area (Honiball & Catling 1998).

1.5.4 Cultural Practices

Orchard sanitation is considered the best way of controlling the Carob moth at the moment (Honiball & Catling 1998). In date gardens of the Coachella Valley, California, over winter dates and in season dates that have fallen to the floor are collected to prevent Carob moth emergence in the next fruiting season (Park & Perring 2010). Cultural practices administered for the control of False Codlin moth, also aid the control of Carob moth. These practices include orchard sanitation by which the fallen fruit and fruit left on the tree are removed from the orchard before the next growing season. These fruit if small and dry can be buried no less than 30cm below the soil. If the fruits are wet they can be finely pulped 50m away from the orchard (Moore 2011).

1.6 Bio Ecology

The general ecology of the Carob moth in citrus orchards is not known for South Africa. Studies in this regard have been undertaken in other parts of the world, on different host plants.

Female moths lay eggs within the pomegranate calyx; from here the larvae develop inside the fruit. In dates the first two instars of the moths develop outside the fruit on the surface of

the fruit skin, and the later instars then penetrate the ripe fruit (Dhouibi & Abderahmane 1996).

The Carob moth completes 4-5 generations per year in Southern California on date palm fruits. As the season progresses and the abundance of fruit increases so do the densities of the Carob moth. The Carob moth over winters in all larval stages in fruits that have fallen to the ground or fruits that were not picked in the preceding season (Gianessi 2009). Three generations of Carob moth can complete development within one abscised fruit (Nay et al. 2006). Seasonal densities of Carob moth are closely related to the seasonal development of the date fruit. Carob moth larvae only move into the fruits when they are ripe (Park & Perring 2010).

Al-Izzi *et al.* (1985) undertook a seasonal study and infestation assessment, to determine the ecology of the Carob moth in pomegranate fruit collected from all areas of Iraq. It was found that there are four to five larval instars that develop on pomegranate fruit. Some of the fourth and most of the fifth instars overwinter within the fallen pomegranate fruits. They also found that Carob moth infestation was dependant on orchard sanitation practices.

The Carob moth was found to diapause in its larval form when the day length became shorter than 13 hours and the temperatures fell to 20°C or below (Cox 1979). Heydari & Izadi (2014) found the Carob moth to diapause in its larval stages by studying the physiological aspects of the larva's survival.

In Cyprus the Carob moth is reported to deposit its eggs on unripe carob pods, from where the larvae bore into the pods and predispose the pods to attack by other lepidopteran pests (Ashman 1968).

In South Africa the Carob moth was mostly found in mature acorns, *Quercus robur* which were found on the ground near to Naval orange citrus orchards in Citrusdal, Western Cape (Stotter 2009).

1.7 Aims and objectives

The aim of this study was to establish some guidelines for the development of an integrated pest management programme, which will enable growers to more effectively manage Carob moth infestations. The objectives were as follows:

1. To collate all available literature for *E. ceratoniae* to make the information more accessible to researchers. Currently this information is hidden in obscure texts and is often contradictory or incomplete.
2. To supplement this information with an assessment of the taxonomic status by means of own descriptions of the morphology of the adult, egg, larva and pupa.
3. To assess the pest status of *E. ceratoniae* relative to *T. leucotreta* within citrus orchards of the Western Cape Province, by monitoring seasonal life cycle patterns with the aid of pheromone traps and fruit damage assessments.

Each chapter is written as individual research papers, therefore some repetition may occur. Some supplementary trials were conducted, which failed to yield usable results, but which are included as addendums for future reference. Addendum 1, rearing the Carob moth, formed part of an attempt at establishing a colony to conduct a life table study. Addendum 2, Female chemical lure trials, attempted to formulate a lure that would attract females, with the hope of them laying fertilized eggs in an attempt at starting a colony.

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Chapter 2

A Review of the Systematics and Morphology of *Ectomyelois ceratoniae* (Zeller)

2.1 Introduction

This chapter deals with the morphology and systematics of the Carob moth, *Ectomyelois ceratoniae* (Zeller) (Pyralidae: Phycitinae). Correct identification and determination of the specific identity of this moth, a prerequisite to any study of this kind, is problematic as various reports provide confusing, if not conflicting, information on its identity and morphological detail (Corbett & Tams 1943; Heinrich 1956).

Establishing the correct identity of many other phycitid moths is a problem generally experienced as, for example, the wing pattern of many species deviates so little from each other that this prompted researchers, for example, Neunzig (1990), to stress the need for detailed study of the genitalia for specific determination. In many cases, species have been described from single specimens, ignoring variability within a species, adding to the overall problematic situation as, for example, in Balinsky (1994). In fact, many publications referring to this moth omit to investigate its proper taxonomic status. In the case of the Carob moth, the original description by Zeller (1839) and the absence of the whereabouts of the type specimen adds to the uncertainty of its specific identity.

Traditional taxonomy is grounded on the basis of subjective visual evaluations to identify species (Mutanen & Pretorius 2007). This is rather time consuming and an expertise intensive task, which is presently becoming difficult due to lack of specialization, shortage of taxonomic expertise and difficulty of accessing relevant, especially historic, taxonomic information (Walter & Winterton 2007). This has led to a strong leaning and dependence towards an integrated taxonomic approach prevalent in the past decade. Taxonomy at present is now a combination of traditional taxonomy, coupled with more modern identification techniques such as DNA barcoding, interactive identification keys and morphometrics, known as integrative taxonomy (Schlick-Steiner *et al.* 2010)

What follows is a review of the main characters at each level, from family to species, into which *E. ceratoniae* is classified.

2.2 Classification and systematics of *Ectomyelois ceratoniae*

2.2.1 Superspecific classification of *E. ceratoniae*

Family and subfamily placement

Ectomyelois ceratoniae is placed in the Pyralidae by virtue of its possession, in the adult moth, of a pair of tympanal organs on the second abdominal sternite. This family, with the other families Hyblaeidae and Thyrididae were considered part of the Pyraloidea (Heppner 1998), but in a phylogenetic analysis of the extant lepidopteran superfamilies, the Pyraloidea are considered to consist of one family, the Pyralidae (Kristensen & Salski 1998). The subfamily Crambinae was raised to family level by Munroe & Solis (1998); this arrangement has generally been accepted by, amongst others, Vári *et al.* (2002). The two families within the Pyraloidea are distinguished from each other by the Crambidae having a praecinctorium (= modification of the ventral tympanal arrangement) present; this structural arrangement is lacking in the Pyralidae (Munroe & Solis 1998: Figs 14.2 G-J).

The Pyraloidea is considered the second largest superfamily of moths and estimated to contain about 25 000 named species worldwide with most species present in the tropical regions (Scoble 1992; Munroe & Solis 1998; Solis 2006). At least four times more species remain to be described (M. Shaffer *in* Scoble 1992).

2.3 Diagnostic features of the Pyraloidea:

Maxillary palpus small to prominent, scaled and generally three- or four segmented. Proboscis scaled at base, sometimes vestigial. Foretibia with epiphysis and tibial spurs normally 0-2-4. Abdomen with paired tympanal chambers, facing anteriorly, on sternite of second abdominal segment. Hindwing with Sc + R1 close to or fused with Rs for some distance beyond discal cell.

Eggs of flat type, ellipsoidal or lenticular, with thin chorion and inconspicuous sculpture (Common 1990; Heppner 1998). The larva of Pyralidae has been described by Common

(1990). Larva usually cylindrical and often slender. Most are without bright colouration or pattern, although some have large pinaculae which appear spotted and others have obscure longitudinal bands. The head is usually prognathous, with the frontoclypeus and adfrontal areas not reaching the epicranial notch. There are six stemmata on the lateral side of the head. Spiracles are usually small, most often oval or elliptical. Ventral prolegs may be short or long and the crochets are usually biordinal, but sometimes triordinal or rarely uniordinal, and are nearly always arranged in a circle or mesal penellipse. On the prothorax the prespiracular L-group is bisetose. The SV group is bisetose on the prothorax, and usually unisetose on the meso- and metathorax. On the abdominal segments 1 to 8 L1 and L2 are close together on the one pinaculum, but on A0 the L setae may vary in number from one to three. The SV group is trisetose on A1 to A6 and unitose or bisetose on A7 to A9 (Common 1990). The pupa with epicranial suture present, except in Epipaschiinae, and lobes representing pilifers, except in Galleriinae (Moscher 1916). The pupa is usually well sclerotized with appendages fused to one another and to the body; maxillary palpus present. Labrum often displaced anteriorly and the pilifers (genae) either meet medially or are separated by a small exposed area of the labial palpus. The antennae are usually long and extend nearly to the wing tips. Proboscis long, usually extending to the wing tips or beyond. The fore femora are exposed and the mid tarsi usually extend to the wing tips. The abdomen is without dorsal spines. A cremaster may be present or absent, and the setae at the posterior end may be straight or hooked (Common 1990).

2.3.1 Characteristics of the Pyraloidea:

Adults very small to large, but typically midsized or smaller moths; forewing length ranging from 5-75 mm, but mostly under 30 mm. Overall build slender, but also thick and robust. Frons smooth or roughly scaled, rounded, flat and oblique, sometimes prominent with spines, ridges or processes present. Erect scaling on vertex. Labial palpus usually prominent and 3-segmented, palpus rarely reduced, sometimes excavated dorsally to receive maxillary palpus [a feature also noticed by Zeller (1839)], in various stances (decurved, porrect, ascending or upturned), scaling well-developed. Maxillary palpus reduced, 1-to 4-segmented, sometimes absent; scaling compressed, plumose or tuft-like. Proboscis sometimes reduced or absent;

eye without macroscopic setae, large, globular. Ocellus often present, near dorsal margin of eye, sometimes reduced or absent. Chaetosoma present as a radiating group of short fine setae near posteroventral angle of vertex. Antenna various, filiform, annulate or laminate, but less commonly uni- or bi-pectinate, antenna often sexually dimorphic. Thorax without primary tympanal organs. Legs slender to thick and robust, smoothly scaled or with thick vestiture, in males often with prominent androconia; foretibia with epiphysis, tibial spurs 0-2-4 (Munroe & Solis 1998).

Wings variable in shape, forewing wide to narrow, hindwing wide with narrow fringe; costa straight or arched, sometimes concave, sinuous or distorted in either fore- or hindwing, especially in males; termen usually convex or sinuate. Forewing primitively with Sc, 5-branched R, R₃ and R₄ stalked, 3-branched M, M₂ closer to M₃ than to M₁, stem of M absent or faintly present, discal cell usually closed, CuA 2-branched, CuP represented by a vein, fold or weak tubular vestige, or absent. 1A well-developed, 2A shorter and weak, often joined to 1A; retinaculum in both sexes on underside of cubital area. Hindwing with Sc+R1 close or fused with Rs for some distance beyond cell, subsequently diverting, Rs unbranched, often short-stalked with M1 from anterior angle of discal cell, M1 sometimes separate from discocellular, M 3-branched, M1-3 arranged as on forewing, CuA 2-branched, CuP usually well-developed, 1A + 2A always and 3A usually present. Discal cell open in Crambinae, but closed in other taxa. Frenulum unisetose in male, multi- or unisetose in female (Munroe & Solis 1998) (Fig. 1).

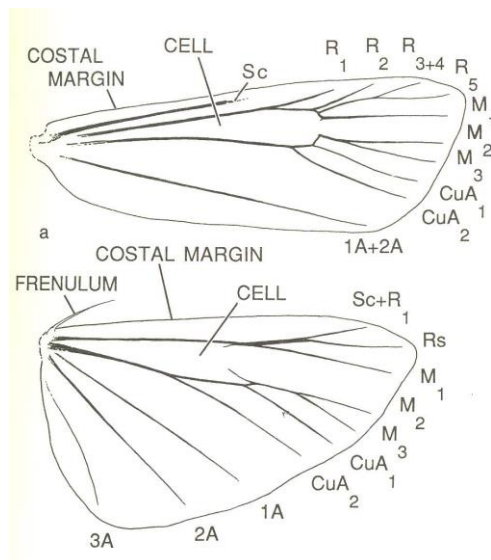


Figure 1: Wing venation schematic of the general Pyraloidea family, including venation names and a general wing outline. (Acquired from Neunzig 1990).

Male genitalia diverse (Fig. 2). Tegumen and vinculum forming a complete ring, vinculum often produced anteriorly into midventral saccus. Uncus present, of various shapes. Gnathos consisting of a pair of lateral arms arising from junction of tegument and uncus, medially fusing to form a posteriorly directed median process. Basal articulations of gnathos either movable or fixed. Transtilla present as a transverse sclerotized band between costae of valvae, sometimes medially narrowed or absent. Aedeagus a sclerotized tube projecting through a membranous manica or sometimes through a tubular anellus. Aedeagus with eversible vesica, generally minutely spinose, often also with single or grouped larger spines, the cornuti. The cornuti are often deciduous, being shed into the female bursa during copulation. Juxta variable in shape and size, ventral to manica or anellus, connecting ventrolaterally with bases of valvae. Valva extremely various in form, primitively with subparallel costal and ventral margins, each somewhat inflated, the inflation termed the saccus, and with rounded or oblique terminal margin; costa articulating basally with transtilla; most of valvar base hinged with vinculum and ventral angles with juxta; inner (mesal) surface generally with variously grouped setae and sometimes spines, often also with ridges and processes; in certain groups the costa and/or saccus produced distally as a free process (Munroe & Solis 1998).

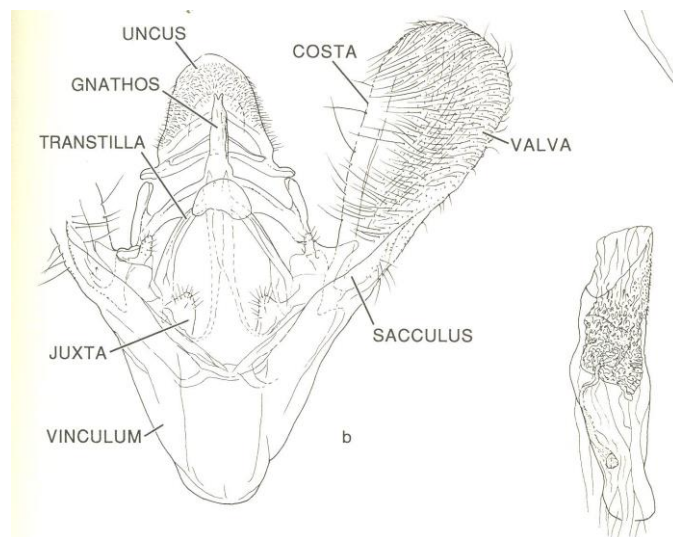


Figure 2: General schematic of the male genitalia of the Pyraloidea family, with labels of all important structures used for diagnostic use. (Acquired from Neunzig 1990).

The female genitalia (Fig. 3) primitively with a pair of membranous setose ovipositor lobes that on each side are supported by a usually T-shaped posterior apophysis. Ostium base simple or variously armed. Corpus bursae variable in shape and armature; ductus bursae usually differentiated from corpus bursa; sometimes an accessory sac arising from corpus bursae; ductus seminalis usually arising from ductus bursae, but sometimes from corpus bursae; ductus bursae often with a collarlike or complex sclerite at or just posterior to opening of ductus seminalis (Munroe & Solis 1998)

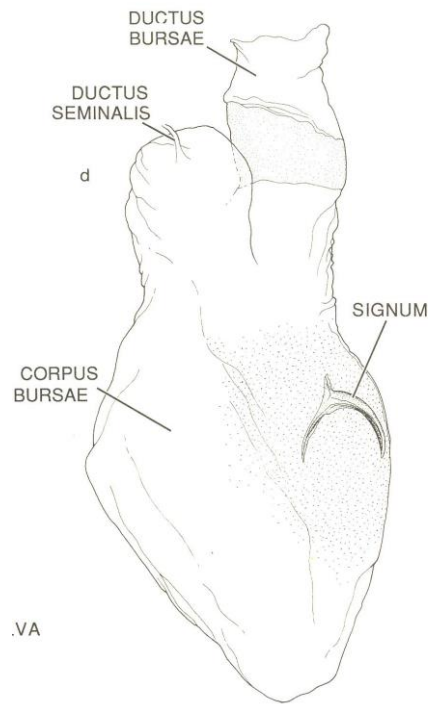


Figure 3: General schematic of the female genitalia of the Pyraloidea family, with labels of all important structures used for diagnostic use. (Acquired from Neunzig 1990).

2.4 Diagnostic features of Pyralidae

Tympanal organs with tympanal case almost completely closed; conjunctiva and tympanum in the same plane; praecinctorum absent (Munroe & Solis 1998: Figs 14.2 G & I); vein R5 of forewing stalked or fused with R 3 + R 4; abdominal segment 8 of larvae almost always with a sclerotized ring around base of seta SD1; male genitalia with uncus arms, a pair of processes arising laterally from the base of the uncus (Hasenfuss 1960).

2.4.1 Characteristics of Pyralidae

Head with frons rounded; scaling of frons usually smooth. Labial palpus almost always 3-segmented, porrect, obliquely ascending or upturned in front of face. Maxillary palpus usually 3-segmented, sometimes 2- or 4-segmented (Heppner 1998), often minute or absent, usually shorter than labial palpus and highly modified in some groups (Munroe & Solis 1998). Proboscis usually well developed, but in various groups reduced or absent. Eye normally large and globular, without macroscopic setae, proboscis scaled, but sometimes naked, filiform, bipectinate or pectinate antennae, one pair of ocelli and chaetosemata which may be absent or present (Heppner 1998).

Wing venation as described for the superfamily. Forewing with R_2 closely apposed to, not usually stalked with, R_3 and R_4 ; R_3 , R_4 , R_5 sometimes reduced to two or single vein, M_1 from near anterior angle of cell. M_2 , M_3 and CuA_1 from posterior angle of cell or near it, M_2 and M_3 sometimes stalked. CuP well developed, or incomplete, only its distal part well developed, or absent, or reduced to a fold. $1A$ strongly developed; $2A$ distally free or connected by a crossvein to $1A$ to form a closed cell. In the hind wing $Sc + R_1$ and R_s may be fused or separate. M_2 and M_3 usually separate, but sometimes fused, the extent of fusion variable within taxa. CuA_1 and CuA_2 usually arising separately from cell; CuA_1 rarely fused with M_3 , CuP and $1A + 2A$ present. Frenulum single in males, mostly multiple in females, but singly in those of Phycitinae. (Common 1990) (Fig. 1).

Legs varying from long and slender to short and stout, often with modified scaling (Munroe & Solis 1998), tibial spurs numbering 0-2-4 (Heppner 1998) (Fig. 4).

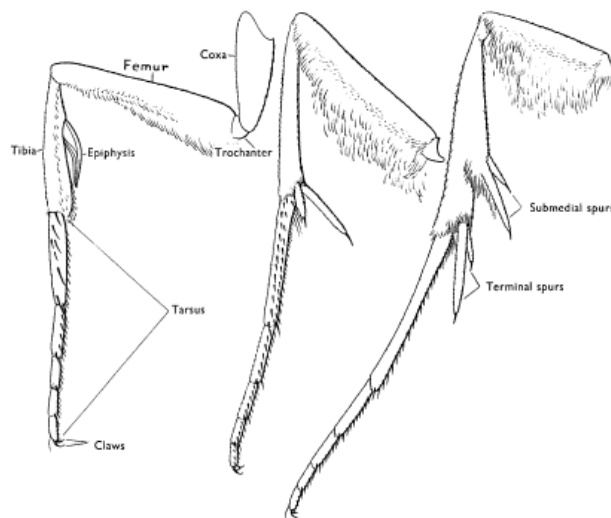


Figure 4: General schematic of the structure of the Pyraloidea legs, including all important diagnostic labels. (Acquired from Zimmerman 1958).

Male genitalia with uncus usually well developed and with ventro-anteriorly elongate arms articulating with base of gnathos; in some phytines these arms are very short or absent. Uncus with many setae caudally. Tegumen in phycitines abutting on only a very small portion of base of uncus (Munroe & Solis 1998). Tegumen complete and gnathos usually present. Distal end of gnathos either a simple hook or curved, s-shaped or often with variously modified apex as

in many Phycitinae. Valva most often simple, but in some groups with modified lobes or setae. Transtilla well developed only in some phycitines. Juxta plate-like, U-shaped, or variously modified. Aedoeagus usually a short cylinder, with or without caecum; cornuti often absent, but present in many phycitines (Fig. 2).

Ovipositor lobes membranous, with many, usually unmodified setae. Lamella antevaginalis membranous, but sclerotized in some species. Ductus bursae usually membranous, but sometimes with sclerotized or scobinate areas. Corpus bursae membranous or spinulose, globular, oval, or wrinkled and contorted, without special sclerotization, or with one or two elongate-conical, spine-like projections, or one or two flat, scobinate patches, or with various, often bizarre spinning or sclerotization. Ductus seminalis usually originating from corpus bursae in Phycitinae, but from ductus bursae in all other subfamilies (Munroe & Solis 1998). The larvae of Pyralidae associated with stored products were described by Hinton (1946a), giving details of larval chaetotaxy, including that of the Phycitinae.

The Pyralidae is subdivided into 11 (Kristensen 1985) or 20 (Heppner 1998) subfamilies; the family is grouped into two series, the Pyraliformis (Gallerinae through Pyralinae) (Pyralinina of Heppner 1998) and Crambiformes (Crambinina of Heppner 1998), on the basis of the absence or presence of a praecinctorium, an intertegumental fold in front of the tympanal organs (Munroe 1972-[4] 1976). Fletcher & Nye (1984) recognized 17 subfamilies. The Pyraliformis contain the subfamily Phycitinae, in which *Ectomyolois ceratoniae* is placed, a major group of the Pyralidae (Scoble 1992) with more than 900 species described so far. For the South African fauna, 73 species of Phycitinae have been described (Vári *et al.* 2002).

Munroe & Solis (1998) provided a key to the subfamilies of the Pyralidae. Diagnostic characters that separate the Phycitinae from all other subfamilies include the following: Praecinctorium absent; tympanal case closed medially and open anteriorly only; tympanum and conjunctiva in the same plane (Munroe & Solis 1998: Figs 14.2 G, I), secondary venulae absent; female frenulum with one bristle; ductus seminalis usually originating from corpus bursae.

2.5 Characteristics of Phycitinae

Usually small moths with long, narrow forewings, some with raised scales. Ocellus present or absent. Chaetosema present. Frenulum with one bristle in both sexes. Uncus arms of male extending at 110 degrees or more from longitudinal axis; tegument abutting a small portion of base of uncus. Ductus seminalis generally originating from corpus bursae, rarely from ductus bursae. Tympanal organs with a circular sclerotization around distal insertion of scoloparium; secondary venulae usually absent, but present in most genera of the Peoriini. Larva with sclerotized area encircling base of seta SD1 of mesothorax (Hasenfuss 1960; Munroe & Solis 1998); bisetose L-group setae on the prothorax, larval prolegs present on abdominal segments A3-6, A 10 (Heppner 1998); pupa with modified spinose cremaster (Heppner 1998; Patočka & Turčáni 2005)

The eggs are of the flat or scale-like type. The phytophagous larvae are usually concealed feeders, making their detection in pods, seeds and fruit often difficult, some in a tube of silk mixed with frass. Others are borers in buds, shoots, cones, fruit, galls, cankers and cambium; leafminers in early instars and in stored products (Munroe & Solis 1998).

Solis & Mitter (1992) *in* Munroe & Solis (1999) estimate the subfamily to include close to 4 000 species globally. A general overview of the Phycitinae was given by Janse (1941, 1942, 1944, 1945), Heinrich (1956), Neunzig (1990), Ragonot (1885-86), Roesler (1988).

Phycitine moths are small to medium sized, ranging in colour from dark red or brown to a dull yellow or orange in a few of the groups; some are brightly coloured or mimetic (Munroe & Solis 1998). The moths prefer warmer areas and enjoy a strictly nocturnal habit (Meyrick 1895). As the Phycitinae hosts many economically important stored product pests worldwide (Aitken 1963), the group has been the subject of many taxonomic and economic studies worldwide.

2.6 Generic placement of *Ectomyolois ceratoniae*

When described by Zeller (1839), the species was initially placed in the Fabrician genus *Phycis* (1798) as *P. ceratoniella* by Schmidt *in lit.* in Fischer von Röslerstamm (1839), but *Phycis*, as indicated by Zeller (1839), was, in fact, already in use, having been assigned already to a genus of fish. Zeller (1839), when dealing with a description of the antennae of male and

female moths and their palpi, also remarked on inconsistencies in the characterization of the genus *Phycis* (of Fabricius) and on this basis selected six genera to cover all described species. These are: *Myelois* Hübner (in both sexes simple straight antennae, indistinct maxillary and distinct labial palpi), *Anerastia* Hübner (antennae also straight, maxillary palpi absent and straight labial palpi), *Phycidea* Zeller (simple antennae which, in the males, are notched beyond the scapus, maxillary palpi small and cylindrical and labial palpi distinctly upturned), *Epischnia* Hübner (male antennae naked, clearly curved, maxillary palpi small and cylindrical and labial palpi straight or upturned), *Nephopteryx* Hübner (male antennae clearly curved, but scaled, maxillary palpi small and cylindrical and labial palpi distinct and upturned, and *Pempelia* Hübner (antennae of male as in *Nephopteryx*, the maxillary palpi brush-like, settling in excavated labial palpi, in female labial palpi close to face). Zeller (1839) furthermore divided *Myelois* in four sections (A, B, C *Acrobasis* Zeller and D – *Zophodia* Hübner), again based on characteristics of the antennae and palpi, but now including features of the wing as well. Using the latter separation, *ceratoniae* Zeller was placed in section B (Zeller 1839).

As shown in the species synonymy of Vári *et al.* (2002) (Table 1), *E. ceratoniae* has been placed in different genera, including *Phycis*, *Phycita*, *Laodamia*, *Myelois*, *Trachonitis*, *Hypsipyra*, *Heterographis*, *Euzophora* and *Spectrobates*. Most of these names were coupled to various specific names giving rise to the Carob moth (Table 1) without recourse to the individual generic characteristics. Heinrich (1956) characterized various genera in the Phycitinae, supplemented by Zimmermann (1958) with a venational key to some phycitine genera. Neunzig (1990) provided a detailed key, based on features of the male and female moths, to various genera in the Phycitinae. Only the couplets (renumbered) dealing with the genus *Ectomyelois* are given below: A key to the genus *Ectomyelois* was also compiled by Balinsky (1994), but with considerably less detail.

Key features of both the male and female adult moths as outlined by Neunzig 1990's key to the genera.

1. Male
2. Antenna with distinct sinus in shaft
3. Labial palpus with second segment not as broadly scaled; hindwing with 6-8 veins
4. Antenna with simple to slightly serrate shaft
5. Antenna without row of sensilla in sinus; forewing with costal fold
6. Forewing with 10 veins
7. Antenna without cluster of dark sensilla at base of shaft
8. Forewing with 8-10 veins
9. Valve without large basal clasper
10. Transtilla without 2 diverging, hornlike, apical elements (if strongly developed apical elements present, they bear numerous, long setae)
11. Valve without a costal hook or with a weakly developed costal hook
12. Valve more elongate; maxillary palpus more robust, squamous
13. Juxta with shorter elements; valve with distal $\frac{1}{2}$ not greatly reduced
14. Uncus not strongly expanded distally
15. Hindwing with 7 veins (1A, 2A and 3A together count as 1 vein)
16. Gnathos with apex simple
17. Juxta with lateral elements robust distally; transtilla distinctly bifid distally

1. Female
2. Corpus bursae with or without signum; signum never clawlike
3. Genital opening without associated large patch, or patches of dense microspines
4. Ductus bursae without transverse wrinkles or ridges anterior of genital opening
5. Genital opening without associated triangular, sclerotized plates
6. Forewing with 11 veins
7. Hindwing with 8 veins (1A, 2A and 3A together count as 1 vein)
8. Ductus bursae usually broader and always shorter, and corpus bursae more slender
9. Signum of corpus bursae and invaginate cup or an elongate patch of scobinations or microspines
10. Corpus bursae with anterior $\frac{1}{2}$ not distinctly more enlarged than posterior $\frac{1}{2}$; signum of larger scobinations or microspines
11. Corpus bursae only slightly broader than ductus bursae

2.7 Specific identity of *Ectomyelois ceratoniae*

Subsequent to the published accounts of Zeller (1839) and Fischer von Röslerstamm (1839), Vaughan (1870) described the Carob moth as *Trachonitis pryrella*, at the same time expressing doubt as to the correct generic placement of the species. Only the female was described, the three specimens all collected in London. No further details were provided. Sorhagen (1881a) added to the nomenclatorial confusion by describing the Carob moth under two different names, viz. *Myelois tuerkheimiella* and *Euzophora zellerella*. He considered his species to be closely related to *E. ceratonia*, basing his description on minor differences in wing colouration, but in a later paper (Sorhagen 1881b) he described the Carob moth again, but now as *Euzophora Zellerella*, referring to differences in the wing pattern of the adult moth and in the larval development. In 1896 Hampson described the Carob moth as *Phycita dentilinea*, synonymized by Vári *et al.* (2002). Unfortunately, the publication in which the description was done, could not be traced. Hampson (1903) again described the Carob moth, but now as *Hypsipyla psarella*, providing only a brief description of the wing pattern of the adult. *Heterographis rivulalis* was the name given to the Carob moth by Warren & Rothschild (1905), also basing their description on the wing colouration and pattern of a single female. Dyer (1911) described the Carob moth as *Myelois oporedestella*, in this case also detailing wing patterns and colouration. *Myelois phoenicis* was the name given to the Carob moth by Durrant (1915), as before, only relying on details of patterns on the wings. However, Heinrich (1956) considers *M. phoenicis* to be only a colour variety or race of *E. ceratoniae*. Lucas (1950), described *Laodamia durandi*, another synonym of *E. ceratoniae*. In this case, not only were details of the wing's pattern and colouration given, but also a brief reference to the general appearance of the moth. Appendix 1 is a collation of all the synonymic authors literature mentioned above, and Appendix 2 contains the individual wing colour and pattern descriptions. From all these descriptions it is clear that no genitalia were used to ascertain the specific identities nor was attention paid to the characteristics of their various genera in which species were placed. Janse (1941), in dealing with a number of phycitine genera, stressed the need for a thorough understanding of generic concepts before allocating a species to a specific genus. Subsequently, Corbet & Tams (1943), in an adjunct study of Hinton (1946a), made

use of genitalia of the male and female moths for specific identification. These authors then provided the first detail of the venation of the wings and morphology of the genitalia, including that of the Carob moth, besides producing a key to facilitate the identification of the Carob moth, amongst others. In a major study on American phycitine moths, Heinrich (1956) provided much needed morphological detail on the systematics of this cohort, relying mainly on genitalia characteristics and provided, amongst others, synonymic detail relating to *E. ceratoniae*.

Modern authors have placed the species *ceratoniae* in four genera throughout its taxonomic history: *Ectomyelois*, *Myelois*, *Spectrobates* and *Apomyelois*. *Ectomyelois* as described by Heinrich in 1956 is the most commonly used generic name, but some authors use it as a synonym of *Apomyelois* (Gilligan & Passoa 2014). Janse 1941 re-described the genus *Myelois* that was first described by Hübner 1825. Hübner's description was not available for examination to aid in this study. Neunzig 1990 provided a key to distinguish between *Ectomyelois* and *Apomyelois*, but also discusses his choice to use *Ectomyelois* instead of *Spectrobates*. Balinsky (1994) also provided a key to the genera where he distinguished between *Ectomyelois* and *Apomyelois*, but does not make reference to any of the other synonym genera.

Solis (2006) provided a key to *E. ceratoniae* based on larval structure, keying down from family and subfamily level to the specific identity of the Carob moth as follows (only the couplets directly applicable are included):

- | | | | |
|----|-------------------------------------------------------------------------------------------------------------------------------|---------------------|---|
| 1. | Sclerotized ring around seta SD1 on A8 (missing in some phycitines);
three (sometimes two) setae in the L group on A9..... | Pyralidae | 2 |
| 2. | Sclerotized ring around seta SD1 on mesothorax, metathorax or
A1...Phycitinae | | 3 |
| 3. | Sclerotized ring around seta SD1 on mesothorax | most
Phycitinae | 4 |
| 4. | Sclerotized ring around seta SD1 on mesothorax | other
Phycitinae | 5 |

5. Prespiracular shield of prothorax never extending below and behind spiracle	6
6. Integument granulose under low magnification	7
7. Prothoracic shield not with black areas on lateral margins and longitudinal black areas on either side midway between center line and lateral margins	8
8. Prothoracic shield yellowish without the pattern above	9
9. Prothoracic shield yellowish without pattern	10
10. Coronal suture absent; A1 to A7 with a crescent shaped patch above seta SD1	11
11. Anal plate with seta SD1 closer to seta D1 than to seta L1; seta SD2 of A8 usually separated from the spiracle by 2 or more times the diameter of the spiracle	<i>Ectomyelois ceratoniae</i>

2.8 History of the species *ceratoniae*

To contextualize the above information, a table was compiled which places the Carob moth in systematic order and contains all the known synonymy of the Carob moth in chronological order.

Table 1: The classification and systematic position of the Carob moth *Ectomyelois ceratoniae* and the historic known synonymy of the Carob moth, in chronological order.

Order	Lepidoptera
Family	Pyralidae
Subfamily	Phycitinae
Tribe	Phycitini
Species	<i>Ectomyelois ceratoniae</i> (Zeller, 1839)
Species synonyms	<i>ceratoniella</i> (Schmidt, 1839) <i>Phycis ceratoniella</i> (Fisher von Roeslerstamm, 1839) <i>Phycis ceratoniae</i> (Zeller 1839) <i>Myelois pryrella</i> (Vaughan, 1870) <i>Trachonitis tuerkheimiella</i> (Sorhagen, 1881) <i>Myelois zellerella</i> (Sorhagen, 1881) <i>Euzophora dentilinella</i> (Hampson, 1896) <i>Phycita psarella</i> (Hampson, 1903) <i>Hypsipyla</i>

	<i>rivulalis</i> (Warren & Rothschild, 1905) Heterographis <i>oporedestella</i> (Dyer, 1911) Myelois <i>phoenicis</i> (Durrant, 1915) Myelois <i>durandi</i> (Lucas, 1950) Laodamia
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2.9 Conclusion

Carob moth taxonomy has a very prolific history, with the moths having many generic and specific names throughout its nomenclatorial history. The misconceptions made by all the authors were because the descriptions were based on wing colouration, which is a poor character trait. The subsequent chapters of this study however will show that for the correct identification of the Carob moth to generic level wing venation should be closely studied, and for identification down to a specific level it is imperative to study the structure of both the male and female genitalia.

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Chapter 3

Morphology of the Carob moth *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae)

3.1 Introduction

The Lepidoptera is a monophyletic lineage defined by more than 20 derived features, the proboscis and scales on the wings being the most noteworthy (Powell 2009). The Lepidoptera is one of the largest orders of insects, with an estimated 160,000 named species (Powell 2009).

Pyralids are the second biggest superfamily within the Lepidoptera. There are an estimated 25 000 species already described and approximately four times as many species still unknown to science (Scoble 1992). The Phycitinae, a subfamily of Pyralidae, contain around 12 genera and almost 784 species with a global distribution (Janse 1941).

The Carob moth (*Ectomyelois ceratoniae*) is widely distributed and occurs in Europe, Africa and Arabia (Catling 1979). It is a phytosanitary pest of a variety of fruit crops as well as of stored food products, such as acorns, macadamia nuts, rotting apples and fruits of wild trees such as *Ximenia caffra* and *Bequaertiodendron magalismontanum* in South Africa (Catling 1979; de Villiers 2001). The larvae are concealed feeders which make them difficult to detect and control (Scoble 1992; Mediouni & Dhouibi 2007). Carob moths in their larval stage bore into fruit. The female moth will lay her eggs in already split fruits or on the skin of the fruit or pod. Upon hatching the larvae will enter the fruit through the split or through a hole previously made by other insects (Neunzig 1979). Fully grown larvae either emerge from the fruit just before pupation to pupate in the soil, or pupate within the fruit itself. The adult is a small, inconspicuous grey moth. The wing markings, body size and genitalia are extremely variable, thus strongly supporting the existence of several races within the species (Honiball & Catling 1998). The wingspan of the moth ranges between 19-26mm; the forewing is light grey in colour with two faint slanted stripes. The hind wing is white to light grey and fringed with long hairs (Honiball & Catling 1998).

Carob moth is often confused with False Codling moth (FCM) in its larval stage (Honiball & Catling 1998). Both are pink in colour and both are found within the same fruit types. There are, however, microscopic differences to identify the individual larvae. Another difference between the two species is that Carob moth larvae bore into the rind and albedo, but do not seem to penetrate far into the flesh, whereas FCM actually penetrate the flesh of the fruit (Honiball & Catling 1998). Rentel (2012) designed a lucid key to distinguish between major Tortricid pests of economic significance in South Africa.

The aim of this chapter was to assess what we recognize as Carob moths in South Africa, with that of the compiled literature to ensure that the taxonomic status is correct. This is important as a first step to ensuring efficient application of integrated pest management methods. To assist accurate monitoring of the pest, an easy to follow pictorial identification key, using information from the existing tortricid key (Rentel 2012), was designed as part of the objective of this chapter.

3.2 Materials and Methods

3.2.1 Preparation of specimens and imaging techniques

3.2.1.1 Immature life stages

3.2.1.1.1 Eggs

A total of 83 eggs of the Carob moth, collected from adult moths emerging from pecan nuts collected in the Vaalharts area (Northern Cape), (28°01'S; 24°43'E) were observed and measured. Three eggs were carefully removed from plastic honey jars on which eggs were laid. Prior to imaging, the sample was mounted on a stub using double sided carbon tape. The sample was then coated with a thin layer of gold to make the sample surface electrically conductive. Imaging of the sample was accomplished using a Leo® 1430VP Scanning Electron Microscope (SEM) at Stellenbosch University. The SEM images indicate the surface structure of material. Beam conditions during surface analysis were 7 kV and approximately 1.5 nA, with a spot size of 135. Egg size was determined using an ocular micrometer.

3.2.1.1.2 Larvae

Three heads of larva collected from pecan nuts in the Groblersdal (Mpumalanga) (25°09'S; 29°23'E) area were removed from final instar larvae. The heads were then dehydrated, using 50%, 70%, 80% and 96% ethanol, respectively. The dehydrated heads were then submerged in xylene for 48 hours, after which they were mounted on a stub, using double sided carbon tape. The sample was coated with a thin layer of gold in order to make the sample surface electrically conductive. The SEM images will indicate the surface structure or features of material. Imaging of the samples was accomplished using similar conditions as described for the egg stages.

3.2.1.1.3 Pupa

Seven pupae were collected from pecan nuts collected in the Vaalharts area in the Northern Cape (28°01'S; 24°43'E) of South Africa. They were stored in Kahle's fluid to maintain their colour and structure. The pupa were positioned in soft wax and then photographed using a Leica MZ 16A automontage microscope for two dimensional image analysis with a Leica DFC 290 fixed digital camera and Leica Application Suite (LAS) v.2.7. Software. Photos were then edited in Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated).

3.2.1.2 Mature life stage

3.2.1.2.1 Wings

The wings of thirty six adults were carefully removed from moths and submerged in 50% ethanol for a few minutes to remove traces of oils and fats. Thereafter wings were placed in an excavated block containing a few drops of household bleach diluted to a 10% solution. Using a dissecting microscope, the scales were gently brushed away using two synthetic hair brushes. After this the wings were washed three times in 30% ethanol, followed by a final rinse in 70% ethanol. The wings were then immersed overnight in an acid fuchsin stain composed of 1g of acid fuchsin dissolved in 100 ml of 50% ethanol mixed with 5ml glacial acetic acid in which 10g of chloral hydrate had been dissolved. Each wing was gently transferred from the stain into an excavated block with 95-100% ethanol and immediately brushed flat. After rinsing the wings a further two times in 95-100% ethanol, more scales were removed if needed. With a rolling brush motion the wings were then dropped onto a dry microscope slide and covered

with a small drop of Euparal as soon as most of the ethanol had evaporated. After gently lowering the coverslip onto the slide, the slides were then weighed down for a few days to keep the wings flattened (Horak 2006).

3.2.1.2.2 Genitalia

The whole abdomen was removed from 28 moths collected from pecan nuts picked in the Vaalharts area in the Northern Cape, by gently pressing abdomen down from pinned specimens or dissected away from the moths, then stored in 50% ethanol. It was then placed in a 10% KOH solution for up to 24 hours. The abdomena were thoroughly rinsed in water and transferred into a 30% ethanol solution for dissection. Using two synthetic hair brushes, the abdomen were cleaned of the scales, and the pelt was separated from the abdomen. Both the abdomen and the genitalia were stained briefly in 70% ethanol containing a 1% solution of Chlorazol black E. After flattening, cleaning and dehydration, the genitalia and pelt were mounted in Euparal on standard microscope slides: voucher material will be deposited in the Entomology museum of the Conservation Ecology and Entomology Department at Stellenbosch University in drawer number 172.

3.2.2 Identification key

A key was produced using the main diagnostic characteristics of the Carob moth and the False Codling moth in all of their respective life stages. In the adult stage, the moths position at rest were observed. Distinguishing setal characteristics were used for the larval stage. The pupal key made use of the presence and absence of the cremaster. The False Codling moth information was sourced from Rentel (2012).

3.3 Results

3.3.1 Immature life stages

3.3.1.1 Eggs

The collected eggs proved to be infertile. An ocular micrometer was used to measure the length and width of 83 eggs. The mean length was 0.063 ± 0.0001 mm and the mean width was 0.0441 ± 0.0001 mm (N=83; Sd 0.000657). The eggs are ovoid in shape and were laid singly or in clumps of two to three eggs (Fig. 1).

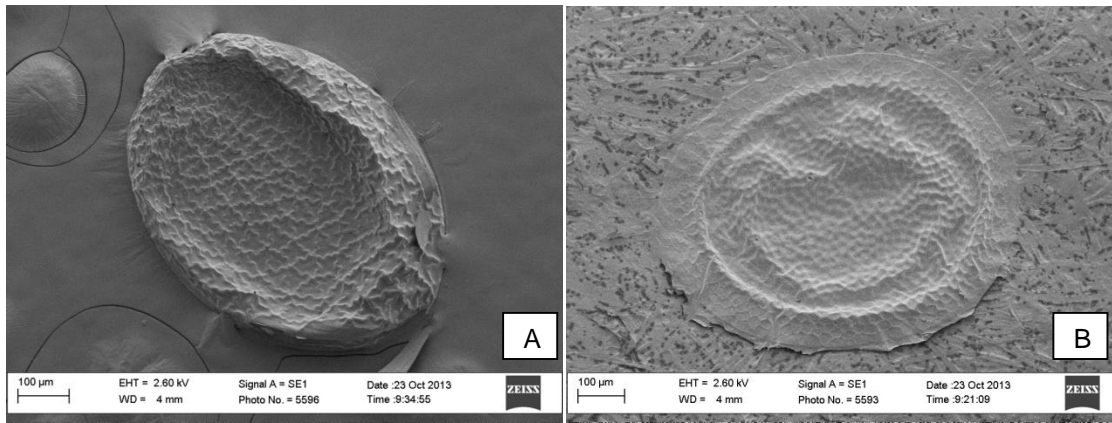


Figure 1: SEM images of an unfertilized Carob moth egg (A) and a fertilized False Codling moth egg (B).

3.3.1.2 Larvae

Larvae are slender, elongate, cream white to light pink colour with rugose integument. Setal pinacular easy to observe standing out by their darker colour from the surrounding integument. The head yellowish red-brown in colour. The prothoracic and anal shield, medium red-brown with dark brown to almost black sclerotized patches.

Head: Hypognathous, with the dorsal surface flattened and broad. The ocellar area is rounded. All six stemmata approximately the same size and irregularly rounded in shape. Stemmata 1 and 2 are closer to each other than 2 and 3 (Fig. 2). Stemmata 2 and 3 are separated by seta S1. Stemmata 3 and 4 are equidistant and 5 lies outside of the semi-circle. Stemmata 5 and 6 are separated by seta S3. Seta S2 approximates to stemmata 1. Seta MD3, MDa, MD2 and MD1 all situated in a vertical line (Fig. 3). Seta P2 is equidistant from MD1 and MD2. Seta AF2 and AFa lie within the adfrontal area and AF1 is close to the frons on the lateral adfrontal suture.

Mandible with 4 teeth, outer three large and usually acuminate. Fourth tooth smaller and flattened with a straight edge (Fig. 4).

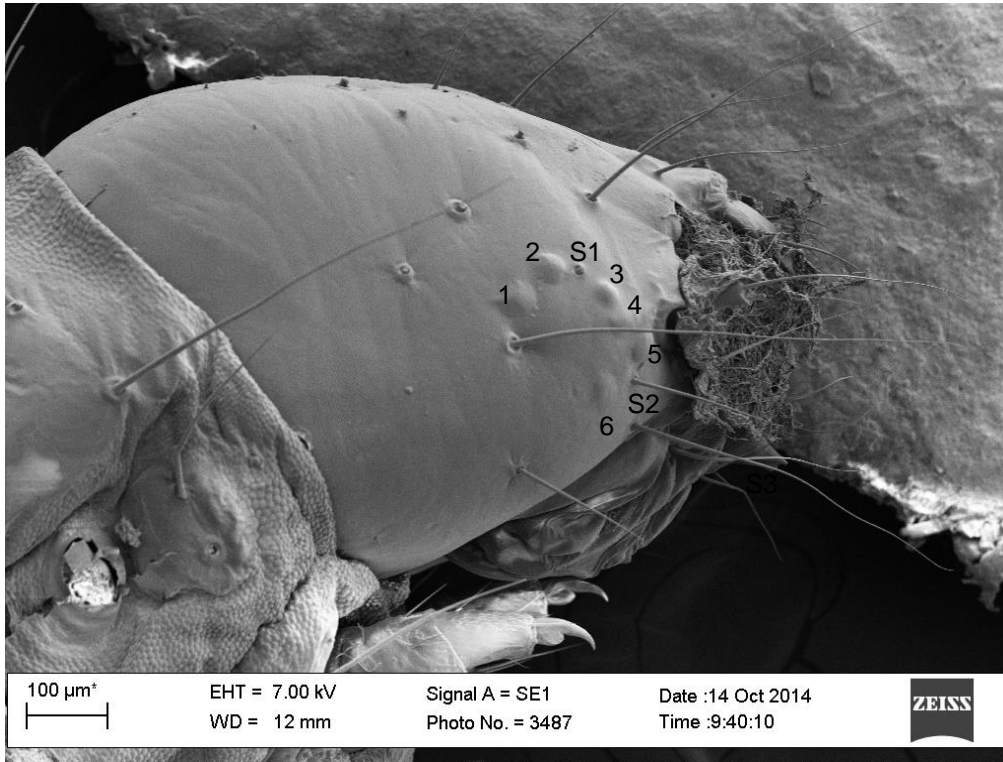


Figure 2: Lateral view of the Carob moth final instar larva head showing the ocelli (1-6) and the seta (S1 and S2).

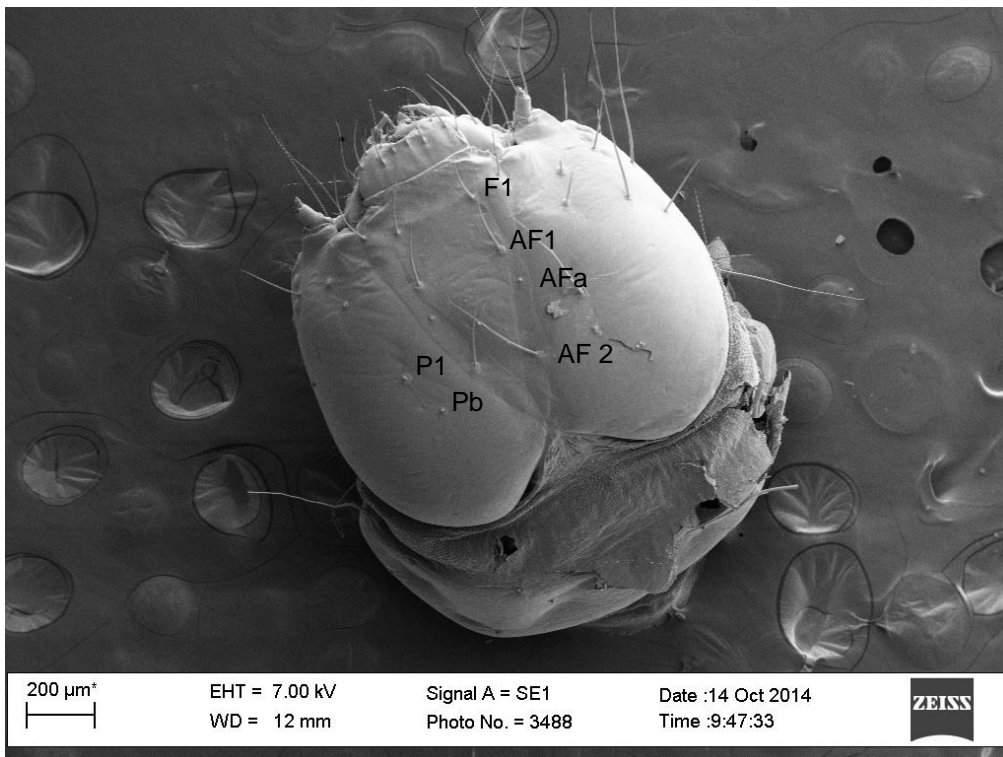


Figure 3: Dorsal view of the Carob moth final instar larval head showing F1, AF1, AFa, AF2, P1 and Pb seta and pinacula.

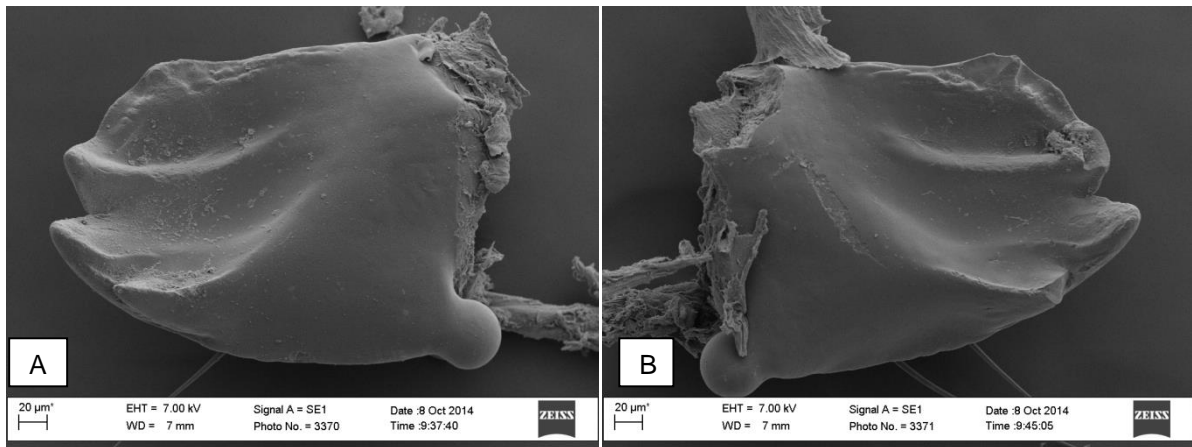


Figure 4: SEM images of the mandibles of a Carob moth larva, (A) is the right mandible inner side and (B) is the left mandible inner side.

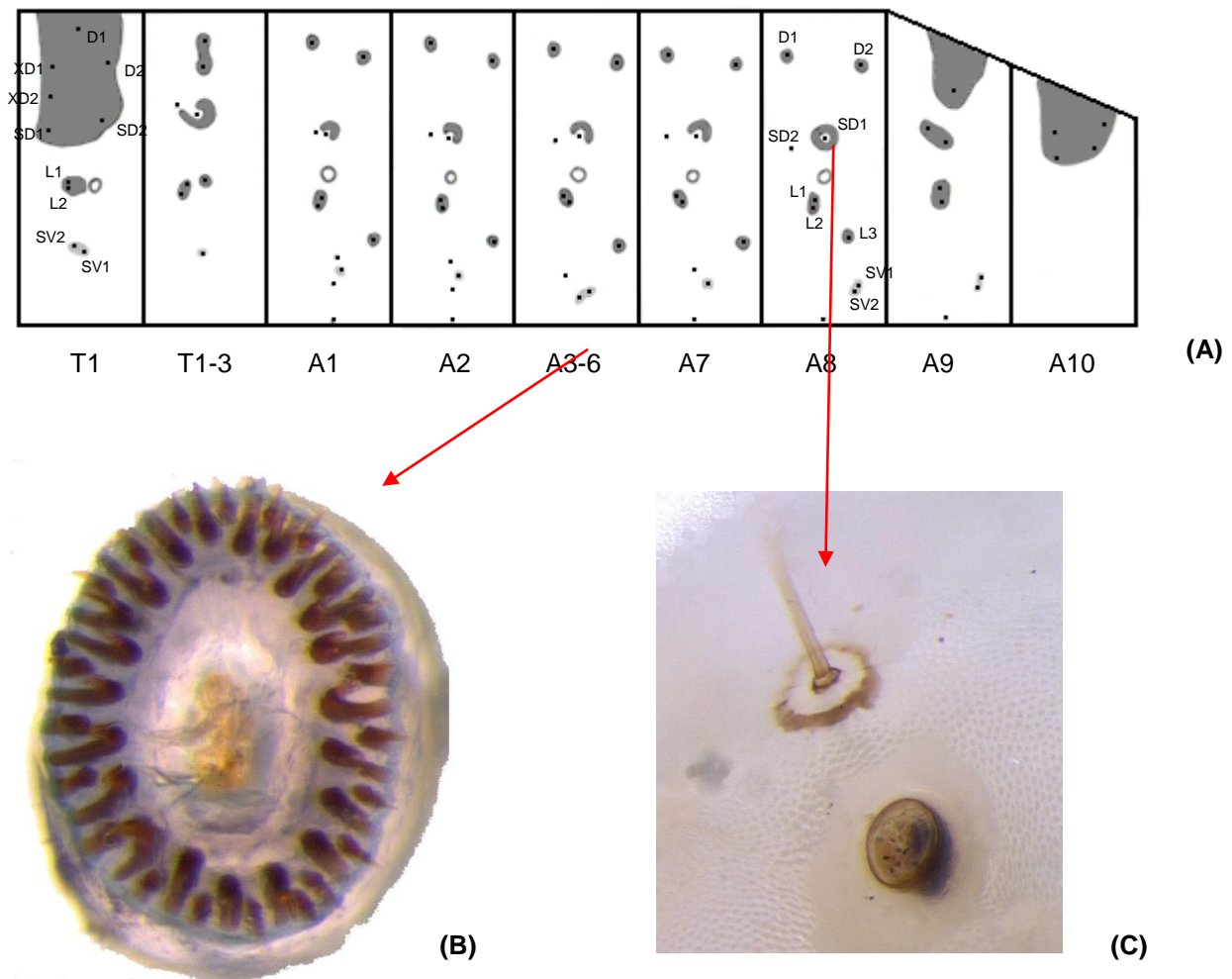


Figure 5: A setal map of the final instar of the Carob moth larva, modified from a sketch, of Gilligan & Passoa (2014) (A). Bilateral crochets on ventral prolegs on abdominal segments 3-6 (B). Pinaculum of seta SD1 surrounded by sclerotized darkened ring (C).

Thorax: Prothoracic shield anterior lateral at obtuse margin, slightly concave and curved at about 1/3 of length, curved convexly towards mid-line. Lateral margin straight and posterior margin evenly rounded. Spiracle circular with seta L1 and L2 bordering a highly sclerotized square patch anterior to spiracle. Seta SD1 closer to XD2 than to SD2. D1 dorsal to D2 and stilted on a slightly darker sclerotized pinacula. Seta SD1 surrounded by a strongly sclerotized crescent ring on T2 and a less sclerotized ring on T3. Thoracic claws small and curved.

Abdomen: Spiracles, medium-sized, round and darkly sclerotized (Fig. 5 (C)). SD1 dorsally situated above the spiracle. Segment A8 complete dark sclerotized ring around pinaculum of

the SD1 seta separated from the spiracle by 1.5 the length of the spiracle (Fig. 5). SV group on A1, 2, 7, 8 and 9 usually 3:3:2:2:2. On A9 D1 and SD1 are on the same pinaculum and L1, L2 and L3 on the same pinaculum whereas on A1-A8 L1 and L2 share the same pinaculum and L3 has own pinaculum.

Anal fork: absent

Anal shield: Tapering posteriorly, evenly rounded along posterior margin. Lateral margin acute, angled anteriorly, anterior margin broadly curved. SD1 and SD2 equidistant from one another as D1 is to D2. SD1 and D1 separated from each other by the same distance as that of SD1 to SD2 and D1 to D2.

Prolegs: Crochets of abdominal prolegs irregularly bi-ordinal, arranged in an ovoid to oval pattern, inner crochets twice the length of the outer crochets 36 and 24 on anal prolegs (Fig. 5 (B)).

3.3.1.3 Pupa

3.3.1.3.1 General

Pupa yellow to red-brown in colour, with darker abdominal marking ventrally. Pupa 11mm in length.

Head: Prominent dark eyes in mature pupae. Frons smoothly rounded, with a pair of sets present on the clypeus. Labial palpi not visible.

Thorax: Metanotum not complete. Notum on dorsal side with a longitudinal ridge with lateral projections.

Abdomen: Spiracles are oval. A 4-9 with a pair of spines dorsally present. Segment 10 delaminated by thick coloured band. Cremaster present and very distinct, with pair of large ventrade projections. Three pairs of thick distinct curled seta on A10 (Fig. 6). Male pupa are identified by abdominal segments 8-10 that are fused with two bullae being present on segment 8. Female pupae are identified by abdominal segments 7-10 being fused and on the 9th segment a slit-like opening is distinguishable.

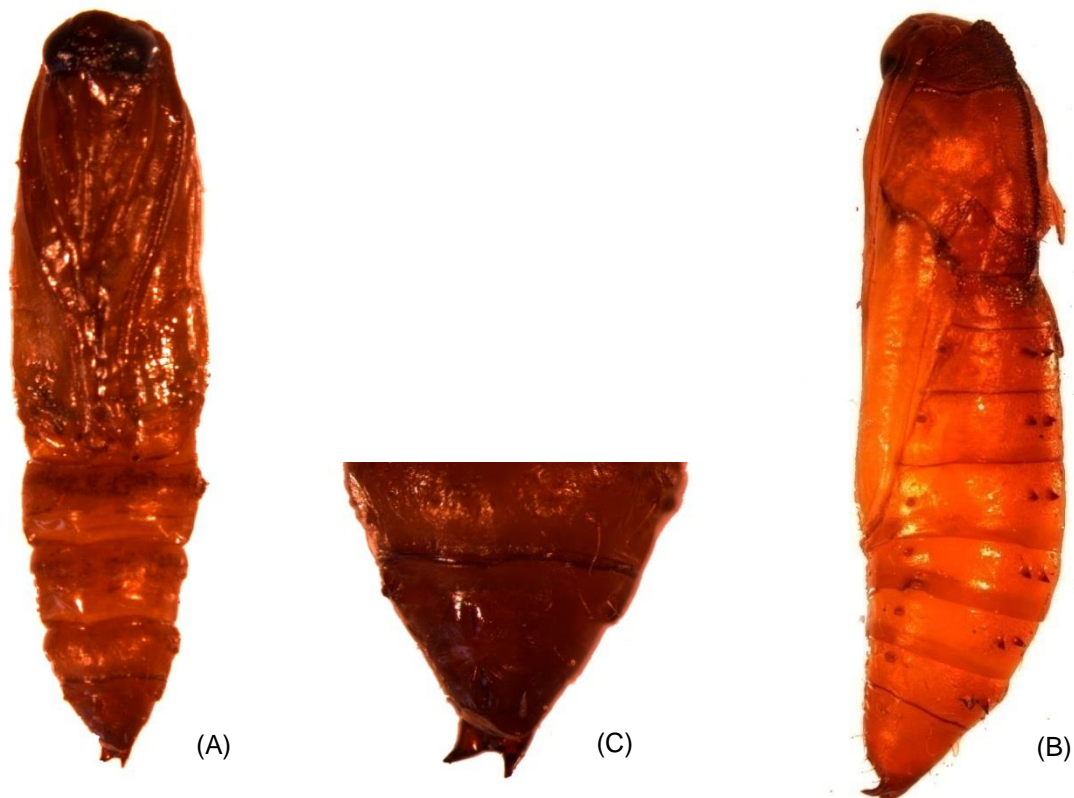


Figure 6: (A) Frontal view, (B) Lateral view, (C) Cremaster (enlarged)

3.3.2 Mature life stages

3.3.2.1 Wings

3.3.2.1.1 Wing venation (Fig. 7)

Forewing ♂: smooth scaled, with 11 veins. R2 branches from cell with R3 and R4 branching from R2. M1 branches from below upper angle of cell. M2 and M3 stalked for more than half their length. CuA1 branches from lower angle of the cell, CuA2 branches before lower angle of cell.

Hind wing ♂: with 10 veins. Sc+R1 branches from upper angle of the cell, with Rs branching off of Sc+R1 near the edge of the wing. M1 also branches from upper angle of the cell. M2 and M3 branched for more than half of their length before the cell. CuA1 fuses with M2 closely approximate to border of the cell. CuA2 branches from well beyond the cell.

Forewing ♀: smooth scaled, with 11 veins. R2 branches from upper angle of cell. R3 runs parallel to R2 with R4 branching off of R3. M1 branches from below upper angle of cell. M2

and M3 stalked for more than half their length. CuA1 branches from lower angle of the cell, CuA2 branches before lower angle of cell.

Hind wing ♀: Sc+R1 branches from well before the upper angle of the cell with Rs branching off of SC+R1. M1 branches off of SC+R1 well before the upper angle of the cell. M2 and M3 branched for less than half the length of the vein from the cell. CuA1 branches from well before the lower angle of the cell. CuA2 branches well beyond the cell.

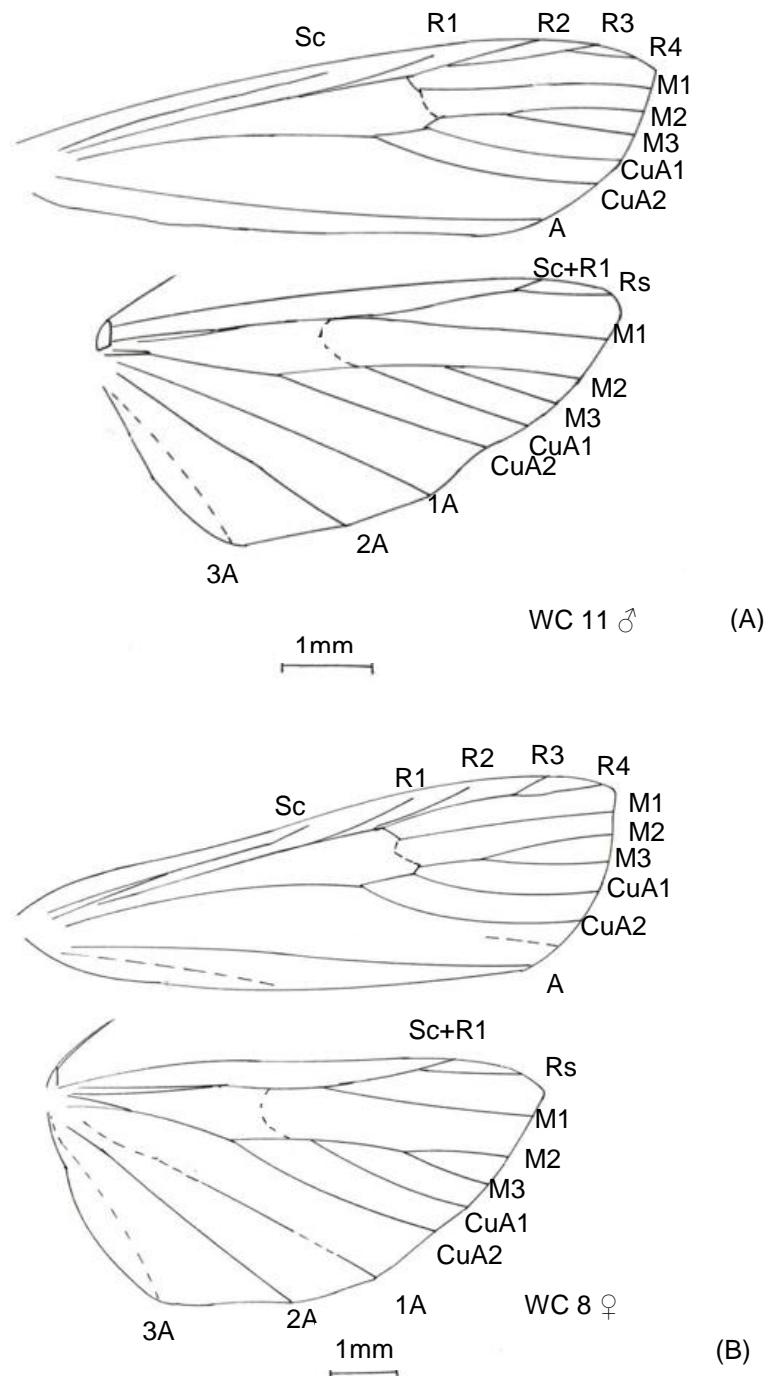


Figure 7: Sketch of the wing venation for both the male (A) and female (B) forewing and hind wing of the Carob moth.

3.3.2.1.2 Wing colouration (Fig.8)

Forewings: Basal part of the forewing dark grey. Subterminal lighter grey line preceded by a dark grey antimedial line. Postmedial dark grey line preceded by lighter submedial line. Terminal end of the wing; darkened triangular spots preceded by a thick fringe of cilia on the

outer margin of the wing. Two defined spots at an obtuse angle to the costa, a third of the distance between the postmedial and antimedial line. Spots appear to distinguish the Carob moth other similar Phycitinae species.

Hind wings: Hind wings cream colour. Shaded marginally, very distinctive long fringe of cilia all around the outer margin of the wing.



Figure 8: Wing coloration and pattern for both the male and female Carob moth adults.

3.3.2.2 Genitalia

There are a few things to consider when comparing a specimen with previously published articles. Problems were encountered with the comparison, as only some of the features observed under the microscope tallied with any one of the studies encountered in the literature. But from all observations it was determined that concentrating on a few structures would be the optimal procedure.

In the male genitalia focus was placed on the structure of the vinculum, transtilla and the aedeagus. For the female genitalia focus was placed on the posterior and anterior apophysis as well as the signa on the corpus bursa.

3.3.2.2.1 Male (Fig. 9 A-E)

Valve with costa straight, many seta on the inside of the valve. Uncus is rounded. Gnathos with darkly sclerotized tip in the form of a strong medial hook (Fig. 9 B). Transtilla and juxta strongly developed, transtilla in the shape of a three-leaf clover, with distal part shallowly bifid. Juxta with robust, club-like outgrowths (Fig. 9 D;E). Vesica produces from tip of aedeagus (Fig. 9 A). Aedeagus with rounded shape 2/3 from the base and at the base of the cylinder.

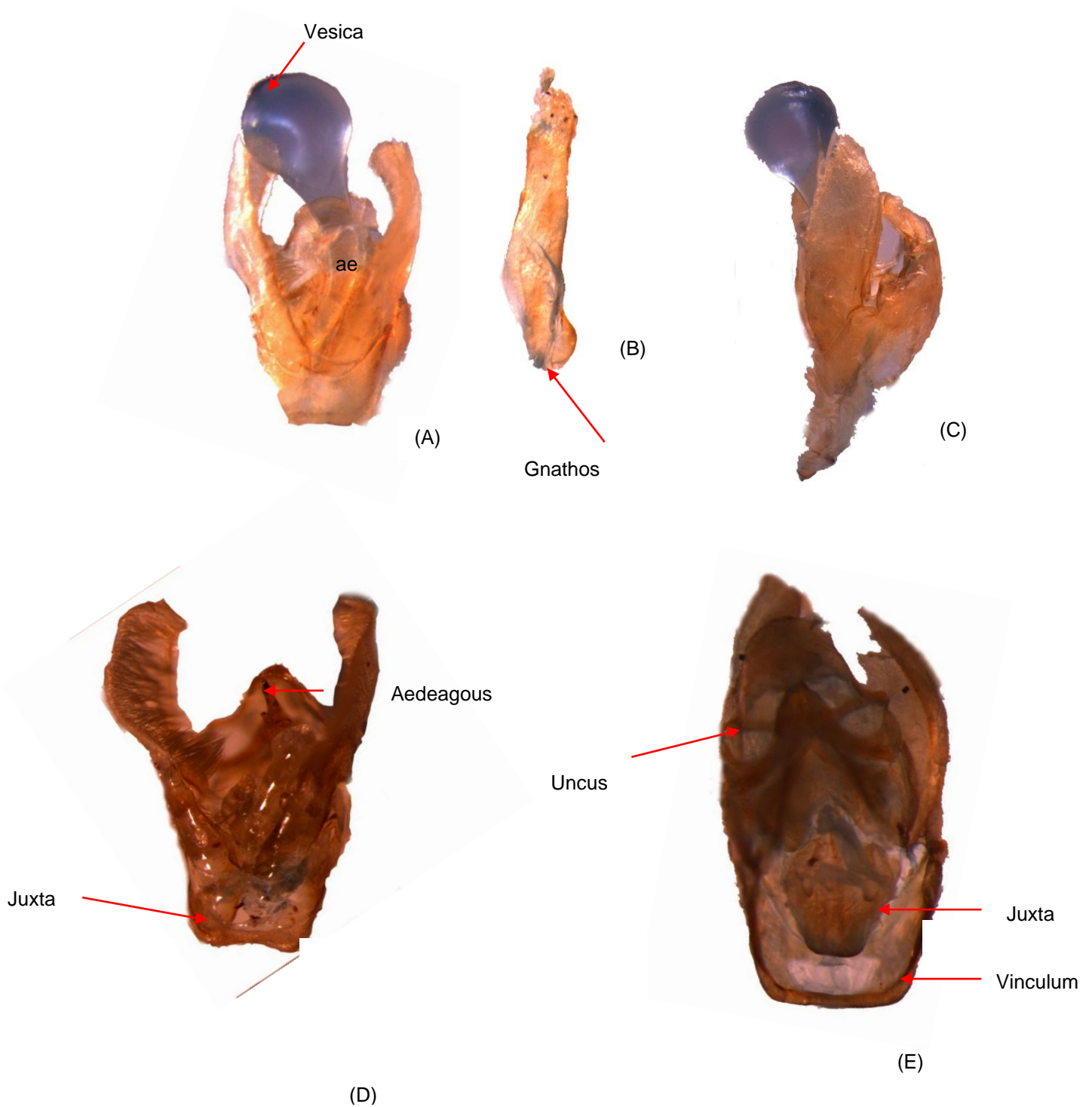


Figure 9: Male genitalia of the Carob moth. (A) Dorsal view of the genitalia highlighting the vesica (ae= aedeagus). (B) Shape and form of the gnathos. (C) Lateral view of the genitalia. (D) Dorsal view of the genitalia with emphasis on the juxta and aedeagus. (E) Dorsal view of the genitalia highlighting the juxta shape and the vinculum shape.

3.3.2.2.2 Female

Ductus bursae and corpus bursae are membranous and in the shape of an elongated conical flask. Signum is present in form of an elongate patch of scobinations or microspines (Fig. 10). Ductus seminalis arises from the juncture of the ductus bursae and corpus bursae. One pair of anterior and one pair of posterior apophysis present, anterior apophysis begins at the base of the posterior apophysis. Papillae anales with a distinct tip.

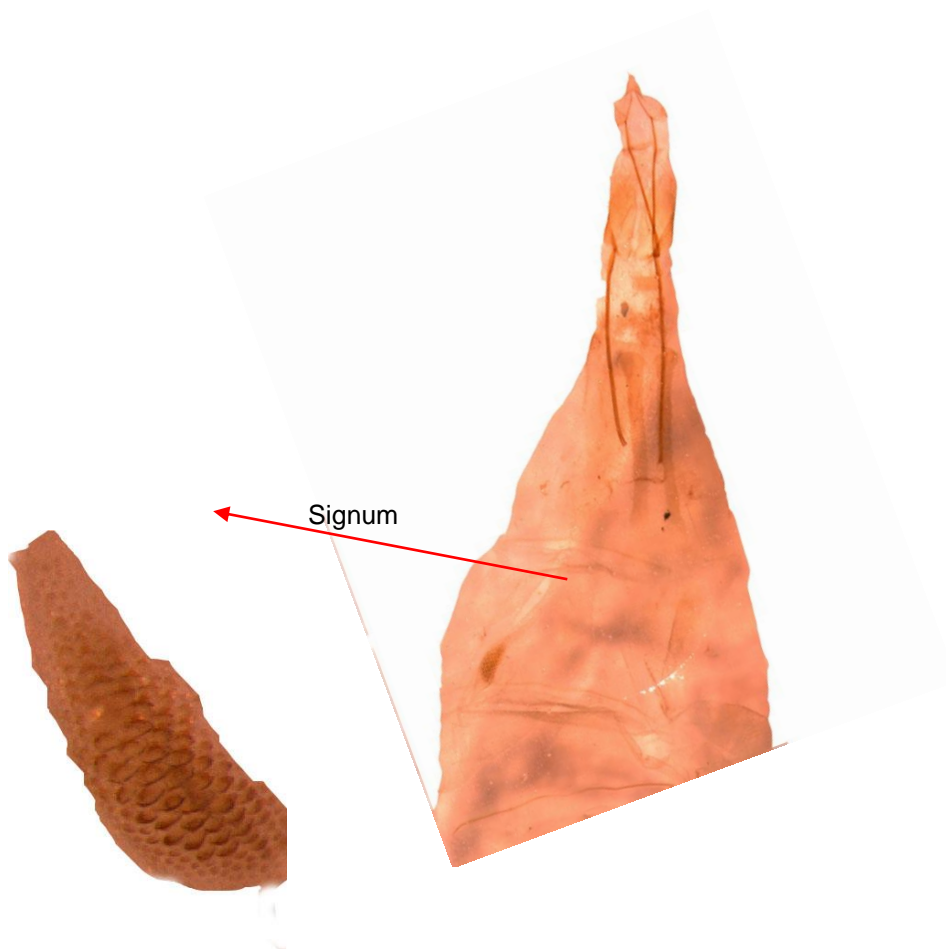


Figure 10: Female genitalia of the Carob moth, with emphasis on the shape of the signa and apophysis.

3.3.3 Molecular study



A molecular study was conducted with the aid of Dr A. Timm at Rhodes University. Samples from a few localities in the Western Cape were analyzed along with samples from Kenya and

Australia. The results of this study are attached to this thesis in Appendix 3, as it was not an integral part of this study.

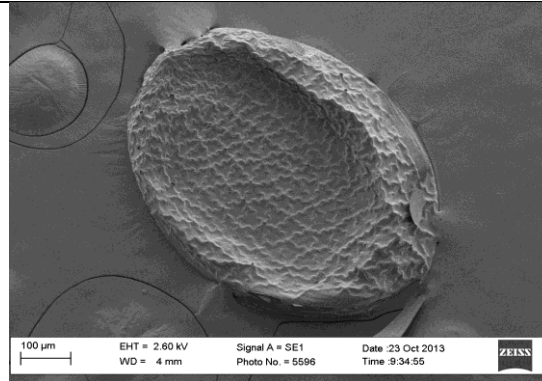
3.6 Key development

By virtue of the Carob moths morphology, the following key was designed to assist advisors and researchers in accurate separation of the two species, Carob moth and False Codling moth (Table 1).

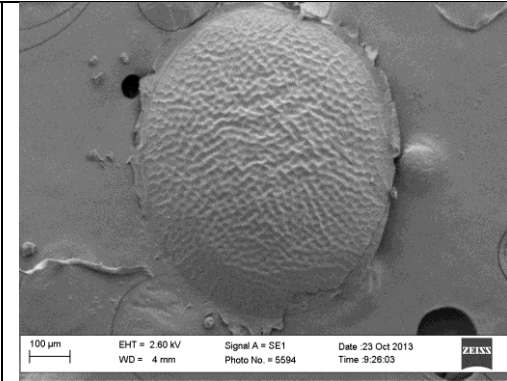
Table 1: A comparison of the most important differences between the Carob moth and False Codling moth in the adult, egg, larval and pupal stages of their respective life cycles.

Carob moth		False Codling moth	
Adult			
<ul style="list-style-type: none"> Wings held flat across the abdomen when in a restive position 	 <p style="text-align: right; font-size: small;">Dr. P. Addison</p>	<ul style="list-style-type: none"> Wings held roof-like across the abdomen when in a restive position ♂ with fluff on hind legs 	 <p style="text-align: right; font-size: small;">Dr. P. Addison</p>
Egg			

- Oval in shape
- Lattice pattern

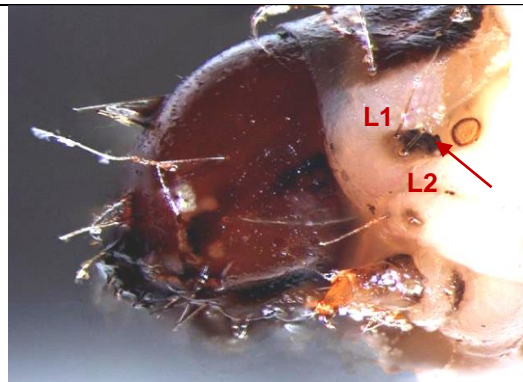


- Slightly oval in shape
- Stippled pattern



Larva

- A dark sclerotized patch on the T1 segment encompassing the L-group of seta
- Only two seta in the L-group on the T1 segment




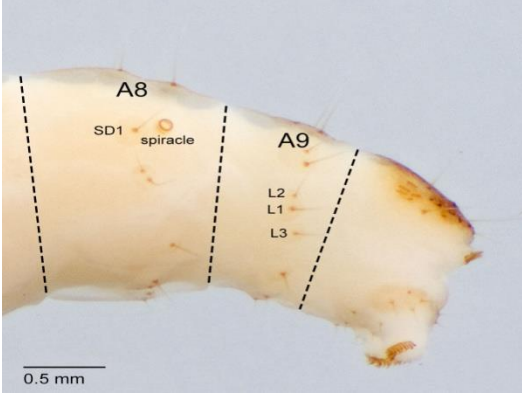




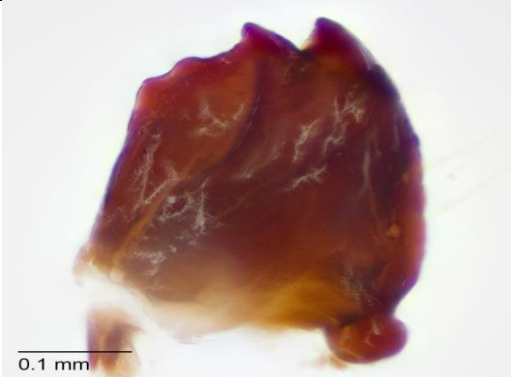

Monique Rentel 2013

- No sclerotized patch encompassing the L-group of seta
- Three seta in the L-group on the T1 segment



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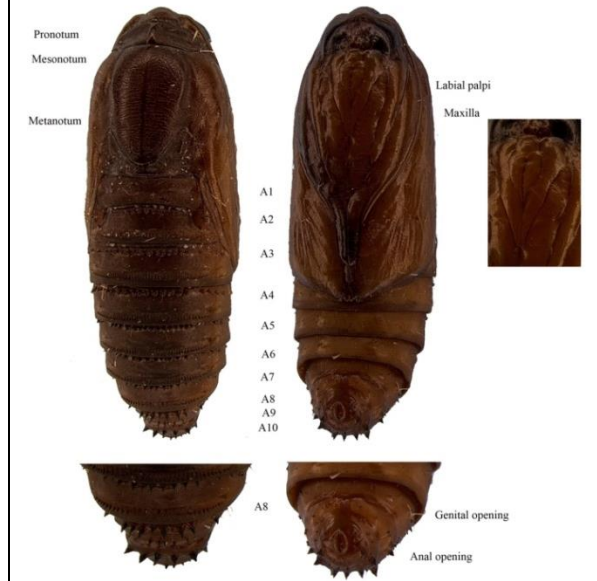
<ul style="list-style-type: none"> • No anal comb on segment A10 	 <p>Monique Rentel 2013</p>	<ul style="list-style-type: none"> • An anal comb is present on segment A10 	 <p>Monique Rentel 2013</p>
<ul style="list-style-type: none"> • There is a sclerotized ring around the SD1 seta on the A8 segment pinaculum • The SD1 seta is situated dorsal to spiracle segment A8 	 <p>0.5 mm http://idtools.org/id/leps/lepintercept/index.html</p>	<ul style="list-style-type: none"> • There is no sclerotized ring around the SD1 seta on the A8 segment • The SD1 seta is situated in line with and anterior to spiracle segment A8 	 <p>0.5 mm http://idtools.org/id/leps/lepintercept/index.html</p>

<ul style="list-style-type: none">• Crochets in the form of an irregular biordinal circle	 <p>0.1 mm http://idtools.org/id/leps/lepintercept/index.html</p>	<ul style="list-style-type: none">• Crochets in the form of an irregularly triordinal circle	 <p>0.1 mm http://idtools.org/id/leps/lepintercept/index.html</p>
<ul style="list-style-type: none">• Four mandibular teeth	 <p>0.1 mm http://idtools.org/id/leps/lepintercept/index.html</p>	<ul style="list-style-type: none">• Five mandibular teeth	 <p>0.1 mm http://idtools.org/id/leps/lepintercept/index.html</p>
<p style="text-align: center;">Pupa</p>			

- Thoracic spiracles present
- Gibba absent
- Cremastral setae slender and hooked with a slightly curved conical protuberance
- Lateral projections on the dorsal side of the notum



- Cremaster indistinct



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3.4 Discussion

3.4.1 Eggs

The eggs are ovoid in shape and are laid singly or in clumps of two to three eggs which concurs with the findings made by Honiball & Catling (1998). The eggs are also clearly marked with a lattice shape pattern, which covers the whole egg. Compared to the eggs of the False Codling moth (FCM), the FCM eggs are more rounded in shape and have a more stippled pattern, not reaching the edges of the egg. When the eggs are not fertilized, Carob moth eggs only collapse in the center and the edges stay intact, whereas FCM eggs flatten completely. When the eggs are fertilized their shape may differ to the above findings.

3.4.2 Larva

Larval descriptions of the Carob moth were only reported by four authors. Hasenfuss (1960) was the first to describe the larval structures of the Carob moth, thereafter followed Aitken (1963), Neunzig (1979) and a key was more recently produced by Solis (2006). The commonality in all these reports is the full sclerotized ring around the SD1 seta on the eighth abdominal segment, which was also found as a distinguishing character when compared to False Codling moth. There was an array of colour variations between larvae; some individuals displayed lighter coloured sclerotized markings than others.

As the Carob moth, in its larval stage, is of importance as a phytosanitary threat on citrus fruit, it is useful to compare it with other pests with which it is commonly confused, in South Africa this is mainly the False Codling moth. For comparison of the Carob moth to the False Codling moth a few more distinguishing larval characters need to be highlighted: firstly the sclerotized block surrounding the L1 and L2 seta on the T1 segment is only present on Carob moth larvae. Secondly the L-group in the Pyralidae only have two seta, whereas, the Tortricidae three seta constitute the L-group. The Carob moth does not have an anal comb, whereas the False Codling moth does. More characters can be seen in the comparison above (Table 1).

3.4.3 Pupa

Neunzig (1979) and Patočka & Turčani (2005) were the only authors to describe the pupal stage of the Carob moth. Both descriptions focused on the structure of the cremasta as being very diagnostic of the Carob moth. Neunzig (1979) also focused on the thoracic crest on the

dorsal side of the pupa. Both these structures were very prominent in the observations of the present study.

The major difference between the Carob moth and False Codling moth pupa is that the Carob moth has a distinct forked cremaster, whereas that of the FCM has no obvious cremaster structure (Patočka & Turčani 2005). The FCM pupa also has a greater number of spines on the dorsal side of the pupa and on the terminal end of the pupa, when compared to the Carob moth.

3.4.4 Adult

Wing venation in moths is characteristic at the generic level. In the case of the Carob moth, the most distinguishing feature, also reported by previous authors was that the M1 vein branches from near the anterior angle of the cell (Munroe & Solis 1998; Janse 1941; Heinrich 1956; Neunzig 1990). This is also evident in the findings above. What is novel to this study is the fact that this study described both the male and female wing venation, (which differs to some extent) whereas none of the previous authors indicated the sex of the wings. The findings here show that male and female Carob moth wing venation differs and this should be taken into consideration when identifying this genus.

The Carob moth has seen many name changes throughout its taxonomic history, which suggests that there must be a great variation within this group. Most of the descriptions were based on wing colouration and pattern variation, which clearly is an unsatisfactory form of species identification. A clear reference of a comparison of wing pattern descriptions from various authors is given in Appendix 2.

Genitalia descriptions are a much more useful taxonomic tool, as there are more tangible differences between genital depictions at a species level. The Carob moth genitalia were first depicted by Corbert and Tams in (1943), they however did not describe their illustrations. Heinrich (1956) gave reliable descriptions, making his study the basis of all further descriptions of the genitalia. Zimmerman (1958); Medvedev (1986) and Neunzig (1990) are the most recent authors to describe and depict the genitalia of the Carob moth. Most of the authors made

reference to the strong juxta, hooked gnathos and distinct transtilla shape when describing the male genitalia. Female genital descriptions all mentioned the elongated signum with the prominent scobinations. All these features are in line with the observations of this chapter.

3.4.5 Molecular study

The molecular study done by Dr Timm, found some interesting results in that the genera *Apomyelois* is a sister clade to *Ectomyelois*, and that the African samples all grouped together as a sister clade to most of the Australian samples. The Western Cape samples (Entsoc) didn't all group together; neither did the Vaalharts, Northern Cape (posted) samples. From the tree it can be deduced that there is a lot of variation within the Carob moth samples analysed.

3.5 Conclusion

The taxonomy of the Carob moth was very difficult to study. Firstly there were so many historical synonyms and the literature describing these different names was not easy to come by. Secondly, but most importantly, there is a lot of variation within the species which made the taxonomic task even greater. This finding was bolstered by the molecular study's findings in Appendix 3. It is suggested that a detailed molecular study should be undertaken to establish the population genetic structure of the Carob moth.

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Chapter 4

Monitoring seasonal history and damage potential of the Carob moth using commercial pheromone lures relative to False Codling moth

4.1 Introduction

The Carob moth (*Ectomyelois ceratoniae*) is widely distributed, occurring on almost all of the continents, where weather conditions permit the adequate growth of their preferred host plants (Table 1 in Chapter 1). Adequate weather conditions include areas where the summer climate is hot and dry, for example Iran, Israel and all Mediterranean areas (Aitken 1963; Gothilf 1984). It is a phytosanitary pest of a variety of fruit crops as well as of stored food products, including carob pods, used as animal fodder, acorns, macadamia nuts, rotting and dry fruit of wild trees, including *Ximenia caffra* and *Bequaertiodendron magalismontanum* (Table 2 in Chapter 1) (Catling 1979; de Villiers 2001). The larvae are concealed feeders, making them difficult to detect, identify and control (Scoble 1992; Mediouni & Dhouibi 2007).

The female moth deposits her eggs in already split fruits or on the skin of the fruit. When emerging, the larvae enter the fruit through open cracks or through holes made previously by other insects (Neunzig 1979). Fully grown larvae either exit the fruit to pupate in the soil or pupate within the fruit itself.

Carob moth larvae are often confused with those of the False Codling moth (FCM) *Thaumatotibia (Cryptophlebia) leucotreta* (Meyrick) (Honiball & Catling 1998). They are both pink in colour and often found within the same type of fruit, but have distinct morphological differences that can be used to distinguish between them. There are also differences in their mode of feeding: larvae of the Carob moth bore into the rind and albedo of fruit and do not penetrate far into the flesh, whereas larvae of the False Codling moth penetrate the flesh of the fruit (Honiball & Catling 1998).

Both species cause economic damage and the occurrence of either on crops is cause for concern to the producer, especially when the crop is destined for the export market as both species being quarantine pests. The greater incidence of Carob moth in citrus orchards could be due to the increased use of synthetic pyrethroids and insect growth regulators used for the control of the citrus thrips, *Scirtothrips aurantii* Faure, and other armoured scale pests (Honiball & Catling 1998). The use of these pesticides can upset the natural balance in an orchard, resulting in outbreaks of mealybug, *Planacoccus citri* Risso, Australian bug, *Icerya purchasi* Maskell, and citrus wax scale, *Ceroplastes brevicauda* (Hall). These sap-sucking insects produce honeydew, which in turn, leads to the development of sooty mold, which attracts greater numbers of Carob moth into the system (Honiball & Catling 1998).

FCM is an indigenous insect which has been reported from many parts of Africa. It is a pest on many indigenous plants as well as cultivated crops (Hofmeyr & Burger 1995). The use of synthetic sex attractants as well as controlled release pheromone dispensers (septums impregnated with pheromone, for purposes of trapping), have been investigated for more effective management of False Codling moth (Hofmeyr & Calitz 1991; Hofmeyr & Burger 1995). No control measures targeting specifically Carob moth are yet available (registered) in South Africa, and control of this pest therefore appears to take place via already existing spray programmes for other pests. In South Africa there are two pheromone lures available for Carob moth, and by comparing their effectiveness in different areas a lure preference can be determined and then suggestions can be made to producers as to which lures are most adequate for a Carob moth monitoring program.

The Carob moth as an insect of concern on citrus was recorded for the first time in South Africa in 1974 when found emerging from ripening navel oranges in the Clanwilliam-Citrusdal region (Honiball & Catling 1998). The biology of the Carob moth in South Africa has not been investigated in detail as yet (Honiball & Catling 1998).

The synthesis of a synthetic sex attractant for attracting male Carob moth was actively studied (Baker *et al.* 1989; Baker *et al.* 1991; Todd *et al.* 1992). Commercial sex pheromone lures for

Carob moth and False Codling moth, are available and are considered suitable to assess the required outcomes (Su *et al.* 2004). Newton & Mastro (1989) evaluated many pheromone trap types for purposes of assessing population size, in a range of colours, and found that the triangular yellow delta trap caught consistently more male moths, however the trap colour is not important.

The present investigation aims to contribute to a better understanding of the Carob moth's biology, population changes and seasonal life history as well as the extent of damage caused to the citrus industry.

4.2 Material and methods

For this study three blocks, each 1 ha in extent, were selected in two separate citrus growing regions, Citrusdal area (S32°35.700'; E018°59.300') and the Robertson/Bonnievale area (S33° 48' 7.43"; E19° 53' 15.15")/ (33° 57' 8.39"S;20° 8' 45.69"E), Western Cape, South Africa (Fig. 1). The areas are about 204km apart and in different geographical settings. Blocks were chosen within citrus orchards in close proximity to known alternative hosts of the Carob moth, for example, oak trees and pomegranate orchards. Both areas apply control measures for False Codling moth. In Citrusdal the producers have access to an SIT program, where sterile moths are released into the orchards to curb the population growth of the wild FCM. In Robertson/Bonnievale the producers make use of pheromone mating disruption to curb their FCM population growth. Both areas supplement with synthetic chemical pesticides.

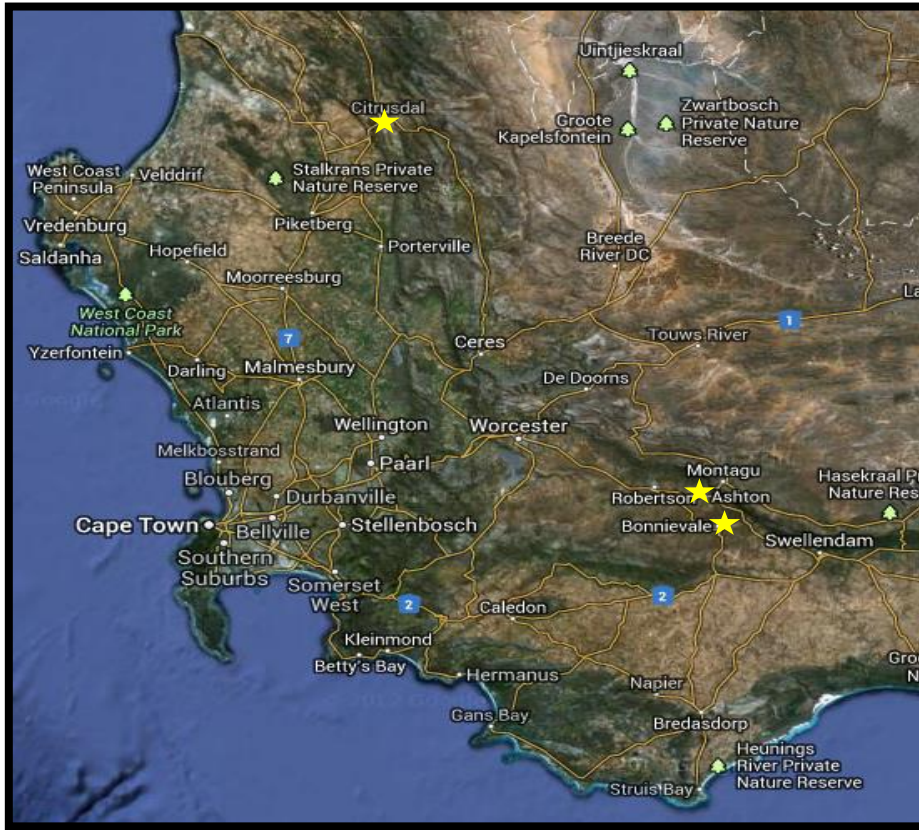


Figure 1: Distribution map of sites within the Western Cape.

4.2.1 Seasonal cycle study

Different commercially available sex pheromone traps were used in this study. Insect Science® Carob lure, containing 7,9,11-dodecatrien-1-ol, formate, (7Z,9E) for attracting males of the Carob moth and Lorelei®, produced by Hendrik Hofmeyr, containing (E)-7-dodecenyl acetate/ (E)-8- dodecenyl acetate/ (Z)-8- dodecenyl acetate, designed for attracting males of the False Codling moth, were used. Two Chempac® yellow delta traps/block were used for trapping because they are highly visible and very simple to use (Chempac (Pty) Ltd., Simondium, South Africa). Traps were suspended in each experimental block (Fig. 2). One trap contained the Insect Science® Carob moth lure, suspended in a septum and stuck to a Chempac® sticky pad. The other trap contained a Lorelei® pheromone dispenser which is a pheromone attractant for FCM, was attached to the inner roof side of the delta trap; this trap also contained a Chempac® sticky pad. Traps were spaced approximately 100 m apart in each block and hung approximately two meters above the ground, in the outer canopy of the tree, so as to be visible and to allow air to flow freely through the trap (Moore 2011). The traps were

hung on the southern side of the tree and orientated in a north-south direction and monitored for 18 months, starting in October 2012 and ending in June 2014. When traps were counted for FCM, sterile moths and wild moths were counted separately. They could be distinguished by the colour of their abdomens. The abdomens of sterile moths when crushed were pink in colour because of the diet they are fed on as larvae contains calco red dye.

Trapping for 18 months constituted two full growing seasons, as well as the six month period in austral winter, when there was either no, or only a few immature fruits present in the orchards. The data was then averaged for each area for further statistical analysis.



Figure 2: Yellow delta trap containing a sticky pad, and baited with moth attracting lure.

The Insect Science® Carob moth lure traps were monitored every two weeks, and the lure was replaced every four weeks during the period October 2012 to June 2013. The Lorelei® FCM lure was also monitored every two weeks, but replaced every six months according to the instructions pertaining to the use of the lure, for the same period. Due to the end of the fruiting season in both regions, traps were monitored subsequent to June 2013 once every four weeks. From October 2013, the fruiting season started again. Due to the low numbers of moth catches recorded in the first season, it was decided to continue monitoring the traps only once every four weeks for the period October 2013 to June 2014. The Chempac® sticky pads were

replaced as needed, particularly in the case of insect or debris catch which rendered the sticky pads less effective.

In addition to the above mentioned monitoring study a list of Carob moths already preserved in the Stellenbosch University museum collection was compiled to observe seasonal patterns. The list collated the dates of when the moths were found or reared from host plants as well as the areas in which the moths were collected.

4.2.2 Alternative host sampling

Alternative hosts such as acorns and pomegranates were sampled on an *ad hoc* basis once a month in both the Citrusdal and Robertson/Bonnievale areas and alternative sites such as Riebeek kastel and Stellenbosch (Western Cape). Damaged pomegranites were collected from the orchard floor and acorns collected from the ground were collected in 2l containers. The fruits were incubated in the laboratory at 25°C with a 12:12 light: dark photoperiod.

4.2.3 Lure comparison

From March 2014 to June 2014 a concurrent study was undertaken to detect whether significant differences in attractiveness between Insect Science® Carob moth lure (Insect Science, Tzaneen) and Chempac® Carob moth lure existed. An additional trap to the already existing seasonal cycle trap was hung in an adjacent citrus orchard block and baited with the Chempac® Carob moth lure. These traps were monitored every four weeks and the lures were alternated between the two Carob moth trap positions on each assessment so as to cancel out the effect of a positional effect on the number of male Carob moths caught in the traps.

4.2.4 Damage assessment

Concurrently to the above experiments, a damage assessment was conducted in the same 1 ha blocks. Once a month, from January 2013 to June 2013, 36 randomly chosen citrus trees were monitored in each block. On six trees in six rows, five randomly selected fruit were assessed for damage, making a total of 180 fruit inspected per block/month, with a total of six blocks inspected each month. If the fruit was found to show any sign of damage, it was collected and incubated in the laboratory at 25°C with a 12:12 light: dark photoperiod. Fruit was also collected from orchard floors and from alternative hosts close by on an *ad hoc* basis

when available. Moths that emerged during the incubation period were collected, counted and identified.

4.2.5 Statistical analysis

The data for each area was pooled, and then the statistical differences were determined for each area. Statistical analysis for between farm variation was not possible because of low sample sizes. For the seasonal cycle data a one-way ANOVA with repeated measures was performed. This compared the 'between treatments' variation with the 'within treatment' variation to assess whether the means were due to chance or treatment effects (Clewer & Scarisbrick 2001). For the lure comparison experiment a two way mixed model of repeated measures (ANOVA) was performed. This analysis compares two independent variables which were the area and lure on their own and one dependant variable which was the relationship between the area and lure together (Clewer & Scarisbrick 2001). All statistical analyses were conducted in Statistica, version 12 (Statsoft Inc. 2013).

4.3 Results

4.3.1 Seasonal cycle

For the entire sampling period (two sampling seasons), Carob moth trap catches totaled 88 moths in Citrusdal and 94 moths in Robertson/Bonnievale (Table 1). The seasonal activity of the Carob moth and False Codling moth started from October within citrus orchards and then progressed with population peaks in January and again in March (Figs. 3 and 4). The trap catches remained constant until June at which time the traps did not catch any more moths. The seasonal activity of the False Codling moth within the same citrus orchards mirrors that of the Carob moth.

The results show that there were no significant differences in Carob moth catches between the two study sites ($F_{(1,4)}=0.00661$; $P=0.94$,) (Fig. 5). Similarly, no significant differences in wild FCM catch between the two study sites (Fig. 6) could be detected ($F_{(1, 4)}=1.2960$, $P=0.32$).

A study of the museum collection at Stellenbosch University led to a greater understanding of when Carob moth is most prevalent in the Western Cape (Table 2). Most of the specimens were caught in the November 2009 period.

4.3.2 Alternative host sampling

No Carob moth was obtained from incubated pomegranites or acorns. The only insects emerging from the acorns were Nitidulidae beetles.

4.3.3 Lure comparison

The attractiveness of the Insect Science® lure and that of the Chempac® lure showed no significant difference in Carob moth trap catches between study areas ($n=36$, $P=0.54085$, $F=0.44599$). There was however a significant difference between the two lures tested ($n=36$, $P=0.007182$, $F= 25.6000$), with the Insect Science lure catching more moths. When the lures and areas were compared it was found that lure efficiency was dependent on area and that the Insect Science® lure was more efficient in the Citrusdal area when compared to the Chempac® lure ($n=36$, $P= 0.003198$, $F= 40.0000$) (Fig. 7).

4.3.4 Damage Assessment

In the laboratory, Carob moths only emerged in the first sampling season from damaged citrus fruit from the Citrusdal study sites. From a total of 80 damaged fruits collected, only three Carob moths emerged in the first season (Table 4).

False Codling moth was more prolific in its emergence from the damaged fruit from both study sites (Table 7). From the same 80 fruits collected in the Citrusdal area, only four False Codling moths emerged from the damaged fruit. In the Robertson/Bonnievale area in the first season 69 damaged fruits were collected yielding five False Codling moths from the damaged fruit. In the second sampling season, no False Codling moths were collected in the Citrusdal area, whereas from the 89 damaged fruits collected in the Robertson/Bonnievale area 35 False Codling moths emerged.

Euzophera cullinanensis (Pyralidae) and *Lobesia vanillana* (Tortricidae) also emerged from the damaged fruit collected in this study. The *E. cullinanensis* was collected from damaged citrus fruit collected in the Robertson/Bonnievale area in the first season of this study, after which it was identified by Prof H. Geerstema. The *L. vanillana* emerged from damaged fruit also collected in the Robertson/Bonnievale area. This moth was attracted to the Carob moth

traps on one of the sites in Bonnievale. It was identified through DNA analysis as *L. vanillana* by Dr A. Timm, Rhodes University.

Table 1: A summary of the total amounts of Carob moth, False Codling moth steriles and False Codling moth wild trap catches for each farm in both Citrusdal and Robertson/Bonnievale.

Area	Farm	Nr. Of Carob moth	Nr. Of FCM sterile moths	Nr. Of FCM wild moths
Citrusdal	1	54	305	1
Citrusdal	2	23	1223	49
Citrusdal	3	11	863	34
TOTAL/area		88	2391	84
Robertson/Bonnievale	1	73	0	24
Robertson/Bonnievale	2	15	0	3
Robertson/Bonnievale	3	6	0	3
TOTAL/area		94	0	30

Table 2: A summary of the details concerning the Carob moth specimens preserved in the Stellenbosch University Entomology museum.

Date Collected	Date Emerged	Nr. Specimens	Locality	Host Plant
January 1962		1	Pretoria	
March 2006		6	Citrusdal	
July 2008		1	Ceres	Peach galls
March 2009		1		Acorns
April 2009		1	Bien Donne	Olives
May 2009		1		Acorns
July 2009		3	Riebeek kasteel	Acorns
October 2009	November 2009	1	Somerset West	Port Jackson Galls
November 2009	November 2009	2	Spier	Port Jackson Galls
November 2009	December 2009	1	Bien Donne	Port Jackson Galls
November 2009	December 2009	3	Bien Donne	Port Jackson Galls
November 2009	November 2009	1	Spier	Port Jackson Galls
November 2009		4	Western Cape	
November 2009	December 2009	1	Spier	Port Jackson Galls
November 2009		3	Western Cape	
November 2009	November 2009	3	Spier	Port Jackson Galls
November 2009		2	Western Cape	
December 2009		1	Western Cape	
December 2009		1	Riebeek kasteel	
December 2009		1	Western Cape	
September 2012	October 2012	3	Swellendam	<i>Pycnantha</i>
June 2013		1	Vaalharts	Pecan nuts

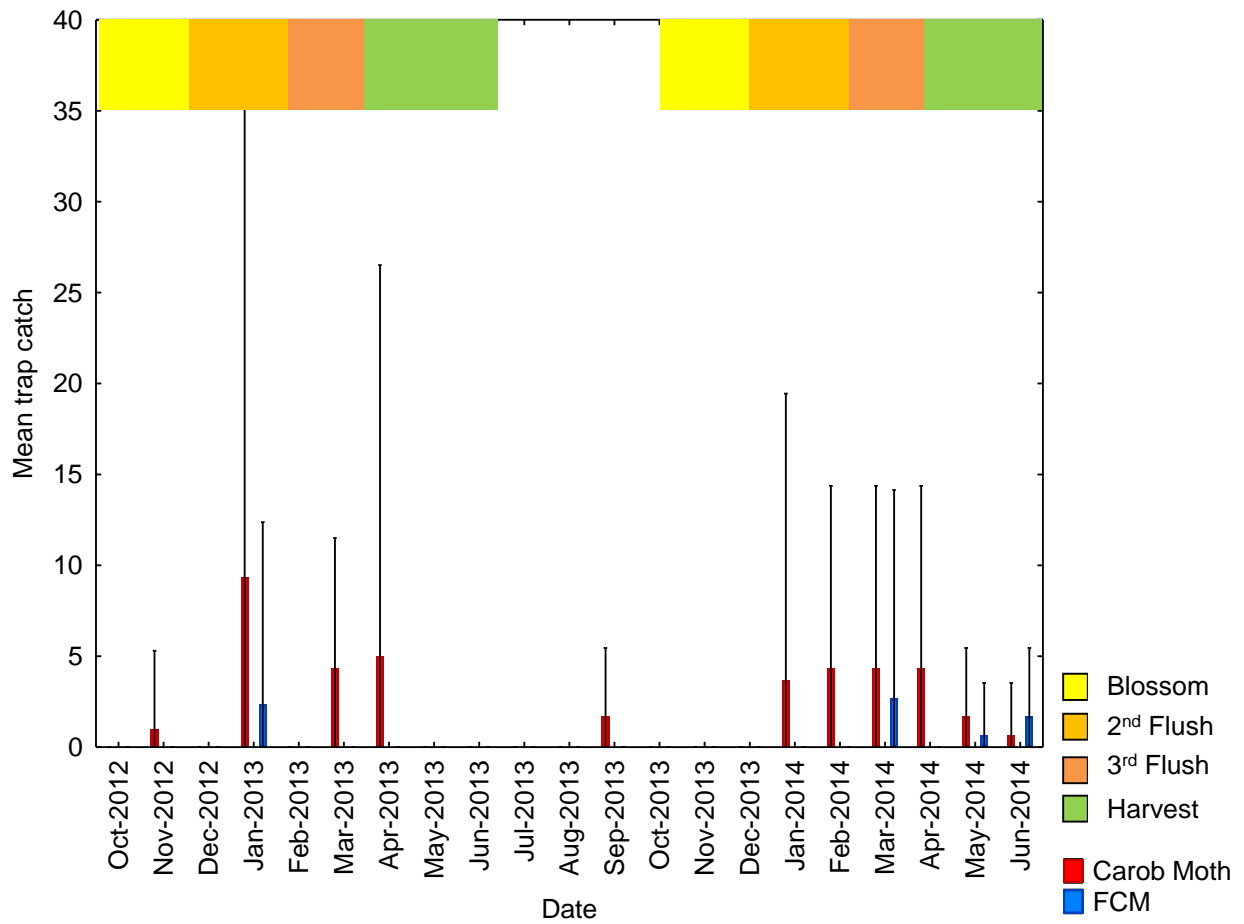


Figure 3: Total trap catches of *Ectomyelois ceratoniae* (red) and *Thaumatotibia leucotreta* (blue) in the Robertson/Bonnievale area for the period October 2012 to June 2014, using pheromone-baited delta traps. Error bars denote the 95% confidence levels.

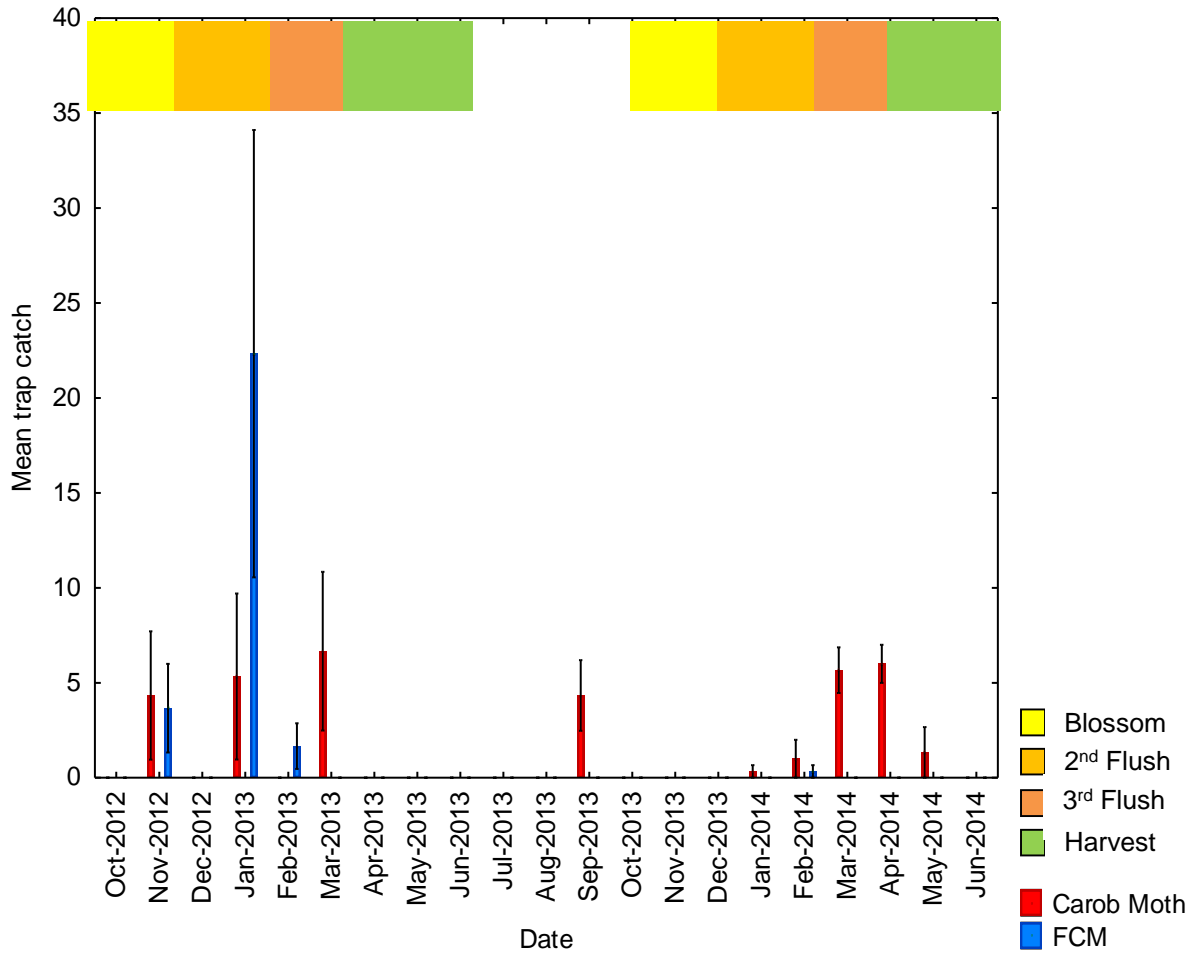


Figure 4: Total trap catches of *Ectomyelois ceratoniae* (red) and *Thaumatotibia leucotreta* (blue) in the Citrusdal area for the period October 2012 to June 2014, using pheromone-baited delta traps. Error bars denote the 95% confidence level.

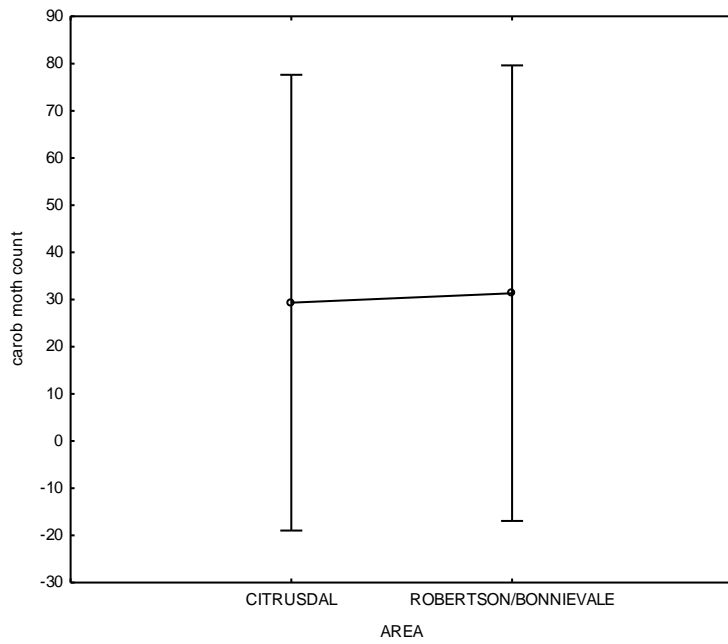


Figure 5: Average number of Carob moths, *Ectomyelois ceratoniae*, caught in the Citrusdal and Robertson/Bonnievale area from October 2012 to June 2014. Error bars denote a 95% confidence levels.

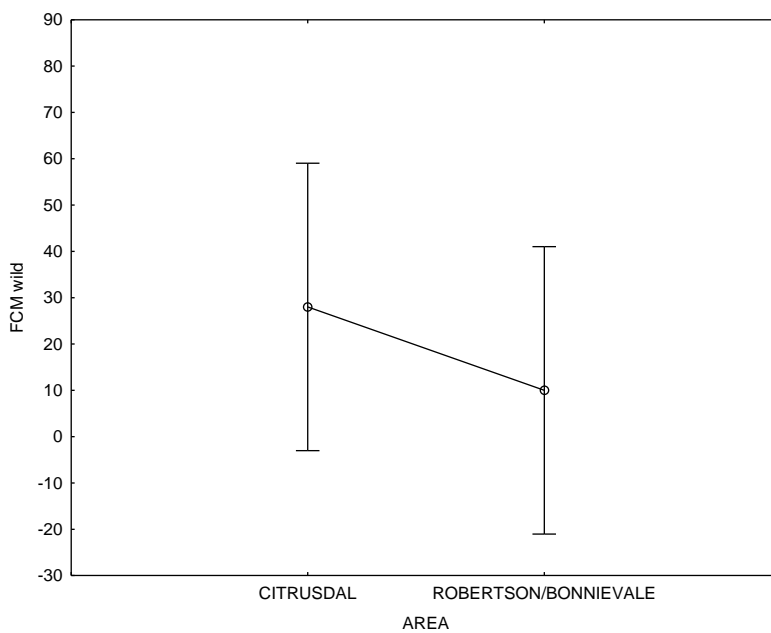


Figure 6: Average number of wild False Codling moth, *Thaumatotibia leucotreta*, caught in the Citrusdal and Robertson/Bonnievale area from October 2012 to June 2014. Error bars denote a 95% confidence levels.

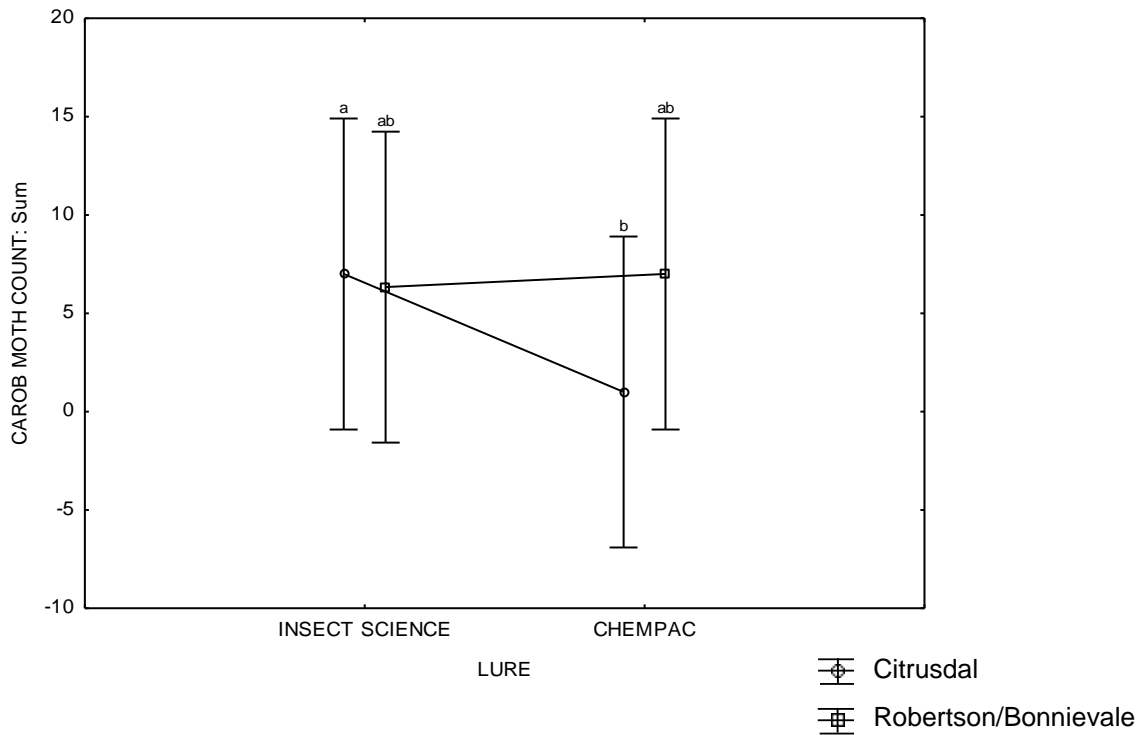


Figure 7: A comparison of Insect Science lure Carob moth and Chempac Carob moth lure efficacy in both the Citrusdal and Robertson/Bonnievale areas. Error bars denote a 95% confidence levels (a and b denotes a significant difference between lures whereas ab denotes no difference).

Table 3: The percentage of moth emergence from damaged citrus fruit collected in the Citrusdal area for the period of February 2013 to April 2014.

	<i>Nr of fruits collected</i>	<i>Thaumatotibia leucotreta</i> %	<i>Ectomyelois ceratoniae</i> %	<i>Euzophera cullinanensis</i> %	<i>Lobesia vanillana</i> %
Feb-13	42	2.38	2.38	0	0
Mar-13	16	6.25	6.25	0	0
Apr-13	22	13.64	4.55	0	0
May-13	26	0	0	0	0
Jun-13	10	0	0	0	0
Feb-14	18	0	0	0	0
Mar-14	11	0	0	0	0
Apr-14	10	0	0	0	0

Table 4: The percentage of moth emergence from damaged citrus fruit collected in the Robertson/Bonnavale area for the period of February 2013 to April 2014.

	<i>Nr of fruits collected</i>	<i>Thaumatotibia leucotreta</i> %	<i>Ectomyelois ceratoniae</i> %	<i>Euzophera cullinanensis</i> %	<i>Lobesia vanillana</i> %
Feb-13	61	3.29	0	0	0
Mar-13	8	12.50	0	0	0
Apr-13	0	25.00	0	50.00	0
May-13	0	0	0	0	0
Jun-13	0	0	0	0	0
Feb-14	28	42.86	0	0	3.57
Mar-14	54	40.74	0	0	1.85
Apr-14	7	14.29	0	0	0

4.4 Discussion

Cumulative trap catches of male Carob moth and False Codling moth individuals showed a considerable amount of variation in population levels between farms. This could be attributed to the different micro habitats on each of the farms, management methods, as well as the differences between fruit cultivars on each of the farms. Naval orange varieties are more susceptible to FCM damage than the Valencia orange and Mandarin varieties (Schwartz 1981) as the naval fruit provides a protected site for egg laying. When the larvae hatch, they bore readily through the rind of the fruit (Bijzet 2006; Newton 1990).

There was also a large variety of alternative crops surrounding the orchards in which the sampling was done. For example, the Robertson Mandarin orchard was surrounded by peach orchards, which could be a determining factor for the above average Carob moth catch in that area (personal observation). It has been found that when border vegetation is botanically related to adjacent crop plants, there is a greater danger that it can serve as a potential source of infestation by injurious insects (Dambach 1964). The abundance and diversity of entomophagous insects within a system is closely related to the vegetation surrounding that system (Altieri & Letourneau 1982).

The total trap catches of both the Carob moth and wild False Codling moth in the Citrusdal area is very similar: 88 Carob moth and 84 FCM. It has to be remembered that the producers in this area practice SIT for the control of FCM. So when looking at the numbers, we can determine that Carob moth is not a major threat in this area, because its numbers are on a par with the strictly controlled FCM pest. In the Robertson/Bonnievale area the total Carob moth and wild False Codling moth counts are different. The Carob moth total is 94 and the FCM total is 30. In this area the producers make use of mating disruption pheromones for FCM control. Although Carob moth numbers are higher than the FCM numbers they are still on a par with the total trap catches in the Citrusdal area, which also seems to indicate that the Carob moth isn't a major pest in the Western Cape. The current economic threshold for FCM is 10 males/trap/week, using a pheromone monitoring system (Hofmeyer 1998), indicating that the

management methods were sufficient for FCM except for one high trap catch in Citrusdal during January 2013.

Both Carob moth and FCM seasonal cycles within citrus orchards show a strong relationship to the phenology of the citrus tree and the prevailing weather patterns. When the tree starts to bud in October, moth activity starts to increase. This is when they lay their eggs close to the flowers. In December-January, there is a peak in moth trap catch, indicating that the first generation has been completed. The second peak is in March-April, indicating the completion of the second generation. In May, weather starts to cool, so trap catch starts to decrease. The fruit is then picked in May- June, and all moth activity ceases for the dormant period of the citrus tree. The life cycle of the Carob moth has not been studied in South Africa, but in Israel it was found to have a 6 week to 5 month generation period, depending on the season (de Villiers 2001). These findings correlate well with the data collected for the seasonal cycle study. Stotter (2009), in his study based in the Citrusdal area, found Carob moth to be the main larvae infesting Acorns when the fruits were mature and had fallen to the floor (August 2007). He also found that FCM were the main larvae attacking Acorn fruits that were still unripe and in the tree (April 2007). This also correlates with our seasonal cycle data, because when the Carob moth pupate from the Acorns the adults would emerge around October (Stotter 2009), which is when the first Carob moths were recorded in the area in the current study.

It is interesting to note that when control measures such as SIT and mating disruption are applied in an area it is expected that trap catches should approach zero. In this study however we recorded 24 FCM in the Citrusdal area and 30 FCM in the Robertson/Bonnievale area.

The dates collated from the Stellenbosch University collection revealed that November and December are the months where the most Carob moth captures or rearing occurred. This trend closely follows the seasonal cycle results, showing a peak in moth trap catch from November to January. It is also interesting to note that the first Carob moth recorded in the collection was in 1965, and yet the first published occurrence of the moth came nine years later in 1974

(Honnibal & Catling 1998). Therefore it appears that the Carob moth has been present in South Africa for a longer time.

Although there was large geographical and ecological variation between farms, there was no statistical difference between Carob moth and FCM trap catches between sample areas. Both areas have their own technique for controlling FCM on an area-wide scale. In Citrusdal, Sterile Insect Technique (SIT) has been practiced since November 2007. In the Robertson/Bonnievale area the farmers practice FCM mating disruption using Isomate® (John Lerm, personal communication). Both control methods as well as a regime of orchard sanitation are used in conjunction with a spray program by each farmer. Because Carob moth is a minor pest, insecticide control is not warranted, therefore by controlling the FCM population in an area the farmers are inadvertently controlling the Carob moth population in that area as well (Honiball & Catling 1998).

A comparison of Chempac® and Insect Science® lures in both the Citrusdal and Robertson/Bonnievale area revealed that lure efficacy was area dependent. In the Robertson/Bonnievale area both lures were equally efficient in their ability to attract the Carob moth. In Citrusdal however, the Insect Science® lure was more efficient at trapping Carob moth than the Chempac® lure. This could be due to environmental differences between the areas. Citrusdal is located in a valley between two mountain ranges, whereas the Robertson/Bonnievale sites are on a flatter plain, and not so secluded. The farmers in both areas also practice different spray regimes, which directly influences the number of moth trap catches. It could be that, based on the huge amount of statistical variation obtained from these trials, that a larger trial is needed, spanning over a longer time period.

One hundred and sixty five damaged fruits were collected throughout the two seasons. Most of the damage could be attributed to fruit splitting. Fruit splitting is a physiological disorder, which occurs because the rind of the fruit doesn't expand fast enough to compensate for the rapidly expanding pulp of the fruit (Garcia-Luis *et al.* 1994). No fruit was collected where there was a clear sign of larval entry through a hole in the rind. Therefore, moths that were collected

in the study area could have been from the moths laying their eggs in already split fruit. There was very low moth emergence during the second season compared to damaged fruit collected in the first season, which indicates that pest management in both areas was relatively good. In the second season, however, there was a surge of FCM emergence in the Bonnievale area. A conversation with a pomegranate farmer in the area, who also experienced a problem with FCM, indicated that the farmers there may have missed crucial sprays which lead to the outbreak. This outbreak had a severe effect on the pomegranate farmers in the area, to the extent that some of the fruit had up to eight FCM larvae in one fruit (personal observation).

The *Lobesia vanillana* (Lepidoptera: Tortricidae) collected in the Bonnievale area was a novel catch, which only seems to have arisen within the last two sampling seasons. Little is known about this moth except for its recorded geographical distribution, including Reunion, Cosmelego, Aldabra, Madagascar and Kenya (Razowski & Wojtusiak 2012; Brown et al. 2014).

The *Euzophera cullinanensis* was also collected in the Robertson/Bonnievale area from damaged citrus fruit. It is of the Pyralidae family and was first described by Balinsky in 1991. Very little is known about this moth, except that it has been recorded as a gall inhabiting moth (Rösch et al. 2001).

It would be recommended to continue monitoring for these two species of moth, in order to establish their future pest status in deciduous fruits and citrus orchards of the Western Cape Province. In future more emphasis should also be placed on the collection of alternative host plants in study areas.

4.5 Conclusion

The low Carob moth trap catch with regard to the strictly controlled False Codling moth trap catch in both study areas is indicative of the Carob moth not being a major pest within the Western Cape area, based on two years of field studies. If FCM is under control in an area, Carob moth populations could inadvertently also be controlled at the same time. However, as pesticides are lost for use by farmers due to residue issues, it is possible that this scenario

could change in future and continued monitoring is important. A species-specific economic threshold could not be determined during the current study due to too many zero trap catches occurring in the data set. This could be revisited should the abundance of this pest increase in future.

The differences in lure attractiveness should be studied further and over a longer time period than in the present study.

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Chapter 5

Discussion and conclusion

The aim of this study was to contribute towards the management of Carob moth (*Ectomyelois ceratoniae*), which is a known threat to the fruit producing industry in South Africa, specifically the citrus industry. It was aimed, firstly, to concisely order and clarify previous studies on all the synonyms of the Carob moth. The second objective was to clarify the morphology of the Carob moth based on own morphological descriptions, to aid in the identification of the moth in all its life stages, and the final objective was to establish a seasonal cycle for the moth within citrus orchards, to aid in control mechanisms. A synopsis of these objectives is outlined below.

Carob moth taxonomy has a very prolific history, with the moths having many generic and specific names throughout its nomenclatorial history. The synonyms recorded for the Carob moth were all based on wing colouration, which is a fickle trait to base any significant description on. Therefore this study endeavored to verify all known synonyms of the Carob moth, by collecting and ordering all the original literature into a unified body of work. This study highlighted all the distinguishing characters that proved invaluable in correct identification of the Carob moth. These included the wing venation, which was useful in identification and generic placement of the Carob moth, and the genitalia descriptions which were useful in identifying the Carob moth down to a specific level.

A detailed morphological study is the keystone to the correct identification of any species. Specific to this study was the fact that the Carob moth and the False Codling moth (*Thaumatotibia (Cryptophlebia) leucotreta*) both infest the same host plant, and therefore a key had to be developed to distinguish the two species, particularly in their larval form. By drawing on previously published literature and the addition of more detailed studies, this study aided in producing a user-friendly key to aid in species identification between the two main phytosanitary pests threatening citrus production in the Western Cape of South Africa.

Identification can easily be done for the final instar larvae, pupae and adult life stages using the provided key and a simple 10x magnifying hand lens. Neunzig (1979 and 1990), was the most accurate source cited for the descriptions of the larva, pupa and adult Carob moth life stages, and most closely corresponded to my own taxonomic analysis.

All authors that described the larval form of the Carob moth made reference to the sclerotized ring around the pinacula on the A8 segment (Neunzig 1979; Hasenfuss 1960; Aitken 1963 and Solis 2006). This was also a distinguishing feature found in my study, along with the sclerotized patch on the T1 segment and the lack of an anal comb.

The pupal descriptions made by Patočka & Turčáni (2005) and Neunzig (1979) highlighted the trait of a prominent hooked cremaster as their distinguishing feature for identifying the Carob moth pupa. This was also a prominent feature in my findings along with the lateral projections on the dorsal side of the notum.

The adult was described by many authors mainly based on wing colouration and pattern, it was however found that to describe the Carob moth down to species level, genitalia descriptions are a more accurate tool. Heinrich (1956) and Neunzig (1990) presented the most accurate description of the genitalia features present on the Carob moth. They both found the structure of the juxta to be a prominent feature along with the transtilla shape, as well as the elongated signa in the female moths. By studying the available material, this study was able to verify these structures as being the most distinguishing characters of the Carob moth adult. When looking at the Tortricidae, other features are used to identify the moths down to species level, for the male moths these include the shape of the valvae, and the absence or presence of the uncus. The most important feature in the female moths is the shape of the corpus bursae and the presence of thorn-like spines is a distinguishing character for this family (Rentel 2012)

An assessment of the pest status through seasonal sampling using pheromone traps and damage assessments revealed relatively low abundance of this pest, in comparison to False Codling moth, if taking species-specific management actions targeting False Codling moth into account. Fruit infestations were comparable in the Citrusdal area, while no fruit infestations

were recorded in the Bonnievale/Robertson area, suggesting that other hosts, such as pomegranates and peaches are potentially more preferred hosts for Carob moth.

General control measures (insecticides) put in place by farmers to control False Codling moth could inadvertently also have had an effect on the presence of Carob moth in the areas, while FCM were controlled using mating disruption and sterile insect technique in addition to a spray regime.

Carob moth was found to be present within orchards throughout the growing season, and, following Stotter (2009), moths then most likely retreated to the alternative host plants when the months got cooler, either acorns in the Citrusdal area or as yet unknown alternate hosts in the Robertson/Bonnievale area or fallen fruits due to poor sanitation practices. Monitoring should continue using a pheromone based system. This monitoring is essential in determining whether the Carob moth numbers are increasing in a specific season and to determine whether control measures should be administered, although an economic threshold is not yet available and could not be determined from the data of this study. Further monitoring can also aid in determining a threshold for the Carob moth, which my study could not achieve because of the low moth abundances (many dates with zero moth catches) within the orchards.

More seasons of data are needed to verify the lure comparison results found in this chapter as well as to verify the sporadic nature of the Carob moth as a pest within citrus orchards, which has been reported by growers and technical advisors.

As verified in the Addenda attached to this thesis, rearing the Carob moth is extremely difficult (Addendum 1). Even when colonies were successfully started in South Africa, the colony only progressed to the third generation after which it collapsed. Therefore it is imperative to concentrate all efforts into finding out how to rear this species, so that the colony can be used to determine life history parameters for the Carob moth, which has not yet been determined. A colony could also be used in laboratory based chemical attractant tests for use in the development of monitoring systems.

Once a colony is available, the female chemical lure trial (Addendum 2), should be attempted again under laboratory conditions, to firstly determine if the formula actually will work and then secondly at what concentration it will be the most effective. A female lure would also be a valuable monitoring tool to have, to establish abundances and activity patterns of the entire population.

As for the field research aspect, I suggest monitoring be expanded to a country wide scale, and trying to establish which host plants are relevant for Carob moth in South Africa. This information should then be modelled based on climate patterns to determine why there is such a sporadic appearance of the moth within different areas. Further, there is a need to develop good management methods, as currently there is nothing registered against Carob moth. Harpaz & Wysoki (1984) have tested the use of *Bacillus thuringiensis* Berliner as a bio-control agent against Carob moth with a 95% success rate in killing fourth instar larvae. In Tunisia an SIT program to control the Carob moth infesting their pomegranate orchards is being initiated and this could be an option for South Africa if Carob moth becomes an increasing problem (Personal Communication with Mr Matthew Addison).

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Addendum 1

Attempts at breeding the Carob moth, for the purpose of establishing a colony

We tried to start a colony for the purpose of doing a life table study on the Carob moth. Infested Pecan nuts were received from the Vaalharts area of the Northern Cape, which yielded the adults that were used for the following attempts we made at starting a colony. None of the attempts were successful, as the females were never fertilized.



Attempt 1: Moths that emerged from the pecan nuts were collected and released under the dome. They were provided with wax paper as an oviposition source, and a cotton ball soaked in water as a water source. They were placed at a window, to simulate natural light.



Attempt 2: A clean glass honey jar was prepared with a folded piece of rough tissue paper as an oviposition source. A moist cotton ball as a source of water and a dried apricot was placed in the jar. Moths emerging from the pecan nut were then sexed under a microscope, a male and female pair was released into the honey jars, which were then closed with mesh and a rubber band. These were also kept near windows to simulate natural light conditions.

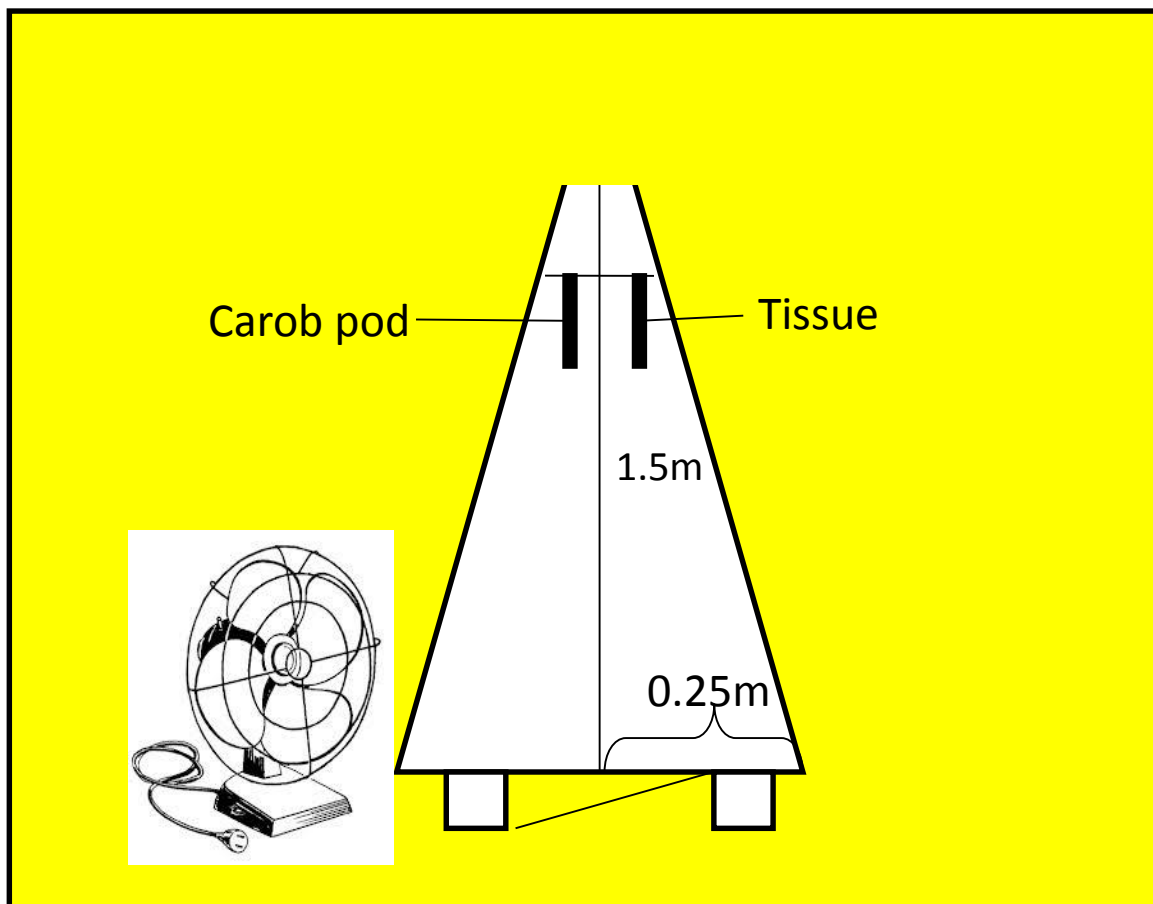


Attempt 3: A glass vial was prepared with a cut bamboo skewer, a moist sponge and a wax paper oviposition site. One male and one female were released into the vial which was then

capped with a modified plastic stopper. The stopper had a hole drilled in the top which is covered with a mesh. This was done to promote ventilation and air movement for pheromone attraction. These vials were kept near windows to simulate natural light conditions. Later they were also kept in front of an artificial light source controlled by a timer to simulate 12:12 light:dark conditions.



Attempt 4: A hole was drilled into modified shoe storage boxes so that a plastic honey jar could easily be attached to the box (top right). A wire was spun across the top of the box so that wax paper and rough tissue paper could be suspended into the box. Carob pods were also suspended in the box. On the bottom of the box wax paper and rough tissue paper were also laid down, on top of which a half orange was put down. Moistened sponge was placed at the bottom of the box. These boxes were kept near a window and in 24 hour dark room.



Attempt 5: 1.5m tall cone was constructed from strong plastic and metal rings. The cone was balanced on an upturned sift grid. Using a fan wind was pumped from the bottom of the cone to the top. Tissue paper and Carob pods were suspended in the cone as oviposition sites. Moistened sponges were also placed on the bottom of the cone. Moths were released into the cone from the bottom after which they settled at the top of the cone. This cone was kept in artificially lit conditions on a 12:12 light: dark cycle. When this did not work the cone was kept outside under natural conditions.

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Addendum 2

Female chemical lure trial

Cossé et al. 1994 studied the chemistry of date volatiles from dates collected in the Coachella Valley in California with the aim of determining which chemicals the Carob moth was most attracted to. They produced a list of 8 chemical compounds. These compounds were then evaluated at different concentrations to test their attractiveness to the Carob moth. From their results we then mixed all the most stimulating compounds together to try and formulate a female chemical lure compound.

The female chemical lure was mixed from 200ml 99.9% Ethanol, 200ml Acetaldehyde and 20ml Ethyl hexanoate (Cossé et al. 1994). From this lure mixture 60ml was mixed with 3ml of glycerene. From this mixture 1.5ml was pipetted into sterile ependorph tips. A cotton wool stopper was then inserted into the tip, after which the tip was sealed. A pin was then used to puncture a hole in the top of the ependorph tip, which was then sealed with a piece of tape to curb evaporation until the lure was placed into the trap in the field.

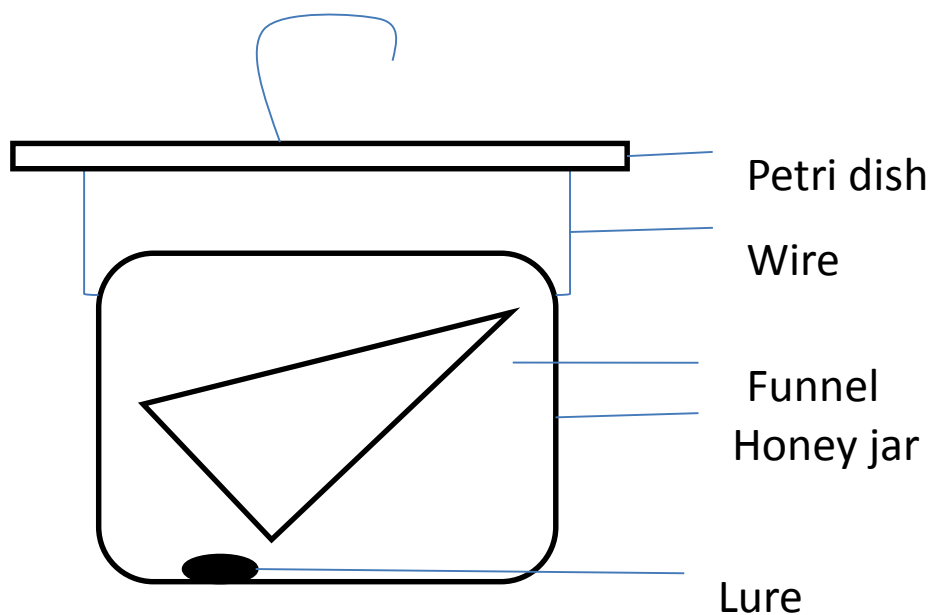


Figure 1. Traps made from honey jars, Petri dishes and funnels, were built for catching specimens with little damage occurring to the moths.

The traps above were then hung in a single row on a farm where Carob moth had previously been caught, in the Citrusdal area. 16 male and 16 female baited traps were hung on either side of the tree. The traps were checked once a week for the whole month of March, at which time the Female lure dispenser was then replaced. Male lure dispensers lasted for 4 weeks. March was chosen, because it represented the second peak for that area from previous seasonal data.

No Carob moth specimens were recorded for this trial. The only insects caught in the trap were a dung fly (Diptera: Scathophagidae) and a hawk moth (Lepidoptera: Sphingidae). No males were caught either using the conventional pheromone lure. There are a few reasons why this might be the case. It could be that the area was saturated with the smell of the lure and therefore the moths could not find the traps. It is also possible that the chemical mixture that we used doesn't actually attract Carob moth in South Africa. Because the chemicals were synthesized from date fruit, it is possible that Carob moths in citrus growing areas aren't attracted to date volatiles. Another reason for not catching any of the moths could hinge on the fact that there were very few moths caught in that area to begin with.

Reference

Cossé, A. A., Endris, J.J., Baker, T.C. 1994. Identification of volatile compounds from fungus-infected date fruit that stimulate upwind flight in femal *Ectomyelois ceratoniae*. *Entomologia Experimentalis et Applicata*. **72** (3), 233-238.

Appendix 1

Table of descriptions of Carob moth, *E. ceratoniae*, life stages listed by authors

	Larvae	Pupa	Adult	Wings
Fischer 1838	<ul style="list-style-type: none"> • Redish colour • Sclerotizations dark brown • Pro-thorasic shield mottled dark brown colour 	<ul style="list-style-type: none"> • Thin brown pupa • 2 strong curved hooks on the end 	<ul style="list-style-type: none"> • Grey palps slightly bent • Head and thorax grey with some white scales • Antennae on ♂ and ♀ brush shaped 	<ul style="list-style-type: none"> • Elongated forewing • Fore- and hind margin slightly rounded • White-grey to almost grey colour. • Just before the mid section of the wing there is a light zig-zaged parallel stripe • Followed by a thicker dark grey stripe • Before the hind margin is a second more finely zigzagged light parallel stripe • Followed by a fine grey seem • Between the two parallel stripes closer to the forewing margin are 3

				rarely 4 grey dots, in a triangle
Vaughan 1870			<ul style="list-style-type: none"> • Stout • Broad thorax • Robust abdomen which tappers to the anal extremity • Filiform, pale grey antennae • Grey head and thorax • Metathorax and patagia tipped posteriorly with long dark grey scales • Abdomen is pale grayish-white 	<ul style="list-style-type: none"> • ♀ wing length 10 to 11 ½ lines • Moderate wing length and relatively broad width • Costa slightly rounded towards the apex • Inner wing margin nearly straight • Forewings ground colour, pale grayish-white, shaded towards the base with dark grey • First line, which is undulating and oblique, passing from the inner third of the costa to the middle of the inner margin shaded with dark grey • Second line shaded to a lesser degree • The stigmata indicated by darker grey markings • Second line denticulate, parallel to the hind margin • Subterminal line very wavy and faintly visible on the paler ground colour • Hind margin dotted with dark grey

				<ul style="list-style-type: none"> • Hind wings silky white and narrowly bordered with fuscous
Hampson 1903			<ul style="list-style-type: none"> • Head, thorax and abdomen grey, mixed with brown • Abdomen has segmental white lines 	<ul style="list-style-type: none"> • Forewing grey, thickly irrorated with brown • Antemedial white line oblique from costa to below cell, where it is acutely angled and then angled inwards on vein 1 • Dark discocellular lunule • Minutely dentate white subterminal line slightly bent outwards at middle • Series of dark terminal points • Hindwing semibyaline white • Termen and costa tinged with fuscous
Dyer 1911			<ul style="list-style-type: none"> • Expanse 16 to 22mm 	<ul style="list-style-type: none"> • Bluish gray • Irregularly shaded • Inner line straight, oblique, whitish with a slight point or projection outwards at its middle • Discal marks slightly indicated by several cloudy points

				<ul style="list-style-type: none"> • Outer line crenulated, excurved mesially, whitish, slender (near margin) • Hindwing whitish, smoky along the veins and in a double marginal line
Durrant 1915			<ul style="list-style-type: none"> • Antennae, Palpi, head and thorax chalky whitish • Alar expanse, 22-24mm • Abdomen and legs chalky whitish 	<ul style="list-style-type: none"> • Forewings narrow • Veins 4-5 stalked • Chalky whitish, sparsely sprinkled with grayish scales, concentrated to form an obscure first line • Outwardly oblique from costa to dorsum and somewhat angulate outward on the fold of the second line, indicated by an obscure costal shade • Few scarcely traceable spots below • Obliquely placed discal spots • Inconspicuous spots indicate the position of a subterminal line • Seven or eight indistinct grey dots occur along the termen • Cilia chalky whitish, traversed by indistinct pale grey lines

				<ul style="list-style-type: none"> • Hindwings thinly scaled, chalky white, slightly brassy sheen • Cilia shining, whitish, with slight indication of a darker shade line
Heinrich 1958			<ul style="list-style-type: none"> • Transtilla of male genitalia more constricted and distinctly narrower • Alar expanse, 16-24mm 	<ul style="list-style-type: none"> • Forewing uniformly gray • Less white dusting on median area • Antemedial line narrower and distinctly notched
Warren and Rothshcild 1905			<ul style="list-style-type: none"> • Head, thorax and abdomen same colour as wings • Wing expanse 19mm 	<ul style="list-style-type: none"> • Forewing olive grey • Speckled with darker olive and rufous scales • First line from $\frac{1}{4}$ of costa to $\frac{1}{3}$ of inner margin • Crinkled line of fine black scales followed by fuscous shade • Outer line pale on both sides, more thickly inwardly • Indented basewards beyond cell and on submedial fold • Some dark scales represent cell-mark • Marginal line of crinkled black scales • Fringe pale grey

				<ul style="list-style-type: none"> • Darker middleline • Hindwing: pearly white, fine grey marginal line, white fringe with grey basal line • Underside glossy, whitish
Sorhagen 1881			<ul style="list-style-type: none"> • Bigger and sleeker build • Abdomen exceeds wing length by half • First ring of abdomen is light grey, nearly white 	<ul style="list-style-type: none"> • Forewing when extended less curved on front and inner edges, not curved downwards at the tip • Light grey, with front dark grey diagonal line not very prevalent • The back line nearly straight, not broken, not serrated, bordered outwards with whitish grey • Dark middle point between the lines, barely prevalent • Fringe slightly lighter than ground colour • Blackish fringe points stand out sharply, small rectangles • Hind wings more dark white, anal region is pure grey
Sorhagen 1881			<ul style="list-style-type: none"> • Head, thorax and long palps are ground colour 	<ul style="list-style-type: none"> • Front diagonal stripe cuts the root obliquely, as it

				<p>runs from 1/3 from the front edge to the inside edge</p> <ul style="list-style-type: none"> • Black line runs parallel to the seam and has a serration in the front edge, directed towards the root • Grey colour shows 7 half moons towards the root • Fringes are long, and ground colour, glossy white at the tips
Lucas 1950			<ul style="list-style-type: none"> • Head, thorax, abdomen grey ochreous, dark • Antennae pale ochreous • Tarsi whitish ochreous 	<ul style="list-style-type: none"> • Two parallel subterminal lines • One central and one basialar • Greyish • Antesubterminal slightly wavy • Underside: shiny, slightly ochreous (lines indistinct)
Zeller 1839	<ul style="list-style-type: none"> • Reddish-white with brown head, neck and prothoracic shield with brownish warts 		<ul style="list-style-type: none"> • Dorsum and head grey with darker posterior margins • Antennae gray • ♂ antennae faintly incised and larger with finer pubescence • Frons covered with fainter, curled scales • Maxillar palps well developed, speckled grey 	<ul style="list-style-type: none"> • Forewings long and narrow, broadening towards back • Ash grey • Wing base darker • First cross band broad before middle, angled, clear grey, longitudinal veins outwardly convex

			<ul style="list-style-type: none"> • Labial palps twice length of eyes, upturned, thin, compressed, brownish-grey, white dusting • Proboscis coiled, scaled • Underside of thorax and inner part of legs gray-white • Outer legs dark gray and dusted • Midtibia distally ringed grey-white proximally with blackish band • Hind legs grey-white • ♀ abdomen grey with prominent ovipositor 	<ul style="list-style-type: none"> • Second cross band close to hind margin, whitish and inwards with darker border, ends close to the second centre spot • Hind margin row of blackish dots • Fringes grey • Second blackish centre spot not always clear • Hind wings narrowed towards the front, convex hind margin, darkish-white, brownish margin line • Underside of fore wing dark grey • Hind wings grey-white on front margin grayish striped
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Appendix 2

Trait comparison of various authors for wing colouration and detail

Trait	Fischer 1838	Vaughan 1870	Hampson 1903	Dyer 1911	Durrant 1915	Heinrich 1958	Warren & Rothschild 1905	Sorhagen 1881	Sorhagen 1881	Lucas 1950	Zeller 1839
Forewing colour	White-grey	Ground colour, pale greyish-white	Grey, thickly irrorated with brown	Bluish grey	Chalky white, sprinkled with grey scales	Uniformly grey	Olive grey	Light grey	Grey	Greyish	Ash grey
Forewing margin shape	Slightly rounded	Nearly straight						Less curved on front inner edges, not curved downwards at the tip			
Stripe placement	Before midsection of wing,	First line passing from inner	Antemedial white line	Outer line	First line outwardly oblique		First line from ¼ to 1/3 of		Front diagonal stripe cuts	Two parallel subtermi	First cross band

on forewing	light serrated parallel stripe, followed by a thicker dark grey stripe, followed by fine grey seem	3 rd of costa to middle of inner margin, second line parallel to hind margin	oblique from costa to below cell, minutely dentate white subterminal line slightly bent outwards	near margin	from costa to dorsa, second line angulate on fold		inner margin		the root obliquely, as it runs from 1/3 from front edge to the inside edge, black line runs parallel to the seam and has a serration in the front edge directed towards the root	nal lines, one central and one basialar	broad before middle, second cross band close to hind margin
Dark spots	Between the two parallel stripes closer to the forewing margin are 3 to 4 dots in a tri-angle	The stigmata indicated by darker grey markings	Dark discocellular lunule	Discal marks slightly indicated by several cloudy pots	Few scarcely traceable spots below second line	Some dark scales represent cell-mark	Dark middle pint between the lines, barely prevalent				Second blackish centre spot not always clear
Forewing hind		Hind margin	Series of dark		Seven or eight		Marginal line of	Blackish fringe	Grey colour		Hind margin

margin markings		dotted with dark grey	terminal points		indistinct grey dots occur along the termen		crinkled black scales	points stand out sharply, small rectangles	shows 7 half moons towards root		row of blackish dots
Fringe					Cillia chalky whitish, transverse by indistinct pale grey lines		Fringe pale grey	Fringe slightly lighter than ground colour	Fringes are long, and ground colour, glossy white at tips		Fringes grey
Hind wings		Hind wings silky white and narrowly bordered with fuscous	Semibyaline white	Whitish, smoky along the veins and in a double marginal line	Thinly scaled, chalky white, slightly brassy sheen, with shiny cilia of a whitish colour with a darker shade line		Pearly white, fine grey marginal line, white fringe with grey basal line, underside glossy and whitish	Hindwings more dark white, anal region is pure grey			Narrowed towards the front, convex hind margin, darkish-white, brownish margin line. Underside of forewing dark grey,

Appendix 3

Molecular tree produced by Dr Alicia Timm from Rhodes University for samples collected from different localities

