Bioecology of lepidopteran pests of *Macadamia integrifolia* in South African production areas

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Abstract

South Africa is the largest exporter of macadamia nuts in the world. Larvae of the Lepidoptera nut borer complex causes economic damage to macadamia in South Africa. Larvae damage both young developing and old hard nuts which then drop from trees and may be unsuitable for processing. The Lepidoptera species that constitute the nut borer complex in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae). Mixed populations of these species are often reported in macadamia orchards in South Africa. Previous surveys of the nut borer complex in Limpopo and Mpumalanga showed that the species complex may change within and between seasons. Limited information is available on the nut borer, both within smaller regions in provinces, as well as in areas such as KwaZulu-Natal. The aims of this study were to determine the moth flight patterns, population structure, phenology, and relative abundance of larvae of the nut borer complex over a growing season at Barberton in the Mpumalanga province of South Africa. In this study, pheromone traps were used to monitor moth numbers during one growing season and nuts were sampled to determine the identity and quantify of larval numbers inside nuts at weekly intervals for 21 weeks after commencement of macadamia flowering at this locality. Identification of larvae was done using both morphological and molecular methods. A rapid assessment of the species complex was also done by means of an area-wide survey at five localities outside of the Barberton area, including the two sites in KwaZulu-Natal. Three of the moth species were captured by means of pheromone traps at Barberton. According to moth catches, *T. leucotreta* dominated *T. batrachopa,* with these two species making up 69 % and 27 % of the trap catches, respectively. No *E. ceratoniae* moths were captured in pheromone traps. Peak moth flight numbers were recorded 12-16 weeks after commencement of flowering. Significant bycatches of *T. batrachopa* were made in the traps put out for *C. peltastica*, and *vica versa.* Larval infestations, which included *E. ceratoniae,* varied during the season and was dominated by *T. leucotreta*, followed by *T. batrachopa*, *E. ceratoniae* and *C. peltastica*. Four other Lepidoptera species were also recorded at Barberton but at very low numbers. These were: *Lobesia vanillana* (de Joannis), *Nola imitate* (Son) (Nolidae), *Janseodes melanospila* (Guenée) (Erebidae), and *Ariathisa* sp. (Noctuidae). In the area wide survey, all the species of the borer complex were collected, except for *C. peltastica*. The most diverse nut borer complex was recorded at Levubu and Nelspruit. *Thaumatotibia batrachopa* was recorded in three of the areas, including KwaZulu-Natal while *T. leucotreta* was only recorded in Mpumalanga and Limpopo. *Ectomyelois ceratoniae* was recorded in Mpumalanga and Limpopo as well as in one locality in KwaZulu-Natal where it was also the only species that was collected from fallen nuts. Monitoring data such as this can be used in decision making regarding the application of various pest management practices. This study highlights the discrepancy between pheromone trap catches and larval numbers and that trap captures do not provide an accurate indication of the larval community composition and the time of infestation.

Key words: Lepidoptera, macadamia pests, nut borer complex, pheromones, surveys

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Chapter 1:

Literature review

1.1 Introduction

South Africa is the world's largest exporter of macadamia nuts, with approximately 98 % of the produce being exported. Larvae of the Lepidoptera nut borer complex causes economic damage to macadamia (*Macadamia integrifolia*) in South Africa. Larvae can damage both young developing nuts as well as old hard nuts (La Croix & Thindwa, 1986). When larvae damage macadamia nuts, it may be unsuitable for processing (La Croix & Thindwa, 1986). When macadamia nut shells are fully developed, damage to husk tissue may result in nuts to drop from trees. Fruit drop is a typical result of damage by the lepidopteran nut borer complex on macadamia (La Croix & Thindwa, 1986).

The complex of indigenous tortricid species that attacks macadamia nuts in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Schoeman, 2016). The carob moth (*Ectomyelois ceratoniae*) (Zeller), (Lepidoptera: Pyralidae) is also of major economic importance but is thought to be native to the Mediterranean basin (Prinsloo & Uys, 2015). The life cycles and behaviour of these closely related species are similar and correct identification, especially of the larval stages, is of significant importance for pest management purposes (Venette *et al.*, 2003). The moth's complex life cycle and behaviour are closely related but discussed separately.

Table 1: Classification of the Lepidoptera species addressed in this study: false codling moth, macadamia nut borer, litchi moth and carob moth.

Family	Tortricidae	Tortricidae	Tortricidae	Pyralidae
Tribe	Grapholitini	Grapholitini	Grapholitini	Phycitini
Genus	*Thaumatotibia*	*Thaumatotibia*	*Cryptophlebia*	*Ectomyelois*
Species	*leucotreta* (Meyrick)	*batrachopa* (Meyrick)	*peltastica* (Meyrick)	*ceratoniae* (Zeller)
Common name	False codling moth	Macadamia nut borer	Litchi moth	Carob moth

1.2 The False codling moth

1.2.1 History and taxonomy

The False Codling Moth (FCM), *T. leucotreta*, was first described as a citrus pest in Natal, South Africa, by Fuller in 1901 (Catling & Aschenborn, 1974) and was classified under the genus *Carpocapsa* (Schwartz, 1981). It was first named the 'Natal Codling Moth' since it was most likely confused with the Codling Moth, *Cydia pomonella* (Fuller) (Lepidoptera: Tortricidae), due to the damage symptoms and larval stages that appear similar (Schwartz, 1981). In 1909, Howard referred to *Enarmonia batrachopa* as the orange codling moth, whereas in 1914, Kelly referred to this pest as the False Codling Moth instead of the Natal Codling Moth (Schwartz, 1981). The term False Codling Moth has been used ever since Meyrick described the moth in 1913 as *Argyroploce leucotreta* (Schwartz, 1981). In 1958, Clarke classified the moth under the genus *Cryptophlebia* (Schwartz, 1981). The False Codling moth was known as *Cryptophlebia leucotreta* until recently, when it was transferred by Komai to *Thaumatotibia leucotreta* (Venette *et al.*, 2003).

1.2.2 Geographical distribution

The False Codling Moth is distributed south of the Sahara throughout Africa and nearby islands in the Atlantic and Indian oceans (Grové *et al.*, 1999a). It is believed that *T. leucotreta* is endemic to Africa (Schwartz, 1981; Grové *et al.*, 1999a). FCM is present in all major citrus and avocado-producing areas of South Africa (Schoeman & Beer, 2009) and damages crops in other countries such as Malawi and Zimbabwe (La Croix & Thindwa, 1986). FCM also occurs in the following African countries: Angola, Benin, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo Democratic Republic, Côte d'Ivoire, Eritrea, Ethiopia, Gambia, Ghana, Kenya, Madagascar, Mali, Mauritius, Mozambique, Niger, Nigeria, Rwanda, Réunion, Saint Helena, Senegal, Sierra Leone, Somalia, Sudan, Swaziland, Tanzania, Togo, Uganda, and Zambia (Stibick, 2010).

1.2.3 Host plants

FCM is highly polyphagous and a well-known citrus pest in South Africa (Adom *et al.*, 2021). It also attacks many other deciduous, subtropical, and tropical fruit tree species

(Grové *et al.*, 1999b; Brown, 2006). FCM has a wide range of natural hosts as well as cultivated crops. The economically important host plants of FCM are listed in table 2.

Table 2. Economically important host plants of *Thaumatotibia leucotreta* (EPPO, 2013).

Popular name	Scientific name
Pepper	*Capsicum* spp.
Mandarin orange	*Citrus reticulata* & hybrids
Orange	*Citrus sinensis* & hybrids
Grapefruit	*Citrus paradisi*
Cotton	*Gossypium* spp.
Litchi, Litchee	*Litchi chinensis*
Macadamia	*Macadamia* spp.
Mango	*Mangifera indica*
Peach	*Prunus persica*
Nectarine	*Prunus persica* var. *nucipersica*
Avocado	*Persea americana*
Guava	*Psidium guajava*
Pomegranate	*Punica granatum*
Oak	*Quercus robur*
Castor oil plant	*Ricinus communis*
Rose	*Rosa* sp.
Eggplant	*Solanum melongena*
Grape	*Vitis vinifera*
Maize	*Zea mays*

1.2.4 Biology and life cycle

Figure 1: Life stages of the false codling moth; A: Larva; B: Adult; C: Egg; D: Pupa (Rentel, 2013).

The different life stages of FCM are illustrated in Figure 1. The complete life cycle of FCM can range from 30 days under favourable conditions to 174 days under poor conditions (Stibick, 2010). With the high variability in the life cycle of FCM, two to ten generations can be expected per annum (Stibick, 2010). Environmental conditions such as temperature, photoperiod, humidity, latitude, and the effect of predators, diseases, food availability and quality thereof can influence the life cycle of FCM. (Stibick, 2010). FCM may be active throughout the year, provided their food supply is uninterrupted (Stibick, 2010). Female moths live longer than males (Daiber, 1980).

1.2.4.1 Egg stage

Female moths are nocturnal, and oviposition on host crops occurs primarily between the hours of 17:00 and 23:00 (Stibick, 2010; De Jager, 2013). Eggs are laid singly or in batches, directly onto the fruit surface, and appear as translucent, flat, and oval with a reticulate sculpture (La Croix & Thindwa, 1986; Stibick, 2010; Grové *et al.*, 2014). Eggs are approximately 1 mm in diameter and are laid randomly on the fruit surface in key areas such as depressions, smooth and non-pubescent surfaces or fallen fruit. Fertile eggs transform into a reddish colour as the embryos develop over time, returning to a black colour shortly before hatching as the head capsule becomes visible through the translucent egg shell (Daiber, 1979b). Females lay 3 to 8 eggs per fruit but can lay up to 800 eggs over her lifespan at the optimal temperature of 25 °C (Stibick, 2010). Multiple females may lay eggs on a single fruit if infestation pressure is high (De Jager, 2013). Egg development rate is strongly influenced by temperature and humidity, and take 2 to 22 days to hatch (De Jager, 2013).

1.2.4.2 Larval stage

FCM larvae penetrate the fruit upon hatching by gnawing a small hole into the fruit (Stibick, 2010). The entrance is easily recognizable with discolouration around the entry hole, or the presence of frass (Stibick, 2010). There are 5 instars and the larvae develop to the final instar inside the fruit (Stibick, 2010). Fully grown larvae are pink in colour and approximately 15 mm in length (Van der Walt, 2012). The larval period can span between 12 to 33 days in warm weather and between 35 to 67 days in cooler conditions (Stibick, 2010). The quality of the fruit greatly influences larval development time, with lower quality food leading to longer development times (Stibick, 2010). Usually only one larva per fruit survives but three larvae per fruit have been reported (Stibick, 2010). The larvae do not pupate inside the fruit but in the soil.

1.2.4.3 Pupal stage

The larvae balloon to the ground by means of silk threads when they reach the fifth-instar and depending whether the fruit has not yet fallen (Stibick, 2010; Prinsloo & Uys, 2015). Larvae then spin a cocoon in which they enter the pupal stage which can last from 2 to 27 days in the soil (Stibick, 2010). The cocoon consists of a silky body substance and often contains soil particles (Stibick, 2010). The prepupa is cream-coloured and soft until it changes into a pupa, turning brown and hardening as chitin forms (Daiber, 1979a). The pupae of female moths are generally larger than those of males (Daiber, 1979a). Males take longer to develop than females, with females taking between 11 to 39 days to emerge (Stibick, 2010). The entire pupal stage can last 13 to 60 days depending on temperature (Stibick, 2010). Development of males lasts 13 to 47 days before they emerge from cocoons (Stibick, 2010). The pupae are sensitive to temperature as well as heavy rainfall which can reduce the population of FCM (Daiber, 1979a; Stibick, 2010).

1.2.4.4 Adult stage

FCM moths have mottled dark grey wings and a wingspan of 16-20 mm (Grové *et al.*, 2014). The males are smaller than the females and are characterised by a greyish anal tuft of scales, a scent organ near the hind wings and densely packed grey-white hair on the hind tibia (Grové *et al.*, 2014). Males live 14 to 57 days and females 16 to 70 days, and often seek shelter in shaded spots on plants during the day (Stibick, 2010). Adults do not move more than a few hundred meters and their activity is influenced by factors such as temperature and host availability (Stibick, 2010).

1.3 Macadamia nut borer

1.3.1 History and taxonomy

The macadamia nut borer, *T. batrachopa,* was originally described from the Eastern Cape Province of South Africa (Prinsloo & Uys, 2015). In 1914, Kelly wrongfully referred to *Enarmonia batrachopa* as the false codling moth (Schwartz, 1981).

1.3.2 Geographical distribution

The Macadamia nut borer (MNB) is native to Africa and is known throughout east, west and southern Africa (La Croix & Thindwa, 1986; Prinsloo & Uys, 2015). This species also occurs on islands surrounding Africa such as Madagascar and São Tomé (Prinsloo & Uys, 2015).

1.3.3 Host plants

The macadamia nut borer has only two known host plant species in South Africa, i.e., Macadamia and litchi (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Rentel, 2013).

1.3.4 Biology and life cycle

The different life stages of MNB are illustrated in Figure 2.

Figure 2: Life stages of the macadamia nut borer; A: Larva; B: Adult; C: Egg; D: Pupa (Bright, 2020).

1.3.4.1 Egg stage

The eggs of *T. batrachopa* are characterised as flat, oval, with a pitted surface and are approximately 0.8 x 0.6 mm wide and 0.2 mm high (La Croix & Thindwa, 1986). Eggs are laid individually on the fruit surface with up to three eggs per fruit (La Croix & Thindwa, 1986). Eggs take approximately 6.8 to 10 days to hatch at 27 °C (La Croix & Thindwa, 1986). Under these conditions females lay on average between 20.8 and 35.2 eggs (La Croix & Thindwa, 1986).

1.3.4.2 Larval stage

Larvae either burrow directly into the nut or fruit below the egg position, or move approximately 4 mm before they burrow into the husk of a nut or fruit (La Croix & Thindwa, 1986). Under controlled laboratory conditions at 27 °C, larvae take between 20.9 to 27.9 days to complete their development (La Croix & Thindwa, 1986). Larvae appear cream to greyish in colour with dark spots on the body and a brown head capsule (Prinsloo & Uys, 2015).

1.3.4.3 Pupal stage

Under controlled laboratory conditions (27 °C), moth emergence from pupae is staggered over time, with 50 % emergence occurring within 9 days and an 80 % emergence at 14 days (La Croix & Thindwa, 1986). Following the 14-day period, emergence is spread over a 4-month period (La Croix & Thindwa, 1986). The pupae range between 6 – 8 mm in length and appear dark brown in colour (Prinsloo & Uys, 2015). The pupa is covered in a cocoon of soil particles and silk when pupation takes place in the soil (Prinsloo & Uys, 2015). When pupation occurs inside the fruit, no encapsulation takes place (Prinsloo & Uys, 2015).

1.3.4.4 Adult stage

MNB moths are small and grey to light brown with distinct darker and paler markings on the wings (Rentel, 2013; Prinsloo & Uys, 2015). Moths are between 16 – 20 mm in length with a characteristic saddle like structure behind the head (Prinsloo & Uys, 2015). A longer, denser anal tuft, which distinguishes it from other species, is present (Rentel, 2013). Adults in captivity live for 3 – 7 days (La Croix & Thindwa, 1986).

1.4 Litchi moth

1.4.1 History and taxonomy

Very little to no information is available regarding the taxonomy and history of *C. peltastica.*

1.4.2 Geographical distribution

The Litchi moth is a very important pest in South Africa (Timm *et al.*, 2007). It is native to Africa (Grové *et al.*, 2014) and occurs in Madagascar, Mauritius, Reunion, and the Seychelles (Manrakhan *et al.*, 2008; Grové *et al.*, 2014).

1.4.3 Host plants

The litchi moth is polyphagous and is of economic importance in litchi, and to a lesser extent on macadamia in southern Africa (Grove, 1999; Prinsloo & Uys, 2015). Other known host plants are listed in table 3.

Table 3. Economically important host plants of *Cryptophlebia peltastica* (Prinsloo & Uys, 2015).

Common name	Scientific name
Jacket plum	*Pappea capensis*
Karoo boer-bean	*Schotia afra*
Pride-of-Barbados	*Caesalpinea pulcherrima*
Flamboyant	*Delonix regia*
Pride-of-De Kaap	*Bauhinia galpinii*

1.4.4 Biology and life cycle

Moths reared under laboratory conditions have a greater emergence rate from litchi fruit exceeding 15 mm in diameter (Manrakhan *et al.*, 2008). The total time to complete the life cycle from neonate emergence to adult stage is reported to be 25 to 29 days at 23.9 - 25.6 °C, respectively, at relative humidity of approximately 80 % (Manrakhan *et al.*, 2008). The different life stages of litchi moth are illustrated in Figure 3.

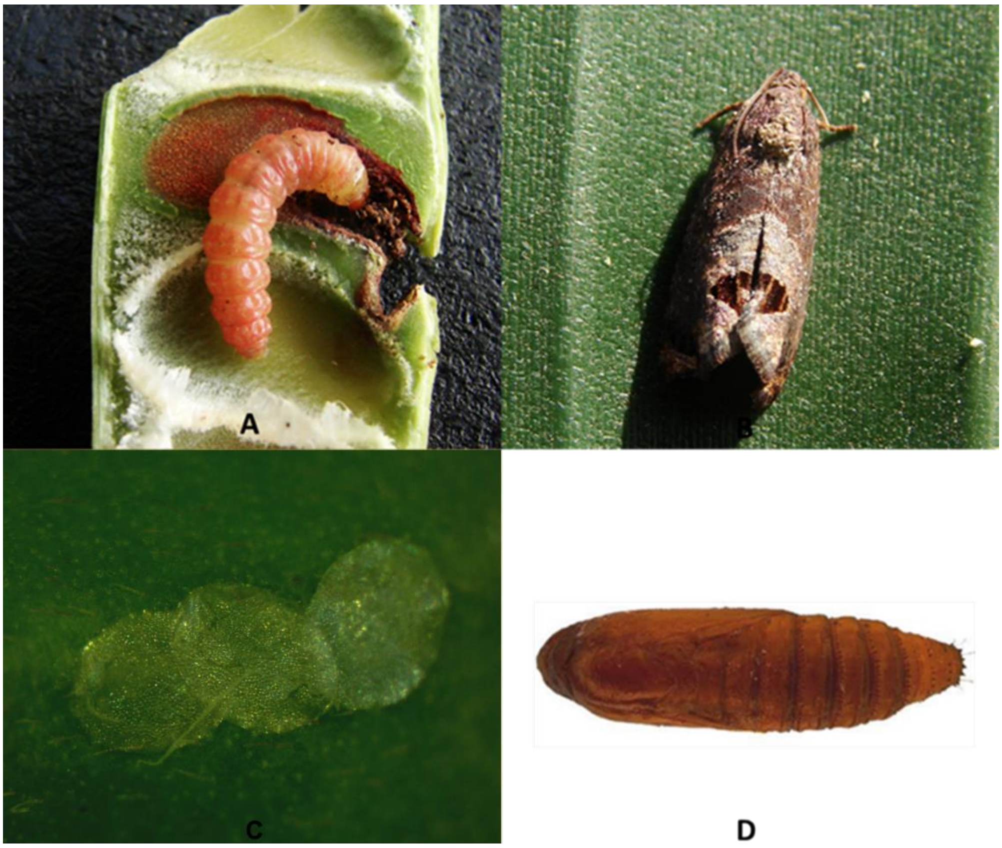

Figure 3: Life stages of the litchi moth; A: Larva; B: Adult; C: Egg D: Pupa (Rentel, 2013).

1.4.4.1 Egg stage

The eggs of *C. peltastica* are flat, oval, and translucent with a reticulate sculpture and measure approximately 1 mm in diameter (Grové *et al.*, 2014; Prinsloo & Uys, 2015). Eggs are laid on the surface of fruit during the development stages (Grove, 1999).

1.4.4.2 Larval stage

Newly hatched larvae eat through the skin of the fruit to penetrate the fruit (Grove, 1999). The final instar is light red to light pink with an elongated body of about 15 mm long (Grove, 1999; Prinsloo & Uys, 2015). The head is brown with darker pigmentation at the ocellar areas and the prothoracic shield being dark brown (Prinsloo & Uys, 2015). The anal shield is yellowish brown with light brown pigmentation (Prinsloo & Uys, 2015). The final-instar larva lacks an anal comb, distinguishing it from the false codling moth and the macadamia nut borer (Prinsloo & Uys, 2015).

1.4.4.3 Cocoon and pupal stage

The pupa of *C. peltastica* is dark brown with darker patches on the pronotum and the frontal region (Timm *et al.*, 2007). The pupa ranges between 8.59 – 10.63 mm in length (Timm *et al.*, 2007). When pupation takes place inside the soil a cocoon is formed of soil particles and debris, however, when pupation occurs inside fruit, no cocoon is present (Timm *et al.*, 2007). A distinct cremaster is absent in the pupa of *C. peltastica* (Grové *et al.*, 2014).

1.4.4.4 Adult stage

Moths of *C. peltastica* are dark brown to greyish in color with a blackish marking near the apex of the forewing. A characteristic black diamond shaped pattern can easily be seen in the tornus of the forewing when closed (Grové *et al.*, 2014). The moth measures about 16 - 20 mm across the spread wings and 8 mm in length when at rest (Bradley, 1953; Prinsloo & Uys, 2015).

1.5 Carob moth

1.5.1 History and taxonomy

The oldest documented record of CM in South Africa dates back to 1962, from an unknown host in Pretoria in the Gauteng Province (Morland *et al.*, 2019).

1.5.2 Distribution and pest status

The CM, *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), has a global distribution and is a field and stored product pest of tree fruit and nuts (Thackeray *et al.*, 2017). This species originates from the Mediterranean basin and is widely distributed in Europe, the Near East, Arabia, Africa, and Australasia (Prinsloo & Uys, 2015; Thackeray, 2016). CM is present in all the major citrus producing areas of South Africa (Honiball & Catling, 1998). Until recently, CM has been considered a minor pest of tree nut crops and citrus in South Africa (Thackeray *et al.*, 2017).

1.5.3 Host plants

The larvae of the CM are polyphagous and 62 known host plants have been documented for this species (Morland, 2015). CM is known to feed on fruit of plants in 13 plant families, with plants in the Fabaceae family being the most common (Nay & Perring, 2008). Over 50 host species have been identified in South Africa, many of which are exotic (Bazelet, 2021). This pest targets different fruit tree species before and after harvest, as well as stored food and wild hosts (Morland, 2015; Hosseini *et al.*, 2017). The host plants of CM are listed in table 4.

Table 4. Economically important host plants of *Ectomyelois ceratoniae* (Bazelet, 2021).

Popular name	Scientific name
Citrus	*Citrus sp.*
Carob tree	*Ceratonia siliqua*
Almond	*Prunus dulcis*
Pistachio	*Pistacio vera*
Dates	*Phoenix dactylifera*
Pomegranate	*Punica malus*
Walnuts	*Juglans* sp.
Pecans	*Carya illinoinensis*
Macadamia	*Macadamia integrifolia*
Pigeon pea	*Cajanus cajan*
Tamarind	*Tamarindus indica*

1.5.4 Biology and life cycle

The different life stages of CM are illustrated in Figure 4.

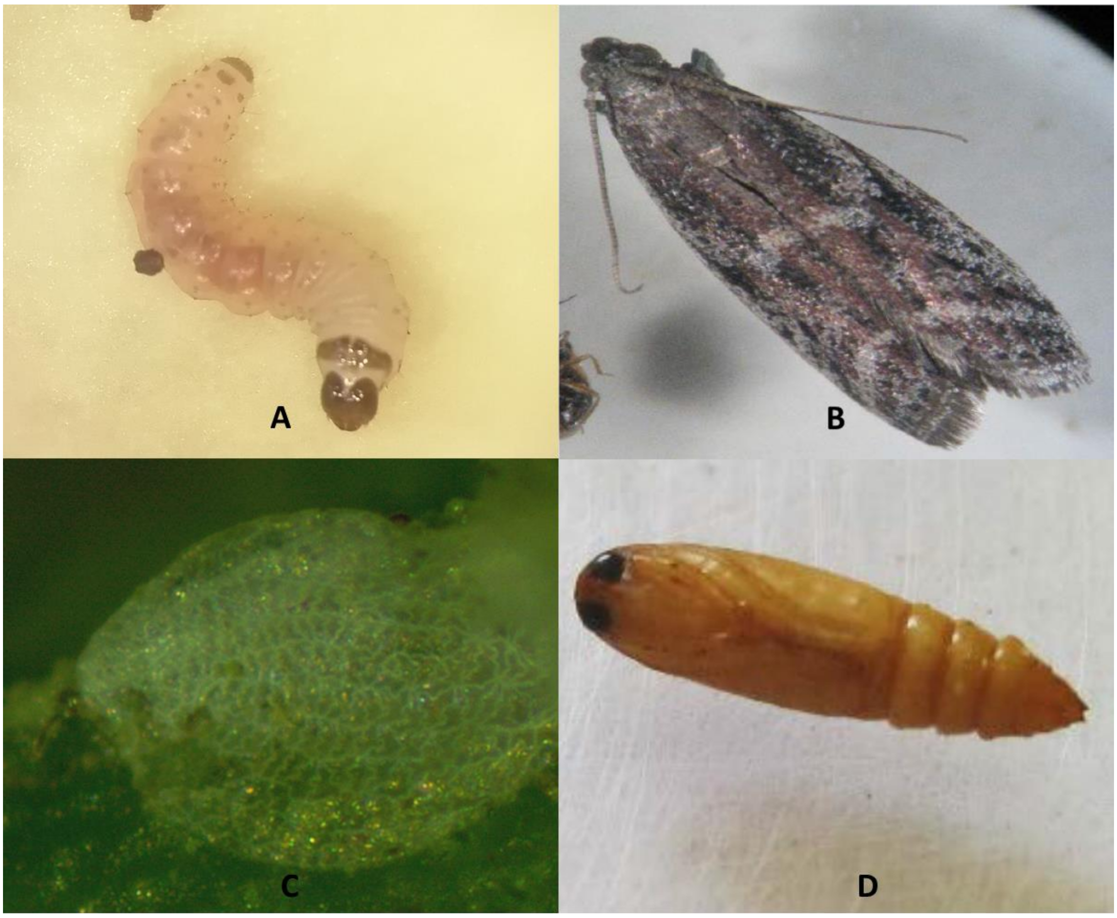

Figure 4: Life stages of CM; A: Larva; B: Adult; C: Egg; D: Pupa (Bazelet, 2021).

1.5.4.1 Egg stage

Eggs are small, flat, and ovoid in shape (Honiball & Catling, 1998; Morland, 2015; Prinsloo & Uys, 2015). The eggs are generally laid singly or in clusters of up to three (Morland, 2015; Prinsloo & Uys, 2015). The eggs are initially white then turn reddish-brown and are approximately 0.7 mm in length and 0.05 mm wide (Morland, 2015; Prinsloo & Uys, 2015). A clear lattice shape pattern covers the entire egg, which distinguishes it from the eggs of FCM (Morland, 2015). Unfertilised eggs collapse only in the centre of the egg with the edges staying intact (Morland, 2015).

1.5.4.2 Larval stage

Larvae are slender, elongate, creamy white to light pink in colour with rugose integument and a brown coloured head-capsule (Morland, 2015; Prinsloo & Uys, 2015). Larvae can attain a length of 18 mm (Prinsloo & Uys, 2015) and an anal comb is absent from segment A10 (Morland *et al.*, 2019).

1.5.4.3 Cocoon and pupal stage

Pupae are generally 10 - 11 mm in length and yellow to red-brown in colour (Prinsloo & Uys, 2015; Thackeray, 2016). Cocoons are formed underneath a web spun over the feeding area by the mature larva to pupate inside the fruit. Larvae may also drop to the soil surface to pupate in the soil (Prinsloo & Uys, 2015; Morland *et al.*, 2019).

1.5.4.4 Adult stage

CM adult is a small inconspicuous grey moth with extremely variable wing markings, body size and genital structures (Prinsloo & Uys, 2015). The wingspan is approximately 19 - 26 mm with the forewings that are grey in colour with two faint and variable oblique stripes (Honiball & Catling, 1998; Prinsloo & Uys, 2015; Thackeray, 2016). The rear wing is light grey to white or cream in colour and have a distinctive long fringe around the outer margins with long hairs (Honiball & Catling, 1998; Prinsloo & Uys, 2015; Thackeray, 2016; Morland *et al.*, 2019). Wings are held flat across the abdomen when in a resting position (Morland *et al.*, 2019). Females lay up to 340 eggs over a 14-day period (Prinsloo & Uys, 2015).

1.6 Macadamia

1.6.1 History and origin of macadamia production

A German explorer, Ludwig Leichhardt was the first person to collect macadamia and following from this, the Director of the Royal Botanical Gardens in Melbourne, Baron Ferdinand von Mueller, was the first to describe the macadamia tree in 1857 taxonomically, and the name macadamia was given to the plant in honour of his friend Dr John Macadam (Stephenson, 2005). In autumn, aboriginal tribes in Australia gathered the nuts as bush food but did not cultivate the plant (Anonymous, 1998). The macadamia was introduced into Hawaii from Australia during the late 1800s but was not commercially grown until the early 1900s (Shigeura & Ooka, 1984; Nagao *et al.*, 1992). Australian farmers were slow to take advantage of macadamia crop production and only realised its potential in 1950, after the successful introduction and plantings in Keaau, Hawaii (Shigeura & Ooka, 1984).

The macadamia nut is the only commercial food crop indigenous to Australia. It originated along the coastal subtropical rainforests of southeast Queensland and northeast New South Wales (Nagao *et al.*, 1992; Stephenson, 2005). *Macadamia* F. Muell, is in the Proteaceae family which occurs predominantly in the Southern Hemisphere (Nagao *et al.*, 1992; Wallace & Walton, 2011). Commercial cultivars of *Macadamia integrifolia* Maiden & Betche, and *M. tetraphylla* L., along with hybrids of these two species, are cultivated widely (Nagao *et al.*, 1992; Wallace & Walton, 2011). Nuts of the wild *Macadamia ternifolia* and *Macadamia jansenii* are bitter and inedible (Dahler *et al.*, 1995; Stephenson, 2005).

1.6.2 Macadamia cultivars in South Africa

Several macadamia cultivars are planted in South Africa, with the most popular cultivars for the 2020 season being Beaumont, A4, 816, Nelmak 2, A16, 814, 788, 849 and 842 (SAMAC, 2020).

The percentage market share of the most popular macadamia cultivars planted in South Africa is presented in Figure 5.

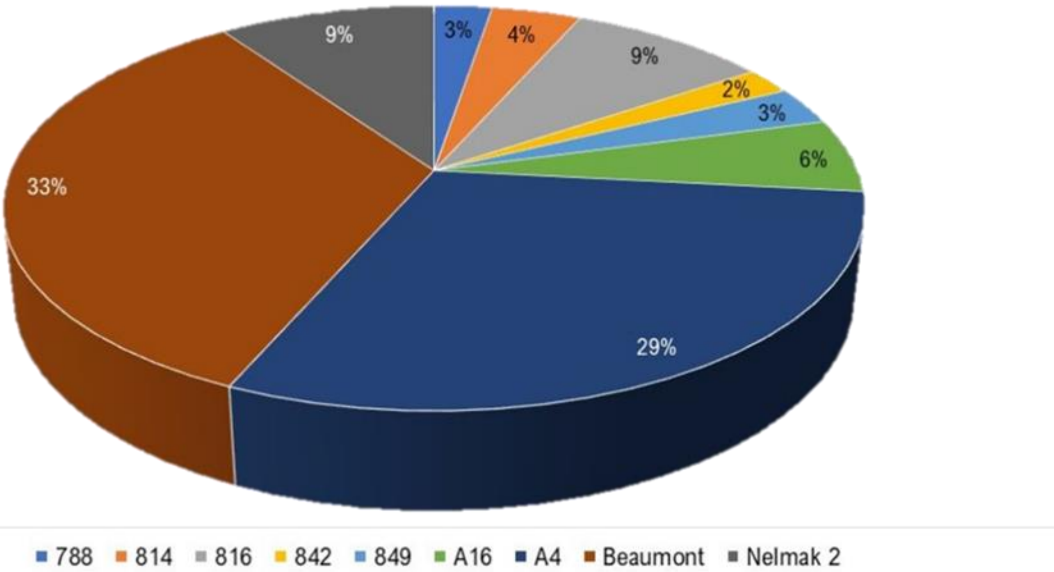

Figure 5: Percentage breakdown of the most popular macadamia cultivars planted in 2020 in all provinces of South Africa (SAMAC, 2020).

1.6.3 Botany

Macadamia trees are medium-sized evergreen trees that can reach a height of over 18 m and a canopy width of 12 m (Hamilton *et al.*, 1983). Trees have shiny, green, holly-like foliage (Hamilton *et al.*, 1983). Macadamias are self-pollinating but yields are much higher when two or more varieties are grown in close proximity (Schoeman, 2009). This clarifies why most orchards consist of a combination of varieties (Schoeman, 2009).

1.6.4 Production and economic importance in South Africa

South Africa produced 48 925 tonnes of nut in shell (NIS) macadamias during the 2020 season (SAMAC, 2020). The 2020 season was not as productive as the 2019 season during which the harvest was 17.1 % higher (SAMAC, 2020). This was due to several reasons including adverse weather conditions during flowering, and early nut formation. South Africa was the largest exporter of NIS macadamia nuts in the world for 2020 and competes with Australia (SAMAC, 2020). Approximately 98 % of macadamia produced in South Africa is exported, which produced a revenue of R4.8 billion in 2020 (SAMAC, 2020). South Africa has a rapidly growing macadamia industry with 50 133 hectares established prior to 2020 and 5 331 hectares that were newly established during 2020 (SAMAC, 2020).

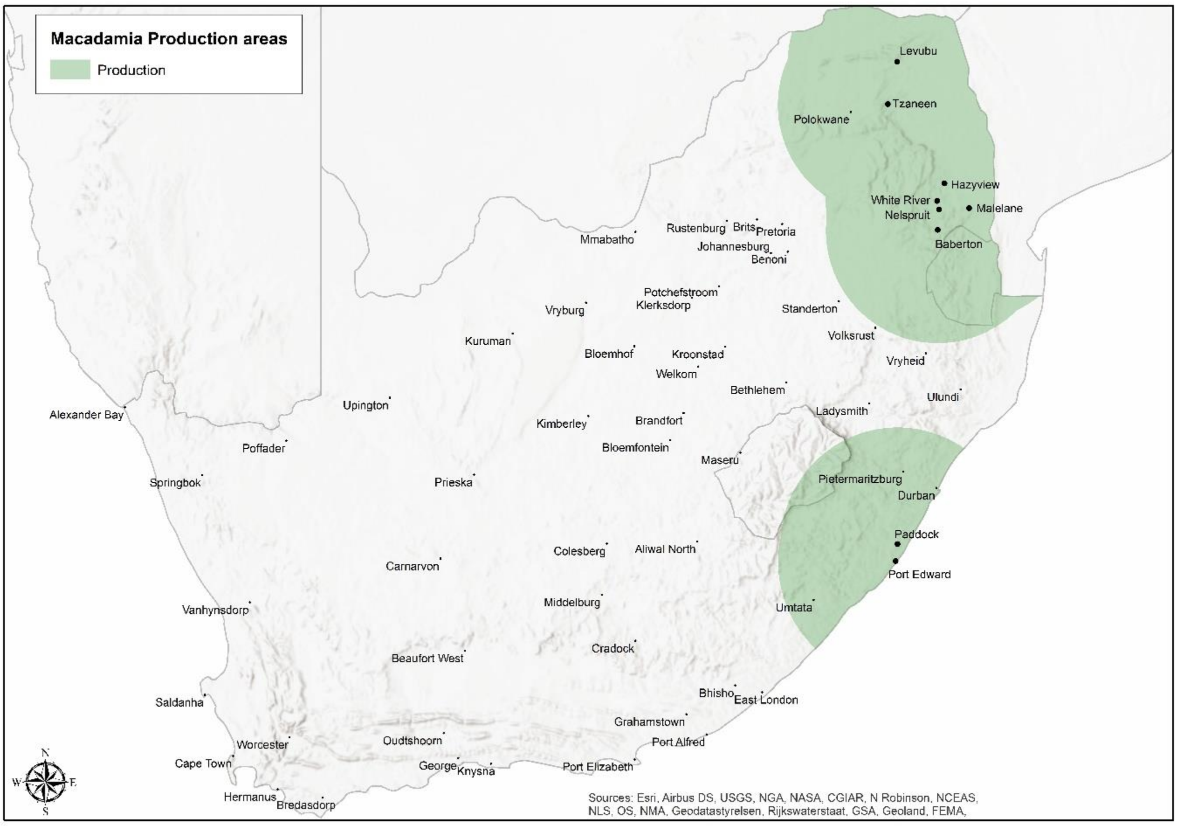

Figure 6: Main macadamia nut production areas in South Africa

1.6.5 Monetary losses due to agronomical and biotic constraints

Crop losses in macadamia was estimated at R337 million and R283 million for the 2019 and 2020 production years respectively (Figure 7).

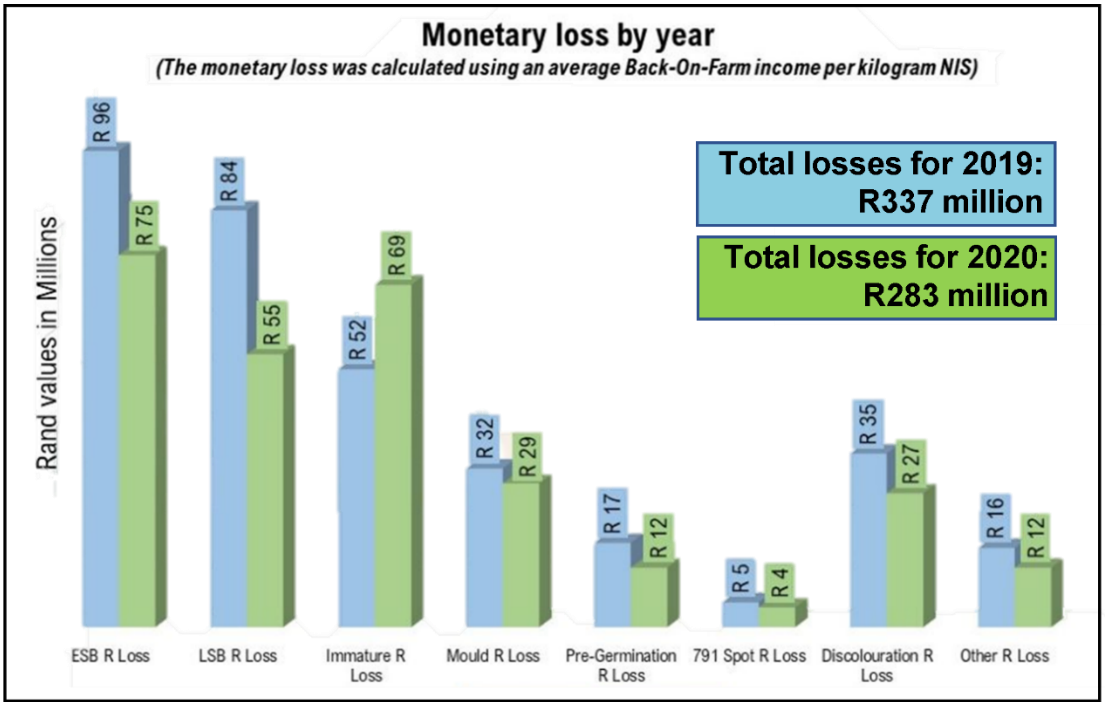

Figure 7: Average monetary loss of nut in shell per kilogram by year due to various constraints. ESB = Early stinkbug, LSB = Late stinkbug (SAMAC, 2020).

Damage caused by the macadamia nut pest complex is not listed among the important factors responsible for causing losses in macadamia in South Africa. This is due to pest damage being regarded as indirect damage and it is rarely highlighted in processor’s reports on account of a low incidence of damaged kernels being delivered to processing plants (Schoeman, 2021). According to Schoeman (2021) infestation levels of active larvae during the 2020 season varied considerably, ranging from 1.6 – 22.2 % with an average infestation of 9.31 %. Calculated at a yield of 2 t/ha and an average price of R80.00 / kg, monetary losses due to the nut borer complex therefore range from R 2 560.00 to R35 520.00 per ha (Schoeman, 2021). Due to insect feeding, the average industry wide monetary losses for this cultivar can be as high as R100 million per year (Schoeman, 2021). The losses for the industry may be far greater as these calculations are only done for a single cultivar and do not consider the losses sustained by other commonly planted cultivars in South Africa.

1.6.6 Phenology

Macadamia trees form large numbers of racemes, up to 2500 per tree, although less than 0.3 % of the flowers produce nuts (Moncur *et al.*, 1985; Moncur, 1988). Each raceme contains between 100 and 300 flowers (Urata, 1954). Approximately 99.8 % of all macadamia flowers fail to develop into mature nuts (Schoeman, 2009).

The young buds are green and usually become creamy white in colour 3 to 4 days before anthesis (Urata, 1954). Under conditions where there is adequate light, racemes normally flower basipetally, but trees may bloom acropetally (Urata, 1954). Initial set of the nut can be recognized by enlarged ovaries on racemes which remain after 10 to 15 days of anthesis and after most of the flowers have been shed (Urata, 1954). The young fruits continuously drop until about 45 to 60 days after anthesis (Urata, 1954), which, in this study, would imply natural nut drop from week 4 (beginning of October) to week 9 or 10 (mid-November).

1.7 Integrated Pest Management

Integrated Pest Management (IPM) can be defined as a decision-based process involving coordinated use of multiple tactics for optimizing the control of all classes of pests (insects, pathogens, weeds, vertebrates) in an ecologically and economically sound manner (Prokopy, 2003). IPM is thus an ecological approach to pest control, based upon sound biological knowledge and principles (Sandler, 2010).

Insect damage can either be direct or indirect (Jones, 2002). Direct damage occurs when a marketable commodity is damaged by insects which degrade the value (Jones, 2002). Nut borer larvae cause direct damage to macadamia nuts (Jones, 2002). Indirect damage occurs when the insects feed on parts of the plant that is not marketed and this type of damage can be tolerated more than direct damage (Jones, 2002). When designing a pest management program, it is essential to take into account that using a pesticide or other control measure to kill the pest, does not undo existing damage to the crop (Jones, 2002). Thus, the primary concern of pest management in terms of a high-quality crop such as macadamia is to prevent damage to the marketable product before it occurs (Jones, 2002).

Insects rarely become pests under natural conditions where they are seldom found at extremely high levels (Jones, 2002). Only when insect population numbers increase and cause economic damage are they considered pests (Jones, 2002). The factors that influence insect populations can be either biotic or abiotic (Jones, 2002). Increased orchard sizes and cultivation development creates a large monoculture environment for insects to thrive in and more energy is spent on reproduction than foraging for food (Jones, 2002).

1.8 Population monitoring and control methods

The commercial control strategies discussed in this chapter are applicable and recommended for all four moth species. International food markets have an ever-increasing demand requiring reduced insect presence and reduced pesticide use in export crops, to limit the ecological damage and exposure to consumers (Witzgall *et al.*, 2010). Consumers have also become increasingly aware of aspects such as pesticide residues on fruit (Witzgall *et al.*, 2010). This movement is driven by social, political, climatic, and even emotional development. In order to keep up with the demands of the markets, farmers need to keep pesticide residues to a minimum and employ/utilise other control methods to produce quality products while still being competitive. Novel methods need to be adopted and strategies developed that combine different control methods to ensure effective pest control (Perring *et al.*, 2015). Insecticide use alone is not an effective long-term option and needs to be managed to prevent the evolution of insecticide resistance by pests (Witzgall *et al.*, 2010).

There are currently 23 active ingredients registered for control of the macadamia nut borer pest complex in South Africa. Five of these can be regarded as biological products and a further six as environmentally friendly pheromone-based products (Schoeman, 2021). The remaining 12 active ingredients are generally regarded as safe pesticides (Schoeman, 2021).

1.8.1 Semiochemicals

Semiochemicals is a broad term that defines signalling compounds that mediate interactions or communication between organisms to modify behaviour or elicit specific responses (Reinhard, 2004; Bruce *et al.*, 2005). It has the advantage of being used over relatively long distances in relation to other means of insect communication (El-Shafie & Faleiro, 2017). Insects use semiochemicals to locate a mate, host, or food source, avoid competition, escape natural enemies, and overcome natural defence systems of their hosts (El-Shafie & Faleiro, 2017).

Semiochemicals are classified on their effect and function in the environment (El-Shafie & Faleiro, 2017). There are two main groups of semiochemicals namely pheromones and allelochemicals (Reinhard, 2004; El-Shafie & Faleiro, 2017). Pheromones are used for interactions and communication among individuals of the same species (intraspecific reactions) and allelochemicals for different species (interspecific interactions); (Reinhard, 2004; El-Shafie & Faleiro, 2017; El-Ghany, 2019). Pheromones are blends of multiple compounds differing in structure and amount (Bakthavatsalam, 2016). The blend of the major and minor chemical compounds causes the species specificity of pheromones (Bakthavatsalam, 2016).

Releaser pheromones are separated according to their function, with the most common being sex pheromones which facilitates interactions between sexes of the same species (Reinhard, 2004; El-Ghany, 2019). The first identification of a sex pheromone was that of the silk moth, *Bombyx mori* in 1959 (Butenandt, 1959). Sex pheromones are highly species-specific (Reinhard, 2004) and are therefore used to monitor flight activity patterns and numbers of individuals over seasons. If economic thresholds based on moth counts are available, these can be used in decision making regarding control methods.

1.8.2 Monitoring

Agricultural monitoring uses synthetic pheromones in traps to determine whether a specific insect species is present, when its seasonal flight period commences, and how numbers vary over time (Witzgall *et al.*, 2010). This strategy is used to time insect sprays accordingly (Witzgall *et al.*, 2010). Population monitoring relates trap captures to the abundance of, or to the damage caused by an insect species (Witzgall *et al.*, 2010). Thresholds are therefore used for making decisions whether or not remedial action is needed, and to determine the timing of control measures (Witzgall *et al.*, 2010).

1.8.3 Mating disruption

Mating disruption works on the principle of flooding an orchard with large amounts of synthetic female sex pheromones to prevent males from locating females for reproduction (Foster & Harris, 1997). It causes communication disruption and disorientation (Witzgall *et al.*, 2010). There is no evidence that pheromones cause adverse effects on public health, non-target organisms, or the environment making it a safe control method (Witzgall *et al.*, 2010). An example of a mating disruption product used for the macadamia nut borer (*T. batrachopa*) and false codling moth (*T. leucotreta*) is X-Mate™, a product registered by Insect Science (www.insectscience.co.za, 2022).

1.8.4 Attract and kill

Attract and kill strategies use similar principles as mating disruption. The use of attract and kill technology implies that products consisting of a formulation of an insecticide and pheromone or infected with a pathogen are applied onto trees in an orchard to attract male moths. These moths are then killed by the insecticide in the product, or infected with a pathogen to kill it at a later stage (Foster & Harris, 1997). An example of a registered attract and kill product for FCM in South Africa is Last Call FCM ® (Stotter, 2009).

1.8.5 Chemical control

Chemical control targets the eggs and the larval stages of pests before entering the fruit. Chemical control practices are complicated by the timing of applications and the small window between emergence and entry of fruit by pest larvae. No pesticides were registered for FCM control until the early 1980s (Stotter, 2009). However, many pesticide formulations have since been registered for control of FCM, macadamia nut borer and litchi moth. One of the challenges with chemical pest control is the evolution of resistance (Tabashnik et al., 1989). Insect resistance creates a treadmill effect where more pesticides are needed to be effective (Malan *et al.*, 2018). Chemical control should therefore only be used when other methods do not provide sufficient control.

1.8.6 Biological control

Biological control forms an important part of natural pest control in orchards. For example, *Thaumatotibia leucotreta* and *C. peltastica* eggs on litchi are parasitized by a *Trichogrammatoidea* sp. (Hymenoptera: Trichogrammatidae); (Newton & Crause, 1990; Grové *et al.*, 2014). *Trichogrammatoidea cryptophlebiae* is successfully used as a biocontrol agent against *T. leucotreta* and *C. peltastica* (Manrakhan *et al.*, 2008). Mass rearing of parasitoids and natural enemies are done to release large numbers of individuals into orchards as part of area-wide management strategies for some of these pests.

1.8.7 Cultural control

This method of control is used in conjunction with other methods to ensure suppression of pest numbers. It can also provide effective long-term control to a certain extent. One of the more effective cultural control methods is orchard sanitation, which implies removing fallen fruit from orchards (Schwartz, 1981; Stibick, 2006). Visibly damaged fruit should also be removed even if it has not fallen yet since it may be infested with larvae and attract moths for oviposition (Schwartz, 1981; Stibick, 2006). The removed fruit should be destroyed either by burning, or crushing, or buried underneath plastic covers which will prevent larvae from pupating in the soil (Schwartz, 1981; Stibick, 2006).

Manipulation of tree size to keep it manageable for pesticide application and harvest can lead to improved efficacy of chemical control (Schoeman, 2009). Trees of some macadamia varieties can be up to 10 m tall and commercial air-assisted sprayers can only effectively reach up to 4 – 4.8 m (Schoeman, 2009). It has been reported that mature and dense trees do not allow effective pesticide penetration into the canopy, which can lead to refuge areas for pests (Schoeman, 2009).

1.8.8 Host plant resistance

Host plant resistance and/or tolerance is the first line of defence against pests and may be a cost-effective strategy to use in macadamia crop protection, similar to its use in the avocado industry in South Africa (Schoeman, 2009). The resistance is largely a function of the combined husk and shell thickness to prevent damage to the kernel (Schoeman, 2009). No literature regarding resistance of macadamia to Lepidoptera pests could be found.

1.8.9 Sterile insect technique

The sterile insect technique (SIT) is an area-wide and long-term management strategy where large numbers of sterile males are released into a specific area to mate with wild females, resulting in the production of unfertilised eggs (Foster & Harris, 1997). The SIT requires rearing large numbers of the target pest species, exposing them to ionizing radiation to induce sexual sterility, and releasing them into the target pest population on an area-wide basis (Klassen & Vreysen, 2021). The SIT principle is dependent on the population dynamics and ratio of over-flooding wild males with sterile males (Nepgen, 2014; Cardé, 2021). The SIT technique is being successfully used in the Citrusdal region in the Western Cape (Hofmeyer *et al*., 2005; Hofmeyer *et al*., 2015) for FCM control. In the 2005-2006 season, a pilot project in the Citrusdal region showed a 95 % reduction in fruit damage by FCM, which resulted in the citrus industry wanting to introduce the program on a commercial scale (Barnes *et al.*, 2015; Boersma, 2021). The program showed that SIT is a sustainable method to suppress pest populations and to limit damage below threshold values (Boersma, 2021).

1.9 Types of damage and economic importance of the nut borer complex

Figure 8: Types of damage caused by larvae of the lepidopteran borer complex to macadamia nuts.

Damage by lepidopteran larvae to nuts are usually visible as small entry holes with frass which can be observed on the husk where the larvae entered the nut (Prinsloo & Uys, 2015). When nuts are small, the entire kernel may be eaten, but in larger nuts, only part of the kernel is eaten, leaving the nut unsuitable for processing (La Croix & Thindwa, 1986). When nuts are small, larvae may need to consume more than one kernel, and to do so, they usually move to adjacent nuts. This means that larvae need to move to the nut's surface and migrate to reach a fresh nut (La Croix & Thindwa, 1986). When macadamia nut shells are fully hardened, the larvae cannot penetrate them and larvae are only able to complete their development inside the husk (La Croix & Thindwa, 1986). If vascular bundle tissue inside fully developed nuts are damaged by larval feeding, all further development is halted (Prinsloo & Uys, 2015). Thus, the fruit drops from the tree when the husk is damaged. The latter type of damage (fruit drop) is typical of the nut borer complex and distinguishes it from damage symptoms of other pests of macadamia (La Croix & Thindwa, 1986).

1.10 Aims of this study

The aim of this study was to investigate the ecology of the lepidopteran nut borer complex of *Macadamia integrifolia* in the Barberton area of South Africa and to determine the geographical distribution of these pest species in macadamia production areas in the country.

The specific aims of this study were to:

- determine moth flight patterns of the nut borer complex in macadamia orchards throughout the season at Barberton
- determine species composition of the Lepidoptera complex inside macadamia nuts in the Barberton area.
- assess the geographical distribution of the nut borer complex in different macadamia production regions of South Africa.

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Chapter 2

The seasonal flight patterns of the Lepidoptera moth borer complex of macadamia nuts in the Barberton area

Abstract

Larvae of the Lepidoptera nut borer complex cause economic damage to macadamia in South Africa. The Lepidoptera species that constitute the nut borer complex in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae). Mixed populations of these species have been reported to occur in macadamia orchards in South Africa. Monitoring of moth flight patterns provides information on when seasonal flight activity commences and provides information about the abundance of moths over time. Monitoring data can then be used in decision-making regarding the application of various pest management practices. In this study, pheromone traps were used to monitor the moth numbers of these four species during one growing season in two macadamia orchards. Three moth species were captured during the 28-week period, with *T. leucotreta* and *T. batrachopa* making up 69 % and 27 % of the trap catches, respectively. No *E. ceratoniae* moths were captured. Peak moth flight numbers were recorded 12-16 weeks after the commencement of flowering, followed by smaller peaks at 22 and 25 weeks. Significant bycatches of *T. batrachopa* were made in the traps intended for *C. peltastica*, and *vice versa.* This may result in an overestimation of their pest status, especially during early season.

Keywords: Macadamia pests, Tortricidae, Pest complex, Pyralidae

2.1 Introduction

The complex of lepidopteran pests that targets macadamia nuts in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (False codling moth, FCM), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) (Macadamia nut borer, MNB), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (Litchi moth, LM) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Carob moth, CM) (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Schoeman, 2016).

While the life cycles of these pests are similar, the peak periods of activity of moths of the different species may vary during the season. Synthetic sex pheromones are used in monitoring programs to determine whether a specific insect species is present, when seasonal flight activity commences, and how individual numbers vary over time (Witzgall *et al.*, 2010). Therefore, monitoring data provides information on the relative abundance of different species of moths during a season and can be used to facilitate effective management of this pest complex. Although monitoring of adults provides information on the presence of species in orchards, it is not always an accurate predictor of the pest status of these species in macadamia orchards (Smith *et al.*, 2022). If the information on seasonal moth flight activity and the abundance of moths can be related to their pest status (Witzgall *et al.* 2010), monitoring data can play an essential role in decision-making regarding control actions such as the application of pesticides and, if available, the timely release of biocontrol agents. For example, La Croix & Thindwa (1986) reported that the maximum incidence of larval damage is reached approximately four weeks after the influx of moths into macadamia orchards.

Pheromone trapping provides information on seasonal patterns of pest species and may also indicate changes within a pest complex over time. For example, in Malawi, long-term monitoring (1980 – 1987) of the lepidopteran pest complex in macadamia found that *C. batrachopa* increased in abundance over the years to become the dominant species, while *C. leucotreta* numbers decreased (La Croix & Thindwa, 1986). Similarly, a recent survey in South Africa (Smith *et al.*, 2022) showed that the dominant lepidopteran pest of macadamia was *T. batrachopa*. This is contrary to results from surveys during the early 2000s in the Levubu region (Limpopo Province), which showed *T. leucotreta* was the dominant species, followed by *T. batrachopa* (Mlanjeni *et al.*, 2002).

A previous study of macadamia pests in South Africa highlighted the confusion regarding the pest community composition and when insecticides should be applied to control the moth borer complex. The latter study reported that some growers commence insecticide applications immediately after the flowering period (September/October), despite reports by Waite *et al.* (1999) and Schoeman (2009), which indicate that this group of insects are primarily pests of larger, older nuts.

Limited information exists on the moth activity patterns in macadamia orchards in South Africa. Monitoring of *T. leucotreta* and *E. ceratoniae* numbers using pheromone traps in citrus in the Western Cape region indicated two periods of activity (summer and early spring), with damage taking place during autumn, close to harvest (Morland *et al.*, 2019). Both CM and FCM seasonal cycles within citrus orchards show a strong relationship with the phenology of the citrus tree and the prevailing seasonal weather patterns (Morland *et al.*, 2019). Similar relationships between tortricid infestation patterns and macadamia tree and nut phenology have been reported by Schoeman (2009). The dynamic nature of pest populations within macadamia orchards necessitates continuous monitoring to tailor control strategies to specific pests that are present and dominant (Smith *et al.*, 2022).

This study aimed to determine the moth flight patterns of the nut borer complex over a growing season in the Barberton area, Mpumalanga, South Africa.

2.2 Material and methods

2.2.1 Study area

This study was conducted over an entire production season, from September 2020 to March 2021. The trial site was located on a commercial farm in the Barberton region, situated ± 40 km from Nelspruit in the Mpumalanga province (25°44'43.3" S, 31°00'51.8" E) (Figure. 2.1). Crop management was done according to local agricultural practices during the growing period. The insecticides used in the orchards are listed in Table 2.1.

Table 2.1. Insecticides, active ingredients, and dates of application for pest control in the macadamia orchard at Barberton.

Date applied	**Week number as indicated in Chapter 2** (or 3)	**Name**	**Active ingredient and insecticide class**	**Group**
07/10/2020	**5**	Chlorpyrifos	Chlorpyrifos (organophosphate)	1B
21/10/2020	**7** (1)	Rossi	Fipronil (phenyl pyrazole) Lambda-cyhalothrin (pyrethroid)	2B/3A
01/11/2020	**9** (3)	Rossi	Fipronil (phenyl pyrazole) Lambda-cyhalothrin (pyrethroid)	2B/3A
09/11/2020	**10** (4)	Methomex	Methomyl (carbamate)	1A
16/11/2020	**11** (5)	Cypermethrin	Cypermethrin (pyrethroid)	3A
23/11/2020	**12** (6)	Methomex	Methomyl (carbamate)	1A
20/12/2020	**16** (10)	Bulldock	Beta-cyfluthrin (pyrethroid)	3
15/02/2021	**24** (18)	Cypermethrin	Cypermethrin (pyrethroid)	3A

The study was conducted in two orchards separated by a narrow dirt road (Figure 2.2). The macadamia cultivar grown in one block was Beaumont, while there were two cultivars grown in the other block (mixed block) (Figure 2.2). The mixed block consisted of alternating rows of cv. 816 and Beaumont (Cultivar code 695). The orchards were established approximately 15 years ago. The tree spacing in each orchard was 8 m x 4 m, which is the standard industry spacing in macadamia orchards (SAMAC, 2020). Treatment blocks consisted of 300 to 330 trees per hectare.

Previous studies showed that planting Beaumont trees in mixed stands with other macadamia cultivars, resulted in reduced levels of nut damage by both Heteroptera and Lepidoptera pests in cv. Beaumont. The presence of a resinous substance in cv. Beaumont may act as a deterrent but it has not been scientifically proven (Schoeman, 2009). In a no-choice situation, pure stands of cv. Beaumont are also attacked by both moth species of the nut borer complex.

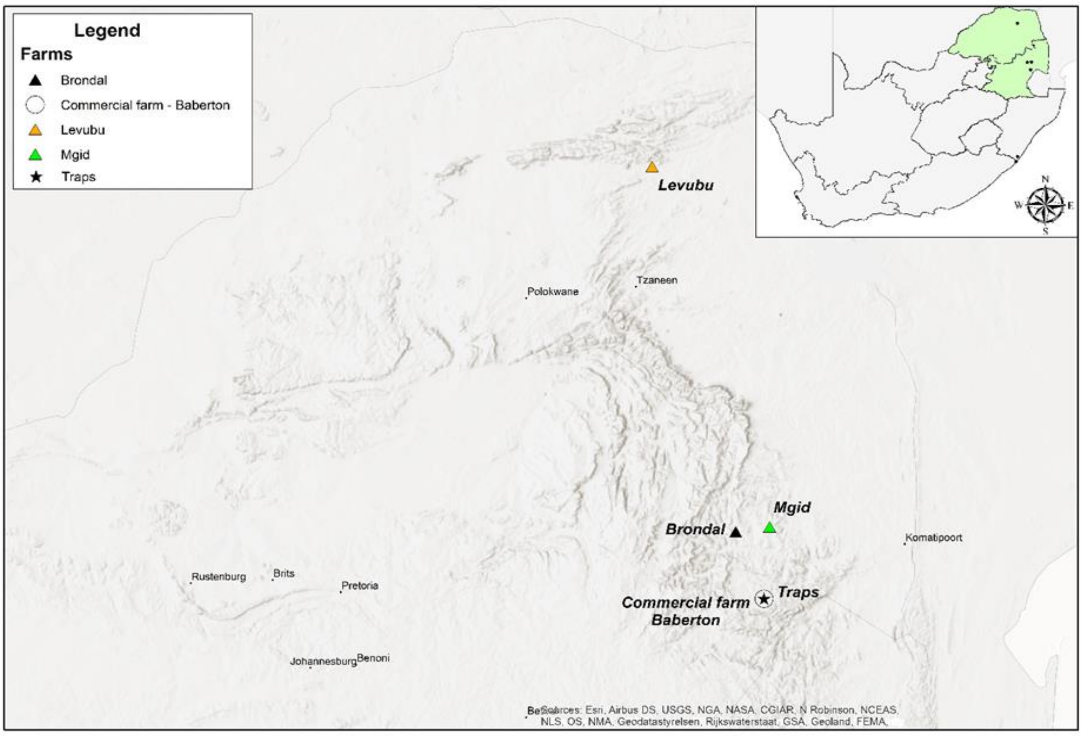

Figure 2.1: Map indicating the locality (star) outside the town of Barberton where this study was conducted.

Figure 2.2: Orchard layout at the Barberton study site. Blue lines indicate rows sampled in the Beaumont block, and orange lines indicate rows of cv. 816 that were sampled in the mixed block.

2.2.2 Pheromone traps

The layout of the pheromone traps in the two different orchards is illustrated in Figure 2.3. Three trapping stations were established for each of the four moth species inside each of the orchards (blocks) (Figure 2.3), according to the specifications provided by the manufacturers. Each trapping station had four delta pheromone traps, one for each of the four Lepidoptera species (Figure 2.4). Moth trapping commenced at the start of flowering (07/09/2020) and was done until harvest (08/03/2021). Peak flowering occurred at week 2 of this study. Traps were spaced 12 m apart (4 trees) with MNB and LFM being at opposite ends of the trap station.

Figure 2.3: Locations of yellow delta pheromone trap stations inside the two macadamia orchards at Barberton.

The pheromone lures used for monitoring the different species and distributors of dispensers are provided in Table 2.2.

Pheromone dispensers and traps were sourced from manufacturers in South Africa (Table 2.2). The FCM and LM pheromone lures were supplied by Insect Science (Pty) Ltd. (Tzaneen, South Africa), while the MNB and CM lures were provided by (Chempac (Pty) Ltd., Simondium, South Africa). Insect Science Yellow delta traps and sticky liners (Insect Science (Pty) Ltd. Tzaneen, South Africa) were used during the study (Figure 2.4). Pheromone dispensers were replaced as indicated by the manufacturer's guidelines (Table 2.2).

Traps were checked at weekly intervals for 28 weeks, and the number of moths of each species were recorded. Sticky liners were replaced each week. The bycatch was also recorded for each of the different traps. Moths of the four species were considered bycatch if they were caught in traps baited with pheromones other than indicated for their species. The number of moths of any of the other three target species, other than the species that the specific pheromone was intended, was considered the bycatch.

Table 2.2. Pheromone lures and trap type used for monitoring of *Thaumatotibia leucotreta*, *Thaumatotibia batrachopa*, *Cryptophlebia peltastica* and *Ectomyelois ceratoniae*, numbers in macadamia orchards in Barberton.

Species	Common name	Provider	Lure replacement intervals	Active ingredient
Thaumatotibia leucotreta	False codling moth	Insect Science, Tzaneen, South Africa.	Not replaced. Lasts 28 – 30 weeks.	E-7-dodecenyl acetate
Thaumatotibia batrachopa	Macadamia nut borer	Chempac, Paarl, South Africa.	Every 6 weeks.	Z-8-dodecenyl acetate
Cryptophlebia peltastica	Litchi moth	Insect Science, Tzaneen, South Africa.	Every 6 weeks.	Z-8-dodecenyl acetate E-8-dodecenyl acetate
Ectomyelois ceratoniae	Carob moth	Chempac, Paarl, South Africa.	Every 6 weeks.	(7Z,9E)-7,9,11-dodecatrienyl formate

Figure 2.4: Two pheromone traps suspended from macadamia tree branches at a trapping station (left) and an example of a sticky liner with moths (right).

2.2.3 Data analysis

The mean number of moths per trap per week was calculated for each species for each of the two orchards. Descriptive statistics were used to illustrate moth flight patterns for each orchard over time. To determine a general flight pattern for the study site, moth catch numbers were also pooled for each species, and a three-week moving average was calculated for the 28-week period. Summary statistics were conducted using Microsoft Excel (Microsoft 365).

2.3 Results

A total of 1673 moths were caught in the yellow delta traps over the 28-week period. No *E. ceratoniae* moths were caught in any traps throughout the season.

The proportions of moths of the different species captured during the season are provided in Figure 2.5. Pheromone trap results indicated that the nut borer complex at the study site in Barberton was dominated by FCM, followed by MNB, which collectively constituted 96 % of the total catch.

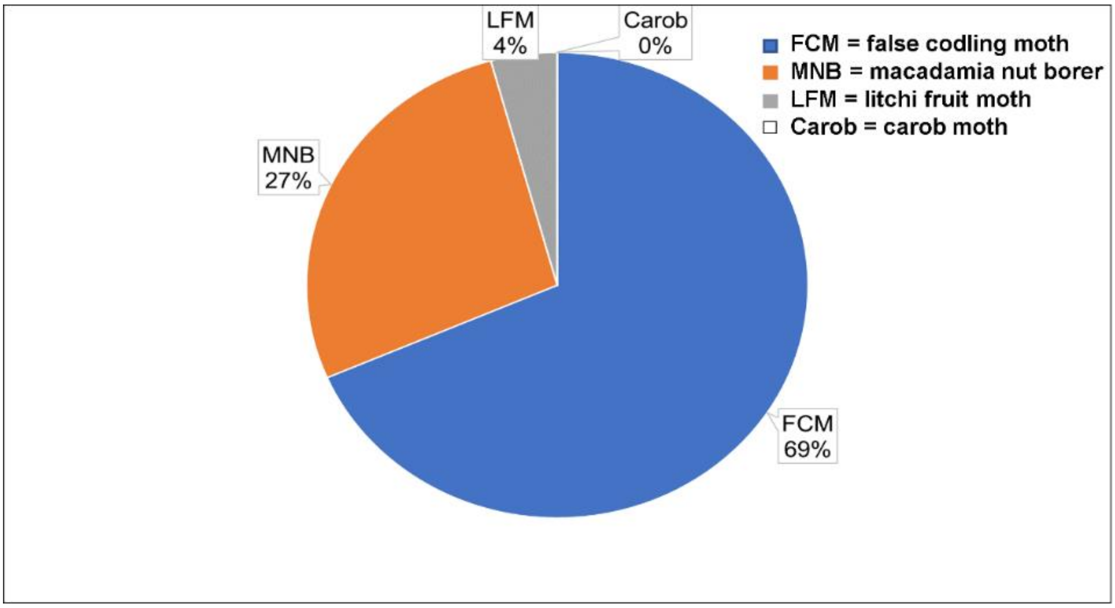

Figure 2.5: Pie chart showing the percentage of moths of each species captured in yellow delta traps during the 28-week trapping period. The total number of moths was 1673.

Results of the mean numbers of moths per trap per week in the Beaumont orchard are presented in Figure 2.6 A. During the first week of monitoring, the first FCM and MNB moths were captured very early in the season. The numbers of MNB moths caught in traps were very low during the beginning of season and only started to increase from week 12 onwards, with a peak in numbers during weeks 12-16 and smaller peaks, at 21 and 25 weeks. FCM moth numbers were also low (5</per trap/week) during the first 11 weeks after which high numbers were recorded from 12 - 16 weeks, followed by three smaller peaks at 21 - 23, 25, and 28 weeks. The numbers of LM moths were very low (<5 moths per trap) throughout the season (Figure. 2.6 A), and virtually no captures were made in the latter part of the season.

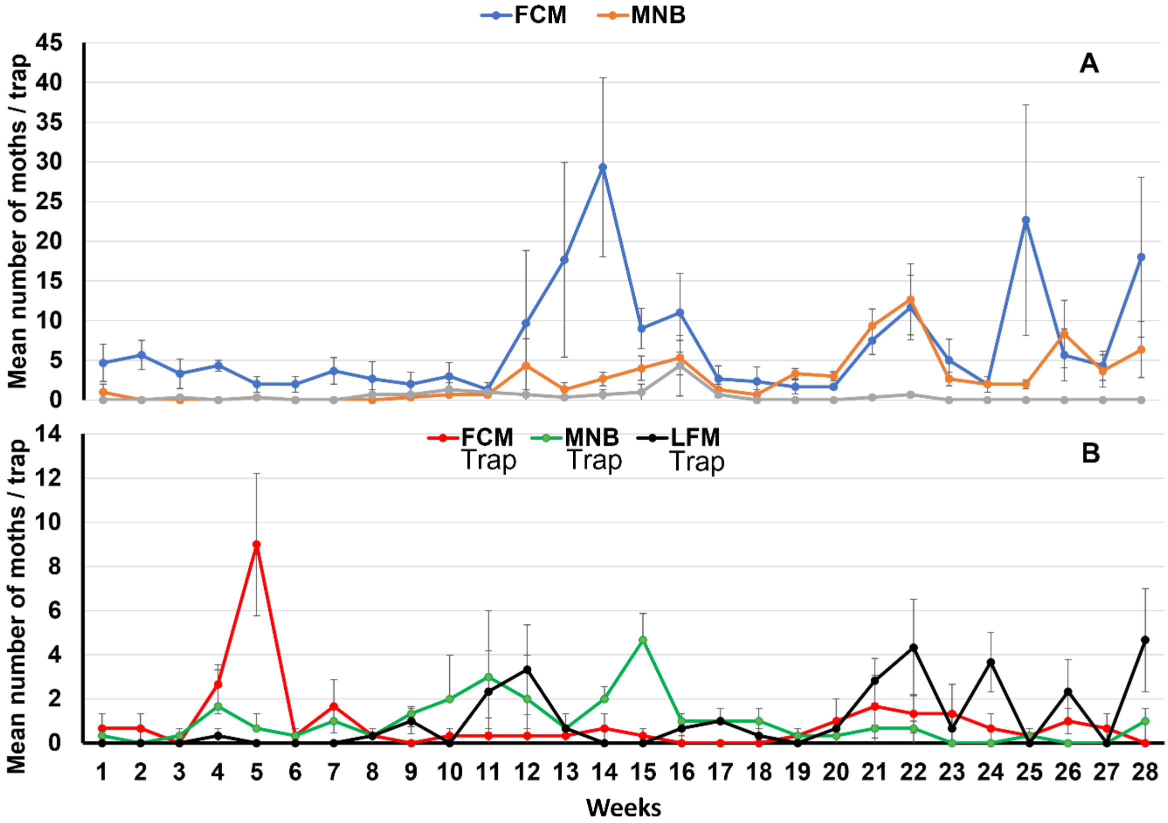

Figure 2.6: Mean number of (A) FCM, MNB, and LM moths per trap, and (B) bycatch of FCM, MNB, and LM moths per trap. Trapping started at week 1, when flowering commenced, and was continued over a 28-week monitoring period in a Beaumont cultivar orchard. Bycatch in FCM traps were limited to moths of different Looper (Geometridae) species. Bycatch in MNB traps was limited to LM moths. Bycatch in LM traps was limited to MNB.

Data on the bycatches of moths in the cv. Beaumont orchard are provided in Figure 2.6 B. Compared to the numbers of moths captured in the designated traps during the monitoring period (Figure. 2.6 A), comparatively high numbers were caught as bycatch. The mean number of MNB moths captured towards the latter part of the season was <1 per trap, while that of LM was comparatively higher toward the end of the season. The mean number of LM moths per trap was 2 or higher on several occasions after week 20. These late-season LM moth numbers in the bycatch of the MNB traps, were mostly higher than those captured in the actual designated LM traps.

Results of the mean numbers of moths per trap per week in the cv. 816 orchard are presented in Figure 2.7 A. The first FCM moths were captured very early in the season.

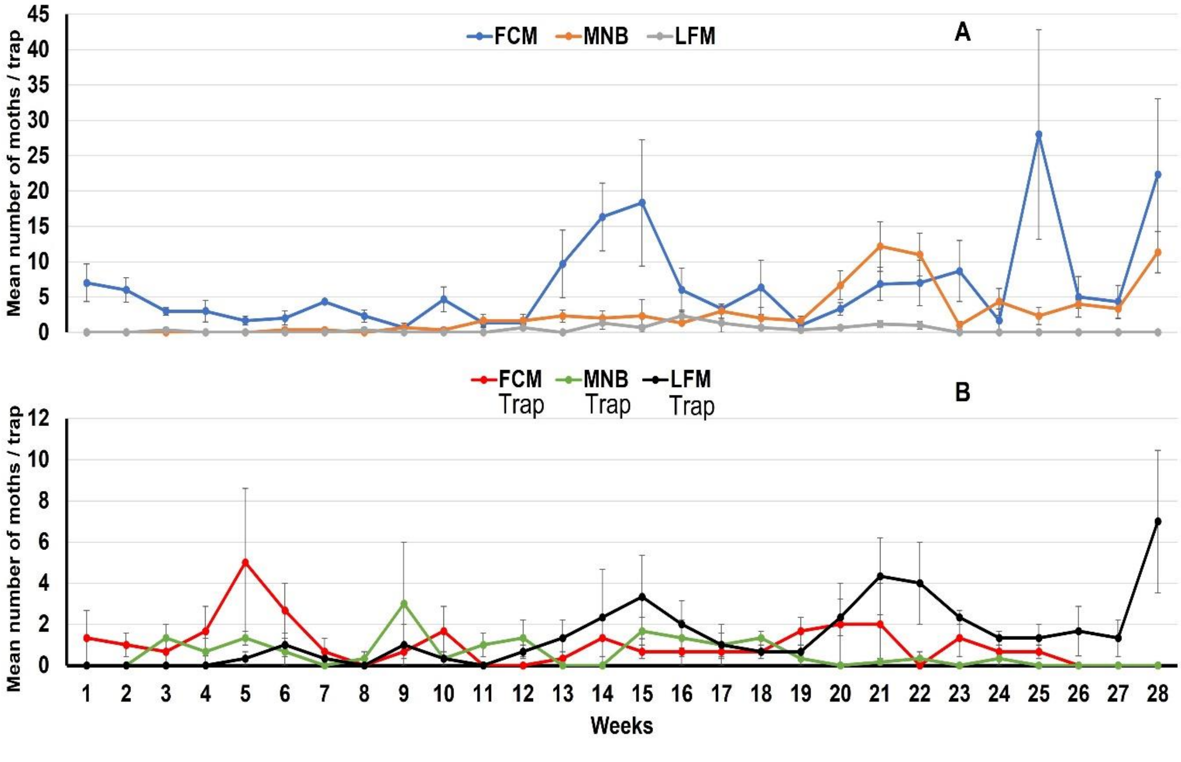

Figure 2.7: Mean number of FCM, MNB, and LM moths per trap. Trapping started at week 1, when flowering commenced, and was continued over a 28-week monitoring period in the cv. 816 orchard. Bycatch in FCM traps were limited to moths of different Looper (Geometridae) species. Bycatch in MNB traps was limited to LM moths. Bycatch in LM traps was limited to MNB.

Mean FCM moth numbers from 3 to 12 weeks were low (≤ 5 per trap/week), after which numbers increased, indicating peak flight periods from week 13 to 15, and once again at week 25. The number of MNB and LM moths captured during the first 19 weeks of the season was extremely low (≤ 2). MNB moth numbers were very low throughout the season, with a slight peak in numbers from week 20 to 22. No LM moths were captured after week 22.

The bycatches of moths in the cv. 816 orchard are provided in Figure 2.7 A. Compared to the numbers of moths captured in the designated traps during the monitoring period (Figure. 2.7 A), comparatively high numbers were caught as bycatch. The mean number of MNB moths captured towards the latter part of the season was very low (<1 per trap), while that of LM was comparatively higher toward the end of the season, with a mean number of 2 or higher being recorded on several occasions after week 19. These relatively high LM moth numbers in the bycatch of the MNB traps were, in many cases, higher than those captured in the designated LM traps. No CM or bycatch for CM traps were caught during the study. FCM trap bycatch were moths of different Looper (Geometridae) species.

The general moth flight patterns of *T. leucotreta*, *T. batrachopa* and *C. peltastica* were calculated using a 3-day moving average of pooled data from the two different orchards and are provided in Figure 2.8. Results show the dominance of *T. leucotreta* moths in traps throughout the season and a distinct peak in activity between week 13 and 17 after the commencement of flowering. The nuts were mature and fully developed when peaks at week 25 and 28 occurred. *Thaumatotibia batrachopa* and *C. peltastica* moth numbers only increased after ten weeks, with a minor peak in *C. peltastica* numbers from 16 to 18 weeks. A notable increase in *T. batrachopa* numbers occurred from weeks 20 to 22.

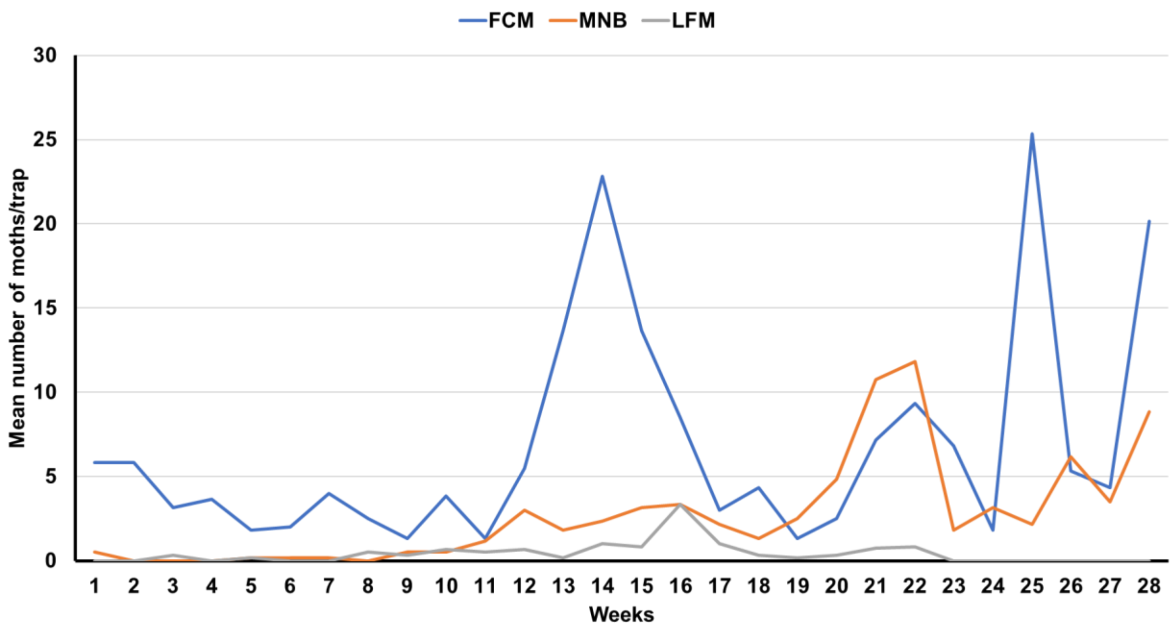

Figure 2.8: Generalised flight patterns of FCM, MNB, and LM moths in two macadamia orchards in the Barberton macadamia production area of South Africa. Flowering commenced at week 1 (07/09/2020). Trapping was done until week 28 (08/03/2021).

2.4 Discussion

The activity patterns of the Lepidoptera nut borer complex moths have not previously been determined in macadamia orchards in South Africa. This study showed that moth numbers increase in orchards at the beginning of the season and that there is a marked increase in moth numbers during the period from 13 to 18 weeks. No *E. ceratoniae* moths were captured. This was ascribed to the lures not being effective since, based on the presence of larvae inside nuts (Chapter 3), moths were present in the orchards. Although the sex pheromone lures of *E. ceratoniae* used in this study were also provided by Chempac, no moths were captured. Morland *et al.* (2019) did however, capture *E. ceratoniae* moths in citrus orchards in the Western Cape, using the same pheromone formulation. In the latter study, both *T. leucotreta* and *E. ceratoniae* showed increased activity in summer and early spring with damage occurring during autumn, close to harvest.

The macadamia industry in South Africa considers *T. batrachopa* to be the most important species in the nut borer complex (Smith *et al.*, 2022). Information on species dominance has largely been based on the occurrence of larvae of these species inside nuts (Mlanjeni *et al.*, 2004; Smith *et al.*, 2022). The latter studies showed that the dominant lepidopteran pest of macadamia in the early 2000s in the Levubu region (Limpopo Province) varied interchangeably between *T. batrachopa* and *T. leucotreta* within a season (Mlanjeni *et al.*, 2004). According to Bruwer (2001), *T. batrachopa* is the most dominant nut borer species, making up to 90 % of the species complex, followed by *T. leucotreta* (8 %) and *E. ceratoniae* (2 %).

However, in the present study, the predominant species, based on pheromone captures in the Barberton region, was *T. leucotreta* which constituted 69 % of the total moths captured, followed by *T. batrachopa* with 27 %. Furthermore, significant bycatches of *T. batrachopa* in traps intended for *C. peltastica,* and *vice versa* were recorded. The respective species were considered bycatches if they were present in traps of the other species monitored in this study. No trap interference was expected in this study since traps were spaced 12 m apart (4 trees) with MNB and LFM being at opposite ends of the trap station.

The capture of species in traps other than those intended, can be ascribed to the similarity of certain compounds in the pheromone blends of these species (Table 2.1). The number of *C. peltastica* moths captured as bycatch in traps for *T. batrachopa* was higher than that of the designated traps on several occasions during the study. This indicates that the *C. peltastica* traps did not effectively capture this species during the late season, although there were still significant numbers of moths present in the orchards. It is essential to be able to distinguish between LM and MNB to ensure the correct control measures are used mainly when highly species-specific products are used, like pheromones. Therefore, a pest species cannot be assumed based on the broader area but must be correctly identified for each individual locality.

Although the population levels of these moths appear to be relatively low in most cases, population outbreaks may occur if the correct combination of host plants and environmental conditions occur (Schoeman, 2009). This could result in increased pheromone trap catches and larval damage on nuts (Schoeman, 2009). This spike in moths can easily be detected when monitored correctly and can be predicted to improve control.

According to Jones (1995), pheromone traps are limited in determining population dynamics in macadamia orchards and are not a reliable source of information to reflect the damage caused by larvae.

Although this study, which shows the dominance of *T. leucotreta,* was only conducted over a single season at one locality, it highlights the importance of relating larval infestation levels to moth flight numbers prior to decision-making on pest control interventions.

2.5 Conclusions

Monitoring of *T. leucotreta, T. batrachopa* and *C. peltastica* can be done commercially by using pheromone traps. Technology intended for monitoring purposes are commercially available, and results from season-long monitoring can be used to facilitate appropriate management actions. For effective decision-making, supporting information regarding flight peaks could be combined with egg counts on the fruit and larval infestation patterns. More information is needed regarding the relationship between moth flight patterns, egg counts and larval infestation levels in macadamia nuts.

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Chapter 3:

The relative abundance of larvae of the Lepidoptera moth borer complex inside macadamia nuts in the Barberton area

Abstract

The different species of the nut borer complex often co-occur in mixed populations in macadamia orchards in South Africa. The species that make up the nut borer complex in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (False codling moth, FCM), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) (Macadamia nut borer, MNB), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (Litchi moth, LM) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Carob moth, CM). Moths of these pests lay eggs on macadamia nut husks throughout the season and larval damage to husk tissue results in nuts to drop from trees. Correct identification, especially of the larval stages is important for pest management purposes where species specific interventions are planned. Although pheromone traps can be used to monitor moth abundance in orchards, trap captures are not always an accurate indication of the egg and larval community composition and the time of infestation. The aims of this study were to determine egg parasitism levels as well as the population structure, phenology, and relative abundance of larvae of the nut borer complex over a growing season. Identification of larvae was done using both morphological and molecular methods. Larvae of the four species mentioned above were recorded at varying levels during the season. The community was dominated by *T. leucotreta*, followed by *T. batrachopa*, *E. ceratoniae* and *C. peltastica*. Four other Lepidoptera species were also recorded in nuts, but at very low numbers. These were: *Lobesia vanillana* (de Joannis), *Nola imitate* (Son) (Nolidae), *Janseodes melanospila* (Guenée) (Erebidae), and *Ariathisa* sp*. (Noctuidae).*

Key words: Macadamia pests, Pest phenology, Species composition, Tortricidae, Pyralidae

3.1 Introduction

South Africa is the largest exporter of macadamia nuts in the world. Larvae of the Lepidoptera nut borer complex can damage both young developing nuts as well as old hard nuts which may be unsuitable for processing (La Croix & Thindwa, 1986). Damage to husk tissue of fully developed nuts may result in nuts to drop from trees, which is a typical symptom of the damage caused by the nut borer complex (La Croix & Thindwa, 1986).

The different species of the Lepidoptera nut borer complex often co-occur in mixed populations in macadamia orchards in South Africa (Smith *et al.*, 2022). The biology of these pests is largely similar and due to their small size and similarity between some of the species, identification is difficult (Morland, 2015). The species that make up the nut borer complex in South Africa are three Tortricidae species, i.e., *Thaumatotibia leucotreta* (Meyrick), *Thaumatotibia batrachopa* (Meyrick), *Cryptophlebia peltastica* (Meyrick). One Pyralidae species, *Ectomyelois ceratoniae* (Zeller) also infest macadamia in South Africa (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Schoeman, 2016).

Previous larval surveys in South Africa showed variable results regarding the nut borer community complex. Surveys conducted over a 3-year period in the Levubu region of the Limpopo Province, showed that *T. leucotreta* was the dominant species, followed by *T. batrachopa* (Mlanjeni *et al.*, 2002). A recent survey in South Africa (Smith *et al.*, 2022) showed that the dominant lepidopteran pest of macadamia was *T. batrachopa*. Knowledge of the larval infestation patterns of these pests and their relative abundance in macadamia orchards can play an important role in pest management decisions. Although pheromone trapping and monitoring of adult numbers provide information on their presence and abundance in orchards (Witzgall *et al.*, 2010), it does not always confirm whether macadamia serves as host for the larval stage of a particular species (Smith *et al.*, 2022). Since pheromone trap catches are influenced by environmental conditions (Jones, 1995; Lösel *et al.*, 2002) as well as bycatches and pheromone efficacy (Chapter 2), larval numbers are considered a more accurate indication of infestation levels of a particular species (Jones, 1994a). It is, however, important to relate the relative seasonal abundance of moths to the occurrence of larvae of the different species, since the mere abundance of moths does not indicate a high pest status. For example, poor correlations between moth catches and larval numbers in nuts were reported for *E. ceratoniae* in citrus (Morland *et al.*, 2019), and certain *Thaumatotibia (Cryptophlebia)* species in lichi and macadamia (Jones, 1995). Monitoring of egg numbers on developing nuts can also provide general information on the time of pest infestation in macadamia but was reported not to be a suitable predictor of damage (Jones, 1994b).

A 3-year study of the community composition of larvae inside nuts, conducted in the Limpopo Province in the early 2000's showed that *T. batrachopa* contributed to approximately 70 % of the species complex (Mlanjeni *et al.*, 2004). During certain periods of the season, only larvae of *T. leucotreta* and *T. batrachopa* were collected from nuts. Bruwer (2001) also reported that these two species were the dominant species in the pest complex. The study by Mlanjeni *et al.* (2004) also reported that *T. peltastica* and *E. ceratoniae* were of minor importance and that it made up only 5 % of the species complex in that region. Species composition of these nut borers within and between growing regions in South Africa have recently been addressed by Smith *et al.* (2022) who reported that *T. batrachopa* represented 95 % of the larvae collected in damaged nuts across all growing regions in the country. Correct identification, especially of the larval stages is of significant importance for pest management purposes (Venette *et al*., 2003). Molecular tools, such as the use of mitochondrial cytochrome c oxidase subunit 1 (COI) can be used to identify species and overcome some of the limitations of morphological taxonomy (Hebert *et al.*, 2004; Timm *et al.*, 2007; Smith *et al.*, 2022). However, such methods are not yet available at farm-level and farmers are mostly guided by moth numbers in pheromone traps, when they make decisions regarding pest control.

The aims of this study were to determine the population structure, phenology, and relative abundance of larvae of the nut borer complex of macadamia, over a growing season. Egg parasitism was also recorded. Identification of larvae was done using both morphological and molecular methods. Possible relationships between nut size, population structure and larval infestation levels were also investigated.

3.2 Material and methods

3.2.1 Study area

This study was conducted on a commercial farm in the Barberton region in the Mpumalanga Province. Details regarding the trial site and different orchards in which the study was done, is provided in Chapter 2.

3.2.2 Abundance of larvae inside nuts

Sampling of nuts was done over a 21-week period, from the commencement of flowering (07/09/2020) to harvest (08/03/2021). Sampling was interrupted for four weeks, due to the presence of cyclone Eloise during January 2021. Fixed rows were used for sampling which was done on every second row in the Beaumont (Cultivar code 695) block and every third row on cv. 816 in the mixed block (Figure. 3.1). There were eight replicates per orchard and each replicate consisted of a row of approximately 94 trees.

Twenty nuts that could be identified as recently fallen, were randomly collected from underneath the trees in each of the eight rows per block (160 nuts per block/week) (total number of nuts = 5440). The most recently fallen nuts were sampled and stored in brown paper bags in a fridge at a temperature of 4-6 ºC. This was done to slow down the development of the larvae and limit the rotting of husks. The nuts were dissected and inspected for the presence of Lepidoptera larvae within two days of collection.

Figure 3.1: Orchard layout at Barberton study site. Blue lines indicate rows in the Beaumont block and orange lines indicate rows of cv. 816 in the mixed block.

The diameter of each nut was measured by means of a digital electronic calliper and the number of Lepidoptera eggs on each nut were recorded. No distinction was made between eggs that hatched or not. Black coloured eggs were recorded as parasitised. Nuts were then carefully dissected, and all larvae removed. The number of dead larvae were recorded separately to determine the incidence of natural mortality. The numbers of dead larvae were included in the calculation of the total number of larvae per nut. The areas where larvae occurred inside the fruit were also recorded (Figure. 3.2). The area where larvae occurred within the nut was recorded either as ''outside the kernel'' (feeding on husk or shell), or ''inside the kernel'', which implies damage to the kernel.

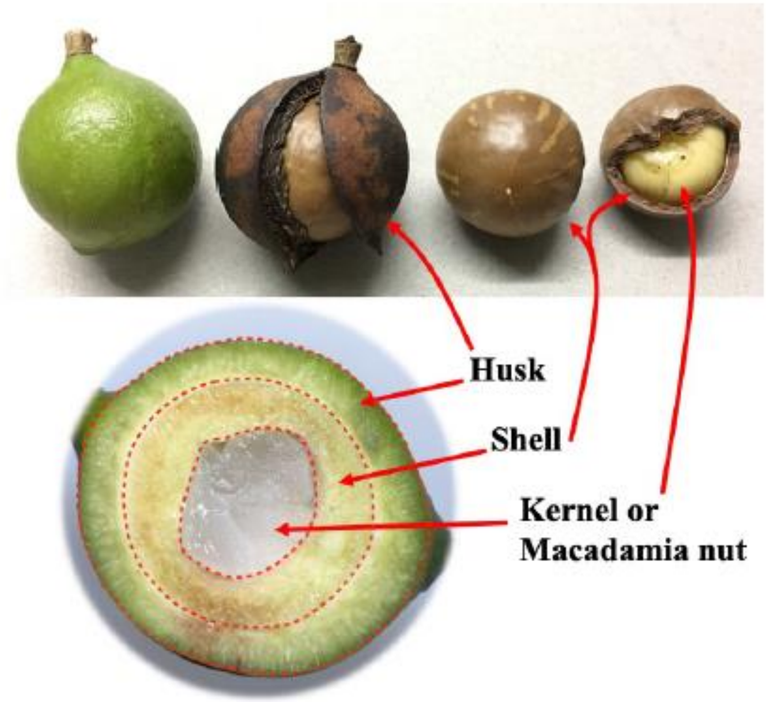

Figure 3.2: Immature macadamia nut cross-section and terminology used for describing mature nuts (Khun, 2021).

3.2.3 Larval identifications

Larvae were individually preserved in 1.5 ml centrifugal tubes filled with 99 % ethanol after which they were identified by means of morphological and molecular methods.

The larvae that could not be identified by means of morphological techniques were identified by means of molecular techniques. Samples were identified at the Department of Agricultural Biology, Colorado State University, Fort Collins, Colorado, USA. Larvae were identified morphologically by using a microscope to examine characters such as the location of primary setae, the arrangement of crochets on abdominal prolegs, the presence or absence of an anal comb and, if an anal comb was present, the number and arrangement of prongs on the anal comb. Since these structures can vary in young instars, only older instars (approximately third-instar and older for Tortricidae) were examined. To confirm morphological identifications a subset of specimens was identified using DNA sequences. DNA was extracted using the Lucigen MasterPure Complete DNA and RNA Purification Kit (Lucigen Corporation, Middleton, WI, USA) by soaking whole larvae in the extraction buffer. After DNA extraction, larvae were removed, rinsed in ethanol, and stored at -80 ºC to retain as voucher specimens. Approximately 658bp of the cytochrome oxidase I (COI) gene was amplified with the primers LepF1/LepR1 (Hebert *et al.*, 2004) using standard PCR protocols. PCR products were purified using ExoSAP-IT® (Applied Biosystems, Foster City, CA). Purified PCR products were sequenced by the University of Chicago Comprehensive Cancer Center DNA Sequencing and Genotyping Facility with an Applied Biosystems 3730XL DNA sequencer (Applied Biosystems, Foster City, California, USA). Sequencing was performed in both directions using the same primers that were used for PCR amplification. The resulting sequences were trimmed, assembled, and aligned using Geneious Prime 2021.0.3 (www.geneious.com). Sequences were identified to species based on comparison to sequences in the BOLD Systems database. A sequence match of at least 98 % was regarded as confirmation of a species, in addition to accurate placement on trees produced by the database using the Kimura Two-Parameter distance model.

3.2.4 Data analysis

Data on nut diameter, total number of eggs and parasitised eggs per nut were analysed by means of descriptive statistics. No distinction was made between newly laid unhatched eggs and eggs that already hatched and which were still visible on the surface of the nuts. Summary statistics were conducted by means of Microsoft Excel (Microsoft 365). The number and position of larvae inside nut (including husk), entry holes into the husk, as well as damage on the inside and outside of each nut were also analysed by means of descriptive statistics.

3.3 Results

A total number of 1449 larvae were collected from nuts over the sampling period. Of these, 528 could be identified to species level, leaving 921 unidentified larvae, and 216 pupae. The larvae that could not be identified are not represented in Figure 3.3. The overall population composition of larvae that were identified, were as follows: FCM = 67 %, MNB = 19 %, LM = 4 % and CM = 10 %. Very low numbers of the following species were also recorded: *Lobesia vanillana* (Tortricidae), *Nola imitate* (Nolidae), *Janseodes melanospila* (Erebidae) and *Ariathisa excise* (Noctuidae).

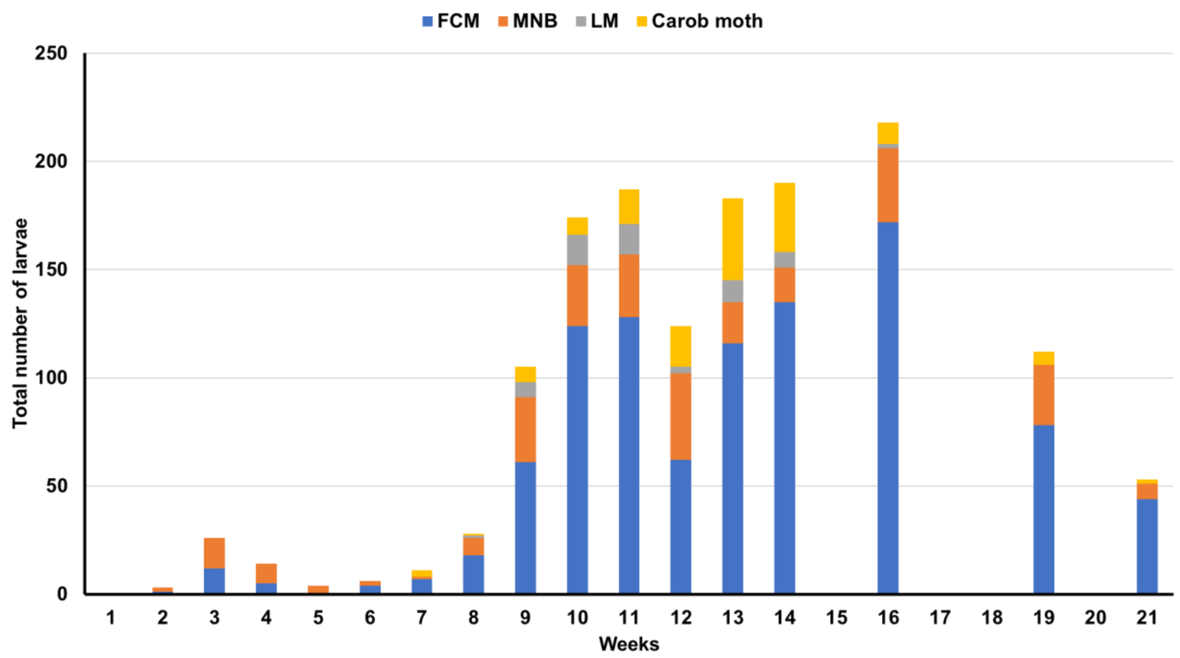

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Figure 3.3. The actual number of identified larvae of each species recorded from 320 fallen nuts, sampled at weekly intervals.

The numbers of larvae per nut was very low during the first few weeks of the season (Figure. 3.3). A sharp increase in larval numbers commenced during week 9 and high numbers remained high until week 19.

For the first four weeks of sampling only two species were present, FCM and MNB (Figure. 3.4). MNB was dominant for the first four weeks after which FCM dominated for the remainder of the growing season. MNB made up 67 %, 54 %, 64 % and 100 % of the population for the first four weeks. For the remainder of the growing season MNB made up a much smaller portion of the population. Approximately 38 % of the population over the first three weeks consisted of FCM. From week five onwards, FCM comprised approximately 67 % of the population.

CM only occurred from week six onwards and remained present for the rest of the growing season. The proportional contribution of CM varied during the season with a high of 27 % of the larvae during week six and a low of 3 % in week seven. CM was only present in very low numbers towards the end of the season. LM larvae did not occur until week seven and no larvae were detected after week 15. The LM population made up the smallest percentage of the pest complex.

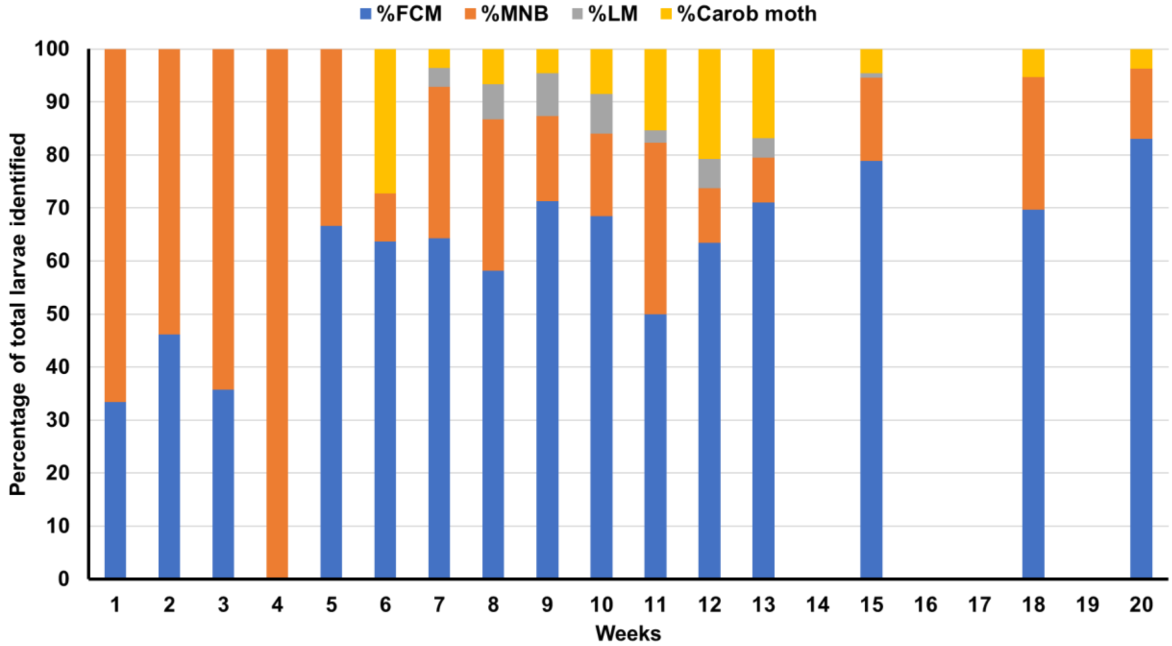

Figure 3.4. Percentage of total FCM, MNB, and LM larvae identified, recorded from 320 fallen nuts, sampled at weekly intervals over a 20-week period.

The mean size of nuts of the two cultivars over time is presented in Figure 3.5. Nut size increased steadily until approximately 16 weeks after commencement of flowering after which they were fully developed. It was only in the period from 9 to 12 weeks that differences in nut size between varieties could be observed with nuts of cv. 816 developing quicker during this period. The mean diameter of nuts of Beaumont and cv. 816 at full maturity was 31.6 mm and 31.7 mm, respectively (Figure. 3.5).

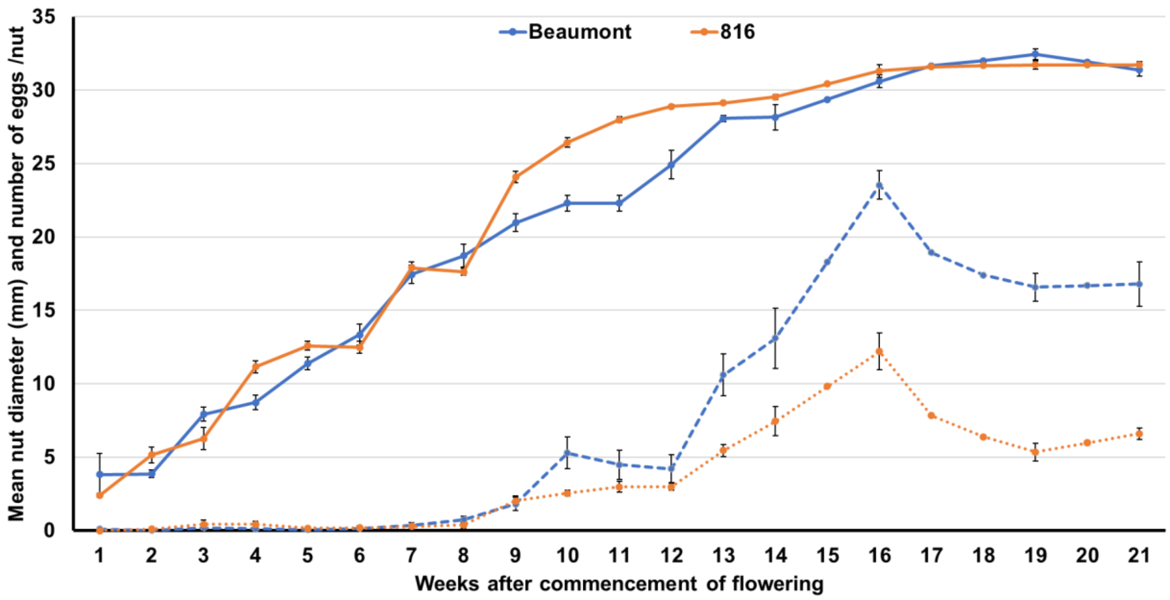

Figure 3.5: Mean diameter of macadamia nuts and mean number of lepidopteran eggs per macadamia nut sampled from Beaumont and cv. 816, over a 21-week period after commencement of flowering. Solid lines indicate nut diameter, dotted lines indicate numbers of eggs. Bars indicate Standard Error.

Very low numbers of eggs (<3 / nut) were detected on nuts during the first nine weeks when nut diameter was < 25 mm (Figure. 3.5). The mean numbers of eggs per nut differed between the two cultivars with higher numbers occurring on Beaumont throughout the sampling period. The mean number of eggs started to increase from week 12 onwards and peaked at week 16, with a mean of 23.5 and 12.4 eggs per nut for Beaumont and cv. 816, respectively. The mean number of eggs per nut decreased late in the season (week 17 onwards).

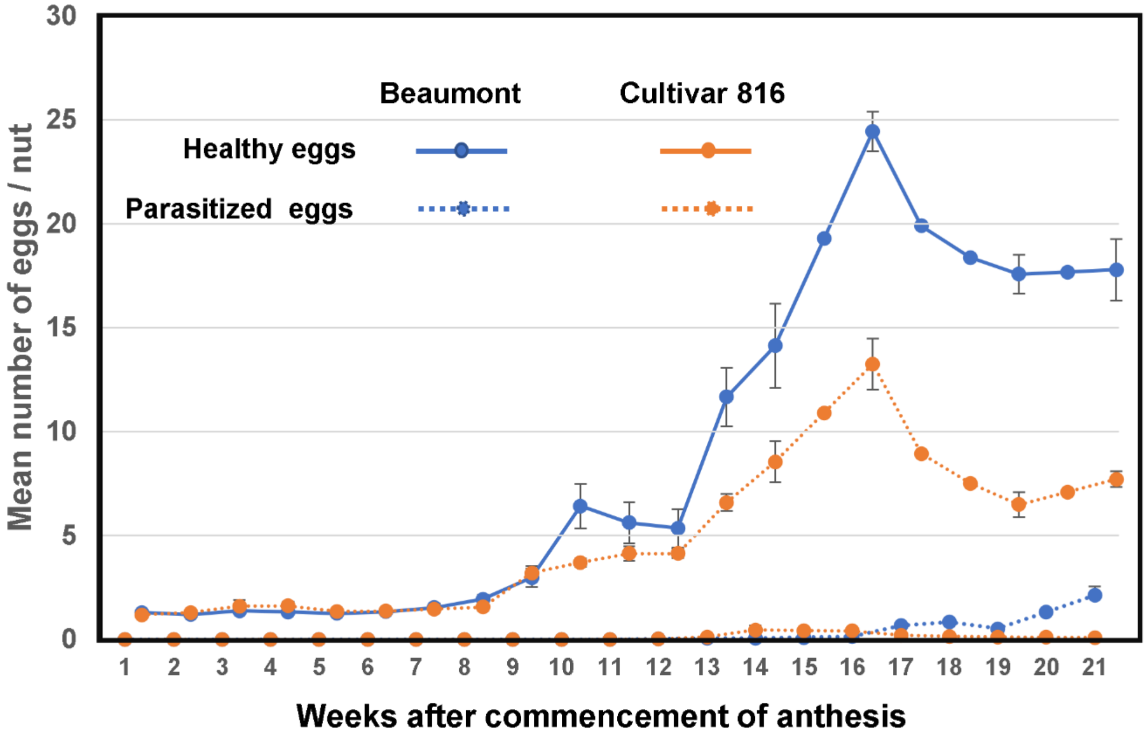

Figure 3.6: The mean number of eggs (hatched + unhatched) and parasitized eggs per macadamia nut sampled from cv. 816 and Beaumont over a 21-week period after flowering. Bars indicate Standard Error.

The mean number of parasitized eggs remained consistently low throughout the season with a slight increase to 2.5 parasitized eggs per nut on Beaumont in the last week of sampling (Figure. 3.6).

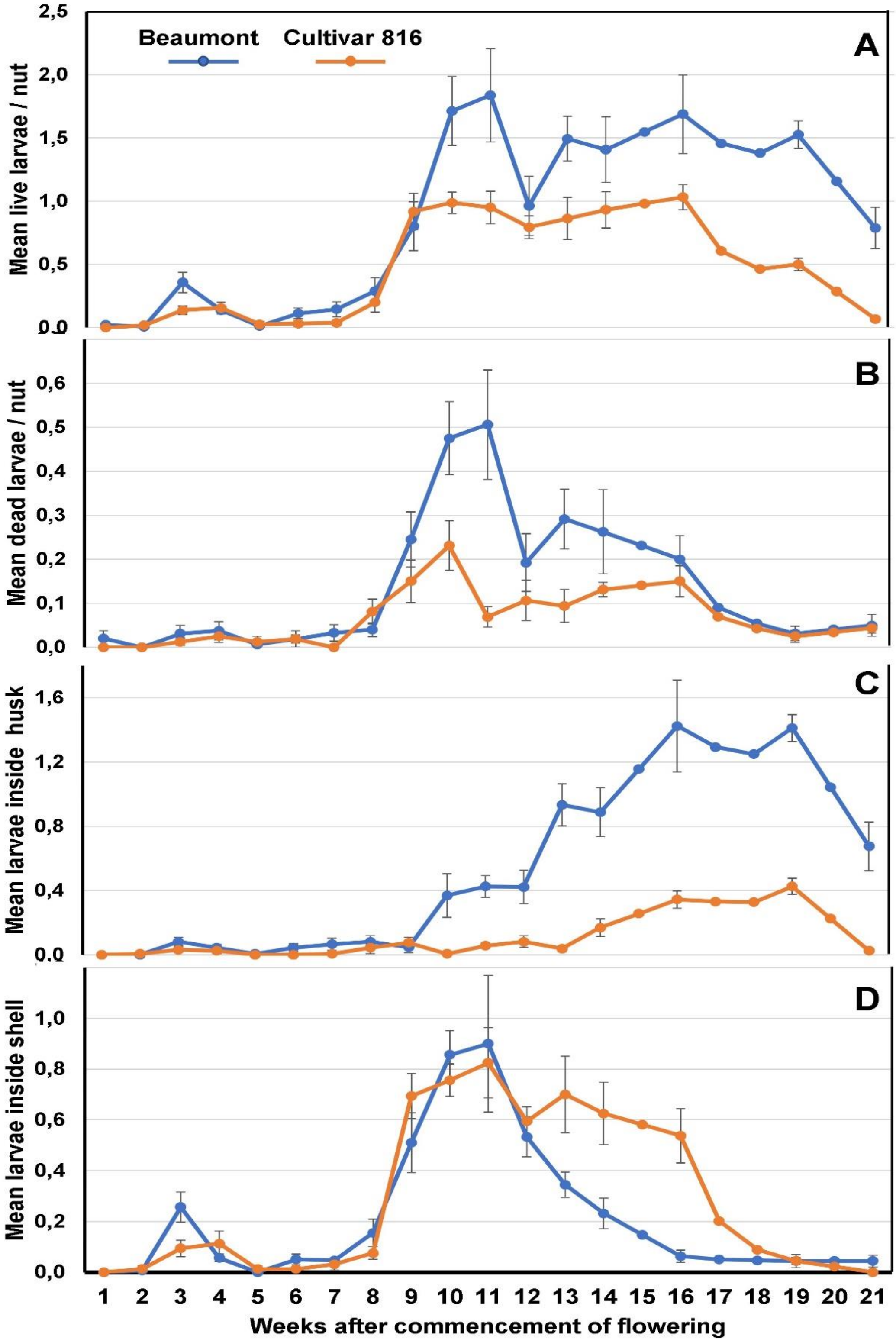

Figure 3.7: Mean number of live and dead larvae per nut, and mean number larvae inside the nut and husk, sampled from cv. 816 and Beaumont over a 21-week period after flowering. Bars indicate Standard Error.

The infestation patterns, indicated by the total number of larvae (live and dead) per nut, were similar for the two cultivars (Figure. 3.7 A, B). However, the number of larvae per nut was generally higher for Beaumont. The mean number of larvae per nut remained low (< 0.5) during the beginning of the sampling period and only started to increase during week 9. The number of larvae per nut in Beaumont ranged from 1.0 to 1.8 from week 9 to 19, after which it decreased markedly. Natural larval mortality patterns, indicated by the mean number of dead larvae per nut, was similar for the two cultivars (Figure. 3.7 B).

The incidence of live larvae inside the husks differed between the two cultivars during the latter part of the growing season (Figure. 3.7 C). The number of larvae inside the husk for Beaumont (Fig. 3.7 C) increased from week 10 onwards and reached a high of 1.4 per husk, at week 16. The highest number of larvae inside the shells of both cultivars was 0.7 and 0.9 (week 9-11) after which the numbers inside shells of cv.816 remained comparatively high until week 16 (Figure. 3.7 D).

The observed patterns of damage (entry/exit holes) to nuts were similar for the two cultivars (Figure. 3.8 A). The holes created by larvae upon entry of nuts of Beaumont showed a small increase at week 3, with a mean number of 0.4 holes per nut. From week 5 to 16 the number of holes increased to 1.7 holes per nut. From week 9 onwards, the mean number of holes per nut increased steadily until the maximum was reached during weeks 16 to 21, when it ranged from 1.4 and 1.7. The incidence of fallen nuts that exhibited damage to husk tissue was low (< 0.2 %) for the first 8 weeks of the sampling period after which there was a rapid increase to between 90 and 100 % from week 13 onwards (Figure. 3.8 B).

The occurrence of nuts that exhibited damage to kernel tissue was low (< 0.4 %) for the first eight weeks of the sampling period after which there was a rapid increase for Beaumont cultivar to between 80 and 100 % from week 11 to 17 (Figure. 3.8 C). The observed patterns were the same until week eight except for a slight increase in the incidence of damaged nuts of Beaumont at week 3. A sharp decrease in the percentage damage to kernels of fallen nuts was observed from week 16 onwards.

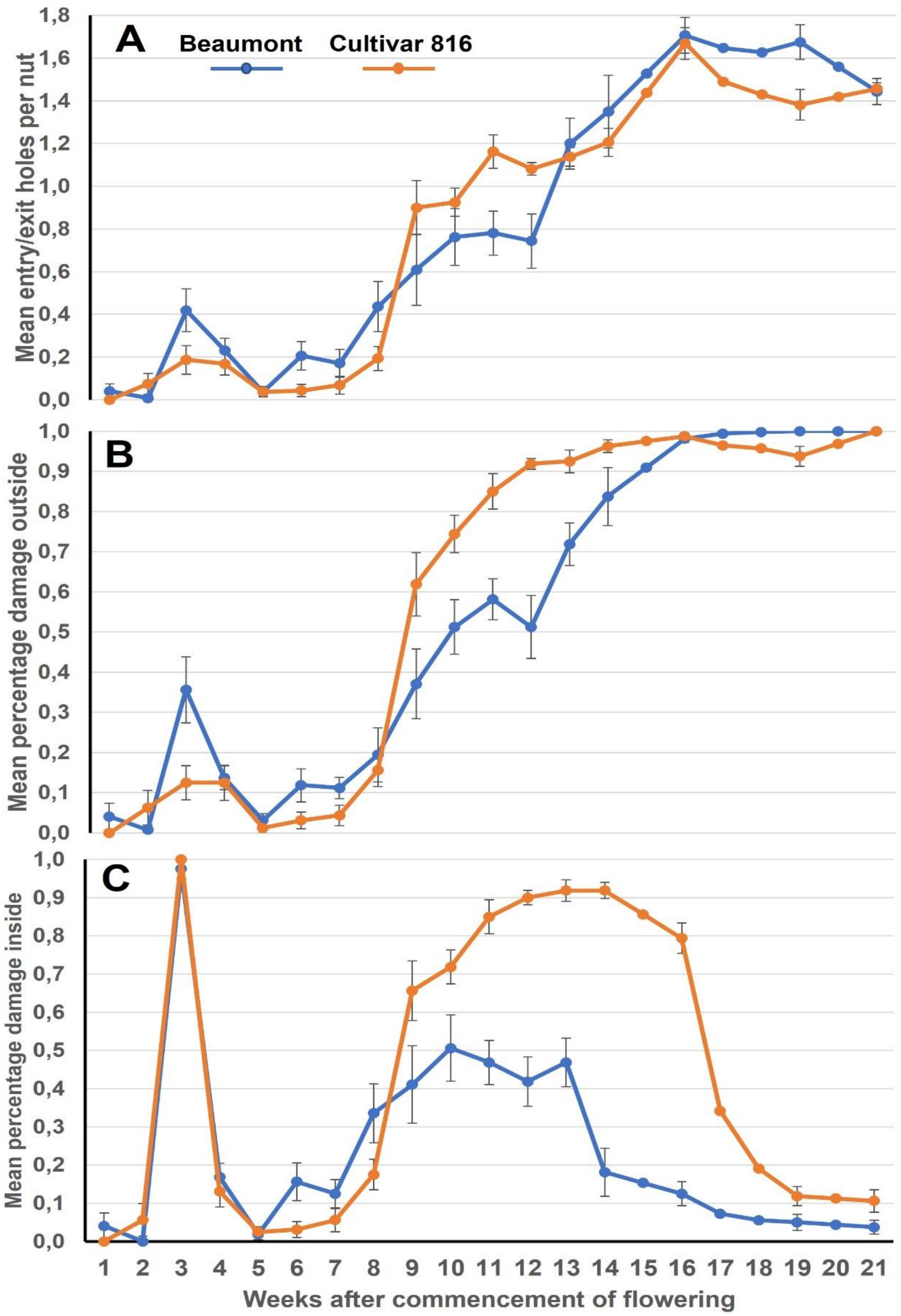

Figure 3.8: The mean number of entry/exit holes, and percentage damage outside and inside the shell per macadamia nut sampled from Beaumont and cv. 816, over a 21-week period after flowering. Bars indicate Standard Error.

3.4 Discussion

This study and that of (Smith *et al.*, 2022) are the first of its kind in South Africa where molecular tools were used to identify species of the macadamia nutborer complex. Due to the small size of larvae, their cryptic feeding habits, and similarity between some of the species, accurate identification to species level is difficult (Morland, 2015). Larval identification by means of molecular methods is the only way to establish the relative dominance of different species of this pest complex within orchards during the season as well as in different regions.

This survey of the Lepidoptera moth nut borer complex of macadamia recorded four species that were present throughout the season and four other species of which only two or three specimens were found. The four species that make up the nutborer complex and which occurred throughout the season were T*. leucotreta, T. batrachopa, C. peltastica* and *E. ceratoniae*. High numbers of *E. ceratoniae* and *C. peltastica* larvae were recorded during this study. This is in contrast to results of (Smith *et al.*, 2022) who did not record any larvae of these two species during a recent survey, which indicates that species complexes vary between macadamia production areas. In South Africa, *E. ceratoniae* larvae have been observed eating small holes on macadamia nuts and that these holes are usually surrounded by larval frass (van den Berg *et al*., 2000). Larval feeding continues between the husk and the brown nutshell once the shell has hardened. Larvae occasionally penetrate through the shell where direct damage to the kernel is caused (Van den Berg, 2001). Gothilf (1970) reported that citrus was the only fruit on which *E. ceratoniae* would lay eggs on undamaged fruit. Under natural conditions, it seldom lays eggs on healthy undamaged fruit and oviposition is largely restricted to damaged pods of host crops. Ghotlif (1970) also reported that *E. ceratoniae* larvae were not able to penetrate healthy fruit from *Acacia farnesiana*.

The other species that were recorded were *Lobesia vanillana* (Tortricidae), *Nola imitate* (Nolidae), *Janseodes melanospila* (Erebidae) and *Ariathisa excise* (Noctuidae). Other Lepidoptera species, additional to the four of the nutborer complex, have previously also been recorded in macadamia nuts in South Africa, for example, Mlanjeni *et al.* (2002) reported four such species, of which two were in the Gracillariidae and Pyralidae, and Smith *et al.* (2022) reported seven.

Thaumatotibia batrachopa was first identified as a macadamia pest in South Africa in 1999 by (De Villiers 2001) and by 2001, it was considered to be the dominant species making up 90 % of the species complex, followed by *T. leucotreta* (8 %), *C. peltastica* and *E. ceratoniae*, which contributed 2 % (Bruwer 2001). A 3-year study into of the community composition of larvae inside nuts, conducted in the Limpopo Province in the early 2000's showed that large intra-seasonal variation in dominance of species may occur. Mlanjeni *et al.* (2002) reported that *T. batrachopa* contributed to between 63 – 76 % of the species complex during certain months, while *T. leucotreta* made up 71 – 55 % during other months. Bruwer (2001) also reported that these two species were the dominant species in the pest complex. The study done in Levubu by Mlanjeni *et al.* (2002) also reported that *T. peltastica* and *E. ceratoniae* were of minor importance and, although *E. ceratoniae* made up 13 % of the species complex during certain periods, the overall contribution to the species complex was low (5 %).

The results on proportional representation of species, as indicated by larval numbers, contrasts with that of moth numbers captured by means of pheromone traps (Chapter 2) and may have important implications for macadamia pest management in different regions. The general perception in the macadamia industry in South Africa is that MNB is the most important species of the nut borer complex. These results question the norm in South Africa, which is that MNB is usually the predominant species which causes damage to macadamia nuts. These results further highlight the importance of long term and regional monitoring to determine the proportional contribution of different species to the nut borer complex. For example, for the first four weeks of this study, *T. batrachopa* larvae were dominant. If a sample would have been taken during that time frame to determine the dominant species in the area the wrong conclusion would have been drawn. Long term monitoring of the species complex and pest status of different species is therefore important to assess which species dominate in which region and during which part of the season.

Similar changes in species dominance in macadamia was reported in Malawi where the lepidopteran pest complex was monitored from 1980 to 1981 (La Croix & Thindwa, 1986). Results of the latter study showed that *T. leucotreta* numbers decreased over years and that *T. batrachopa* became the dominant species. *Thaumatotibia batrachopa* increased in its proportional abundance over years, from about 15 % in the early years (La Croix & Thindwa, 1986) to about 50 % in 1987, while *T. leucotreta* numbers decreased (La Croix & Thindwa, 1986; La Croix, 1990).

Very low numbers of eggs were detected when nut diameter was less than 5 mm and the number of eggs only started to increase once nuts reached a mean diameter of 20 mm. Studies in Hawaii, Australia, and Malawi indicated that direct damage to macadamia kernels declined following shell hardening (Namba, 1957; La Croix & Thindwa, 1986; Ironside, 1988; Jones & Caprio, 1992), and that husk damage is responsible for higher than normal nut abscission rates (Jones, 1994a). When nut diameter is <20 mm they are still developing and are, therefore, susceptible to direct kernel damage. Scouting and monitoring of infestation levels do, therefore, not have to be implemented until nuts are 15 mm in diameter. Similar to what was observed in this study, *Cryptophlebia* females only oviposit to a very limited extent on macadamia and litchi fruit that are smaller than 15 mm (Jones, 1995). The number of eggs per nut started to increase from week 12 onwards and peaked when nuts reached their maximum size (31 mm diameter; week 16). Since peak flowering normally occurs during mid-September (week 2 of this study, Figure. 2.6) the low number of eggs recorded during the first 9 weeks can be ascribed to the occurrence of early-season nuts. These 'early' nuts present fruit of a preferred size for females to oviposit on (Jones, 1998 a).

The mean numbers of eggs per nut differed between the two cultivars with higher numbers occurring on Beaumont throughout, supporting the findings of Schoeman (2009). Peaks of egg-laying were apparent during weeks 8-9 (in early December) and weeks 12-14 (early-January), after which no distinct pattern was discernible, similar to what was reported by Daiber (1980). Previous studies by (Jones, 1994b), show that macadamia nuts are most susceptible to direct kernel damage when they are between 20 and 30 mm in diameter. Nuts <20 mm in diameter are seldomly selected as oviposition sites, and nuts >30 mm generally have a hardened shell, which protects the kernel from larval feeding (Jones, 1994a).

Egg parasitism was very low (<2.5 %) throughout the season and probably does not contribute significantly to suppression of pest numbers in orchards. Mlanjeni *et al.* (2002) also suggested that natural enemies do not seem to suppress nutborer populations because the abundance of parasitic wasps was very low. Bruwer (1999) reported that the abiotic and biotic factors early in the season are not favourable for parasitoids. La Croix & Thindwa (1986) reported that the egg parasitoid, *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) parasitizes *Cryptophlebia* spp. in Malawi but that pest levels are not suppressed sufficiently.

The larval infestation patterns were similar for the two macadamia cultivars although number of larvae per nut was generally higher for Beaumont. As was observed for oviposition, the mean number of larvae per nut also started to increase during week 9, when nut diameter increased to more than 20 mm and nuts started to mature. The incidence of live larvae inside the husks was higher on Beaumont and remained high for most of the sampling period. The numbers of larvae inside nuts were, however, similar for the two cultivars during the peak infestation period between 9 and 12 weeks, after which it remained higher on cv. 816 for an additional period of approximately 5 weeks. This could probably be ascribed to cv. 816 having thinner shells and larger kernels than the Beaumont cultivar, leaving a longer window of opportunity for the larvae to bore through the shell as it hardens. The larger kernel size allows larvae to remain in the kernel for longer as there is more sustenance. In Kenya, Mailu (1997) noted differences in the feeding preferences of nutborers for various macadamia cultivars, but found no significant differences in susceptibility of macadamia cultivars and species to nutborers (Schoeman, 2009). Tunnelling by *Thaumatotibia* spp. larvae inside nuts causes direct kernel damage if it occurs before shell formation (Namba, 1957; Ironside, 1988; Jones & Caprio, 1992) and early nut abortion if it occurs after shell formation (Jones *et al.*, 1991; Jones, 1994b). The rate of shell hardening therefore influences the incidence of direct damage to the kernel, which is reduced in cultivars of which the shell hardens quicker.

Although both cultivars in this study were susceptible to damage, the incidence of damaged kernels in Beaumont started to decline four weeks earlier than that on cv. 816. Cultivar 816 seem more prone to direct kernel damage. Beaumont nuts had more husk damage than cv. 816 throughout the season, which could leave the impression that nuts of Beaumont remain largely undamaged. The increased levels of husk damage that was observed in Beaumont causes premature abscission and poor nut development if the vascular tissue is severely damaged.

The larval infestation patterns observed in this study showed that moths only commenced with significant oviposition activity, 8 weeks after flowering started and when nuts size approached 20 mm. This result supports the finding of Schoeman (2009) who reported that the optimum time to apply insecticide sprays against the borer complex is approximately 9 weeks after anthesis, when nuts attain a medial diameter of 20 mm (Schoeman, 2009). This spray application is usually done towards the end of November (week 13 in this study). The moth borer complex is difficult to control because the window period for effective chemical control is very narrow (Schoeman, 2009), and because of their mining habit and the dispersal of eggs over the crop (La Croix & Thindwa, 1986). Since larvae are concealed feeders and difficult to detect and control (Mediouni & Dhouibi, 2007), chemical sprays must be directed against recently-eclosed first-instar larvae before they burrow into the nut (Schoeman, 2009). Although newly hatched larvae may burrow directly into the husk after hatching, they usually move an average of 4 mm on the surface before they do so (La Croix & Thindwa, 1986), leaving them exposed for a short period of time.

Overall, Beaumont is considered by the macadamia industry to suffer less damage than other cultivars and it is therefore not always treated the same with regards to pest control strategies. According to Schoeman (2009), Beaumont is less prone to nutborer damage than other cultivars such as Cultivar 816. However, similar to what was observed in this study, (Schoeman, 2009) also reported nut damage in pure stands of Beaumont trees. Beaumont nuts contain substantial amounts of sticky resin which do not occur in such large quantities in other cultivars, making it less likely to be damaged (Schoeman, 2009). This was contrary to reports by De Villiers (2001) in South Africa, that susceptibility to attack by *Cryptophlebia* spp. differs among macadamia cultivars. These factors influence pest management decisions, but, since no comparative studies have been conducted, cultivar-specific information for use in IPM programs is very limited.

Monitoring for the presence of tortricids on fallen nuts can easily be done but the decision to spray or not should only be taken if nuts on the trees are also sampled for eggs (Ironside 1988). Although there may be a correlation between egg numbers and damage, the presence of eggs is not a good indicator of damage (Jones, 1995). For example, (Jones, 1995) reported that even when the mean number of eggs per nut was 3 or higher, less than 60 % of the nuts had larvae inside or exhibited larval damage symptoms. In the study by Jones (1995) the presence of eggs on nuts coincided with larval presence (or larval damage) in only <50.5 % of the cases. Jones (1995) and Ironside (1988) designed sequential sampling techniques to facilitate spray decisions. Bruwer (2002) and Haaksma (1993) indicated that spray timing and coverage are critical and proposed that broad spectrum insecticides with long residual action, be sprayed during November or early December (Schoeman, 2009). This would ensure that the window of larval susceptibility to insecticides is maximally utilised.

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Chapter 4:

A preliminary assessment of the macadamia nut borer complexes in different production regions of South Africa

Abstract

The four Lepidoptera species that make up the nut borer complex in South Africa are *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) (False codling moth, FCM), *Thaumatotibia batrachopa* (Meyrick) (Lepidoptera: Tortricidae) (Macadamia nut borer, MNB), *Cryptophlebia peltastica* (Meyrick) (Lepidoptera: Tortricidae) (Litchi moth, LM) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae) (Carob moth, CM). Previous surveys of the nut borer complex in Limpopo and Mpumalanga did not always record all four of these species in all the macadamia growing areas, and reports further showed that the species complex may change over time. Limited information is available on the nut borer complex of macadamia, both within smaller regions in provinces, as well as in areas such as the Eastern Cape and Western Cape where macadamia production is not yet well established. The aim with this survey was to assess the species complexes of macadamia borers in different areas of South Africa. Nuts were collected on farms at five localities and the lepidopteran larvae identified to species level where possible. All the species of the nut borer complex were collected, except for *C. peltastica*. The most diverse nut borer complex was recorded at Levubu and Nelspruit. *Thaumatotibia batrachopa* was recorded in three areas, including the KwaZulu-Natal, while *T. leucotreta* was only recorded in Mpumalanga and Limpopo. *Ectomyelois ceratoniae* was recorded in Mpumalanga, the KwaZulu-Natal and Limpopo. The result of this survey highlights the importance of monitoring and surveillance information in macadamia pest management.

Key words: macadamia pests, South Africa, survey

4.1 Introduction

South Africa is the largest exporter of this crop in the world and the area under cultivation is continuously increasing in the country. South Africa produced 48 925 tonnes of nut in shell (NIS) on approximately 55 00 ha for the 2020 season (SAMAC, 2020). The main macadamia production region in the country is in the Mpumalanga province, which generates approximately half of the total annual kernel production, followed by KwaZulu-Natal and Limpopo (SAMAC, 2022). Other macadamia growing provinces include the Eastern Cape, Western Cape, and Gauteng (SAMAC, 2022). Macadamia production has also commenced in the Eastern Cape and Western Cape which are the newest macadamia growing area.

Research on macadamia pests has largely focused on the Limpopo and Mpumalanga regions and limited information is available on its pests in other areas of the country. The pest complex of crops is determined by various biotic and abiotic conditions, such as the presence of suitable host plants and suitable climatic conditions. Several of the lepidopteran pest species of macadamia also occur on other tree crops which may contribute to the presence and possible pest status of these pests in other regions of South Africa where macadamia is, and in future may be cultivated. For example, the False codling moth (*Thaumatotibia leucotreta*) also attacks subtropical and tropical fruit tree species such as litchi (*Litchi chinensis*), mango (*Mangifera indica*), and grapes (*Vitis vinifera*) (EPPO, 2013), which is grown in other regions of the country and which may maintain populations of this pest. The macadamia nut borer (*Thaumatotibia batrachopa*) has only two known host plant species in South Africa, i.e., macadamia and litchi (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Rentel, 2013) and may therefore be expected to be of lesser importance in other macadamia production regions. The litchi moth (*Cryptophlebia peltastica*) which is of limited importance on macadamia, also occurs on several garden tree species that are cultivated throughout South Africa (Grove, 1999; Prinsloo & Uys, 2015). CM (*Ectomyelois ceratoniae*), which is polyphagous, largely feeds on fruit of the Fabaceae (Nay & Perring, 2008) but also occurs on citrus species, pistachio nuts (*Pistacio vera),* walnuts (*Juglans* sp.), pecans (*Macadamia integrifolia*) and dates (*Phoenix dactylifera*) (Bazelet, 2021). Many of the crop species are cultivated to varying degrees is areas where macadamia is, and in future may be cultivated, and may influence the pest status of these pest species.

The aim of this study was to do a preliminary assessment of the species composition, infestation levels and damage caused by the nut borer complex in the macadamia production regions in South Africa. Egg parasitism was also recorded. Identification of larvae was done using both morphological and molecular methods.

4.2 Material and methods

4.2.1 Study area

Nuts were sampled at five sites in three provinces of South Africa (Figure. 4.1). The localities were Levubu in the Limpopo Province, Nelspruit (Brondal) and White River in the Mpumalanga Province, and Paddock and Port Edward in the KwaZulu-Natal province. The location of the sites is indicated in Figure 4.1.

Sampling was done once for each locality from end of January (29/01/2021) to beginning March (10/03/2021) 2021 since Tortricidae activity is usually high during this period. Access to farms with cv. 816 was difficult to obtain in the White River area. Cultivar 816 was sampled at all sites. At the Paddock locality, orchards of cv. 816 also had cv. 863 and 788 mixed in between. In Paddock and Port Edward the nuts mature earlier in the season, resulting in early nut drop and harvesting. During the growing period, crop management was done according to local agricultural practices.

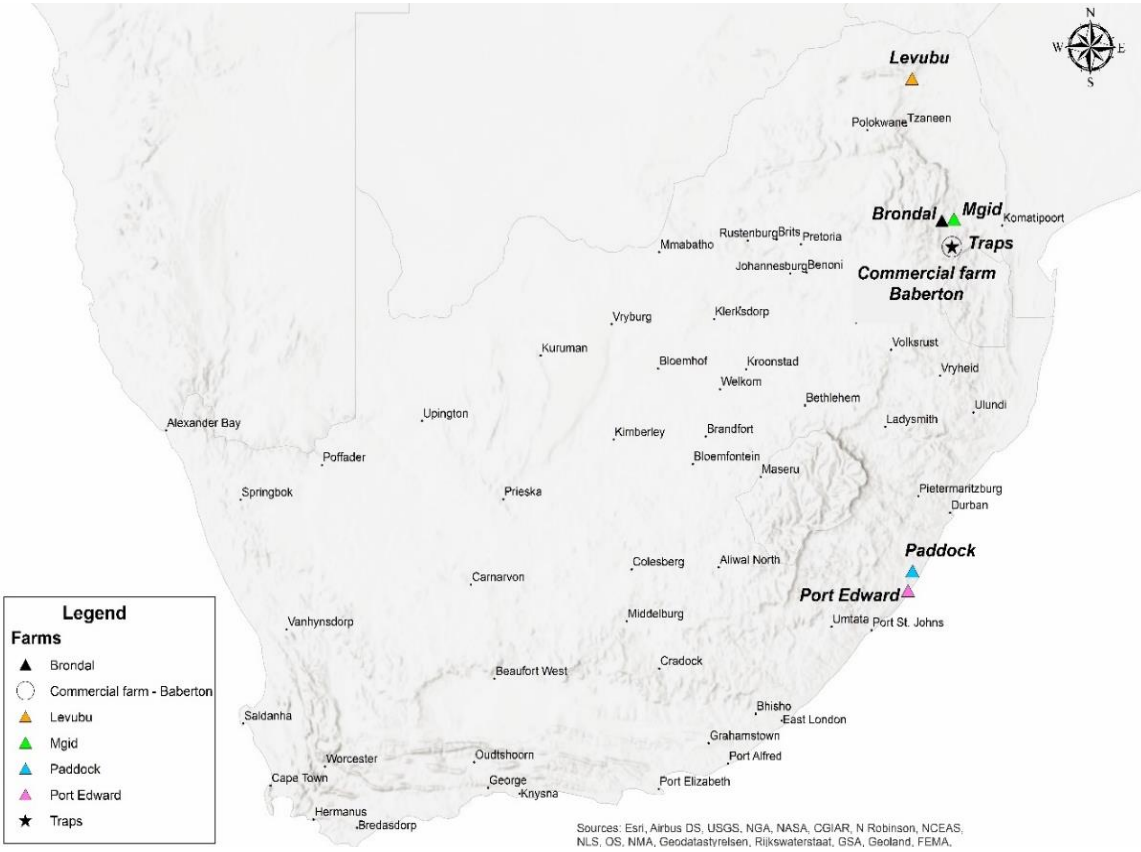

Figure 4.1: Sites where sampling of nuts was done on six commercial farms in some of the macadamia production areas of South Africa. The Barberton site was not included in this survey.

4.2.2 Experimental layout and data collection

Sampling was done in well-established orchards of cv. 816. Nuts were sampled once at each site. Between 17 and 300 nuts were picked up randomly in an orchard on each farm. The large variation in number of nuts sampled per site was due to farmers reluctance to give away mature nuts close to harvest. Nuts were transported to a laboratory in Mbombela.

Nut diameter as well as the numbers of hatched and unhatched per nut were recorded. The numbers of larvae of each species at each locality was used to assess the species composition of the nut borer complex at that locality. The incidence of damaged nuts was determined and the position of larval damage inside nuts recorded either as ‘’outside the kernel’’ (feeding on husk or shell), or ‘’inside the kernel’’, which implies damage to the kernel. Data were recorded on Excel spread sheets for each sampled nut from each locality.

Larvae from each nut were individually preserved in 1.5 ml centrifugal tubes with 99 % ethanol. Each tube was marked according to the farm where samples were collected. The number of larvae per nut was recorded for each species. Larvae were identified as described in Chapter 3. Briefly, where possible, identifications were done using morphological characteristics. The larvae that could not be identified by means of morphological features were identified by means of molecular techniques.

4.2.3 Data analysis

Data on nut diameter, total number of eggs and parasitised eggs per nut were analysed by means of descriptive statistics. No distinction was made between newly laid unhatched eggs and eggs that already hatched and which were still visible on the surface of the nuts. Summary statistics were conducted by means of Microsoft Excel (Microsoft 365). The number and position of larvae inside nut (including husk), numbers of entry holes into the husk, as well as damage on the inside and outside of each nut were also analysed by means of descriptive statistics.

4.3 Results and Discussion

Research on macadamia pests has largely focused on the Limpopo and Mpumalanga regions and limited information is available on its pests in other areas of the country (Mlanjeni *et al.*, 2002; Mlanjeni *et al.*, 2004; Schoeman, 2009; Smith *et al.*, 2022). The only other locality that was previously sampled outside of the above-mentioned provinces was Ramsgate in KwaZulu-Natal (Smith *et al.*, 2022).

Three of the four species that make up the nut borer complex were recorded during this area wide survey. These were: *T. leucotreta*, *T. batrachopa* and *E. ceratoniae.* No larvae of *C. peltastica* were recorded. Smith *et al.* (2022) also did not recover any *C. peltastica* in their survey at farms near Ramsgate in the KwaZulu-Natal province, White River and Kiepersol in the Mpumalanga province and Levubu in the Limpopo province.

The relative contribution of the different species to the nut borer complexes at the different sampling sites are provided in Figure 4.2. The most diverse nut borer complex was recorded at Levubu and Nelspruit, with three species, i.e., *T. batrachopa, T.*

leucotreta and *E. ceratoniae* (Figure. 4.2). At Levubu and Nelspruit, respectively 71 % and 66 % of nuts were infested with *T. batrachopa.*

At three of the sampling sites, only a single species of the nut borer complex was recorded. *Thaumatotibia batrachopa* was the only species collected at Paddock. *Ectomyelois ceratoniae* was the only moth species recorded at the Port Edward locality, while in Levubu it infested 9 %, and in Nelspruit, 6 % of the nuts. *Thaumatotibia leucotreta* was only recorded at Levubu and Nelspruit, in 29 % and 26 % of the sampled nuts, respectively. *Ectomyelois ceratoniae*, which has previously been described as a pest of macadamia in South Africa (De Villiers, 2001; Manrakhan *et al.*, 2008), was collected in significant numbers in this study. However, in a recent country-wide survey by Smith *et al.* (2022), no larvae of this species were collected. These results suggest that this species may only be of sporadic occurrence, as suggested by Smith *et al.* (2022), but that it may attain high pest status in some areas, for example in the KwaZulu-Natal province (Port Edward).

Thaumatotibia batrachopa was recorded at four of the five sites and it was also the dominating species at these sites (Figure. 4.2). This dominance of *T. batrachopa* in the nut borer complex was also reported by Smith *et al.* (2022), who reported that it comprised over 95 % of the larvae obtained from macadamia nuts throughout the 2017/2018 and 2018/2019 growing seasons in Mpumalanga, Limpopo, and KwaZulu-Natal. However, the relatively high incidence of *T. leucotreta* at two sites sampled in this study (Nelspruit and Levubu) (Figure. 4.2) are in contrast to that of Smith *et al.* (2022) who isolated only five *T. leucotreta* larvae over a 2-year period from a country-wide survey. The result of the current study is, however, supported by those of Mlanjeni *et al.* (2002) which reported that *T. leucotreta* comprised 60.7 %, and *T. batrachopa* 34.0 % of the lepidopteran larvae collected at Levubu.

The limited species richness recorded at the two KwaZulu-Natal sites cannot easily be explained. Several factors could have contributed to these low numbers, notably low numbers of nuts sampled in these areas and possibly also the time of sampling. Sampling at these two sites were done late in the season. Macadamia nuts in KwaZulu-Natal mature earlier than Limpopo and Mpumalanga, and are harvested earlier. Most of the nuts sampled in the two KwaZulu-Natal sites were mature and ready for harvest. Results of the weekly surveys done at the Barberton locality

(Chapter 3) showed that the species distribution differs within a season, which may explain the results recorded at Paddock and Port Edward. Similarly, only *T. leucotreta* was recorded at the White River locality. Van den Berg (2001) and Bedford *et al.* (1998) reported that the diversity of pest species may also be influenced by host plant diversity at the landscape level, and that several host plants of *T. leucotreta*, *T. batrachopa* and *C. peltastica* are cultivated in close proximity to macadamia orchards in Mpumalanga.

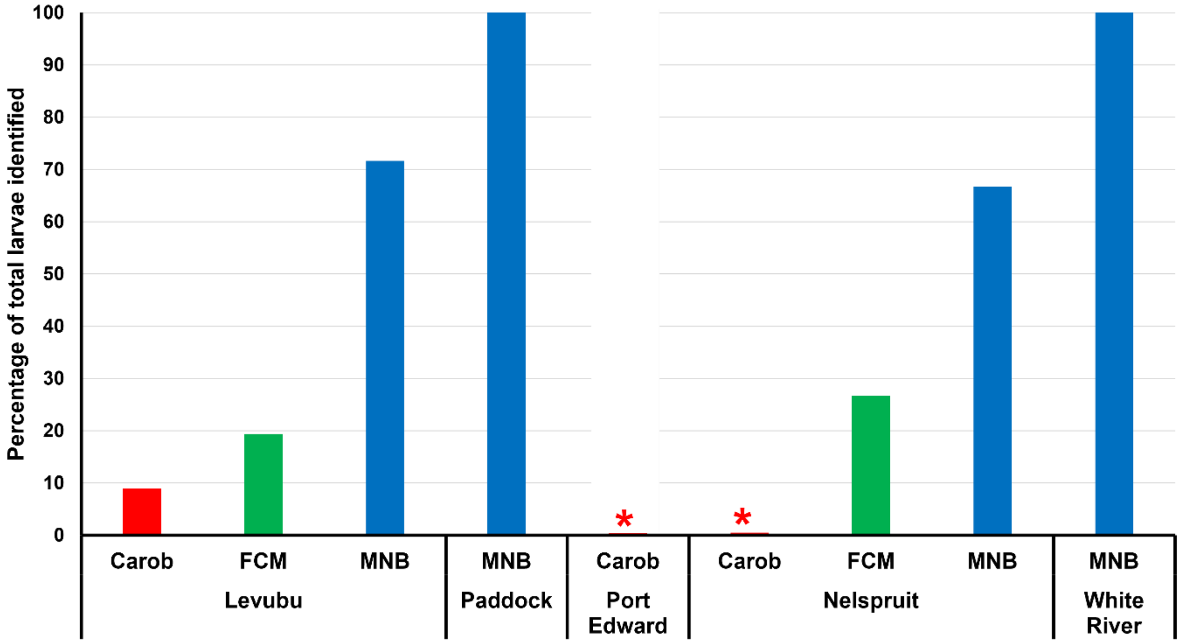

Figure. 4.2. Percentage of the total number of FCM, MNB, and CM larvae identified from macadamia nuts collected at each of five sites in three provinces of South Africa. Limpopo: Levubu; KwaZulu Natal: Paddock, Port Edward; Mpumalanga: Nelspruit, White River. *Asterisk indicates that percentage was not calculated since only a single larva of CM was recorded at each of these two sites.

The species composition of the nut borer complex within and between growing regions in South Africa remains unresolved (Smith *et al.*, 2022). The pest species complexes of crops and their pest status in different geographical areas are influenced by environmental conditions such as climate and the presence of a range of host plants (Skendžić *et al.*, 2021). Results of this survey are by no means representative of the macadamia nut borer complex in South Africa but shows interesting patterns in terms of possible species distribution and pest complexes in different areas.

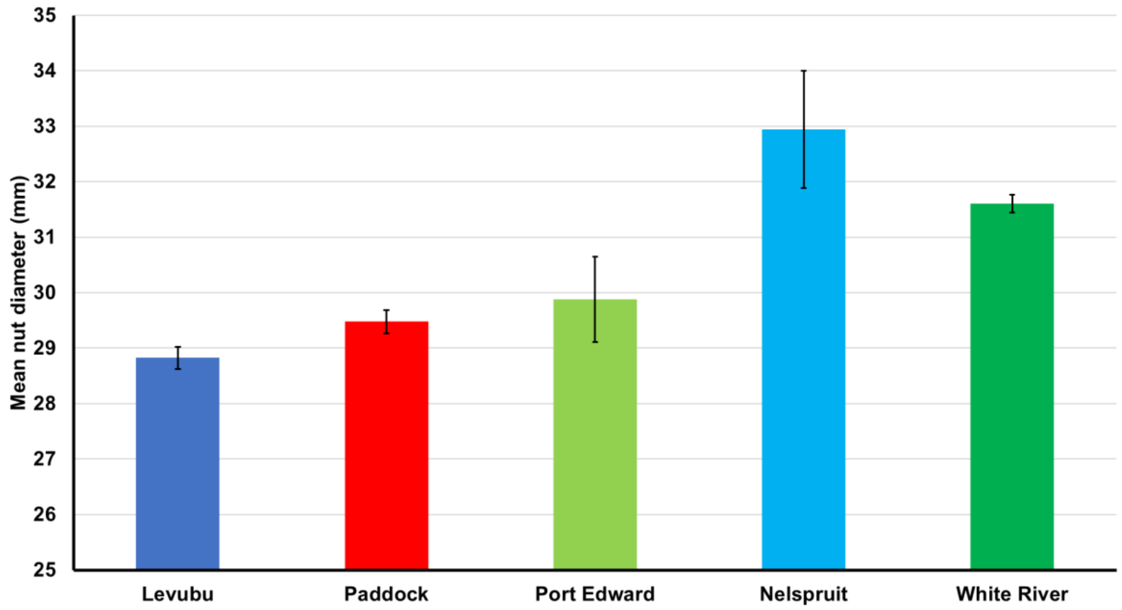

Figure. 4.3. Mean diameter of macadamia nuts sampled from cv. 816 in the main production regions of South Africa. Bars indicate Standard Error.

The mean diameter ranged between 28.8 and 32.9 mm for the different sampling sites. Nuts sampled at the Nelspruit site were the largest followed by those from White River (Figure. 4.3). Nuts collected at Levubu were the smallest (28.8 mm). The sizes of the nuts were all above that where it becomes suitable for moth oviposition. Jones (1994) as well as Chapter 3 of this dissertation showed that nuts <20 mm in diameter are seldomly selected as oviposition sites. Nuts >30 mm generally have a hardened shell, which protects the kernel from larval feeding (Jones, 1994). Although nut size is a dominant factor in oviposition-site choice by moths of the nut borer complex (Jones, 1994) it most likely did not have any influence on the larval species complex recorded in this study.

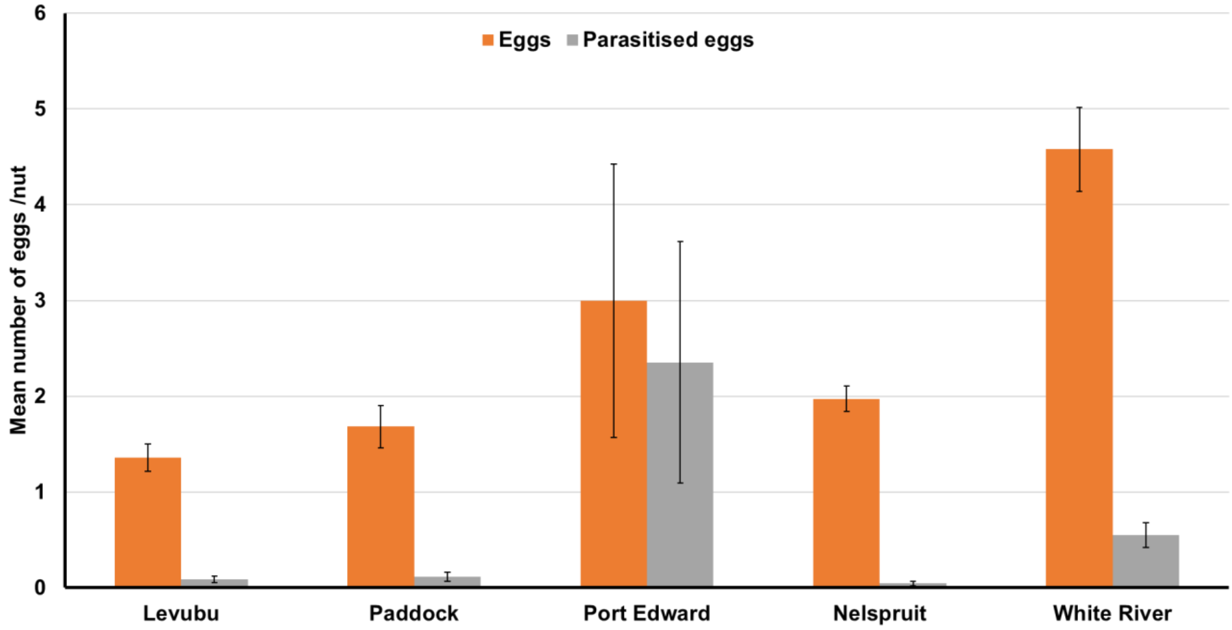

Figure 4.4. The mean total number of eggs (hatched + unhatched) and parasitized eggs per macadamia nut sampled from cv. 816 at different localities in South Africa. Bars indicate Standard Errors.

The mean numbers of eggs per nut differed between the areas (Figure. 4.4). Those collected at White River had the highest number of eggs per nut (4.5) while those from Levubu had the lowest (1.4).

The incidence of parasitized eggs was low at all sites, except at the Port Edward site where 78.4 % of eggs were parasitized. Egg parasitism level at Levubu was 6.6 %, Paddock, 10.5 %, Nelspruit 3.3 % and White River 12.2 %. It is highly likely that nearly all the eggs recorded at Port Edward was that of *E. ceratoniae,* since it was the only species of which larvae were recovered from the nuts (Figure. 4.2). Because of the co-occurrence of the different species of the nut borer complex and the extreme difficulty to distinguish between eggs of the different species, it is not possible to separate egg parasitism levels for the different pest species. However, in this case, where only *E. ceratoniae* occurred, results seem to indicate that egg parasitism levels for this species is very high. This may also explain the variation in pest status of this species in different macadamia areas of South Africa.

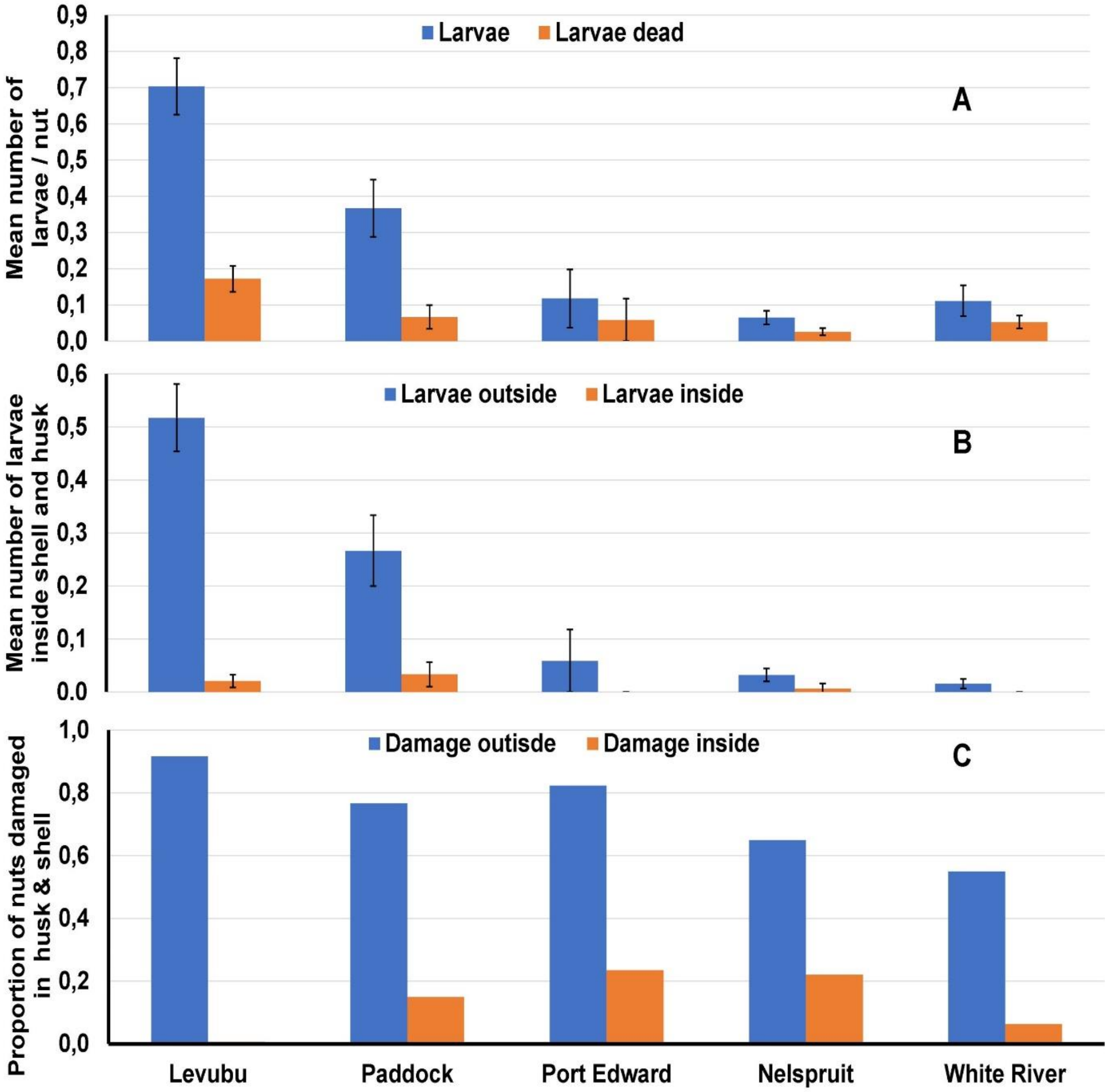

Figure 4.5. Mean numbers of live and dead larvae per nut, mean number of larvae inside the nut and husk, and incidence of damage outside and inside the shell of macadamia nuts sampled from cv. 816 at five localities in South Africa.

The infestation levels (mean total number of larvae per nut) ranged from 0.1 and 0.7 between the different localities (Figure. 4.5 A). The highest infestation level was recorded at Levubu with a mean number of 0.7 live, and 0.17 dead larvae per nut (Figure. 4.5 A). The second-highest infestation level was recorded at Paddock in the KwaZulu-Natal, and the lowest at Nelspruit.

The locality with the highest incidence of larvae outside the shells was at Levubu and the lowest at White River (Figure 4.5 B). At Port Edward and White River, no larvae were recorded inside the shells, although several were present outside of the shells, inside the husks. The incidence of damage inside the husk (outside the shell) was high (>55 %) in all the areas (Figure. 4.5 C). The highest incidence of nuts with damage to the husks (92 %) was at Levubu, while the lowest was at White River (65 %). The incidence of nuts with damage inside the shells was the highest at Port Edward (24 %) and the lowest at Levubu (1 %).
Although the incidence of damaged shells at the Port Edward locality was high, only one CM larva was recovered. This could probably be ascribed to the late sampling of nuts at this locality and the movement behaviour of larvae. CM larvae are known to move from nuts into the soil to pupate, or pupate on the fruit surface from where they could be detached during sampling.

4.4 Conclusions

Macadamia cultivation in South Africa is expanding rapidly and new plantations are being established every year. This rapid expansion leads to new areas being used for macadamia cultivation which creates its own challenges in terms of pest management. Although only a limited number of localities were sampled during this survey, it highlighted differences in pest complexes between regions. Further surveys should be conducted to include representative samples in all macadamia regions of South Africa.

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Chapter 5:

Conclusion and recommendations

South Africa is the largest macadamia producer in the world and production regions continue to expand at a rapid rate. The main insect pests of macadamia in South Africa are the stink bug- and macadamia nut borer complexes (Schoeman, 2009).

Various factors contribute to the difficulty of managing the macadamia nut borer complex. These factors include the difficulty to identify the larvae of the different species, poor relationships between moth flight patterns and larval infestation patterns, unreliable pheromone trapping methods, and variation in pest complexes in different geographical regions. The co-occurrence of the four species of the macadamia nut borer complex, and the variation in their pest status provide further challenges to their management. Schoeman (2009) highlighted the influence of uncertainty regarding pest community composition on pest management decisions such as insecticide applications. The latter study reported that some growers start applying insecticides immediately after flowering commences.

While all four Lepidoptera species that constitute the nut borer complex occur in most of the macadamia production regions in the country, significant differences in pest complexes may occur, which complicates pest management programs. For example, in some regions where *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) dominated the pest complex in the past, *Thaumatotibia batrachopa* (Lepidoptera: Tortricidae) became the dominating species (La Croix & Thindwa, 1986; Mlanjeni *et al.*, 2004). Similarly, a recent survey in South Africa (Smith *et al.* 2022) showed that the dominant species was *T. batrachopa,* which is contrary to what was reported during the early 2000's in the Levubu region, which showed *T. leucotreta* was the dominant species (Mlanjeni *et al.*, 2002). The pest status of *Cryptophlebia peltastica* (Lepidoptera: Tortricidae) and (La Croix & Thindwa, 1986; Timm *et al.*, 2007; Schoeman, 2016) *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) is generally lower than that of the other two species but they are also of major economic importance (Prinsloo & Uys, 2015). This study found *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) dominated the nut borer complex at Barberton in 2020/2021 season. With the expansion in macadamia production to new regions in South Africa, movement of certain pest species by means of host material and subsequent changes in the nut borer complex in some regions may also be expected (Smith *et al.*, 2022).

Various pest management tools are used to facilitate pest management decisions in macadamia orchards in South Africa. Due to the similarity and small size of larvae of the nut borer complex, accurate species identification is difficult. For this reason, pheromone traps which are largely species specific, are used in orchards to provide indications of moth flight patterns which is used as a guideline for the application of control measures.

While this study used pheromone trapping as a monitoring tool for all four species of the nut borer complex, in practice, this is only done for *T. batrachopa* and *T. leucotreta.* However, pheromone trap data are not always reliable. Jones (1995) showed that pheromone traps have a limited use for determining population dynamics in macadamia orchards. In field trials conducted in Hawaii, increases in trap catches did not always correspond with increases in husk damage and *vice versa*. Jones & Caprio (1992) also reported that catches of another tortricid pest (*Cryptobhlebia illepida*) in macadamia was not related to egg numbers on nuts and when moth numbers increased, egg deposition decreased. Results of this study also showed discrepancies between moth activity patterns and larval infestation levels. Furthermore, it showed that pheromone trapping of *E. ceratoniae* moths was highly unreliable since no CM were captured, despite high larval numbers occurring in nuts. This study also showed that ‘by-catches’ could result in over estimation of moth numbers in traps of certain species. This study also shows the value of monitoring for species other than *Thaumatotibia* spp. For example, in this study the overall numbers of moths captured in pheromone traps showed that together, *T. batrachopa* and *T. leucotreta* made up 96 % of the total catch, while their larvae made up 86 % of those that were identified. Although these numbers of moths and larvae show total dominance of the pest complex by *Thaumatotibia* spp. at the target site, it is not an accurate reflection of the within-season dynamics. Larval numbers during the season showed that significant numbers of *C. peltastica* and *E. ceratoniae* occurred inside nuts during the season. Also, the initial dominance of the complex by larvae of *T. batrachopa* during the first five weeks after commencement of flowering, was not reflected in the moth flight data which showed that *T. leucotreta* dominated. It is recommended that in future, where possible, studies be done at more localities and in different regions, to determine intra-seasonal variation in moth flight patterns of the different species. Furthermore, in cases where traps are deployed for monitoring of more than one species of the nut borer complex, the placement of pheromone traps of different species should be investigated. To improve trap reliability it may be possible to allocate FCM and MNB traps to a specific area, further away from LM traps, since this study showed that bycatch occurred between MNB and LM traps. This would indicate if there were interference between pheromones which could influence moth catches and especially 'by-catches'.

This study showed that nut size was an important factor in oviposition and that, similar to what was reported in Chapter 3, as well as by Waite *et al.* (1999) and Jones (1994), that nuts <20 mm in diameter are seldomly selected as oviposition sites. The practice of insecticide application once flowering commences (Schoeman, 2009) is therefore not justified. Nuts >30 mm generally have a hardened shell, which protects the kernel from larval feeding Jones (1994). According to Schoeman (2009) aborted nuts smaller than 15 mm, contain tortricid larvae, but only during the early part of the flowering period (September) when all the nuts are small. In this study larvae were also found during the early part of the monitoring period (October - November) as a result of early flowering, providing out of season nuts. The eggs of *Thaumatotibia* (*Cryptophlebia* spp.) may be difficult to see, may fall off the nuts, be eaten or parasitized, or larvae emerging may not be able to penetrate the husk (Jones, 1995). Easy monitoring can be done for the presence of tortricid eggs on fallen nuts, however, pest management decisions should only be taken if nuts are also sampled from trees (Ironside, 1988). The current study suggests that egg counts would not be suitable for pest-management decision making. To accurately determine population dynamics of *Thaumatotibia*, larval sampling is the most reliable method (Jones, 1995).

Egg parasitism levels recorded during this study ranged from 3.5 % in areas where species occurred in a mixed complex, to 78 % where only *E. ceratoniae* larvae were recovered from nuts. This study allowed the opportunity to evaluate the level of egg parasitism for one of the species of the nut borer complex, i.e., *E. ceratoniae.* Because of the co-occurrence of the different species at most localities and the extreme difficulty to distinguish between eggs of different species, it is not possible to separate between egg parasitism levels for different pest species. However, in this study, only *E. ceratoniae* larvae were recovered from nuts at the Paddock locality, possibly indicating that all or most of the eggs on nuts were also that of this species. Although the sample numbers were low and sampling was only done once during the season, high parasitism levels may indicate the importance of egg parasitism in the suppression of *E. ceratoniae* numbers in South Africa. The pest status of this species in South Africa is low and varies largely between different macadamia regions. Gothilf (1970) reported that *E. ceratoniae* seldom lays eggs on healthy undamaged fruit and that oviposition is largely restricted to damaged fruit.

Tortricid control in macadamia may often be problematic. The window of opportunity for effective control is very narrow and depends on flight- and oviposition peaks of moths. The problems with egg sampling and the deficiencies of pheromone traps, larval sampling remains the most reliable method of assessing the within-season population dynamics of the nut borer complex. More information is needed in new production areas like the Eastern Cape and Western Cape Provinces.

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