The biology and ecology of *Mussidia* spp. (Lepidoptera: Pyralidae) and associated natural enemies in Kenya.

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DEDICATION

To my wife Florence Kalimi and our daughter Jedidah Makaa who had to abide with the profound time load of this work.

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ABSTRACT

Mussidia nigrivenella Ragonot (Lepidoptera: Pyralidae), an important pest of maize, cotton and *Phaseolus* bean in West Africa, has never been described as a crop pest from East and southern Africa (ESA). It was hypothesized that in ESA it was either kept under control by natural enemies or that there exist several populations of *M. nigrivenella* with different host plant ranges. Another possibility is the mis-identification of the *Mussidia* species in ESA. Studies were conducted in Kenya between 2005 and 2007 to assess the species diversity and host plant range of *Mussidia* spp. and spatial distribution studies were done on selected host plants. Later, based on the results of host plant range, surveys were conducted between 2006 and 2007 in mid-altitude coastal Kenya to establish a catalogue of parasitoids associated with *Mussidia* spp. The suitability of stem borers found in Kenya for development of *Trichogrammatoidea* sp. nr *lutea* Girault (Hymenoptera: Trichogrammatoidea) and the factors affecting the bionomics of *Mussidia* sp. in the laboratory were examined. Eight plant species were found to host two *Mussidia* spp. and six putative morphospecies, which occur sympatrically in the coastal region. The two *Mussidia* spp. were *Mussidia fiorii* Ceconni and de Joannis and *M.* nr *nigrivenella*. Only one *Mussidia* sp., *M. fiorii*, was found attacking one host plant species in the mid-altitude regions. In general, the host plant range was much narrower than in West Africa. *Mussidia* nr *nigrivenella* and *Mussidia* "madagascariensis" larval distribution was aggregated on *Canavalia cathartica* Thouars. (Fabaceae) and *Strychnos madagascariensis* Poir. (Loganiaceae), respectively, while the distribution of *M. fiorii* adults on *Kigelia africana* (Lam.) Benth. (Bignoniaceae) was regular. Eight parasitoid species were recovered from *Mussidia* spp. eggs and larvae and include the trichogrammatid

egg parasitoid *Trichogrammatoidea* sp. nr *lutea* Girault, a braconid egg-larval parasitoid, *Phanerotoma* sp., the bethylid *Goniozus* sp. and the braconid *Apanteles* sp. Moreover, the ichneumonid larval parasitoid *Syzeuctus* sp. was obtained from *M. fiorii*, while the tachinid *Leskia* sp. was obtained from *M.* "madagascariensis". *Trichogrammatoidea* sp. nr *lutea*, the only parasitoid species which was successfully reared in the laboratory, successfully attacked and developed on eggs of six lepidopteran hosts indicating its potential to exploit other alternate lepidopteran pests of maize in West Africa. Like the parasitoid species, only one *Mussidia* sp., *M. fiorii*, was successfully reared in the laboratory and it developed on maize seed-, *Canavalia enseiformes* L. DC (Fabaceae) seed- and maize leaf-based diets while it could not develop on *Mucuna pruriens* L. DC (Fabaceae) seed- and *C. cathartica* seed-based diets. The lower developmental thresholds for *M. fiorii* eggs, larvae, pupae and egg to adult were found to be 12.8±0.25°C, 14.4±0.27°C, 11.0±0.03°C and 13.5±0.21°C, respectively, while the thermal constants were 82.0±1.61, 384.6±9.43, 144.9±6.84 and 588.2±10.81 degree days, respectively. Adults started emerging during the last hour of photophase and peak emergence was observed in the 2nd hour of scotophase. Mating activity largely took place between the 4th and 5th hour of scotophase. It can be concluded that there exist several *Mussidia* spp. in Africa that vary in their host plant range. Overall, mortality caused by parasitoids was negligible hence they were unlikely to explain the population dynamics of the *Mussidia* spp. in Kenya. The fact that *Trichogrammatoidea* sp. nr *lutea* successfully attacks and develops in six lepidopteran hosts, including two *Mussidia* spp. indicates its potential for use as a biological agent against *M. nigrivenella* in West Africa. *Mussidia fiorii* was able to develop on diets based on maize and *C. enseiformes*. The knowledge on dietary and thermal requirements would optimize mass production of the host and natural enemies. The present study revealed again a serious bottleneck for biocontrol worldwide, namely the

proper identification of the pest and natural enemy species as a result of an ever dwindling number of taxonomists. We therefore suggest that molecular (DNA) techniques should be used in addition to detailed morphological examination. In view of the fact that natural control will not be effective in case of accidental introduction of the West African *M. nigrivenella* into Kenya, we suggest stringent precautions during movement of grains especially maize between the West Africa region and Kenya.

Key words: *Mussidia* spp., parasitoids, East and southern Africa (ESA), West Africa, alternate species, bionomics.

UITTREKSEL

Mussidia nigrivenella Ragonot (Lepidoptera: Pyralidae), 'n belangrike plaag van mielies, katoen en *Phaseolus*-bone in Wes-Afrika, is nog nooit voorheen as 'n plaag van gewasse in Oos- en suider-Afrika (OSA) beskryf nie. Die hipoteses was dat hierdie plaag deur natuurlike vyande in OSA onder beheer gehou word of dat daar verskillende *M. nigrivenella* populasies met verskillende gasheerplantreekse bestaan. 'n Ander moontlikheid is die verkeerde identifikasie van *Mussidia* spp. in OSA. Studies is gedoen in Kenia tussen 2005 en 2007 om die spesiediversiteit en gasheerplantreeks asook die ruimtelike verspreiding van *Mussidia* spp. op sekere gasheerplante te bepaal. Verdere studies, gebaseer op die resultate van die gasheerplantreeks-opnames is tussen 2006 en 2007 in Kenia gedoen om die diversiteit van die parasitoïed-kompleks van *Mussidia* spp. te bepaal. Die geskiktheid van stamruspers wat in Kenia voorkom vir ontwikkeling van *Trichogrammatoidea* sp. nr *lutea* Girault (Hymenoptera: Trichogrammatoidea) en faktore wat die ekologie van *Mussidia* spp. beïnvloed is onder laboratoriumtoestande bestudeer. Daar is gevind dat agt plantspesies gasheer is van twee *Mussidia* spp. asook ses moontlike morfospesies wat simpatries in die kusstreek voorkom. Hierdie twee *Mussidia* spp. is *Mussidia fiorii* Ceconni de Joannis en *M.* nr *nigrivenella*. Slegs een *Mussidia* sp., *M. fiorii*, het voorgekom op een gasheerplantspesie in die middelland-streek. Oor die algemeen was die gasheerplantreeks in OSA baie nouer as in Wes-Afrika. Die larvale verspreiding van *Mussidia* nr *nigrivenella* en *Mussidia* "madagascariensis" was geaggregeer op *Canavalia cathartica* Thouars. (Fabaceae) en *Strychnos madagascariensis* Poir. (Loganiaceae) terwyl die verspreiding van *M. fiorii* volwassenes op *Kigelia africana* (Lam.) Benth. (Bignoniaceae) reelmatig was. Agt parasitoïed-spesies van *Mussidia* spp. eiers en larwes is gevind. Hierdie spesies sluit in die

trichogrammatid eierparasitoïed *Trichogrammatoidea* sp. nr *lutea*, 'n braconid eier-larf parasitoïed, *Phanerotoma* sp., die bethylid, *Goniozus* sp., en die braconid *Apanteles* sp. Die ichneumonid larfparasitoïed, *Syzeuctus* sp., is uitgeteel vanuit *M. fiorii*, terwyl die tachinid *Leskia* sp. verkry is uit *M.* "madagascariensis". *Trichogrammatoidea* sp. nr *lutea*, die enigste van bogenoemde parasitoïedspesies wat suksesvol in die laboratorium geteel kon word, het die eiers van ses ander Lepidoptera-gashere aangeval en suksesvol daarin ontwikkel. Dit dui aan dat hierdie spesie oor die potensiaal beskik om ander nie-teiken Lepidoptera-plae van mielies in Wes-Afrika as gasheer te benut. Soos met die parasitoïedspesies, kon slegs een *Mussidia* sp., *M. fiorii*, suksesvol in die laboratorium geteel word op mieliesaad-, *Canavalia enseiformes* L. DC (Fabaceae)- en mielieblaar-gebaseerde dieete. *M. fiorii* kon egter nie suksesvol op dieete van *Mucuna pruriens* L. DC (Fabaceae) saad asook *C. cathartica* saad-dieete oorleef nie. Die laer-ontwikkelingsdrempel vir *M. fiorii* eiers, larwes, papies en eier tot volwassene was 12.8±0.25°C, 14.4±0.27°C, 11.0±0.03°C en 13.5±0.21°C, respektiewelik, terwyl die temperatuurkonstante respektiewelik 82.0±1.61, 384.6±9.43, 144.9±6.84 en 588.2±10.81 graad-dae was. Volwassenes het tydens die laaste uur van die ligfase verskyn en die piek-verskyningsperiode is waargeneem in die tweede uur van die skotofase. Paringsaktiwiteit het grootliks plaasgevind tussen die 4^e en 5^e uur van die skotofase. Die gevolgtrekking is dat daar verkeie *Mussidia* spp. in Afrika voorkom en dat hul gasheerplantreekse verskil. In die geheel gesien was die mortaliteit wat deur parasitoïede veroorsaak is uiters laag en kan dit daarom nie die populasiedinamika van *Mussidia* spp. in Kenia verklaar nie. Die feit dat *Trichogrammatoidea* sp. nr *lutea* suksesvol oorleef op ses Lepidoptera spesies (twee *Mussidia* spp. ingesluit) dui aan dat dit moontlik as biologiese-beheeragent van *M. nigrivenella* in Wes-Afrika benut kan word. *Mussidia fiorii* het oorleef op dieete wat gebaseer is op mieliesaad en *C. enseiformis*. Hierdie kennis aangaande dieet-

en temperatuurvereistes kan gebruik word om die massaproduksie van hierdie gasheer- en sy natuurlike vyande te optimaliseer. Hierdie studie het weereens gewys dat die wêreldwye tekort aan taksonome 'n ernstige beperking plaas op biologiese beheer in terme van korrekte identifikasie van plaag- en natuurlike vyandkomplekse. Om hierdie rede word voorgestel dat molekulêre (DNA) tegnieke gebruik word ter ondersteuning van detail morfologiese ondersoeke. Gesien in die lig dat natuurlike beheer van *M. nigrivenella* nie effektief sal wees indien dit per abuis na die OSA-streek gebring word nie, word voorgestel dat streng maatreëls getref word tydens vervoer van grane tussen die twee streke.

Sleutelwoorde: *Mussidia* spp., parasitoïede, oos- en suider-Afrika (OSA), Wes-Afrika, nie-teikenspesies, ekologie.

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CHAPTER ONE

1. General introduction and literature review

1.1 Introduction

Agricultural productivity in Africa is severely limited by a high number of biotic constraints such as arthropods, nematodes, disease, rodents and birds as well as abiotic constraints such as drought, soil infertility and mineral toxicity.

Maize (*Zea mays* L.) is a cereal crop grown throughout the world and plays an important role in the diet of millions of Africans due to its high yields per hectare, its ease of cultivation and adaptability to different agro-ecological zones, versatile food uses and good storage characteristics (Asiedu, 1989). The average yield of maize in Africa in 2004 was estimated to be 1.5 tons/ha (FAO, 2004).

In sub-Saharan Africa (SSA), the most important field pests of maize are lepidopteran stem- and ear-borers belonging to the families Noctuidae, Crambidae and Pyralidae (Polaszek, 1998). These pests reduce both quality and quantity of yield and affects the sustainability of maize production. The problem is particularly acute in the small-scale, resource-poor systems under which maize is typically grown in SSA. Yield losses due to borers occur as a result of leaf feeding, dead hearts, stem tunneling, direct damage to grain and increased susceptibility of attacked plants to stalk rots and lodging. In areas with chronic borer problems losses vary between 10-70% (Bosque-Pérez and Mareck, 1991; Gounou et al., 1994; Cardwell et al., 1997; Sétamou et al., 2000a). In addition, grain damage by lepidopterous borers predisposes maize to pre- and post-harvest infestations by storage beetles, infections by *Aspergillus flavus* Link (Deuteromycetes: Monoliales) and *Fusarium*

verticillioides Sacc. (Nirenberg) (Hypocreales), and to subsequent contamination with mycotoxins such as aflatoxin and fumonisin (Sétamou et al., 1998; Cardwell et al., 2000; Schulthess et al., 2002).

In West Africa, the most commonly reported stemborer species are the noctuids *Sesamia calamistis* Hampson and *Sesamia botanephaga* Tams and Bowden and the pyralids *Eldana saccharina* (Walker) and *Mussidia nigrivenella* Ragonot, (Schulthess et al., 1997; Sétamou et al., 2000a). The noctuid *Busseola fusca* (Fuller) is generally of less importance but in Cameroon, Central Africa, it is a predominant species (Ndemah et al., 2001b). In Kenya, *B. fusca* and the crambid *Chilo partellus* (Swinhoe) are economically the most important stemborers while *S. calamistis*, *Chilo orichalcociliellus* Strand and *E. saccharina* are minor pests (Songa et al., 2001).

1.2 Literature review

The ear-borer *M. nigrivenella* is one of the most important pests of maize in West Africa (Moyal and Tran 1991a; Sétamou et al., 2000a). It was first described by Ragonot in 1888 and its geographical distribution is limited to SSA (Moyal, 1988). It has been reported from different parts of the African continent but the borer is particularly abundant in West Africa (Bosque-Pérez and Mareck, 1990; Shanower et al., 1991; Silvie, 1993). *Mussidia nigrivenella* is a highly polyphagous herbivore (Moyal, 1988; Sétamou, 1996) and in addition to maize, it attacks cotton (*Gossypium hisurtum* L.), Cocoa (*Theobroma cacao* L.), Lima bean (*Phaseolus lunatus* L.), Jack bean [*Canavalia enseiformes* (L.) DC.], Velvet beans (*Mucuna pruriens* DC), the néré tree [*Parkia biglobosa* (Jacq) Benth.], the Shea butter [*Butyrospermum parkii* (G. Don) Kotschy], Baobab (*Adansonia digitata* L), Tallow tree

(*Detarium microcarpum* Gill and Perr), *Piliostigma thonningii* (Schumach) Milne-Redh, Winged bean (*Psophorcarpus tetragonolobus* L. DC), *Sesbania exaltata* (Rafin), *Tephrosia candida* DC, Cowpea (*Vigna unguiculata* L. Walpers), Tamarind (*Tamarindus indica* L), *Musa* spp., Wild olive (*Ximenia americana* L.), *Gardenia sokotensis* Hutch, Common gardenia (*Gardenia ternifolia* Schum and Thonn.), Mopopaja tree (*Sterculia cordifolia* Cav. R. Br.) (Moyal, 1988; Silvie, 1993; Sétamou, 1996; Sétamou et al., 2002). While host plants attacked by *M. nigrivenella* are diverse, only five species of wild host plants appear to be key in harboring populations of *M. nigrivenella* in Benin. These include *P. biglobosa*, *A. digitata*, *X. americana*, *G. sokotensis* and *G. ternifolia* (Sétamou et al., 2000b). However, M. Nuss (personal comm., Museum für Tierkunde, Dresden, Germany), pointed out the possibility of mis-identification of the insect which could greatly reduce the number of the host plants reported.

Mussidia nigrivenella females commence oviposition on the day of adult emergence without any pre-oviposition period and lasts for 5-6 days though some females oviposit for 10-12 days (Sétamou et al., 1999). The peak of egg laying is on the second day of the oviposition period and females lay more than 90% of their egg compliment during the first three days (Bolaji and Bosque-Pérez, 1998; Sétamou et al., 1999). On maize, the moths oviposit on silk and husk leaves covering the maize ear (Moyal, 1988; Bosque-Pérez and Mareck, 1990) and after hatching, the larvae migrate to the ear where they feed on the grain (Sétamou et al., 1999). Incubation period ranges from 6 to 7 days depending on temperature (Moyal and Tran, 1991a). Females also lay eggs on fruits of wild host plants and the first instars bore into the fruits where they feed (Sétamou, 1996). Newly emerged females have a potential fecundity of more than 650 eggs (Moyal and Tran, 1991a). The mean larval and pupal development period is about 18.4 and 10.1 days respectively, depending on

temperature and pupation takes place in a cocoon (Moyal and Tran, 1991a; Bolaji and Bosque-Pérez, 1998). Before pupating, final instars bore exit holes into the pericarp or husks (Sétamou, 1996). No diapause phase has been observed (Moyal and Tran, 1991b). Although only one generation of the borer generally occurs on maize, two to six generations per year were recorded on different wild host plant species in Benin (Sétamou et al., 2000b).

Yield losses caused by *M. nigrivenella* on maize vary between 5-25% (Moyal and Tran, 1991a; Sétamou et al., 2000a). Sétamou et al. (2000a) observed that although the damage potential of *M. nigrivenella* is moderate (perhaps due to the short period in which the borers feed on maize grain) multiple infestations of the same ear can occur over a prolonged period, thereby increasing the damage potential of the borer in case of late harvest. *Mussidia nigrivenella* continues to feed on maize grains in storage leading to additional 10% yield loss (Sétamou, 1996). Moreover, damage by *M. nigrivenella* also predisposes maize to pre-harvest and post-harvest infestations by storage beetles, infection by *A. flavus* and subsequent aflatoxin contamination (Sétamou et al., 1998). The borer usually damages maize from the tip of the kernels (Moyal, 1988), where natural infection of *A. flavus* occurs and thus could easily promote the spread of the fungi as it eats a channel through a whole line of seeds (Moyal and Tran, 1991a). Also because of their large numbers, *M. nigrivenella* larvae tend to be highly destructive, and thus favour establishment and spread of *A. flavus* in grain (Sétamou et al., 1998). Furthermore, *M. nigrivenella* females prefer to oviposit on maize ears infected by *F. verticillioides* over non-infected ones (Ako et al., 2003). Since *M. nigrivenella* is an ideal vector of *A. flavus*, the concomitant increase in ear feeding also increases mycotoxin levels in grain (Sétamou et al., 1999) such as fumonisin which promote esophageal cancer in humans (Rheeder et al., 1992). In addition, losses of up to 15% have been observed due to an increase in the storage beetles *Carpophilus* spp. (Coleoptera:

Nitidulidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) when ears were damaged by *M. nigrivenella* (Sétamou, 1996). Damage from *M. nigrivenella* can easily be detected by the conspicuous amount of silky frass produced by the larvae as it bores into the grains (Sétamou, 1996). Work by Sétamou et al. (2000b) in Benin and by Bosque-Pérez and Mareck (1990) in Nigeria showed that *M. nigrivenella* was the most abundant lepidopteran maize ear-borer species throughout all agro-ecological zones while in Cameroon, *M. nigrivenella* was among the predominant species attacking maize (Ndemah et al., 2001b).

Various control strategies have been tried, with partial success, but all have limitations and none has provided a complete solution. Chemical control, using systemic insecticides provide only protection against early attacks but not against borers feeding in the ear (Sétamou et al., 1995; Ndemah and Schulthess, 2002). Surveys on wild and cultivated host plants of *M. nigrivenella* in West Africa yielded a small number of natural enemy species and with low levels of parasitism (Moyal 1988; Ndemah et al., 2001a; Sétamou et al., 2002).

Though described from wild host plants in East and southern Africa (ESA), *M. nigrivenella* has never been reported as a pest of annual crops outside West Africa. Moreover, with the exception of *M. nigrivenella*, which was the only species identified by Ragonot in West Africa so far, no information is available about the biology and ecology of other *Mussidia* spp. It was hypothesized that, in East and southern Africa *Mussidia* spp. was either under natural control on wild hosts or the locally occurring geographic race did not attack crop plants. Thus, information on the host plant range and on the diversity of *Mussidia* spp. and associated natural enemies could help explain the differences in pest status between the ESA and the West African regions and also give an insight into the possibility of natural

enemies' redistribution (exchange of natural enemies' species and races between regions of a continent) or new association biological control approaches (controlling a pest by natural enemies that have not originally co-evolved with that particular pest) for control of *M. nigrivenella* on crops in West Africa.

The overall aim of this study was therefore to determine the diversity of *Mussidia* species, associated host plants and natural enemies in Kenya. In the following chapter (chapter two), we attempted to evaluate the diversity of *Mussidia* spp. and their host plant range in Kenya through surveys in the mid-altitude and coastal regions of Kenya. Chapter three describes the natural enemies' complex attacking *Mussidia* spp. in Kenya while chapter four reports on the performance of *Trichogrammatoidea* sp. nr *lutea* Girault (Hymenoptera: Trichogrammatidae) reared from *Mussidia* spp. eggs. We report on the factors affecting bionomics of *Mussidia* spp. in the laboratory in chapter five.

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CHAPTER TWO

2. Host plants and species diversity of *Mussidia* (Lepidoptera: Pyralidae) in Kenya[1]

2.1 Abstract

Mussidia nigrivenella (Lepidoptera: Pyralidae), an important pest of maize, cotton and *Phaseolus* bean in West Africa, has never been described as a crop pest from East and southern Africa (ESA), although it was reported to exist in the wild. Generally, little is known about the host plant range and the diversity of *Mussidia* spp. in Kenya. Thus, surveys were carried out in Kenya between 2005 and 2007 to assess the species diversity and host plant range of *Mussidia*. Eight plant species were found to host two *Mussidia* spp. and six morphospecies, which occur sympatrically in the coastal region while only one *Mussidia* sp. was found attacking one host plant in the mid-altitude region of the country. In addition, the spatial distribution of *Mussidia* nr *nigrivenella*, *Mussidia* "madagascariensis" and *Mussidia fiorii* was studied using Taylor's power law. *Mussidia* nr *nigrivenella* and *M.* "madagascariensis" larvae showed to be aggregated on *Canavalia cathartica* and *Strychnos madagascariensis*, respectively, while the distribution of *M. fiorii* adults on *Kigelia africana* was regular. Sampling plans were developed for three *Mussidia* spp. on their respective host plants, which allow for estimation of pest densities with a certain precision level. Whether *M. nigrivenella* occurs in Kenya could not be determined in the present study with absolute

[1]Muli, B.K., Schulthess, F. and Van den Berg, J. (2009). Host plants and species diversity of *Mussidia* (Lepidoptera: Pyralidae) in Kenya. *International Journal of Biodiversity Science and Management* (in press).

certainty and molecular tools might be required to separate the different morphospecies into species.

2.2 Introduction

The ear-borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae), is one of the most important pests of maize in West Africa (Moyal and Tran, 1991; Sétamou et al., 2000a). In the field, yield losses vary between 5-25% (Moyal and Tran, 1991; Sétamou et al., 2000a). In addition, infestation by *M. nigrivenella* predisposes maize to attack by pre- and post-harvest storage beetles *Carpophilus* spp. (Coleoptera: Nitidulidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) leading to further losses of up to 15%.

Surveys in West Africa revealed 20 host plants of *M. nigrivenella* including cultivated crops such as maize, cotton and *Phaseolus* bean (Sétamou et al., 2000b). It was collected from maize from the lowland tropics up to mid-altitude areas (Oigiangbe et al., 1997; Sétamou et al., 2000a, b; Ndemah et al., 2001a). By contrast, *M. nigrivenella* has never been reported as a pest of crops from East and southern Africa (ESA), where, it has however been reported to occur on wild host plants (Janse, 1941; LePelley, 1959). It was hypothesized that in ESA *M. nigrivenella* was either under natural control on wild hosts or the locally occurring geographic race did not attack maize (Ndemah et al., 2001b; Sétamou et al., 2002). Thus, in a first step, the *Mussidia* spp. diversity and their host plant range were assessed in lowland and mid-altitude areas in Kenya.

2.3 Materials and methods

2.3.1 Host plants and *Mussidia* species diversity

Based on results of preliminary surveys during 2001 (F. Schulthess, unpubl. data), surveys for *Mussidia* spp. and their associated host plants were undertaken in the coastal lowlands and mid-altitude regions of Kenya. These zones also correspond to the ecoregions where *M. nigrivenella* occurs in West Africa (Sétamou et al., 2000b). In total eleven surveys carried out between 2005 and 2007, two to four months between consecutive surveys, mature fruits or pods from plant species in families reported as hosts of *M. nigrivenella* in West Africa (Sétamou et al., 2000b) were sampled. A tree, shrub or vine was considered a sampling site and was selected from afar based on whether it had fruits. At close range, it was examined for mature fruits. Fruit samples were randomly selected from the accessible parts (Sétamou et al. 2000c). Fruits were collected from the lower, middle and upper canopy. The plant was roughly divided into four quadrants and fruits were randomly collected from each quadrant. At times, ripe fallen fruits were collected from the ground. Since fruit density varied with plant species, the number of fruits collected from each plant also varied. Fruits were hand-picked and if necessary harvested using a 7m telescoping pole. For trees with fruits >10 cm in diameter, at most 10 fruits were collected while for trees with fruits <10 cm in diameter, as many as 20 fruits were collected per tree. Where many trees of the same species were found in the same locality, a distance of at-least two Kilometers was covered before the next sampling site. Due to the differences in fruiting phenologies, fruits were collected during both the wet and dry seasons. In some cases, some sites were sampled more than once depending on the availability of fruits. Each sample was labeled according to location using a geographic position system (GPS) (Garmin - Geko 201), plant species, and date of collection.

All fruits or pods were taken to the laboratory at the International Centre of Insect Physiology and Ecology, in Nairobi, in 15cm by 15cm by 20cm plastic containers, of which lids were well ventilated to prevent excessive humidity build-up. Containers were kept at 25-30°C for up to eight weeks to ensure that all insects in them emerged as adults. From the emerged adults, some of those specimens having the characteristics of *Mussidia* spp. (Moyal, 1988) were used to produce eggs for starting laboratory colonies on artificial diet described by Onyango and Ochieng-Odero (1994) while some were exposed to ethyl acetate in a 6.5cm diameter by 12cm height killing jar, mounted and sent for identification by M. Nuss at the Museum für Tierkunde, Dresden, Germany, where voucher specimens are kept. For the production of eggs, adults were offered a piece of multipurpose laboratory towel (Sétamou et al., 1999), about 22cm by 48 cm folded quarterly, vertically placed in a 9cm diameter by 16.5 height plastic jars covered with a well ventilated lid to prevent the insects from escaping. The set-up was kept in an incubator at a temperature of 27±1°C and 60-80%RH. The adults were added to the jars as they emerged and irrespective of the sex since mating was assumed to occur immediately after emergence. The adults were fed on 20% honey/water solution which was changed daily until the adults died. Eggs were collected on daily basis and were incubated at 27±1°C, 60-80% RH. Emerged neonates were put on artificial diet in a 9cm diameter by 16.5 height plastic jar using a fine camel hair brush. About 0.3 litres of the diet was used per jar and at-most 50 larvae were used put in a jar. The set up was kept in an incubator at 27±1°C, 60-80% RH. In subsequent surveys, fruits were first visually examined all over for eggs or first-instar larvae suspected to be *Mussidia* spp. (preliminary observation showed that 24 hr-old *Mussidia* spp. eggs and 1st instar larvae were red in colour). Larvae were put on artificial diet while the eggs were incubated in 2.5cm by 7.5cm transparent glass vials at the conditions stated above until larval emergence. At most 100 eggs were put in one

vial. Emerging larvae were reared on artificial diet until adulthood to ascertain the species. When fruits were collected from an unknown plant, a sample of leaves and/or flowers, if available, were preserved in a pressing board for identification at the Kenya Forestry Research Institute (KEFRI) or by Mr. Mathenge, University of Nairobi. Only plants whose fruits yielded adult *Mussidia* spp. were considered as host plants. According to Wiklund (1974), the presence of an egg or larva does not necessarily indicate suitability of the host for completion of lifecycle of an insect.

In addition, two herbaceous legumes, the perennial *Canavalia enseiformes* L. DC (Fabaceae) and the annual *Mucuna pruriens* DC (Fabaceae), which commonly harbor *M. nigrivenella* in West Africa (Sétamou et al., 2000b), were planted under irrigation at the Kenyan coast during 2006-2007 to attempt to trap *Mussidia* spp. Both were spaced 20cm (intra row) by 30 cm (inter row) in plots of 3m by 4m. Planting was done in a staggered manner (after every three weeks) to ensure availability of mature pods through out the year. Between 50 and 100 dry pods per plot were randomly harvested at least once every two months.

2.3.2 Spatial distribution of *Mussidia* spp. and development of sampling plans

To determine the spatial distribution of *Mussidia* spp., fruits from *Kigelia africana* (Lam.) Benth. (Bignoniaceae), *Adansonia digitata* L. (Bombacaceae), *Canavalia cathartica* Thouars. (Fabaceae), *Canavalia enseiformes* L. DC (Fabaceae), *Afzelia quanzensis* Welw. (Fabaceae), *Strychnos spinosa* Lam. (Loganiaceae), *Strychnos madagascariensis* Poir. (Loganiaceae) and *Tamarindus indica* L. (Fabaceae), found harboring *Mussidia* spp. in the previous study, were collected for extraction of larvae of *Mussidia* spp. Mature fruits of each

host plant were randomly hand-picked or harvested using a 7m telescoping pole from the accessible parts of trees/ vines. For *K. africana* and *S. madagascariensis*, at times, ripe fallen fruits were collected from the ground. Since fruit density varied with plant species, the number of fruits collected from each plant also varied: for *K. africana* and *S. spinosa*, at most 10 fruits were collected per tree while for *S. madagascariensis*, *A. digitata*, and *A. quanzensis*, between 10 and 20 fruits were examined. For *C. cathartica*, *C. enseiformes* and *T. indica*, depending on the availability of mature dry pods, between 10 and 50 pods were examined. Like for the host plant surveys, sampling was done during both the wet and dry seasons and subject to the availability of fruits, some sites were sampled more than once. Except for *K. africana* whose fruits are not easy to dissect without destroying the immature stages, fruits were dissected and inspected for the presence of larvae usually found feeding on the seeds. While *C. carthartica, C. enseiformes, A. quanzensis* and *T. indica* pods were longitudinally dissected, fruits of *S. madagascariensis, S. spinosa* and *A. digitata* were not dissected in any particular manner. Larvae obtained were reared on an artificial diet until adulthood to confirm the identity of the species. The larvae were put individually on artificial diet in 2.5cm by 7.5cm glass vials which were then plugged with cotton wool to prevent the larvae from escaping. They were incubated at 27±1°C, 60-80%RH until adult emergence. For *K. africana*, the fruits were brought to the laboratory and kept in 30cm by 30cm by 70 cm screen cages at 25-30°C until adults emerged. The total number of adults emerging per fruit was recorded. Sufficient data (at least 10 sites with infested fruits) for the assessment of the spatial distribution of *Mussidia* spp. were available for three host plants only, namely *K. africana, C. cathartica* and *S. madagascariensis*. While spatial distribution of *Mussidia* spp. on *C. cathartica* and *S. madagascariensis* were based on larval densities, that of *Mussidia* spp. on *K. africana* was based on densities of the emerging adults.

2.3.3 Data analysis

Taylor's (1961) power law was used to describe the distribution of *Mussidia* sp. larvae on *C. cathartica* and *S. madagascariensis* and adults on *K. africana*. This law postulates a consistent relationship for a species between variance (s^2) and the mean (m):

$$s^2 = am^b$$

where b is a measure of distribution of the species, with $b > 1$ indicating an aggregated, $b = 1$ random, and $b < 1$ regular distribution, while a is considered a mere scalar factor without biological meaning. These coefficients were computed by regressing the natural logarithm of the within-plant variance ($\ln s^2$) against the natural logarithm of mean density ($\ln m$), for each plant. In our case, m is the mean number of individuals per fruit of a plant. A t-test was used to determine if b was significantly different from 1.

To obtain optimal enumerative sample size curves, Wilson and Room (1983) incorporated the estimated variance (s^2) from Taylor's (1961) power law into the general distribution equation by Karandinos (1976):

$$n = (Z_{\alpha/2} / D)^2 s^2 / m^2$$

resulting in

$$n = (Z_{\alpha/2} / D)^2 am^{b-2}$$

where n is the number of samples to be taken, $Z_{\alpha/2}$ is the standard normal deviate ($Z_{\alpha/2}$= 1.96 for n<30) and D is the reliability level for a fixed proportion of the mean. Two

reliability levels, (D = 0.2 or 0.3), were chosen depending on the accuracy of the density estimate required.

2.4 Results

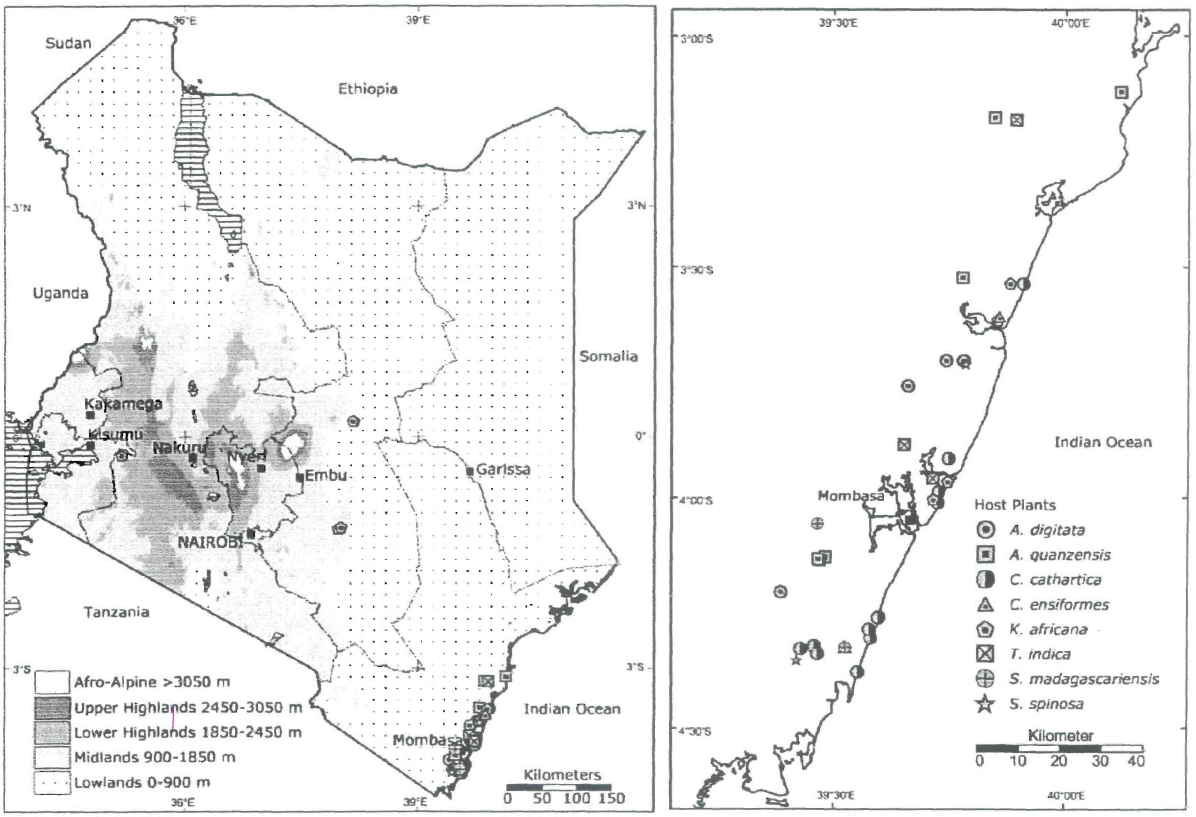
2.4.1 Host plants and *Mussidia* spp. diversity

Mussidia species were obtained from eight plant species all found along the Kenyan coast (Table 2.1, Fig. 2.1). Three plant species were also found in the mid-altitude areas (Fig. 2.1) but *Mussidia* spp. was recovered from only one of them. Seven plant species from four families reported as hosts of *M. nigrivenella* in West Africa were sampled during the current study (Table 2.2).

Table 2.1: Host plant range of *Mussidia* spp. along the Kenyan recorded during the period 2005-2007.

Host plant family	Host scientific name	Host common name	Agro-ecozone host found	*Mussidia* sp.
Bignoniaceae	*Kigelia africana* (Lam.) Benth.	African sausage tree	Mid-altitude and coastal lowlands	*Mussidia fiorii*
Bombacaceae	*Adansonia digitata* L.	Baobab	Mid-altitude and coastal lowlands	*Mussidia* "digitata"
Fabaceae	*Tamarindus indica* L.	Tamarind	Mid-altitude and coastal lowlands	*Mussidia* "indica"
	Afzelia quanzensis Welw.	Pod mahogany	Coastal lowlands	*Mussidia* "quanzensis"
	Canavalia cathartica Thouars.	Maunaloa vine	Coastal lowlands	*Mussidia* nr *nigrivenella*
	Canavalia enseiformes L. DC	Jack bean	-	*Mussidia* "enseiformes"
Loganiaceae	*Strychnos madagascariensis* Poir.	Black monkey orange	Coastal lowlands	*Mussidia* "madagascariensis"
	Strychnos spinosa Lam.	Green monkey orange	Coastal lowlands	*Mussidia* "spinosa"

Nb: - indicates that the plant species was not found in the wild.



a **b**

Figure 2.1: Distribution of host plants attacked by *Mussidia* spp. along the Kenyan coast and the mid-land regions during 2005-2007. **a** shows the whole map of Kenya while **b** is a section of the Kenyan coast at a different scale.

Table 2.2: Some plant species reported as hosts of *Mussidia nigrivenella* in West Africa and were also examined along the midland and coastal regions of Kenyan for *Mussidia* spp. in the current study (Source: Sétamou et al., 2002).

Family	Scientific name	Common name
Caesalpiniaceae		
	Piliostigma thorningii (Schum.) Milne-Redh.	
Fabaceae		
	Mucuna pruriens (L.) DC	Velvet bean
	Canavalia enseiformes (L.) DC.	Jack bean
	Tamarindus indica L.	Tamarind
	Vigna unguiculata L. Walpers	Cowpea
Poaceae		
	Zea mays L.	Maize
Bombacaceae		
	Adansonia digitata L.	Baobab

Plant species that were sampled in the current study and did not yield *Mussidia* spp. are shown in Table 2.3. Two *Mussidia* spp. and six putative *Mussidia* morphospecies, which occurred sympatrically, were obtained from the different host plants (Table 2.1) in the coastal area. However, due to nomenclatural problems, only one species (*M. fiorii* Cecconi and de Joannis) was identified with certainty while another one was close to the West African *M. nigrivenella* (M. Nuss, Museum für Tierkunde, Dresden, Germany). Henceforth, the latter species will be referred to as *Mussidia* nr *nigrivenella* while the other morphospecies will be identified by the species name of the host plant from which they were collected e.g. *Mussidia* collected from *Adansonia digitata* L. (Bombacaceae) therefore becomes *Mussidia* "digitata". Besides, emerging moths were considered to be a morphospecies based on their similarities

in scale appearance. In the mid-altitudes, only *M. fiorii* was recovered from *K. africana*, the only host plant found attacked. Fifty percent of the host plants from which *Mussidia* spp. were reared belonged to the family Fabaceae (Table 2.1). *Mussidia* spp. eggs were found on the surface of the mature fruits or pods, mostly laid in batches and in many cases more than one egg batch were found per fruit. Except for *M. fiorii* attacking *K. africana*, whose mature fruits have a high moisture content, *Mussidia* spp. eggs were collected on drying or dry fruits. Eggs were also found on fruits harboring larvae or pupae, or that had exit holes. *Mussidia* larvae were found feeding on seeds, producing copious amounts of silk and pelleted frass, especially the species feeding on *Afzelia quanzensis* Welw. (Fabaceae), *Strychnos spinosa* Lam. (Loganiaceae), *S. madagascariensis*, *A. digitata* and *C. cathartica*. *Mussidia* "quanzensis" larvae were found feeding on the seed aril before they moved to the rest of the seed. Dissection of *K. africana* fruits, from which *M. fiorii* adults emerged, revealed that their larvae also fed on the seeds. Except for *M. fiorii*, whose cocoons were found singly near individual exit windows, at least three pupae were found near an exit window with their cocoons joined. However, for *M.* "quanzensis", although pupae were found in groups, exit windows were rare and most of the emerging adults were thought to escape through the open suture of the dry pods. The host plant species attacked by the different *Mussidia* spp. had different fruiting phenologies, hence, fruits suitable for attack (mature drying or dry) were available at different times of the year (Fig. 2.2).

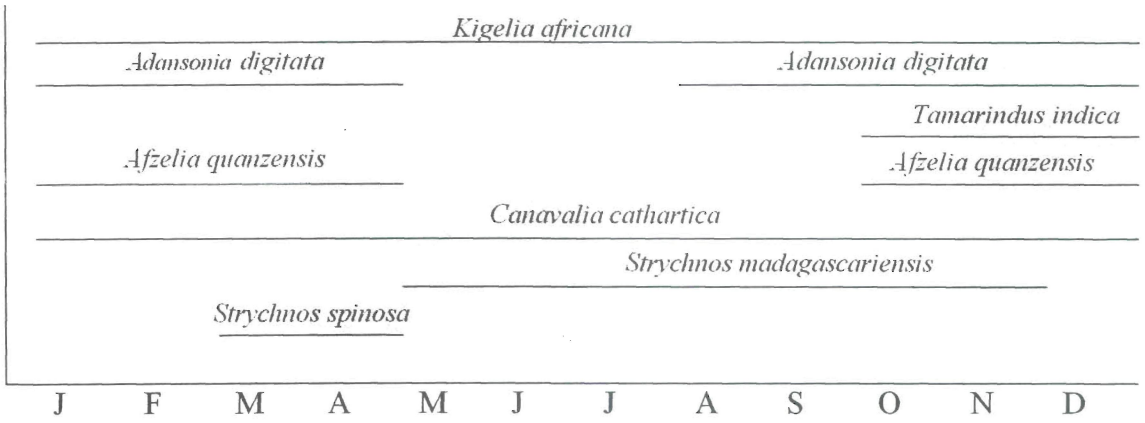


Figure 2.2: Periods when mature fruits of different host plants were available for attack by *Mussidia* spp. at the Kenyan coastal lowlands and mid-altitude regions during 2005 - 2007. The rainy-season months are shaded.

Table 2.3: Plant species from which *Mussidia* spp. were not recovered from their fruit in Kenya between 2005 and 2007.

Family	Scientific name	Common name
Anacardiaceae	*Sclerocarya birrea* (A. Rich) Hochst.	
	Anacardium occidentale L.	Cashew nut
Bignoniaceae	*Jacaranda mimosifolia* D. Don.	
Caesalpiniaceae	*Erythrophloeum suaveolens* Guill. and Perr.	
	Piliostigma thorningii (Schum.) Milne-Redh.	
Combretaceae	*Terminalia brownii* Fres.	
Euphorbiaceae	*Croton megalocarpus* Hutch.	
Fabaceae	*Senna bicapsularis* L.	Butterfly bush
	Delonix regia (Bojer)	Flamboyant
	Cajanus cajan L.	Pigeon pea
	Dolichos lablab L.	Hyacinth bean
	Acacia nilotica L.	
	Acacia stuhlmannii Taub.	
	Gliricidia sepium (Jacq.) Walp.	
	Mucuna pruriens (L.) DC.	Velvet bean
	Mucuna gigantea (Willd.) DC.	
	Senna spectabilis DC.	
	Senna singueana (Delile) Lock	
	Parkia filicoidea Welw. ex Oliver	
Meliacea	*Melia volkensii* Gürke	
Mimosaceae	*Albizia anthelmintica* Brongn.	
Moraceae	*Ficus benjamina* L.	Weeping fig tree
	Ficus sycomorus L.	Fig tree
Moringaceae	*Moringa oleifera* Lam.	
Papilionoideae	*Erythrina abbyssinica* (A. Rich)	
Poaceae	*Zea mays* L.	Maize
Rhamnaceae	*Ziziphus mauritiana* Lam.	Chinese apple
Rubiaceae	*Vangueria infausta* Burch.	Wild pear
Solanaceae	*Solanum incanum* L.	Sodom apple
Sterculiaceae	*Dombeya rotundifolia* Hotchst.	
	Sterculia appendiculata K. Schum.	

2.4.2 Spatial distributions and sampling plans of *Mussidia* spp.

Mean densities of *M. fiorii* adults, *M.* "madagascariensis" and *M.* nr *nigrivenella* larvae were 55.9 ± 7.6, 3.93 ± 0.64 and 0.73 ± 0.14. The Taylor's (1961) power law regressions yielded slopes, *b*, greater than 1 for *M.* nr *nigrivenella* and *M.* "madagascariensis" larvae on *C.*

cathartica and *S. madagascariensis*, respectively, indicating an aggregated distribution. By contrast, *Mussidia fiorii* adults on *K. africana* yielded a slope less than 1, indicating a regular distribution (Table 2.4). Student's *t*-test (Sokal and Rohlf, 1995) showed that all slopes were significantly different from unity (t-value = 13.68, $P<0.0001$; t-value = 4.064, $P=0.0023$ and t-value = 4.29, $P<0.0007$ for *C. cathartica*, *S. madagascariensis* and *K. africana*, respectively).

The optimal sample size curves (i.e. the optimal number of samples to be taken to estimate a given density for a given precision level) for the different *Mussidia* species are shown in Fig. 2.3. *Mussidia* "madagascariensis" required the highest number of fruits, at-least 78 and 30 fruits at reliability level of D=0.2 and D=0.3, respectively, to estimate the population of a site with a density of 1 larvae per fruit.

Table 2.4: Taylor's a and b coefficients and r^2 for three *Mussidia* species each on their main host plants.

Species	Host plant	Taylor's parameters			
		N	*a*	*b*	r^2
Mussidia nr *nigrivenella*	*Canavalia cathartica*	24	0.9125±0.074	1.58±0.115	0.89
Mussidia "madagascariensis"	*Strychnos madagascariensis*	12	0.8792±0.168	1.16±0.284	0.62
Mussidia fiorii	*Kigelia africana*	23	1.4347±0.364	0.94±0.218	0.47

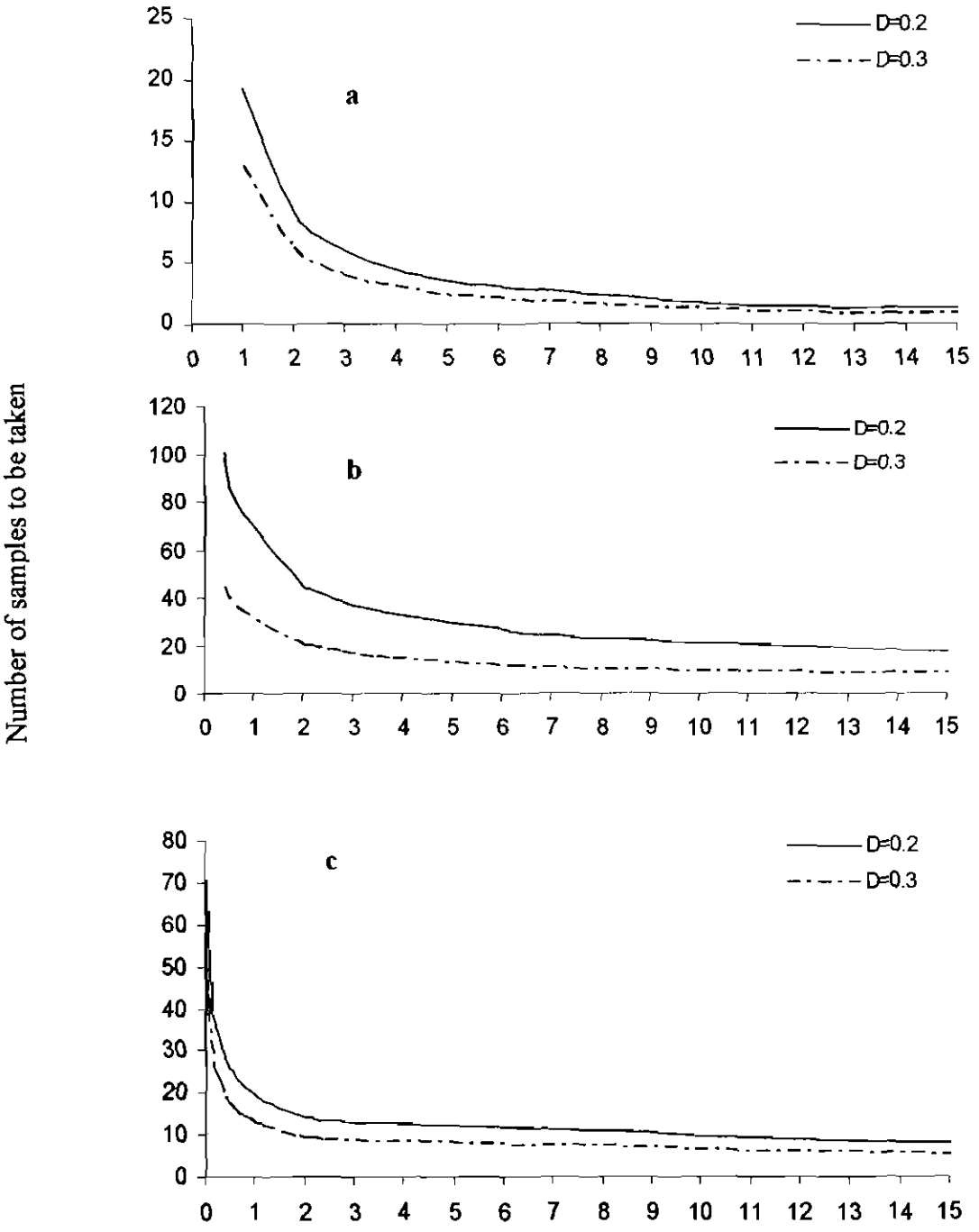


Figure 2.3: Optimal number of samples to be taken to estimate mean densities of a) *Mussidia fiorii* b) *Mussidia* "madagascariensis" and c) *Mussidia* nr *nigrivenella* with reliability levels of D=0.2 and D=0.3.

2.5 Discussion

The host plant range of *Mussidia* spp. were much narrower than for *M. nigrivenella* in West Africa where Sétamou et al. (2000b) identified 20 plant species from 11 plant families hosting the pest. In contrast to West Africa, in the current study, attempts to sample maize cobs yielded no *Mussidia* spp. As also found by Sétamou et al. (2000b) for West Africa, the Fabaceae family yielded the highest number of host plant species. All these species are of economic or agronomic importance, however, and with exception of *C. enseiformes*, they are not cultivated. The wild perennial *C. cathartica* is an efficient nitrogen fixer and its seeds and immature pods are edible while leaves serve as livestock feed (Seena and Sridhar, 2006). In West Africa, the related *C. enseiformes,* which is cultivated as a cover crop or as a green manure (Milne-Redhead and Polhill, 1971), was among the most suitable hosts of *M. nigrivenella* and it was heavily attacked in the field (Sétamou, 1999). *Canavalia enseiformes* is not commonly used in Kenya though it was among the legumes screened by the Legume Research Network Project during 1995 and 1996 for use in soil improvement. It was found to perform well at all sites below 1900 m a.s.l. (Mureithi et al., 1998). *Tamarindus indica* was among the plants reported by Sétamou et al. (2000b) to host *M. nigrivenella* in West Africa. Similarly, it was found to host *Mussidia* sp. in the current study. Sétamou et al. (2000b) found that *A. digitata* was a suitable host of *M. nigrivenella* in West Africa. This was corroborated in the current study where it was found harboring *Mussidia* sp. Its parts have been used for food (Nordeide et al., 1996; Venter and Venter, 1996) besides having some medicinal value. Though reported harboring *Mussidia* spp. in the current study, *S. madagascariensis, S. spinosa, A. quanzensis* and *K. africana* were not among the hosts reported by Sétamou et al. (2000b) to host *M. nigrivenella* in West Africa. Fruits of *S.*

madagascariensis and *S. spinosa* are edible (Mwamba, 2006) while those of *K. africana* have medicinal value (van Wyk et al., 1997) and are fermented for honey beer (Beentje, 1994). Seeds of *A. quanzensis* are used for decorative purposes (Joker and Msanga, 2000; Palgrave, 2002). Owing to the seasonality of fruiting, the current study could not establish how *Mussidia* species survive periods of non-availability of suitable fruiting structures.

Various studies have shown the dispersion of *M. nigrivenella* larvae to be aggregated on maize (Schulthess et al., 1991; Sétamou et al., 2000c; Ndemah et al., 2001c) and on wild host plants (Sétamou et al., 2000c). An aggregated distribution was also found for *Mussidia* sp. larvae on *C. cathartica* and *S. madagascariensis*. This might be attributed to the oviposition behavior of the adults as suggested by Cole (1946) and Sétamou et al. (2000c), whereby eggs are laid in batches, which favors aggregation of larvae in the fruits. By contrast, *M. fiorii* adults showed a regular distribution on *K. africana*. Unlike the other *Mussidia* species, whose pupal cases were found aggregated around the exit windows, only one pupal case was found per exit window on *K. africana* fruits. This indicates a fierce intraspecific competition by *M. fiorii* larvae, probably induced by the high per fruit densities, explaining the regular distribution of adults and, thus, pupae. Sétamou et al. (2000c) suggested that because of the exceedingly cryptic larval feeding behaviour of *Mussidia* spp., emphasis should be given to finding egg and pupal parasitoids. An aggregation of pupae around exit holes should also improve host finding and parasitism efficiency of pupal parasitoids.

The current study indicated that several *Mussidia* spp. exist in Kenya attacking fruits and pods of various wild hosts. However, none of the *Mussidia* species was found attacking maize. Although one *Mussidia* spp. which is close to the West African *M. nigrivenella* was recovered during this particular study, whether *M. nigrivenella* occurs in Kenya could not be

determined with absolute certainty. Molecular tools might therefore be required to separate the different morphospecies into species.

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CHAPTER THREE

3. Parasitoids associated with *Mussidia* spp. (Lepidoptera: Pyralidae) in mid altitude and coastal Kenya[2]

3.1 Abstract

The pyralid *Mussidia nigrivenella*, a pest of cotton, maize and *Phaseolus* bean in West Africa, has never been reported as a crop pest in East and southern Africa, though it is reported to exist in the wild. It is hypothesized that the difference in pest status of *M. nigrivenella* between West and East Africa was either due to differences in natural enemy compositions or that there exist several populations and/or species of *Mussidia*, which vary in their host plant range. Thus, a catalogue of parasitoids of *Mussidia* spp. was established through surveys in mid-altitude and coastal regions of Kenya, between 2006 and 2007. *Mussidia* spp. eggs, larvae and pupae were collected from fruits of host plants known to host *Mussidia* spp. and were examined for parasitoid-related mortality. The trichogrammatid *Trichogrammatoidea* sp. nr *lutea* was obtained from eggs of *Mussidia* spp. found on *K. africana*. A braconid egg-larval parasitoid, *Phanerotoma* sp. was reared from the larvae of three *Mussidia* spp. found on *S. madagascariensis*, *A. quanzensis* and *K. africana* while the bethylid *Goniozus* sp. and the braconid *Apanteles* sp. were obtained from *Mussidia* spp. collected from *C. cathartica*. Moreover, the ichneumonid larval parasitoid *Syzeuctus* sp. was

[2]Muli, B.K., Schulthess, F. and Van den Berg, J. (2009). Parasitoids associated with *Mussidia* spp. (Lepidoptera: Pyralidae) in Kenya. *Phytoparasitica* 37:55-60.

obtained from *Mussidia* spp. attacking *K. africana*, while the tachinid *Leskia* sp. was obtained from *Mussidia* spp. found on *S. madagascariensis*.

3.2 Introduction

In West Africa, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) is a pest infesting cotton bolls [*Gossypium hirsutum* L. (Malvaceae)], maize ears [*Zea mays* L. (Poaceae)] and common bean pods [*Phaseolus vulgaris* L. (Leguminoseae)] (Gounou et al., 1994; Moyal, 1988). Although pest incidence in maize fields is usually more than 50%, yield losses are relatively low ranging between 5 to 25% (Moyal and Tran, 1991; Sétamou et al., 2000a). However, *M. nigrivenella* continues to feed on maize grains in stores leading to an additional 5% loss (Sétamou, 1996). In addition, damage by the borer predisposes maize grain to pre- and post-harvest infestations by storage beetles, *Carpophilus* spp. (Coleoptera: Nitidulidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), infection by *Aspergillus flavus* Link (Deuteromycetes) and *Fusarium verticillioides* Sacc. (Nirenberg) (Hypocreales) and subsequent aflatoxin contamination (Sétamou et al., 1998). Increases of up to 15% in yield loss due to storage beetles were reported in the presence of *M. nigrivenella* (Sétamou, 1996). Besides cotton, maize and beans, *M. nigrivenella* also attacks pods and fruits of wild plants (Sétamou et al., 2000b). Eggs are laid in batches primarily on mature fruit, and the first instars bore into the fruit where they feed cryptically until pupation (Sétamou, 1996; Muli et al., 2009).

Currently, no technologies are available to achieve satisfactory control of *M. nigrivenella*. As shown by Sétamou et al. (1995) and Ndemah and Schulthess (2002), even

systemic insecticides have no effect on pests feeding in the ear. Pesticides have to be directed against the earliest instars before they penetrate the plant, which requires monitoring by the farmer. Intercropping maize with both host and non-host companion plants showed that oviposition and numbers of larvae on maize can be significantly reduced but the results were highly variable (Agboka et al., 2006).

Mussidia nigrivenella has never been reported as a pest of maize in East and southern Africa though it was reported from wild host plants (Janse, 1941; Lepelley, 1959; Waiyaki, 1973). Countrywide surveys in maize fields in Ghana and Benin yielded low egg parasitism by trichogrammatid and scelionid parasitoids (Moyal, 1988) and no larval and pupal parasitoids were recovered (Shanower et al., 1991; Gounou et al., 1994). In contrast, in Cameroon, five parasitoid species were obtained from parasitized larvae and pupae collected from maize (Nonveiller, 1984; Ndemah et al., 2001a). Surveys conducted in different agro-ecological zones of Benin on cultivated and wild host plants during 1994–1997 revealed one egg parasitoid, three larval parasitoids and one pupal parasitoid attacking *M. nigrivenella*. While egg parasitism was exceedingly low, larval and pupal parasitism was usually less than 10% and varied with host plant species. Both larval and pupal parasitoids were rare and mostly absent in field grown and stored maize (Sétamou et al., 2002). It was hypothesized that the difference in pest status of *M. nigrivenella* between West and East Africa was either due to differences in natural enemy compositions or that there exist several populations adapted to different host plants or even due to misidentification of the species. Thus, Sétamou et al. (2002) proposed exploration for natural enemies on various wild host plants in East Africa. Because of the exceedingly cryptic larval feeding behaviour of *Mussidia* spp., it was suggested to give emphasis to egg and pupal parasitoids and to include all *Mussidia*

species because, if they occupy similar ecological niches on the plant, they might share the same natural enemies (Hokkanen and Pimentel, 1989).

In previous surveys in coastal Kenya, several *Mussidia* species were identified on wild host plants but again none from maize (Muli et al., 2009). The present work presents the natural enemy complex obtained from these hosts in subsequent surveys.

3.3 Materials and methods

The search for natural enemies associated with *Mussidia* spp. was conducted on eight host plants namely *Kigelia africana* (Lam.) Benth. (Bignoniaceae), *Adansonia digitata* L. (Bombacaceae), *Afzelia quanzensis* Welw (Fabaceae), *Strychnos madagascariensis* Poir. (Loganiaceae), *S. spinosa* Lam. (Loganiaceae), *Tamarindus indica* L. (Fabaceae), *Canavalia cathartica* Thouars (Fabaceae) and *Canavalia enseiformes* L. DC (Fabaceae). The host plants had been found hosting *Mussidia* spp. in a previous study (Muli et al., 2009). The target zone was the coastal lowlands and mid-altitude regions of Kenya, which is climatically similar to the ecological zone in West Africa and Cameroon, where *M. nigrivenella* was reported as a crop pest (Ndemah et al., 2001b; Sétamou et al., 2002). In seven surveys between January 2006 and November 2007, undertaken between two to four months from each other, eggs, larvae and pupae of *Mussidia* spp. were collected from mature fruits randomly selected from the accessible parts of the host plants or collected from the ground. The number of fruits collected per plant varied depending on their availability. For *K. africana* and *S. spinosa*, at most 10 fruits were examined per tree while for *A. digitata*, *A. quanzensis* and *S. madagascariensis* between 10 and 20 fruits were examined. For *C. cathartica* and *T. indica*, between 20 and 50 pods were examined. Fruits were hand picked and if necessary harvested using a telescoping pole, which could be extended to 7 m. Fruits were inspected for eggs

which resembled those of *Mussidia* spp. One-day old *Mussidia* spp. eggs are red in colour and are laid in small batches on the surface of mature drying or dry fruits (Muli et al., 2009). Fruit showing signs of *Mussidia* spp. infestation, circular exit windows, and characteristic silk and pelleted frass, were also inspected for eggs since *Mussidia* spp. also oviposits on previously infested fruits (Muli et al., 2009). The eggs were counted and then carefully excised from the fruit using a sharp blade and kept in clean transparent 2.5cm by 7.5cm vials plugged using cotton wool. They were brought to the laboratories of the International Center of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, where they were kept at room temperature and checked daily until neonate or parasitoid emergence. Emerging neonates were reared on artificial diet described by Onyango and Ochieng-Odero (1994) in a 9cm diameter by 16.5cm height clear plastic jar covered with a well ventilated lid at 27±1°C, 60-80% RH, and a 12:12 L:D photoperiod, till adulthood. At most 50 larvae were put in 0.3 litres of diet per jar. Field collected larvae were reared individually on artificial diet described above in 2.5cm by 7.5cm glass vials plugged with cotton wool under the conditions described above until death, parasitoid emergence or pupation. Field collected pupae were put in 2.5cm by 7.5cm transparent glass vials plugged with cotton wool and monitored in the laboratory for pupal death, parasitoid or adult moth emergence. Adult *Mussidia* spp. were pinned and sent to Dr. M. Nuss (Museum für Tierkunde, Dresden, Germany) for identification. Larvae were assumed to be *Mussidia* spp. based on their feeding habit characterized by copious production of silk and pelleted frass (Sétamou, 1996; Muli et al., 2009) while pupae, each enclosed in a cocoon, were mostly found joined together near an exit window (Muli et al., 2009). Fruits of *K. africana* are large and difficult to dissect without killing the larvae or pupae. They were brought to the laboratory and kept until parasitoid or adult moth emergence. In all cases, the geographic co-ordinates (altitude, longitude and

latitude), from which fruits were collected, were recorded using a portable geographic positioning system (Garmin – Geko 201).

Percentage parasitism for each life stage and *Mussidia* spp. collected on a particular host plant was calculated as the number of parasitized hosts over the total number of *Mussidia* sp. in that particular stage multiplied by 100. Dead larvae, from which no parasitoid emerged, were not included in the calculation of parasitism. Due to low rates of parasitism, data were pooled across the years. Since larvae were not extracted from *K. africana* fruits, it was not possible to directly calculate parasitism. The trichogrammatid egg parasitoids were identified by Joseph Baya (ICIPE, Kenya) while the hymenopteran parasitoids were identified by Gérard Delvare (Cirad, France). A Dipteran larval-pupal parasitoid was identified by David Barraclough (University of KwaZulu-Natal, South Africa). Though the genus *Mussidia* had been identified with certainty using the key described by Moyal (1988), due to nomenclatural problems, only one *Mussidia* sp., i.e., *M. fiorii* Cecconi and de Joannis (Lepidoptera: Pyralidae), was identified to species level (M. Nuss, Museum für Tierkunde, Dresden, Germany). The other *Mussidia* spp. different from *M. fiorii* and the West African *M. nigrivenella* could not be classified and were subsequently named after the host plant, from which they were recovered, as morphospecies. Due to the fact that it is not easy to differentiate immature stages of different *Mussidia* spp. and the possibility that there can be multiple infestations on one fruit, parasitism levels were related to the host plants from which *Mussidia* spp. were collected.

3.4 Results

The number of *Mussidia* spp. eggs, larvae and pupae collected from different host plants and examined for parasitoids is shown in Table 3.1.

Table 3.1: Number of *Mussidia* spp. eggs, larvae and pupae collected and examined for parasitoids from different host plants at the coastal and mid-altitude regions of Kenya between 2006 and 2007.

Host plant spp.	Host plant family	Eggs	Larvae	Pupae
Kigelia africana	Bignoniaceae	2050	+	+
Adansonia digitata	Bombacaceae	100	120	24
Canavalia enseiformes	Fabaceae	-	270	180
Afzelia quanzensis		7	115	25
Tamarindus indica		5	-	-
Canavalia cathartica		31	400	145
Strychnos madagascariensis	Loganiaceae	300	825	197
Strychnos spinosa		-	-	-

+: indicates that the *Mussidia* spp. host stage could not be extracted from the fruit

-: indicates that the *Mussidia* spp. host stage was not collected in this study

Parasitoids were recovered from *Mussidia* species attacking four host plants in three different families (Table 3.2) found at an elevation of between 9 and 1330 m above sea level. Generally, egg parasitism was negligible. Only one species, the trichogrammatid *Trichogrammatoidea* sp. nr *lutea* Girault, was recovered from *Mussidia* spp. eggs found on *K. africana*.

Similarly, larval parasitism was low and parasitoids were reared only from *Mussidia* spp. found on *C. cathartica*, *S. madagascariensis*, *A. quanzensis* and *K. africana*. The bethylid *Goniozus* sp. and the braconid *Apanteles* sp. were recovered from *Mussidia* spp. larvae found on *C. cathartica*. The egg-larval braconid parasitoid *Phanerotoma* sp.1 was recovered from larvae found on *S. madagascariensis* and from *K. africana* while a second one, *Phanerotoma* sp. 2, was recovered from larvae collected from *A. quanzensis*. The ichneumonid larval parasitoid *Syzeuctus* sp. emerged from fruits of *K. africana*. Moreover,

Leskia sp. Robineau-Desvoidy (Diptera: Tachinidae) was recovered from larvae collected from *S. madagascariensis* (Table 3.2).

Table 3.2: Host plants from which parasitoids attacking *Mussidia* spp. were collected at the coastal region and mid-altitude regions of Kenya between 2006 and 2007.

Host plant	Family	Parasitoid	Agro-ecological zone	Host stage attacked	% parasitism
Kigelia africana	Bignoniaceae	*Trichogrammatoidea* sp. nr *lutea*	Coastal	Egg	0.3
		Phanerotoma sp. 1	Coastal	Larva	-
		Syzeuctus sp.	Mid-altitude	Larva	-
Canavalia cathartica	Fabaceae	*Goniozus* sp.	Coastal	Larva	0.3
		Apanteles sp.	Coastal	Larva	0.5
Strychnos madagascariensis	Loganiaceae	*Phanerotoma* sp. 1	Coastal	Larva	0.2
		Leskia sp.	Coastal	Larval-pupal	6.3
Afzelia quanzensis	Fabaceae	*Phanerotoma* sp. 2	Coastal	Larva	3.0

-: indicates that it was not possible to calculate parasitism levels

From *A. digitata*, *C. enseiformes*, *T. indica* and *S. spinosa* no parasitoids were recovered from any *Mussidia* spp. life stage.

3.5 Discussion

From the eight host plants reported to be attacked by *Mussidia* spp. in Kenya, only four yielded parasitoids. These host plants were not reported as hosts of *M. nigrivenella* by Sétamou et al. (2002) in West Africa. The parasitoid species diversity was low as also observed on maize and on wild host plants by Sétamou et al. (2002) on *M. nigrivenella* in West Africa. Working on maize in Cameroon, Ndemah et al. (2001a) obtained fewer parasitoid species from *M. nigrivenella* but the levels of parasitism were higher. With the exception of *Syzeuctus* sp. and *Apanteles* sp., the other parasitoids found attacking *Mussidia* spp. in this study have not been reported in West Africa. Nonveiller (1984) found *Syzeuctus* sp. attacking *M. nigrivenella* on maize in Cameroon while Sétamou et al. (2002) found *Apanteles* sp. attacking *M. nigrivenella* on three host plants including maize. In the current study, *Syzeuctus* sp. was obtained from *M. fiorii* attacking *K. africana* while *Apanteles* sp. was obtained from *M.* nr *nigrivenella* attacking *C. cathartica*.

In Cameroon, most *M. nigrivenella* parasitoids are polyphagous and have been reported attacking other lepidopteran hosts in the same field. For example, the eulophid *Tetrastichus atriclavus* Waterston has been obtained from the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson) as well as the pyralid *Eldana saccharina* (Walker). The braconid *Bracon sesamiae* Cameron was reported from *B. fusca* and *S. calamistis* (Ndemah et al., 2001a). Similarly, *T.* sp. nr *lutea* found in the present study has been reported attacking eggs of noctuid, pyralid and crambid cereal stemborers (Muholo,

2002; Baya et al., 2002). However, as the parasitoid complexes of cereal stemborers in Africa are very well known (Polaszek, 1998), it is suggested that the species obtained in the present study are not attacking stemborers. Moreover, most parasitoids could only be described to genus level, thus, they might be new to science.

As for *M. nigrivenella* in West Africa, the immatures of *Mussidia* spp. identified at the coast and mid-altitude regions of Kenya spend most of their life feeding inside fruits or pods. Because of this cryptic feeding behaviour Sétamou et al. (2002) suggested to concentrate on egg and pupal parasitoids when exploring for natural enemies in eastern Africa. However, as reported by Sétamou et al. (2002) in West Africa, parasitism of all stages was also low.

In view of the difficulties with the classification of *Mussidia* spp. the question arises if the various reports from outside of West Africa dealt with the same species (Lepelley, 1959; Waiyaki, 1973). However, there might also exist several populations of *M. nigrivenella* in Africa, which are adapted to different host plant species. For example, recent phylogenetic studies showed that in Africa there exist various populations of cereal stemborers such as the noctuids *B. fusca* and *S. calamistis*, and the pyralid *E. saccharina*, which vary in their host plant and climatic affinities (Ndemah et al., 2001b; Assefa et al., 2006; Sezonlin et al., 2006; Ong'amo et al., 2008,). Thus, the accidental introduction of these populations - including *M. nigrivenella* - into areas where they are not present could have serious implications for crops such as maize, sorghum, cotton or *Phaseolus* beans. The present study revealed again a serious bottleneck for biocontrol worldwide, namely the proper identification of the pest and natural enemy species as a result of an ever dwindling number of taxonomists. Moreover, phycitine pyralids are a taxonomically difficult group and we suggest that molecular (DNA) techniques should be used in addition to detailed morphological examination.

The current study indicated that although *Mussidia* spp. are attacked by natural enemies in Kenya, the levels of parasitism could not account for the differences in pest status observed between the West African *M. nigrivenella* and the East African *Mussidia* species.

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CHAPTER FOUR

4. Performance of *Trichogrammatoidea* sp. nr *lutea* Girault (Hymenoptera: Trichogrammatoidea) collected from *Mussidia* spp. in Kenya on eggs of six lepidopteran hosts

4.1 Abstract

The ear-borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae), an important pest of maize in West Africa has never been reported as a pest of crops from East and southern Africa (ESA). In Kenya, exploration for natural enemies associated with *Mussidia* spp. yielded a trichogrammatid egg parasitoid, *Trichogrammatoidea* sp. nr *lutea* Girault. The ability of *T.* sp. nr *lutea* to attack alternate species was studied using the eggs of six lepidopteran hosts found in Kenya, namely the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson), the crambid *Chilo partellus* (Swinhoe) and the pyralids *Eldana saccharina* Walker, *Mussidia fiorii* Cecconi and de Joannis and *Mussidia* "madagascariensis". *Trichogrammatoidea* sp. nr *lutea* successfully attacked and developed on all six hosts indicating its potential to exploit alternate lepidopteran pests of maize in West Africa. Results indicate that *T.* sp. nr *lutea* had the highest intrinsic rate of increase (r_m), net reproductive rate (R_o) and finite rate of increase (λ) when reared on *B. fusca* followed by *S. calamistis*, *M. fiorii*, *E. saccharina* and lowest with *C. partellus*. The doubling time (t) was highest when the parasitoid was reared on *C. partellus* followed by *E. saccharina*, *M. fiorii*, *S. calamistis* and was least when the parasitoid was reared on *B. fusca*. The rearing history of the parasitoid had an effect on the success of *T.* sp. nr *lutea* female parasitoids. Age of the

host egg had an effect on the number of parasitized eggs and total parasitoid progeny and was least at the oldest age. It was concluded that the ability of *T.* sp. nr *lutea* to exploit lepidopterans which are also pests of maize in West Africa may have a synergistic effect on the biological control of *M. nigrivenella* and it should be considered for translocation to that area from Kenya.

4.2 Introduction

The ear-borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae), is one of the most important pest of maize in West Africa (Moyal and Tran, 1991; Sétamou et al., 2000a; Ndemah et al., 2001a). Yield losses caused by *M. nigrivenella* on maize in the field vary between 5-25% (Moyal and Tran, 1991; Sétamou et al., 2000a) with an additional 10% loss occurring in storage (Sétamou, 1996). In addition, damage by *M. nigrivenella* predisposes the ear to attack by pre- and post-harvest storage beetles *Carpophilus* spp. (Coleoptera: Nitidulidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) leading to additional losses of up to 15%. Furthermore, damage by *M. nigrivenella* predisposes maize to infection by *Aspergillus flavus* Link (Deuteromycetes) and *Fusarium verticillioides* (Sacc.) Nirenberg (Hypocreales), which produce mycotoxins (Sétamou et al., 1998; Fandohan et al., 2005).

Mussidia nigrivenella is highly polyphagous and besides maize, it infests crops like cotton, *Phaseolus*, velvet and jack beans as well as fruits and pods of wild host plants, which are of local economic importance (Sétamou et al., 2000b; Agboka et al., 2006). In West and Central Africa, *M. nigrivenella* occurs from the lowland tropics up to the mid-altitudes (Oigiangbe et al., 1997; Sétamou et al., 2000a, b; Ndemah et al., 2001a). By contrast, *M.*

nigrivenella has never been reported as a crop pest from East and southern Africa (ESA), where, however, it appears to occur on wild host plants (Janse, 1941; LePelley, 1959; Entwistle, 1972; Waiyaki, 1973). Sétamou et al. (2002) hypothesized that either the *M. nigrivenella* population in ESA does not feed on crops or it was under natural control in the wild habitats, keeping their populations below outbreak levels. The latter would open up opportunities for biological control of *M. nigrivenella* in West Africa through redistribution or new association approaches. It was suggested that because the *Mussidia* spp. occupy similar ecological niches, they would share common natural enemies (Sétamou et al., 2002). Thus, surveys for parasitoids associated with the genus *Mussidia* were carried out in Kenya between January 2006 and November 2007. Included in the list of parasitoids found attacking different *Mussidia* species in Kenya, was the egg parasitoid *Trichogrammatoidea* sp. nr *lutea* Girault which was obtained from *M. fiorii* eggs collected on *Kigelia africana* (Lam.) Benth (Bignoniaceae) fruits along the Kenyan coast (Muli et al. 2009a).

Trichogrammatid egg parasitoids have been used throughout the world as biological control agents against a variety of lepidopteran pests (Wajnberg and Hassan, 1994; Smith, 1996). In East Africa, *T.* sp. nr *lutea* has been reported attacking crambid, pyralid and noctuid stemborer eggs (Baya et al., 2002; Muholo, 2002; Bruce, 2008). In South Africa, *T. lutea*, was reported attacking pyralids and noctuids on maize and cotton (Nargakatti and Nagaraja, 1977; van Hamburg and Kfir, 1991; Kfir, 1995) and was also a major parasitoid of the false coldling moth, *Crytophlebia leucotreta* (Meyr.) (Lepidoptera: Tortricidae), on citrus and apple orchards (Catling and Aschenborn, 1974; Annecke and Moran, 1982). However, catalogues of natural enemies of cereal stemborers and *M. nigrivenella* from West Africa do not list *T.* sp. nr *lutea* (Bosque-Pérez et al., 1994; Polaszek, 1998; Ndemah et al., 2001b; Sétamou et al., 2002).

The Food and Agricultural Organization (FAO) Code of Conduct outlines the conditions to be fulfilled before an anticipated introduction of a biological control agent (Kairo et al., 2003) and requires a thorough analysis of the host specificity of the biological control agent and any potential hazards to non-target hosts (FAO, 1997). Most of the hosts reported to be attacked by *T.* sp. nr *lutea* in East Africa (Baya et al., 2002; Muholo, 2002; Bruce, 2008), and *T. lutea* in South Africa (Nagarkatti and Nagaraja, 1977; Kfir, 1995; are also important pests of maize and sorghum (Whitney, 1970; Schulthess et al., 1997; Ndemah et al., 2001a) and cotton (Angelini and Couilloud, 1972; Martin et al., 2000; Bre'vault et al., 2008) in West and Central Africa. We therefore tested the parasitoid's performance on eggs of these other pest species: the noctuids *Busseola fusca* (Fuller) and *Sesamia calamistis* (Hampson), the pyralids *Eldana saccharina* Walker, *Mussidia fiorii* Cecconi and de Joannis and un-identified *Mussidia* species (which we are calling *Mussidia* "madagascariensis"), and the crambid *Chilo partellus* (Swinhoe), which only occur in ESA. *Chilo partellus* was first reported in Malawi in the early 1930's (Tams, 1932) from where it spread to nearly all countries in ESA (Nye, 1960; van Hamburg, 1980) but it has not yet reached Central and West Africa. While several *Mussidia* spp. were recovered on wild host plants during field surveys in Kenya (Muli et al., 2009b), *M. fiorii* and *M.* "madagascariensis" were the only *Mussidia* spp. whose eggs could be obtained. Muli et al. (2009b) reported two *Mussidia* species and six morphospecies, including *M.* nr *nigrivenella* but most of them could not be reared in the laboratory. Due to its polyphagous nature and the ability to develop in *M. fiorii* eggs, it was assumed that the parasitoid would also be able to attack and develop in eggs of the West African *M. nigrivenella*. It has been shown that the host species exploited by a parasitoid has an effect on the realized fecundity and longevity of its offspring (Lewis et al., 1976), while host age is known to influence its acceptance as well as parasitism (Pak, 1986;

Strand, 1986; Hinz and Andow, 1990; Reznick and Umarova, 1990; Ruberson and Krin, 1993). This study was therefore aimed at evaluating the effect of the rearing history and host eggs age on parasitism and fecundity of the *T.* sp. nr *lutea* since the two are important quality control parameters should mass rearing of this parasitoid become an option.

4.3 Materials and methods

4.3.1 Insects

Trichogrammatoidea sp. nr *lutea* was obtained from *M. fiorii* eggs collected on *Kigelia africana* (Lam.) Benth (Bignoniaceae) fruits along the Kenyan coast collected as described by Muli et al. (2009b) in 2007. Newly emerged *T.* sp. nr *lutea* adults were identified by J. Baya, ICIPE and voucher specimens were deposited at ICIPE. The parasitoid was maintained on *M. fiorii* eggs for more than 10 generations before use in experiments. Three times a week, on Monday, Wednesday and Friday, eggs freshly laid on a 22cm by 48cm multipurpose paper towel were supplied to *T.* sp. nr *lutea* in a transparent Perspex cage (20cm by 20cm by 20 cm). Before exposure of new eggs, the eggs from the previous exposure, presumably parasitized, were removed, the all purpose paper towel cut into smaller strips which were then put in 2.5cm by 10 cm glass vials which were plugged using cotton wool and kept at room temperature until parasitoids emerged from parasitized eggs. Emerged parasitoids were either used for experiments or returned into the rearing cage for the propagation of the colony. A 10% honey/water solution soaked in cotton wool and placed in a small plastic cup, 1.5cm (diameter) by 0.5 cm (height), was provided as diet for the adults in the rearing cage and was changed daily to avoid fermentation.

Colonies of *B. fusca*, *S. calamistis*, *E. saccharina* and *M. fiorii* had been maintained in the laboratory at ICIPE for several generations at 26±1°C, 50-70% RH using artificial diet described by Onyango and Ochieng-Odero (1994) while *C. partellus* was reared on a diet developed by Ochieng et al. (1985). *Busseola fusca* originated from Kitale (Rift valley province), while *E. saccharina* stemmed from Mbita (Nyanza province) and *S. calamistis* from Machakos (Eastern province). The *C. partellus*, *M. fiorii* and *M.* "madagascariensis" populations originated from the Kenyan coast. *Mussidia* "madagascariensis" adults reared from field collected *Strychnos madagascariensis* Poir. (Loganiaceae) fruits collected from the field at the Kenyan coast provided sufficient eggs for host suitability studies. The fruits were collected as described by Muli et al. (2009b) and were brought to the laboratory in 15cm by 15cm by 20cm plastic containers whose lids were adequately ventilated to avoid excessive humidity build up and were held at 25-30°C and 50-80%RH until adult emergence.

Mussidia "madagascariensis" and *M. fiorii* adults were provided with 22cm by 48 cm all purpose laboratory towels folded into quarter and placed vertically in a 9cm diameter by 16.5cm plastic jar for oviposition. To prevent the adults from escaping, the open end of jar was covered with a well ventilated lid. *Busseola fusca*, *S. calamistis*, *E. saccharina* and *C. partellus* were offered rolls of paper (about 1cm in diameter) simulating maize stem, made from 10cm by 75cm strips of wax paper, as oviposition substrate in a 70cm by 40cm by 40cm cage with three sides made of wire mesh and a solid sliding door. The oviposition substrate was removed everyday and examined for eggs which were used for experiments. Adults were allowed to oviposit until death and the dead adults were removed everyday. The adults were fed on 20% honey/water solution soaked in cotton wool which was placed in a 1.5cm height by 8.5cm diameter plastic petri dish. The honey/water solution was changed every day. The number of adults varied depending on daily emergence.

4.3.2 Evaluation of the reproductive potential of *Trichogrammatoidea* sp. nr *lutea* on eggs of different hosts

To determine the influence of the host species on the reproductive potential of *T.* sp. nr *lutea*, eggs of *B. fusca*, *S. calamistis*, *M. fiorii*, *M.* "madagascariensis", *E. saccharina* and *C. partellus* were used as hosts of *T.* sp. nr *lutea* females. *Trichogrammatoidea* sp. nr *lutea* females used for the experiment were reared on *M. fiorii* as described in section 4.3.1 above and upon emergence, adult parasitoid wasps were allowed to remain in the glass vials for about 24 hours to allow mating. The glass vials were plugged with cotton wool to prevent the parasitoids from escaping. Thereafter, the females were isolated by scattering the adults on a white sheet of paper and covering individual parasitoid wasps with 2.5cm by 7.5 cm glass vials. Once the wasp walked up the glass vial, the open end was plugged using cotton wool. Adults were sexed under the microscope using antennal characters (Pinto, 1998). The females were then offered the eggs of the different hosts for parasitization.

The number of eggs offered to a single *T.* sp. nr *lutea* female was determined in a preliminary study, which showed that in 24 hrs, a female *T.* sp. nr *lutea* parasitized at most 25 eggs (<24-h old) of the host species. Thus, batches containing at-least 40 eggs (<24-h old) oviposited on the respective substrate, described in section 2.1 above, were offered to a single naïve (without oviposition experience), mated female *T.* sp. nr *lutea* in a 2.5cm by 7.5 cm glass vial plugged using cotton wool to prevent the females from escaping. Eggs were replaced every day until the wasp died, and the parasitized batches were kept individually in glass vials at 26±1°C and 60-80% RH until stemborer larvae or parasitoid emergence. Hoffmann et al. (2001) highlighted the importance of using unfed wasps for fitness studies since in nature food and water might not be easily available. Thus, all the experiments were

conducted using unfed wasps. Eggs were considered to be parasitized when they turned black (Flanders, 1937) and suitable if the blackened eggs subsequently led to parasitoid emergence. The percentage of females which oviposited was determined by dividing the number of females that oviposited at-least once by the total number of females tested multiplied by 100. The total number of parasitized host eggs per female and total progeny per female were counted and the progeny per host egg was calculated as the total progeny per female divided by the number of eggs which produced parasitoid progeny. Female longevity and pre-imaginal development time were recorded while sex ratio was calculated as the proportion of females to males. Pre-imaginal development time, daily fecundity, sex ratio and longevity data were used for the calculation of life table parameters. Thirty females were used for each host species.

4.3.3 Influence of the rearing history on the performance of *Trichogrammatoidea* sp. nr *lutea* parasitizing eggs of different hosts

The influence of rearing *T.* sp. nr *lutea* on the eggs of two alternate hosts namely, *B. fusca* and *S. calamistis* was evaluated using the eggs of *M. fiorii*, *B. fusca* and *S. calamistis*. The three were selected because of their high suitability to the parasitoid's development demonstrated in the previous study in section 4.3.2 above. Besides, *T.* sp. nr *lutea* had been collected in the field attacking eggs of *B. fusca* and *S. calamistis* (Bruce, 2008) and *M. fiorii* (Muli et al., 2009a). Prior to being offered alternate host eggs, the parasitoids had been reared on *B. fusca* and *S. calamistis* for six generations. For each host species, thirty 24-h old females were isolated as described in section 4.3.2 above and kept individually in 2.5cm by 7.5 cm glass vials plugged with cotton wool to prevent the parasitoid from escaping. Each

female *T.* sp. nr *lutea* was supplied daily with at-least 40 eggs of *M. fiorii* until the parasitoid died. Before fresh eggs were introduced, the previous ones were removed and kept in a 2.5cm by 7.5cm glass vial. The vials were plugged with cotton wool and the eggs were then incubated at 26±1°C and 60-80% RH until stemborer larvae or parasitoid emergence. The number of parasitized eggs per female and number of offspring per female were counted. The progeny per host egg was calculated as the total progeny per female divided by the number of eggs which produced parasitoid progeny. Sex ratio was determined as the proportion of females to males and pre-imaginal development time recorded.

4.3.4 Suitability and the effect of host egg age for the development of *Trichogrammatoidea* sp. nr *lutea*

Suitability of host age was studied using eggs of *B. fusca*, *S. calamistis* and *M. fiorii*. At least 40 eggs of each age category [i.e., 0 (less than 24 h old), 1, 2, 3 and 4-day old] were exposed to a 1-day old mated, naïve (without oviposition experience) *T.* sp. nr *lutea* female in a 2.5cm by 7.5 cm glass vial. The eggs were presented as described in section 4.3.2 above and the vials were plugged using cotton wool to prevent the females from escaping. After 12 hours of exposure, female parasitoids were removed from the vials and exposed eggs incubated at 26±1°C and 60-80% RH until stemborer or parasitoid emergence. The number of parasitized eggs and total progeny per female counted while the progeny per egg calculated as total progeny per female divided by the number of eggs which produced parasitoid progeny. The sex ratio was determined as the proportion of females to males and the pre-imaginal development time was recorded. Each treatment was replicated 30 times.

4.3.5 Data analysis

Data on the mean number of parasitized eggs, progeny per female, pre-imaginal development time (in days) and the proportion of female to male progeny on the different host species were subjected to analysis of variance (ANOVA), using the general linear model (GLM) procedure of SAS while progeny per host egg species attacked was compared using *generalized linear model with a similar Poisson distribution and log link function* (SAS institute, 1997). Where ANOVA was significant at $P \leq 0.05$, means were separated using Student-Newman Keuls test (SNK). Counts were log (x + 1) transformed while proportions were arcsine transformed before analysis (Zar, 1999). We present back-transformed data in this case. Life table statistics were calculated according to Hulting et al. (1990) using the jackknife program. Differences in the intrinsic rates of increase (r_m) and the net reproduction rate (R_o) were calculated following the protocol of Dixon (1987) and compared with Newman-Keuls sequential tests (Sokal and Rohlf, 1995) based on jackknife estimates of variance for r_m values (Meyer et al., 1986). For any difference between two r_m from the sequence to be significant at the α level, the difference must be equal to or greater than

$$LSR = Q_{\alpha[K,V]} \sqrt{S_{av}^2} \sqrt{\frac{n_i + n_j}{2n_i n_j}},$$

where K is the number of r_m values in the set whose range is tested, and V is the degrees of freedom. The n_i and n_j are the sample sizes of the two r_m values, and $Q_{\alpha[K,V]}$ is a value from the table of Studentized range. S_{av}^2 is the weighted average variance of r_m and it is calculated as

$$S_{av}^2 = \frac{\sum \alpha(n_i - 1)S_i^2}{\sum \alpha(n_i - 1)},$$

where α equals the number of r_m values to be tested, the sample size of the ith r_m is n_i, and S_i^2 is the jackknife estimate of the variance for the ith r_m.

4.4 Results

4.4.1 Evaluation of the reproductive potential of *Trichogrammatoidea* sp. nr *lutea* on eggs of different hosts

The parasitoid attacked and oviposited in all host species tested but the percentage of ovipositing parasitoid females differed among species and was highest with *B. fusca* and lowest with *C. partellus* as the host (Table 4.1).

Table 4.1: Percentage of *Trichogrammatoidea* sp. nr *lutea* females (ex *Mussidia fiorii* eggs) ovipositing on eggs of six lepidopteran host species including *Mussidia fiorii* presented in laboratory bioassays.

Host family	Host species attacked	Females ovipositing (%)	N
Noctuidae	*Busseola fusca*	93.3	30
	Sesamia calamistis	86.7	30
Pyralidae	*Mussidia fiorii*	86.7	30
	Mussidia "madagascariensis"	90.0	30
	Eldana saccharina	30.0	30
Crambidae	*Chilo partellus*	23.3	30

There were significant differences between host species in the number of eggs parasitized by *T.* sp. nr *lutea* and the total progeny per female and they were highest with *B. fusca* and lowest with *C. partellus* as the host (Table 4.2). There were also significant differences in pre-imaginal development time, being shortest with *M.* "madagascariensis" than any other borer tested. Adult longevity was longest with *B. fusca* than any other host species, whereas sex ratio did not vary significantly among host species (Table 4.2). The average number of progeny per egg did not vary among the host species tested (df = 5, $\chi^2 = 4.61$, $P = 0.4656$) and it was 2.3 ± 0.08, 1.9 ± 0.05, 1.6 ± 0.42, 1.6 ± 0.07, 1.9 ± 0.13 and 1.6 ± 0.11 for *B. fusca*, *S. calamistis*, *M. fiorii*, *M.* "madagascariensis", *E. saccharina* and *C. partellus*, respectively).

Table 4.2: Suitability of eggs of six different lepidopteran hosts presented to *Trichogrammatoidea* sp. nr *lutea* females (n=30; means ± SE, ex *Mussidia fiorii*) as determined in laboratory bioassays.

		Parameter				
Host family	Host species attacked	Total parasitized eggs/ female	Total progeny/ female	Development time (days)	Sex ratio	Female longevity (days)
Noctuidae	*Busseola fusca*	17.2 ± 0.81a	38.8 ± 1.80a	10.0 ± 0.05a	0.90 ± 0.01a	1.7 ± 0.15a
	Sesamia calamistis	16.9 ± 1.04a	32.7 ± 2.36a	10.1 ± 0.05a	0.91 ± 0.01a	1.2 ± 0.09b
Pyralidae	*Mussidia fiorii*	16.8 ± 1.11a	15.9 ± 2.01b	9.8 ± 0.08a	0.84± 0.04a	1.1 ± 0.07b
	Mussidia "madagascariensis"	11.2 ± 1.54b	17.2 ± 2.34b	9.3 ± 0.11b	0.77 ± 0.05a	1.0 ± 0.03b
	Eldana saccharina	5.7 ± 0.47c	10.1 ± 0.42b	10.0 ± 0.17a	0.76 ± 0.10a	1.0 ± 0.03b
Crambidae	*Chilo partellus*	4.3 ± 0.68c	3.9 ± 0.67c	10.0 ± 0.22a	0.79 ± 0.07a	1.0 ± 0.03b
	F-value	27.76	41.68	10.03	2.49	9.32
	df	5,113	5,113	5,113	5,113	5,169
	P	<0.0001	<0.0001	<0.0001	0.359	<0.0001

Means within a column followed by same lower case letters are not significantly different (Student-Newman Keuls test, P≤0.05).

Sex ratio was expressed as a proportion of females.

The intrinsic rates of increase (r_m) and net reproductive rates (R_o) were greater on eggs of *B. fusca*, *S. calamistis* and *M. fiorii* than on *E. saccharina* and *C. partellus* eggs as hosts (Table 4.3). Doubling time (t) was lowest on eggs of *B. fusca* followed by *S. calamistis*, *M. fiorii*, *E. saccharina* and *C. partellus*. The mean generation time (G) was highest on eggs of *S. calamistis* while the finite rate of increase (λ) was highest on *B. fusca* eggs as the host (Table 4.3).

Table 4.3: Life table statistics of *Trichogrammatoidea* sp. nr *lutea* reared on eggs of *Busseola fusca*, *Sesamia calamistis*, *Mussidia fiorii*, *Eldana saccharina* and *Chilo partellus* (r_m, jackknife estimate of the intrinsic rate of increase; t, doubling time (day); Ro, net reproductive rate; G, mean generation time (day); λ, finite rate of increase for population).

Parameters	*Busseola fusca*	*Sesamia calamistis*	*Mussidia fiorii*	*Eldana saccharina*	*Chilo partellus*
r_m	0.349 ± 0.008a	0.295 ± 0.010a	0.232 ± 0.016b	0.081 ± 0.035c	0.046 ± 0.034c
R_o	28.0 ± 1.9a	21.9 ± 2.2a	8.98 ± 1.4b	1.9 ± 0.6c	1.8 ± 0.5c
t	2.0	2.4	3.0	10.0	11.7
G	9.6	10.5	9.5	9.5	9.6
λ	1.42	1.34	1.26	1.07	1.06

. Within rows, means followed by a different lower case letter are significantly different (Student-Newman Keuls test, $P<0.05$).

4.4.2 Influence of the rearing history on the performance of *Trichogrammatoidea* sp. nr *lutea* parasitizing eggs of different hosts

Trichogrammatoidea sp. nr *lutea* reared on *B. fusca* successfully attacked and developed in the eggs of *S. calamistis* and *M. fiorii* (Table 4.4a). The number of eggs parasitized per female, pre-imaginal development time and sex ratio did not differ with the host species attacked. However, the total progeny per female and the progeny per egg were significantly different among the host species attacked and was lowest when *M. fiorii* was the host. Like for *B. fusca*, the total progeny per female and progeny per egg were significantly different amongst the host species attacked when *T.* sp. nr *lutea* was reared on *S. calamistis* and was lowest when *M. fiorii* was the host (Table 4.4b). However, unlike when the parasitoid was reared on *B. fusca*, the number of parasitized eggs, pre-imaginal development time and the sex ratio were significantly different amongst the host species attacked when the parasitoid was reared on *S. calamistis*. The number of parasitized eggs and the sex ratio were least when *M. fiorii* was the host while the development time was longest when *B. fusca* was the host (Table 4.4b).

Table 4.4: Suitability of eggs of *Busseola fusca*, *Sesamia calamistis* and *Mussidia fiorii* for attack by *Trichogrammatoidea* sp. nr *lutea* females reared on a) *Busseola fusca* and b) *Sesamia calamistis* for six generations as determined in laboratory bioassays.

Host species attacked	Parameter				
	Parasitized eggs/female	Progeny /female	Progeny/ egg	Development time (days)	Sex ratio
a)					
Busseola fusca	9.3 ± 0.96a	18.2 ± 1.78a	2.1 ± 0.11a	10.3 ± 0.45a	0.79 ± 0.05a
Sesamia calamistis	9.6 ± 1.22a	19.8 ± 2.29a	2.2 ± 0.21a	10.1 ± 0.19a	0.69 ± 0.08a
Mussidia fiorii	7.0 ± 1.19a	6.8 ± 1.19b	1.4 ± 0.17b	10.0 ± 0.24a	0.80± 0.07a
F-value	1.81	16.68	8.68	0.10	0.80
df	2,60	2,60	2,60	2,60	2,60
P	0.1721	0.0001	0.0005	0.9090	0.4549
b)					
Busseola fusca	9.0 ± 0.97a	19.4 ± 2.40a	2.2 ± 0.14a	10.8 ± 0.17a	0.83± 0.04a
Sesamia calamistis	11.2 ± 1.03a	24.0 ± 2.10a	2.3 ± 0.15a	10.0 ± 0.17b	0.76 ± 0.06a
Mussidia fiorii	5.1 ± 0.86b	4.8 ± 0.95b	1.1 ± 0.05b	10.2 ± 0.32b	0.50± 0.12b
F-value	10.5	36.64	24.35	5.43	3.91
df	2,53	2,53	2,53	2,53	2,53
P	0.0002	0.0001	0.0001	0.0073	0.0263

Means within a column followed by a different lowercase letter are significantly different (Student-Newman Keuls test, $P \leq 0.05$).

4.4.3 Suitability and the effect of host egg age on the reproductive performance of *Trichogrammatoidea* sp. nr *lutea*

The mean number of parasitized eggs per female differed significantly among the host species for 3 and 4- day old eggs ($F = 7.21$; $df = 2,75$; $P = 0.0014$ and $F = 16.78$; $df = 2,71$; $P < 0.0001$, respectively) and they were lowest with *M. fiorii* as the host. By contrast, the total progeny produced per female parasitoid varied significantly among the host species for all age classes and it was lowest with 4-day old eggs ($F = 24.65$; $df = 2,71$; $P < 0.0001$). It was also lowest with *M. fiorii* as the host for all the ages. The pre-imaginal development time significantly varied among host species for 0-day ($F = 14.51$; $df = 2,77$; $P < 0.0001$) and 2-day old eggs ($F = 36.63$; $df = 2,73$; $P < 0.0001$) but there was no particular trend. Likewise, the sex ratio significantly varied with host species for 0, 2, and 4 day-old eggs and again there was no discernable trend. For all host ages, the number of progeny per egg significantly varied among host species but without particular trend. The number of progeny per egg was lowest with *M. fiorii* and highest with *B. fusca* as the host (Table 4.5).

With *B. fusca*, host age had an effect on the number of parasitized eggs ($F = 8.86$; $df = 4,137$; $P < 0.0001$), total progeny per female ($F = 14.89$; $df = 4,137$; $P < 0.0001$) and the number of offspring per egg ($F = 6.46$; $df = 4,137$; $P < 0.0001$), and they were lowest for 4-day old eggs (Table 4.5), while pre-imaginal development time did not vary with host age. Host age also affected sex ratio ($F = 9.32$; $df = 4,137$; $P < 0.0001$) but there were no discernable trends. With *S. calamistis*, host age significantly affected the number of parasitized eggs ($F = 9.63$; $df = 4,113$; $P < 0.0001$), total progeny per female ($F = 5.78$; $df = 4.113$; $P = 0.0003$) and the number of offspring per egg ($F = 4.41$; $df = 4,113$; $P = 0.0005$) and they were again lowest with 4-day old eggs. There were also differences in development

time (F = 10.68; df = 4,113; $P < 0.0001$) and sex ratio (F = 5.07; df = 4,113; $P = 0.0009$) (Table 4.5). With *M. fiorii* as the host, host age significantly affected the number of parasitized eggs (F = 40.87; df = 4,129; $P < 0.0001$) and the total progeny per female (F = 17.30; df = 4,129; $P < 0.0001$), and they were lower on 3 to 4 than 0 to 2-day old eggs. There were also significant differences in pre-imaginal development time (F = 7.52; df = 4,129; $P < 0.0001$) and the number of offspring per egg (F = 3.77; df = 4,129; $P = 0.0062$), while sex ratio was not affected by host age (Table 4.5).

Table 4.5: Effect of age of host eggs of *Busseola fusca*, *Sesamia calamistis* and *Mussidia fiorii* on parasitism success of *Trichogrammatoidea* sp. nr *lutea* as determined in laboratory bioassays.

Age (days)	*Busseola fusca*	*Sesamia calamistis*	*Mussidia fiorii*
	Number of parasitized eggs		
0.5	16.8±0.78aA	17.8±1.02aA	17.2±1.32aA
1.5	17.7±0.80aA	16.0±1.06aA	16.4±0.88aA
2.5	14.6±0.87aA	16.5±1.53aA	13.1±0.87aA
3.5	17.6±0.71aA	17.3±1.93aA	10.2±1.08bB
4.5	11.9±1.32aB	11.9±1.41aB	4.3±0.64bC
	Total progeny per female		
0.5	38.6±1.98aA	33.2±2.10aA	15.6±2.48bA
1.5	38.8±1.55aA	32.3±2.61bA	16.1±1.57cA
2.5	33.4±1.98aA	28.0±2.54aA	12.6±1.47bA
3.5	36.5±1.68aA	31.3±3.15bA	6.8±0.96cB
4.5	21.3±2.29aB	18.0±2.61aB	4.6±0.52bB
	Development time		
0.5	10.1±0.07aA	10.2±0.07aB	9.6±0.10bB
1.5	10.1±0.05aA	10.1±0.05aB	10.1±0.07aA
2.5	10.0±0.04bA	10.8±0.11aA	9.7±0.08cB
3.5	10.0±0.07aA	10.0±0.13aB	10.0±0.13aA
4.5	10.2±0.07aA	10.3±0.11aB	10.1±0.05aA
	Sex ratio		
0.5	0.91±0.01aA	0.93±0.01aA	0.79±0.05bA
1.5	0.91±0.01aAB	0.90±0.01aAB	0.87±0.02aA
2.5	0.87±0.01aB	0.80±0.05bC	0.88±0.01aA
3.5	0.90±0.01aAB	0.86±0.02aBC	0.82±0.03aA
4.5	0.82±0.02bC	0.84±0.03abBC	0.87±0.03aA
	Progeny per egg		
0.5	2.3±0.06aA	1.9±0.04bAB	1.4±0.15cA
1.5	2.3±0.08aA	2.0±0.06bA	1.1±0.05cAB
2.5	2.3±0.10aA	1.8±1.12bAB	1.1±0.04cAB
3.5	2.1±0.05aA	1.9±0.99aAB	1.1±0.05bB
4.5	1.9±0.10aB	1.7±0.06aB	1.3±0.06bA

Means within a row followed by different lower case letters and means within a column followed by different uppercase letters are significantly different (Student-Newman Keuls test; $P \leq 0.05$). Ages 0.5, 1.5, 2.5, 3.5 and 4.5 were used because the eggs were exposed to the parasitoid for 12 hours.

4.5 Discussion

Trichogrammatid egg parasitoids are generally polyphagous (Fulmek, 1955; Hirai, 1988; Kot, 1964) with lepidopterans being the main hosts (Thomson and Stinner, 1989). The present findings corroborate results of previous work that *T.* sp. nr *lutea* is highly polyphagous and successfully attacks and develops in the eggs of several lepidopteran pest species in the families Noctuidae, Crambidae and Pyralidae (Baya et al., 2002; Muholo, 2002).

The suitability of the host species eggs attacked by *T.* sp. nr *lutea* varied with host species. Eggs of *B. fusca* and *S. calamistis* were most suitable for this parasitoid. Similarly, Bruce et al. (2006), working on *Trichogramma bournieri* Pintureau and Babault (Hymenoptera: Trichogrammatidae) found that the number of progeny per female varied among the host species and attributed this to differences in host egg size (*C. partellus* =107086.2μm^3; *E. saccharina*=302944.1μm^3; *B. fusca*=341974.3μm^3; *S. calamistis* =336781.5μm^3). This might explain the observed higher parasitism of *S. calamistis* and *B. fusca* eggs compared to their counterparts, *E. saccharina* and *C. partellus*. According to Schmidt and Smith (1985), differences in the surface area of the host eggs determine its acceptability to parasitoids with eggs with more neighbors, and thus reduced exposed surface area being allocated fewer progeny. Owing to their overlapping nature, eggs of *C. partellus* and *E. saccharina* have a reduced surface area compared to their counterparts, *B. fusca*, *S. calamistis* and *M. fiorii*, whose eggs are mostly not overlapped. This might explain the reduced parasitism observed when *E. saccharina* and *C. partellus* were hosts. Honda and Luck (2000) attributed differences in acceptability and suitability to differences in the embryological development between host species. In the current study, the slightly shorter embryological development of *C. partellus* and *E. saccharina* (i.e., 6.0±0.02 and 6.3±0.12,

respectively, at 26±1°C, 60-80% RH) compared to that of *B. fusca* and *S. calamistis* (6.7±0.06 and 7.1±0.03, respectively, at 26±1°C, 60-80% RH) (Muli, ICIPE, unpubl. data) might indicate limited food reserves for their larvae and thus, parasitoid progeny. This may explain their poor acceptability expressed as the number of eggs parasitized per female wasp. Similarly, differences in the number of parasitized eggs observed between *B. fusca, S. calamistis* and *M. fiorii* at ages 3 and 4 could be attributed to the differences in their embryological development as indicated by the differences in their egg stage duration. While *M. fiorii* and *M.* "madagascariensis" egg stage duration (6.2±0.02 and 6.3±0.05 days, respectively, at 26±1°C, 60-80% RH) (Muli, ICIPE, unpubl. data) is not different from that of *C. partellus* and *E. saccharina*, their higher number of parasitized eggs might indicate that they are of superior quality to *C. partellus* and *E. saccharina.*

The present findings indicate that the host the parasitoid was reared on had an effect on the realized fecundity of its offspring corroborating results by Lewis et al. (1976), who showed that the host species on which the parasitoid was reared had a significant effect on the fecundity and longevity of the parasitoid. However, some other studies have shown that the rearing history of the parasitoid had no effect on the reproductive performance of the parasitoid progeny e.g. Bigler et al. (1987) working on *Trichogramma maidis* Pintureau and Voegele (Hymenoptera: Trichogrammatidae) and Corrigan and Laing (1994) working on *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae).

Host age is known to influence host acceptance of the adult Trichogrammatid female and successful development of the parasitoid's offspring (Hinz and Andow, 1990; Reznick and Umarova, 1990; Ruberson and Kring, 1993). Several studies showed that female egg parasitoids lay more eggs and oviposit more readily in younger than older hosts, and that suitability decreases with host age (Vinson and Iwantsch, 1980; Juliano, 1982; Pak and

Oatman, 1982; Chabi-Olaye et al., 1997, 2001, 2004) supporting results of the present study. Safavi (1968) attributed this to the hardening of chorion, which makes it difficult for the parasitoid to probe.

In western Africa, multiple species infestations of maize are common (Schulthess et al., 1997; Ndemah et al., 2001a; Buadu et al., 2003). In contrast to *E. saccharina*, which oviposits on tasseling and post-tasseling plants (Atachi et al., 2005), noctuids oviposit on all the growth stages of maize (Swaine, 1957; Kaufmann, 1983; Ndemah et al., 2000; Ndemah et al., 2002; Chabi-Olaye et al., 2005) while *M. nigrivenella* infests the ears (Whitney, 1970; Moyal and Tran, 1991; Sétamou et al., 1999). This therefore will ensure adequate host resource for the parasitoid before *M. nigrivenella* attacks.

The noctuids appeared to be highly acceptable and suitable as host of *T.* sp. nr *lutea* and, thus, would help to build up parasitoid populations in a maize field. Agboka et al. (2006) showed that by acting as trap plants, the intercropping or planting border rows with, for example, *Canavalia enseiformes* L. and cowpea *Vigna unguiculata* L.(Walpers) (both Leguminosae) significantly reduced the number of eggs laid on maize by *M. nigrivenella.* Eggs laid on companion crops could also be a reservoir for the parasitoid before *M. nigrivenella* attacks. In addition, the fact that the parasitoid attacks *M. fiorii* and *M.* "madagascariensis", which are found attacking wild fruits indicates that it would also search for hosts in wild habitats.

In West and Central Africa, other egg parasitoids have been reported attacking noctuid and pyralid stemborer eggs (Ndemah et al., 2001b; Sétamou et al., 2002). It is likely that interspecific competition will occur between the candidate parasitoid and the native egg parasitoids, if introduced in the region. It is therefore recommended that interspecific

competition studies be carried out to elucidate whether *T.* sp. nr *lutea* would be able to co-exist with the native parasitoids.

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CHAPTER FIVE

5. Factors affecting the bionomics of *Mussidia fiorii* Cecconi and de Joannis (Lepidoptera: Pyralidae) in the laboratory

5.1 Abstract

The pyralid ear-borer, *Mussidia nigrivenella* Ragonot, an important pest of maize, cotton and *Phaseolus* bean in West Africa, has never been reported as a pest of crops in East and southern Africa (ESA), though it exists in the wild. It was hypothesized that in ESA, *M. nigrivenella* was under natural control in its natural habitats. Surveys for *Mussidia* spp., their associated host plants and natural enemies in Kenya revealed several *Mussidia* and parasitoid species. Of all the *Mussidia* species only *M. fiorii* Cecconi and de Joannis was successfully reared in the laboratory, thus making it a suitable host to rear natural enemies of *Mussidia* spp. Suitability of five artificial diets for the development of *M. fiorii* was studied in the laboratory. In addition, the effect of temperature and humidity on the development of *M. fiorii* was studied. *Mussidia fiorii* successfully developed on maize leaf-, maize seed- and *Canavalia enseiformes* L. DC seed-based diets. The lower developmental thresholds for the egg, larvae, pupae and egg to adult were 12.8 ± 0.25°C, 14.4 ± 0.27°C, 11.0 ± 0.03°C and 13.5 ± 0.21°C, respectively, while the thermal constants were 82.0 ± 1.61, 384.6 ± 9.4, 144.9 ± 6.8 and 588.2 ± 10.8 degree-days, respectively on *C. enseiformes* seed-based diet. Adults started emerging the last hour of photophase and peak emergence was during the 2nd hour of scotophase while mating largely took place between the 4th and 5th hour of scotophase.

Information on dietary and thermal requirements besides the reproductive behavior will be used to optimize mass production of the host and the natural enemies.

5.2 Introduction

The ear-borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae), an important pest of maize in West Africa causes yield losses ranging from 5-25% in the field (Moyal and Tran, 1991; Sétamou et al., 2000a; Ndemah et al., 2001) and 10% loss in storage (Sétamou, 1996). *Mussidia nigrivenella* damage also predisposes maize ears to attack by pre- and post-harvest storage beetles, *Carpophilus* spp. (Coleoptera: Nitidulidae) and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), leading to additional losses of up to 15% and to infection by *Aspergillus flavus* Link (Deuteromycetes) and *Fusarium verticillioides* (Sacc.) Nirenberg (Hypocreales), which produce mycotoxins (Sétamou et al., 1998; Fandohan et al., 2005). Besides maize, *M. nigrivenella* has been reported attacking cotton, *Phaseolus*, velvet and lima beans, and fruits and pods of wild host plants (Sétamou et al., 2000b; Agboka et al., 2006). *Mussidia nigrivenella* has never been reported as a crop pest from East and southern Africa (ESA), where, however, it appears to occur on wild host plants (Janse, 1941; LePelley, 1959; Entwistle, 1972; Waiyaki, 1973). Sétamou et al. (2002) hypothesized that in ESA, *M. nigrivenella* was under natural control in the wild habitats. This would open up opportunities for biological control of the pest in West Africa. Thus, surveys were carried out for *Mussida* spp. and associated natural enemies in Kenya. Two *Mussidia* spp. and six morphospecies were recovered from fruits of wild host plants (Muli et al., 2009a). Several parasitoids were recovered from *Mussidia* spp. including the egg parasitoid *Trichogrammatoidea* sp. nr *lutea* Girault (Hymenoptera: Trichogrammatoidea) (Muli et al.,

2009b), which was considered a potential biocontrol agent for *M. nigrivenella* in West Africa. Of all the *Mussidia* species recovered in Kenya only *M. fiorii* Cecconi and de Joannis was successfully reared in the laboratory. *Mussidia fiorii* was therefore used to rear the *T.* sp. nr *lutea* at ICIPE.

It has been shown that the bionomics of *M. nigrivenella* was significantly influenced by the diet type (Bolaji and Bosque-Pérez, 1998; Sétamou et al., 1999). In addition, van Huis and de Rooy (1998) suggested that high-quality plants may support development of herbivores with high nutritional quality that ultimately support high-quality parasitoids. Also, according to Hunter (2003), the host diet is of importance to the nutritional quality of host eggs and the survival of *Trichogramma* and other egg parasitoids used as biological control agents. Thus, several diets were tested here for development of *M. fiorii*.

Temperature is the single most important factor in determining the developmental rate of insects and its interaction with moisture can affect the life history of insects (Howe, 1967; Odum, 1983). Eggs of *M. fiorii* are laid in batches on the surface of the fruits (Muli et al., 2009a), hence, they are subjected to fluctuating temperatures and humidities. Thus, to determine the optimum conditions for rearing of *M. fiorii*, the effect of temperature and humidity on *M. fiorii* egg hatch and development, and temperature on larval and pupal development was studied. In addition, the window of adult eclosion and mating in *M. fiorii* was determined to help in synchronizing adult emergence for successful mating.

5.3 Materials and methods

Experiments were conducted at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. A laboratory colony of *M. fiorii* initiated from individuals reared

from *Kigelia africana* (Lam.) Benth. (Bignoniaceae) fruits collected along the Kenyan coast was maintained in the laboratory on artificial diet described by Onyango and Ochieng-Odero (1994) at 27±1°C, 60-80% RH and 12:12 photoperiod as described in section 3.3.

5.3.1 Effect of temperature and humidity on *Mussidia fiorii* egg stage duration and hatchability

The effect of temperature and humidity on *M. fiorii* egg hatching was studied at seven temperature (15, 18, 21, 24, 27, 30 and 33 ± 1°C) and two humidity (40-50 and 70-80% RH) regimes. For each temperature by humidity combination, at least 200 less than 14 hours old eggs were incubated in a vial. Eggs that were red in colour 24 hours later were considered fertilized (Muli et al., 2009a). The fertilized eggs were counted under a microscope and if they were more than 100, they were divided into 10 groups of at-least 10 eggs and each was put into a 2.5cm by 7.5cm glass vial then returned to the 30cm by 30cm by 30cm perspex experimental cages and observed daily until the larvae hatched. Where less than 100 eggs were found to be fertilized, the procedure was repeated until enough fertilized eggs were obtained. Humidities were maintained according to the procedures described by Hodgman (1948). Calcium chloride and ammonium chloride, both dry, were used to maintain RH at 40-50% and 70-80%, respectively, at the lower temperatures, while ammonium chloride and water were used to maintain 40-50% and 70-80% RH, respectively, at higher temperatures. The cages were kept closed and the lids were sealed with Vaseline petroleum jelly. A thermo-hygrometer was used to monitor temperature and humidity.

5.3.2 Suitability of different diets for the development *Mussidia fiorii*

Five artificial diets were tested for the development of *M. fiorii*. The diets included the standard maize-leaf-powder based stemborer diet modified from the sorghum-leaf based diet described by Onyango and Ochieng-Odero (1994) (Table 5.1), and other four diets where maize leaf powder was replaced by *Canavalia cathartica* Thouars. (Fabaceae), *Canavalia enseiformes* L. DC (Fabaceae), *Mucuna pruriens* L. DC (Fabaceae) or maize seed flour. *Canavalia enseiformes*, *M. pruriens* and maize seeds were selected because of their suitability as hosts of *M. nigrivenella* in West Africa (Sétamou et al., 2002), while *C. carthartica* was found to host *Mussidia* spp. in Kenya (Muli et al., 2009a). One day old larvae were offered diet individually in 2.5cm by 7.5 cm glass vials and incubated at 27±1°C. A third of the vial was filled with the diet for one larva. Larva was introduced into the diet using a soft camel hair brush and the vial was plugged with cotton wool to prevent the larva from escaping. For each diet, 120 larvae were tested. They were divided into twelve groups each having ten larvae. Each group of ten larvae was considered a replicate. Larval, pupal and adult development were recorded. Pupal weight was determined using an electronic weighing balance. Development time of *M. fiorii* from larvae to adult on artificial diet was compared with those reared on *K. africana* fruits. Five mature fruits were each infested with 50 one day old larvae. Fruits were artificially infested with larvae by cutting two circular rings 2.5 cm in diameter and about 2 cm deep on the surface of each fruit. The open ends of two vials, each containing 25 larvae, were attached each to a circular ring on the fruit. The vials were secured using Parafilm adhesive and the set-up was left for about 5 days for the larvae to penetrate the fruit. As the immature stages live cryptically inside the fruits and direct observations are not possible (Muli et al., 2009a), infested fruits were kept in perspex

cages (60cm by 30cm by 30cm) in the laboratory at 27±1°C until adult emergence. Total development time from one-day-old larvae to adult was recorded.

An attempt was also made to determine whether there were differences in male and female larval and pupal development times, and pupal weights. *Canavalia enseiformes* seed-based diet was used. *Canavalia enseiformes* was selected because it was reported harboring *M. nigrivenella* in West Africa (Sétamou et al., 2002) and another *Mussidia* sp. in Kenya (Muli et al., 2009a). One day old larvae were offered diet individually in 2.5cm by 7.5 cm glass vials and incubated at 27±1°C. Hundred and twenty (120) larvae were used in this study and were divided into twelve groups each having ten larvae. Each group of ten larvae was considered a replicate. On pupation, larval and pupal development times were recorded. Each individual pupa was sexed and its weight determined using an electronic weighing balance. The pupae were returned each in individual 2.5cm by 7.5 cm glass vials and returned to the same environmental conditions. The vials were plugged using cotton wool. On adult emergence, pupal development time was recorded and the adults were returned to the stock colony for its maintenance.

Table 5.1: Ingredients of an artificial diet for rearing non-diapausing *Busseola fusca* (Source: Onyango and Ochieng'-Odero, 1994).

Ingredients	Quantity per 2 litre diet
Fraction A	
Distilled water	800 ml
Brewer's yeast	45.0 g
Sorbic acid	1.3 g
Methyl *p*-hydroxybenzoate	2.0 g
Ascorbic acid	5.0 g
Vitamin E capsules (300 i.u.)	4.2 g
Sorghum leaf powder	50.0 g
Bean (*Phaseolus vulgaris*) powder	175.0 g
Sucrose	70.0 g
Fraction B	
Agar (tech. No. 3) powder	25.0 g
Distilled water	800 ml
Fraction C	
Formaldehyde 40%	2 ml

5.3.3 Effect of temperature on development of immature *Mussidia fiorii* reared on *Canavalia enseiformes* seed-based diet

To investigate the effect of temperature on larval development, 1st instar larvae of *M. fiorii* were individually kept on *C. enseiformes* seed-based diet in 2.5cm by 7.5 cm glass vials kept in an incubator at 18, 21, 24, 27, and 30 ± 1°C till adulthood. The vials were plugged with cotton wool to prevent the larvae form escaping. Relative humidity was kept above 60% using water put in an open 15cm by 15cm by 20cm plastic container placed in the incubator. A thermo-hygrometer was used in the incubator to monitor temperature and humidity. For each temperature, a total of 120 larvae were used. The number of larvae surviving to pupae was counted and the larval duration was determined and recorded. Pupae were carefully removed from their cocoons and were each kept in a 2.5cm by 7.5 cm glass vials. The vials

were plugged with cotton wool and were returned to the experimental temperature and monitored daily to determine the pupal period. Emerging females were retained in the vials at the same environmental conditions and were fed on distilled water until their death and their longevity recorded.

5.3.4 Window of adult emergence and mating of *Mussidia fiorii*

Pupae reared on *C. enseiformes* seed-based artificial diet described above were segregated by sex and were pre-conditioned under a reversed experimental photoperiod (12: 12 light: dark), 27±1°C, 60-80 % RH with scotophase starting at 0700h and ending at 1900h during which they were observed. Male (N=100) and female (N=100) pupae were kept in a 15cm by 15cm by 20cm transparent plastic cages with a well ventilated lid and after five days they were observed hourly during scotophase for adult emergence. Emerged adults were moved to an empty screen cage (30cm by 30cm by 60cm) for observation. Since calling behavior of *M. fiorii* was not known, an attempt to describe it was made by observing the moth activity e.g. wing raising, abdomen raising, ovipositor protrusion and retraction etc, at 15 minutes intervals until mating started. However, since male and female moths were in the same cage and were involved in some casual movements especially the males, it was not easy to quantify events preceding copulation. When mating started, pairs in-copula were removed from the cage and each was placed in a 2.5cm by 7.5cm glass vial and the open end was plugged using cotton wool and the duration of copulation was determined using a stop watch. Mated adults were returned into a different empty screen cage (30cm by 30cm by 60cm) and were observed in the subsequent scotophases for any calling behavior and subsequent mating and after onset of copulation, pairs were separated into 2.5 by 7.5 cm glass vial, the open end

plugged using cotton wool and copulation duration determined as described above. The observations were facilitated with a 5W red light.

5.3.5 Data analysis

Data on mean larval, pupal and larval to adult period, and pupal weight on different diets and larval, pupal and adult development times at different temperatures were subjected to analysis of variance (ANOVA). Where significant differences were obtained, the means were separated using Student-Newmans Keuls (SNK) test ($P<0.05$) (Zar, 1999). Counts were log (x + 1) transformed while percentages were arcsine transformed before analysis. Back-transformed data is presented in this case. Only insects which developed up-to adult were considered when assessing the effect of temperature on development.

For each temperature in °C (T), development rates [$r(T)$] of *M. fiorii* eggs, larvae, pupae and adults were calculated as reciprocals of development time (t) of individual *M. fiorii* stages i.e. $r(T) = 1/t$. The model of Campbell et al. (1974) based on the linear regression equation $r(T) = a + bT$ was used to estimate the lower temperature threshold (c) and the thermal constant (k) of *M. fiorii* life stages. The lower temperature threshold was calculated as $c = -a/b$ and the thermal constant as $k = 1/b$. The standard error of the lower temperature threshold (*SEc*), was calculated as:

$$SEc = \frac{\bar{r}}{b}\sqrt{\frac{s^2}{N \times \bar{r}^2} + \left[\frac{SEb}{b}\right]^2}$$

while that of the thermal constant (*SEk*), was calculated as:

$$SEk = \frac{SEb}{b^2}$$

SEb is the standard error of the slope (*b*), s^2 is the residual mean square of *r(T)*, $\bar{r}$ is the sample mean, and *N* is the sample size (Campbell et al., 1974). For calculation of the lower temperature thresholds and thermal constants, development rates of *M. fiorii* life stages were based on development times for both sexes pooled.

A t-test was used to compare the copulation durations between *M. fiorii* pairs mating in the first and those mating in the second scotophase.

5.4 Results

5.4.1 Effect of temperature and humidity on the duration of embryonic development and hatchability of *Mussidia fiorii* eggs

Temperature had a significant effect on the duration of embryonic development for both humidity regimes and was shortest at 30°C for 70-80 % RH and 27°C for 40-50% RH (Table 5.2). Except for 33°C, humidity had a significant effect on duration of embryonic development and was significantly longer at 40-50% RH for all temperatures except at 27°C (Table 5.2).

Table 5.2: *Mussidia fiorii* egg stage duration (mean ± SE) at seven different temperatures and two humidity regimes.

	Days to hatch				
Temp. (°C)	70-80% RH	40-50% RH	*t*-value	df	*p*
15	23.5±0.07a	24.9±0.09a	11.84	197	<0.0001
18	13.7±0.06b	14.7±0.09b	8.72	236	<0.0001
21	10.8±0.06c	11.4±0.06c	7.83	186	<0.0001
24	7.6±0.05d	8.1±0.04d	7.84	224	<0.0001
27	5.4±0.04f	5.1±0.03g	4.11	244	<0.0001
30	5.0±0.01g	5.7±0.07f	14.05	173	<0.0001
33	5.7±0.08e	6.2±0.17e	1.18	118	0.2396
F-value	6751	8480			
df	6,792	6,598			
p	<0.001	<0.0001			

Means within the same column followed by the same lower case letter are not significantly different at (Student-Newman Keuls, $P \leq 0.05$).

Under both humidity regimes, temperature had a significant effect on percentage egg hatch and it was the highest at 18°C and lowest at 33°C (Table 5.3). Except at 18°C, humidity also affected the percentage egg hatch but, there was no specific trend (Table 5.3).

Table 5.3: *Mussidia fiorii* percentage egg hatch (mean ± SE) at seven different temperatures and two humidity regimes.

	% hatch				
Temp. (°C)	70-80 % RH	40-50 % RH	t-value	df	*P*
15	96.4±1.46ab	78.4±2.74c	5.80	18	<0.0001
18	99.0±1.00a	99.3±0.71a	0.24	18	0.8157
21	94.9±1.41abc	63.1±1.81d	13.81	18	<0.0001
24	93.1±1.15bc	98.3±1.11a	3.27	18	0.0043
27	97.0±1.23ab	90.8±1.93b	2.70	18	0.0147
30	96.4±1.46ab	61.2±2.01d	14.19	18	<0.0001
33	89.1±2.34c	4.4±1.67e	29.46	18	<0.0001
F	4.28	186.76			
df	6,69	6,69			
P	0.001	<0.0001			

Means within the same column followed by the same lower case letter are not significantly different at $P<0.05$, Student-Newman Keuls test.

5.4.2 Suitability of different diets for the development *Mussidia fiorii*

While *M. fiorii* completed development on maize leaf-, maize seed- and *C. enseiformes* seed-based diets, it could not develop on *C. cathartica* seed- and *M. pruriens* seed-based diets. The survival of *M. fiorii* larvae was significantly affected by the diet and was nil with *C. cathartica* and *M. pruriens* seed-based diets (Table 5.4).

Table 5.4: Suitability of five diets for the development of *Mussidia fiorii* larvae at 27±1˚C.

Diet type	Percentage larval survival
Canavalia enseiformes seed based	86.7±3.76a
Maize leaf based	70.0±4.44b
Maize seed based	82.5±3.50a
Canavalia cathartica seed based	0.0±0.00c
Mucuna pruriens seed based	0.0±0.00c
F	177.35
df	4,59
P	<0.0001

Means within the same column followed by the same lower case letter are not significantly different at $P<0.05$, Student-Newman Keuls test.

Insects reared on the maize-leaf based diet had the shortest larval development period and lowest pupal weight while those reared on maize seed-based diet had the shortest pupal period. Larva to adult development time was shortest with *K. africana* and similar with the remaining diets (Table 5.5).

Table 5.5: Effect of diet type on mean (± SE) larval period, pupal period, pupal weight and larval to adult period of *Mussidia fiorii* on four diets including the natural diet, *Kigelia africana* fruits at 27±1°C.

Diet	Larval period (days)	Pupal period (days)	Pupal weight (grams)	Larva to adult (days)
Canavalia enseiformes	42.5±0.40 a	10.0±0.13a	0.15±0.00 a	53.6±0.59 a
Maize leaf	40.7±0.40 b	9.7±0.16a	0.13±0.00 b	51.2±0.65 a
Maize seed	43.3±0.49 a	9.0±0.27b	0.15±0.00 a	52.8±0.55 a
Kigelia africana	-	-	-	48.3±0.75 b
F-value	8.54	6.53	8.30	11.35
df	2,278	2,148	2,252	3,293
p	0.0003	0.0019	0.0003	<0.0001

Means within the same column followed by the same lower case letter are not significantly different at $P<0.05$, Student-Newman Keuls test.

With *C. enseiformes* seed-based diet, female larval and pupal periods were significantly longer and female pupae significantly heavier than their male counterparts (Table 5.6).

Table 5.6: Male and female *Mussidia fiorii* (mean ± SE) larval period, pupal weight and pupal period reared on *Canavalia enseiformes* seed-based diet.

Sex	Larval period	Pupal weight	Pupal period
Male	41.11±0.5249	0.11±0.0039	9.66±0.1808
Female	43.74±0.5422	0.16 ±0.0036	10.30±0.1755
t-value	3.47	5.25	2.54
df	100	97	54
p	0.0008	<0.0001	0.0141

5.4.3 Effect of temperature on immature development of *Mussidia fiorii* reared on *Canavalia enseiformes* based diet

Development rates of eggs, larvae and pupae increased linearly with temperature ($r(T) = -0.1561 + 0.0122T$, $R^2 = 0.91$, $P<0.0001$; $r(T) = -0.0373 + 0.0026T$, $R^2 = 0.87$, $P<0.0001$; $r(T) = -0.0758 + 0.0069T$, $R^2 = 0.72$, $P<0.0001$ and $r(T) = -0.0229 + 0.0017T$, $R^2 = 0.93$, $P<0.0001$ for egg, larvae, pupae and egg to adult, respectively). *Mussidia fiorii* eggs required 82.0 ± 1.61 degree-days above a thermal threshold of 12.8 ± 0.25°C to develop into larvae, while larvae required 384.6±9.43 degree-days above 14.4±0.27°C thermal threshold to pupate. Pupae had the lowest thermal threshold of 11.0±0.03°C and required 144.9±6.84 degree-days to develop into adult. From egg to adult, *M. fiorii* required 588.2±10.81 degree-days above a thermal threshold of 13.5±0.21°C.

Larval and pupal development time decreased linearly between 18°C and 30°C. However, adult longevity initially increased and then decreased in a curvilinear pattern as temperature increased, and it was longest at 21°C (Table 5.7).

Table 5.7: *Mussidia fiorii* larval, pupal development times and adult longevity [mean ± SE (days)] at five different temperatures on *Canavalia enseiformes* seed-based diet at RH >60%.

Temp. (°C)	N	Larval period	Pupal period	Adult period
18	17	125.2±2.22a	20.5±0.97a	9.2±1.06b
21	54	49.9±0.51b	15.3±0.27b	12.6±0.43a
24	51	44.9±0.53c	10.8±0.29c	6.9±0.36c
27	59	35.6±0.35d	9.6±0.31d	6.5±0.30c
30	61	23.2±0.17e	7.6±0.09e	3.5±0.17d
F-value		2155.66	202.52	82.55
df		4,241	4,241	4,241
p		<0.0001	<0.0001	<0.0001

Means within the same column followed by the same lower case letter are not significantly different (Student-Newman Keuls test, $P<0.05$). Adult periods presented in the table were for un-mated adults.

5.4.4 Window of adult eclosion and initiation of mating of *Mussidia fiorii*

For both sexes, emergence started during the last hour of photophase and continued during the scotophase until the 11 hour with the highest emergence rate occurring during the first and the second hour of scotophase (Fig. 5.1).

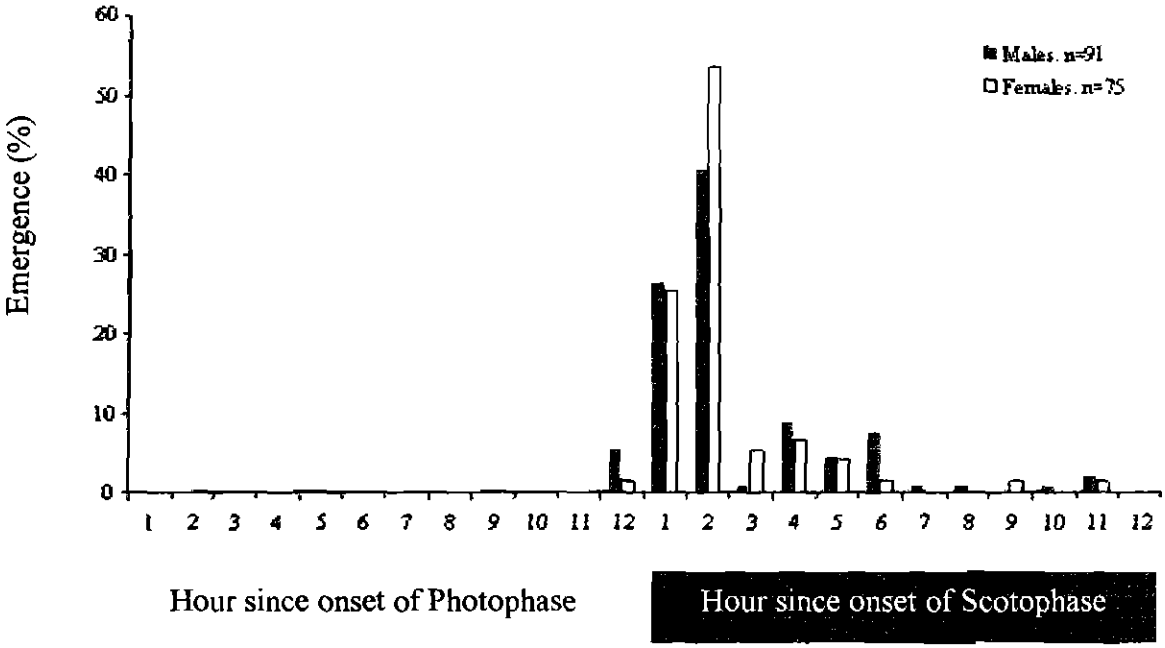

Figure 5.1: Emergence of *Mussidia fiorii* recorded at hourly intervals.

Mating occurred between the 4th and the 8th hour of the 1st scotophase with a peak occurring during the 5th hour (Fig. 5.2). Before initiation of mating females were stationary and held wings close to their body while they rhythmically protruded and retracted the ovipositor beyond a raised abdomen. Agitated males vigorously fanned their raised wings with occasional walking. While fanning the wings, a male tried to antennate the female from the posterior end and if she remained stationary, the male mounted her facing the opposite direction. The females' posture was therefore regarded to portray "calling behavior". Mated females resumed calling the 2nd scotophase and multiple mating was observed on 48% of the females mated during the 1st scotophase. During the 2nd scotophase, mating occurred between the 3rd and the 7th hour of the scotophase with the peak occurring during the 4th hour (Fig. 5.2). Copulation time did not vary significantly between matings in the 1st and the 2nd scotophase (t = 1.45; df = 44; P= 0.1540) and it was 112.94±8.87 minutes (N=31) and 138.27±17.90 minutes (N=15) for the 1st and 2nd scotophase, respectively. Dissection of

females that mated during the 1st and 2nd scotophase revealed two spermatophores, indicating successful multiple mating. About 4% of the pairs of *M. fiorii* were unable to disentangle themselves from the mating posture and ultimately died without uncoupling. For all the females observed, oviposition commenced on the day of emergence.

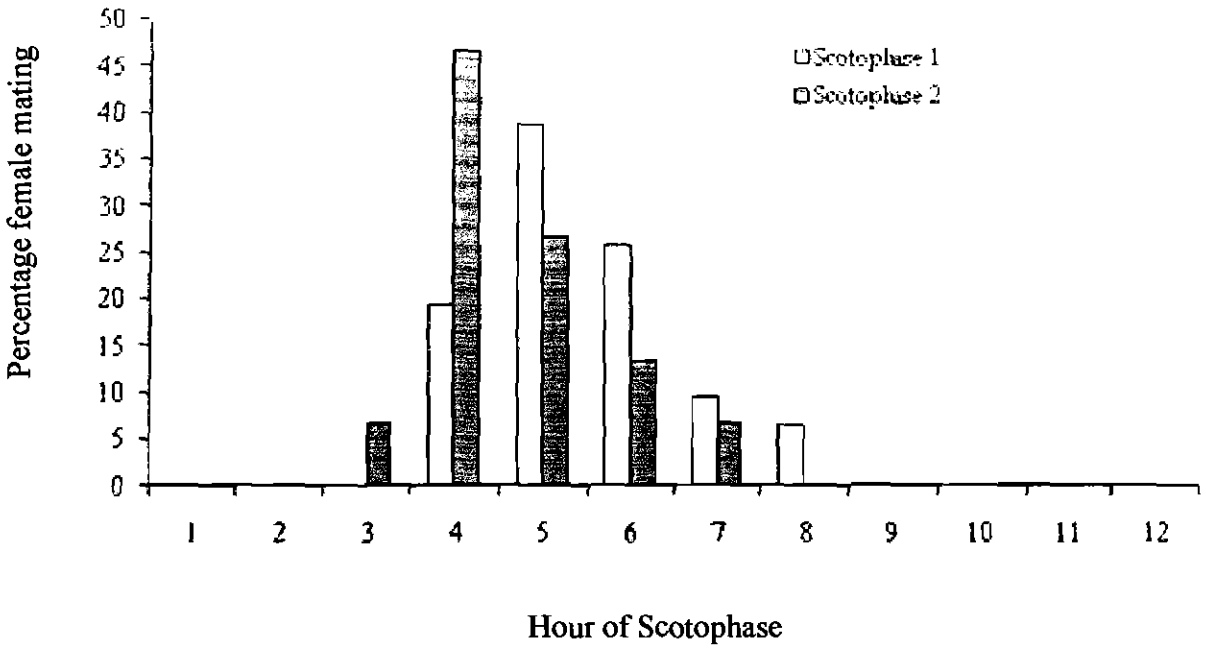

Figure 5.2: Initiation of mating by *Mussidia fiorii* females during the 1st and 2nd scotophase (N=31 and 15 females for 1st and 2nd scotophase, respectively).

5.5 Discussion

The ability of *M. fiorii* to develop on diets based on host plants from different families further confirms the polyphagous nature of species in the genus *Mussidia* as observed by Sétamou et al. (2000b) and Agboka et al. (2006). However, the development time of *M. fiorii* from larvae to adult was shorter when reared on the natural diet *K. africana*, than on artificial diets. Similar results have been reported for other lepidopteran maize pests, e.g. the crambids *Diatraea saccharalis* (Fabricius) (Isa, 1961), *Chilo partellus* (Swinhoe) and *Chilo*

orichalcociliellus (Strand) (Mbapila et al., 2002), and the noctuid *Sesamia calamistis* (Hampson) (Shanower et al., 1993) suggesting that the natural diets were nutritionally superior to the artificial diets. Thus, the prolonged development time might have been a result of compensatory feeding of the larvae on nutritionally inferior diet. The survival of *M. fiorii* larvae reared on maize leaf based-diet was lower than their counterparts reared on *C. enseiformes* seed-based and maize seed-based diets indicating that maize leaf might be nutritionally inferior to *C. enseiformes* and maize seeds.

Results of the current study show that female larval development time was longer than for males and female pupae heavier than the males. Bolaji and Bosque-Pérez (1998) working on *M. nigrivenella* also found female pupae to be heavier than the males. According to Raven (1961) and Slansky and Scriber (1985), higher weight in females compared to males is a trait adapted to the storage of nutrients for egg production. Mackey (1978) and Lederhouse et al. (1982) pointed out that female larvae achieve a larger size by feeding and developing for a longer period. Positive correlations between female body size and fecundity have been reported in many insects (Bessin and Reagan, 1990; Honěk, 1993; Mária, 1996; Calvo and Molina, 2005). This is of advantage in mass production of egg parasitoids. The lower pupal weights for insects reared on maize leaf based diet might be attributed to the shorter larval period which might indicate that the food ingested was little. Lower pupal weight might also indicate that the food was of poorer nutritional quality. *Canavalia enseiformes* and *M. pruriens* are among the most suitable host plants of *M. nigrivenella* in West Africa (Sétamou et al., 1999; Sétamou et al., 2000b; Agboka et al., 2006) while *C. cathartica* was found harboring *M.* nr *nigrivenella* in Kenya (Muli et al., 2009a). In the current study, while *M. fiorii* was found to successfully develop on *C. enseiformes* seed-based diet, it could not develop on *C. cathartica* and *M. pruriens* seed-based diet which

might indicate major differences in the nutrient composition amongst host plants exploited by different *Mussidia* spp. Like *M. nigrivenella* which feeds on maize in West Africa (Sétamou et al., 2000b; Ndemah et al., 2001; Agboka et al., 2006), *M. fiorii* successfully developed on maize based diet. According to Price et al. (1980), natural enemies of phytophagous insects function and develop in a multi-trophic context. Their behavior is therefore influenced by many factors and stimuli derived from the plant and the phytophagous host (Vinson, 1976; Takayabashi et al., 1991). Also, Sétamou et al. (2005) found that the performance of *Cotesia flavipes* Cameron (Hymenoptera: Braconidae) was influenced by the diet on which its host *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) was fed. Owing to the polyphagous nature of *M. fiorii* and *M. nigrivenella*, further studies using *M. fiorii* and the West African *M. nigrivenella* population are necessary to determine whether the performance of *T.* sp. nr *lutea* would be influenced by the diet on which the hosts were reared.

Results of the current study indicate that hatchability of *M. fiorii* eggs was significantly reduced at the lower humidity regime at extreme high temperature corroborating work by Adkisson (1959) on the pink bollworm, *Pectinophora gosypiella* (Saunders) (Lepidoptera: Gelechiidae). According to Bursell (1974) low ambient humidity may indirectly affect the egg development by disturbing the water balance due to the loss of water molecules through the permeable chorion of the egg. A reduced amount of water in the protoplasm slows down metabolic rates and this may result in either the retardation of embryonic development, or to the death of the egg if water loss exceeds certain physiological limits (Wigglesworth, 1965, 1974). Working on *Mamestra configurata* Walker (Lepidoptera: Noctuidae), Jones and Heming (1979) found that low hatchability of eggs at reduced humidities is partly due to the desiccation of the chorion, which became too hard to be broken by the young larva. This could explain the findings of the current study where

dissection of eggs incubated at the lower humidity regime at the highest temperature and from which no larvae emerged revealed that the larvae had died at the black head stage.

In the current study, the threshold temperature value for the biological cycle (egg to adult) was 13.5°C. Studies on other tropical insects have also shown lower threshold temperatures between 11.0 and 15.0 (Honék, 1996). In mass rearing of the host and the parasitoids, delaying growth of insect stages might be required to synchronise, for instance, emergence of adult moths for mating or with emergence of the parasitoid. Knowledge of the lower temperature thresholds would facilitate this.

It is well documented that many behavioral activities in lepidopteran insects are restricted to certain times of the day (Teal et al., 1981; Pope et al., 1984; Raina et al., 1986), usually the scotophase. The results of the present study are in agreement with the previous findings since adult emergence and mating of *M. fiorii* was restricted to certain hours of the scotophase. A series of behavior patterns precede mating in moths. In females, these behaviors include calling which leads to emission of sex pheromones for attraction of potential mates, and the acceptance of males that attempt mating (Itagaki and Conner, 1988; Kingan et al., 1993). In the current study, before mating, female moths were involved in active pulsation of their terminal abdominal segments, with their ovipositor extruded beyond a raised abdomen tip. At the same time, males were highly agitated and eventually, mating occurred. We therefore interpreted the females' behavior as "calling". According to Shorey and Gaston (1965), calling occurs at a restricted time during the day-night cycle, and is synchronized with the time of maximum male responsiveness. This is corroborated by the findings of the current study since calling and the subsequent mating of *M. fiorii* occurred between the 3rd and the 8th hr of scotophase with a peak at the 4th and 5th hours. According to Matthews and Matthews (1988), insects that have a short lifespan mate shortly after

emergence. *Mussidia fiorii* adults are short lived with an average of 6.5 days at 27±1°C and this might explain why mating occurred the same scotophase of emergence. Sétamou et al. (1999) observed that oviposition in *M. nigrivenella* commenced on the day of adult emergence without any pre-oviposition period. In the current study, *M. fiorii* females were also observed to commence oviposition on the day of adult emergence. This is logical because the two species belong to the same genus. Working on gypsy moths (Lepidoptera: Lymantriidae) and silk moths (Lepidoptera: Bombycidae), Giebultowicz et al. (1991) and Karube and Kobayashi (1999), respectively, concluded that presence of sperm in the spermatheca is required to switch off female receptivity and stimulate oviposition and egg maturation in many lepidopteran species. However, the current study is not in agreement with this since mated females were observed re-mating the 2nd scotophase while even unmated *M. fiorii* females were observed ovipositing. Besides, multiple mating has been observed in several lepidopteran species (Delisle and Hardy, 1997; Landolt, 1997; Simmons, 2001) which indicate that the presence of spermatophore had no effect on subsequent receptivity.

Results of the current study show that that *M. fiorii* is capable of developing on different diet types and temperature regimes. The versatile diet breadth, the knowledge of dietary and thermal requirements and the reproductive behavior of *M. fiorii* would be useful in further optimizing of mass production of the host and the natural enemies.

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CHAPTER SIX

6. General discussion, conclusions and recommendations

6.1 Discussion

Previous work by Sétamou et al. (2002) hypothesized that either the *M. nigrivenella* population in Eastern and southern Africa (ESA) does not feed on crops or it was under natural control in the wild habitats (see chapter 2, section 1.2). The latter would open up opportunities for control of *M. nigrivenella* in West Africa through redistribution of natural enemies or new association biological control approaches. Thus, Sétamou et al. (2002) proposed exploration for natural enemies on various wild host plants in eastern Africa. Because of the exceedingly cryptic larval feeding behaviour of *Mussidia* spp., it was suggested to give emphasis to egg and pupal parasitoids, since eggs are laid in batches exposed on the surface of the fruits and pupation takes place near the fruit surface, and to include all *Mussidia* species because, if they occupy similar ecological niches on the plant, they might share the same natural enemies (Hokkanen and Pimentel, 1989). Besides, the observed differences in pest status might even be due to misidentification of the species. The main objective of the studies presented here was therefore to understand the bio-ecology of *Mussidia* spp. in Kenya. Data on host plant range, *Mussidia* spp. diversity and natural enemies associated with the *Mussidia* spp. was therefore collected in Kenya. Besides, the performance of the natural enemies associated with the *Mussidia* spp. and the factors affecting the bionomics of the *Mussidia* spp. were investigated in the laboratory.

6.1.1 Host plants and species diversity of *Mussidia* in Kenya

Results from the surveys on host plants and species diversity of *Mussidia* indicate that two *Mussidia* spp. and six putative morphospecies were obtained from eight plant species in four families all found along the Kenyan coast. The host plant range was however, much narrower than for *M. nigrivenella* in West Africa where Sétamou et al. (2000) identified 20 plant species from 11 plant families hosting the pest. In contrast to West Africa, attempts to sample maize cobs in Kenya did not yield any *Mussidia* spp. (Muli et al., 2009a).

6.1.2 Natural enemies associated with *Mussidia* spp. in Kenya

From the results of surveys for natural enemies associated with *Mussidia* spp., parasitoids were recovered from *Mussidia* species attacking four host plants in three different families. Generally, egg and larval parasitism were negligible while although parasitism by the larval-pupal parasitoid was higher than the former two, parasitism was low. These host plants were not reported as hosts of *M. nigrivenella* by Sétamou et al. (2002) in West Africa. The parasitoid species diversity and rates of parasitism were as low as observed on maize and on wild host plants by Sétamou et al. (2002) on *M. nigrivenella* in West Africa.

6.1.3 Performance of *Trichogrammatoidea* sp. nr *lutea* on alternate lepidopteran hosts

Among the parasitoids recovered attacking *Mussidia* spp. was a trichogrammatid egg parasitoid, *Trichogrammatoidea* sp. nr *lutea* Girault, found attacking *M. fiorii* (Muli et al., 2009b). Catalogues of natural enemies of cereal stemborers and *M. nigrivenella* from West Africa do not list *T.* sp. nr *lutea* (Bosque-Pérez et al., 1994; Polaszek, 1998; Ndemah et al., 2001; Sétamou et al., 2002). It was therefore viewed as a candidate biocontrol agent for *M.*

nigrivenella in West Africa. Studies on the suitability of alternate hosts indicated that while *T.* sp. nr *lutea* successfully attacked and developed on all six hosts tested, *E. saccharina* and *C. partellus* were poor hosts. The noctuids appeared to be highly acceptable and suitable as host of *T.* sp. nr *lutea*.

In West Africa, multiple species infestations of maize are common (Schulthess et al., 1997; Ndemah et al., 2001; Buadu et al., 2003) and the noctuids oviposit on all the growth stages of maize (Swaine, 1957; Kaufmann, 1983; Ndemah et al., 2000; Ndemah et al., 2002; Chabi-Olaye et al., 2005) unlike *M. nigrivenella* which infests the ears (Whitney, 1970; Moyal and Tran, 1991; Sétamou et al., 1999). The noctuids would thus help to build up parasitoid populations in a maize field and might have a synergistic effect on the biological control of the target pest. However, other egg parasitoids have been reported attacking the noctuid stemborer eggs in West and Central Africa (Ndemah et al., 2001a). It is likely that interspecific competition will occur between the candidate parasitoid and the native egg parasitoids. It is therefore recommended that interspecific competition studies be carried out to elucidate whether *T.* sp. nr *lutea* would be able to co-exist with the native parasitoids. The finding that parasitoids reared on *M. fiorii*, a close relative of *M. nigrivenella*, have a higher biotic potential implies effective control.

6.1.4 Factors affecting the bionomics of *Mussidia fiorii* in the laboratory

Mussidia fiorii was able to develop on diets based on host plants of *M. nigrivenella* in West Africa. Thus, *M. fiorii* is ideal for the rearing of the parasitoid in Kenya. The knowledge on its dietary, thermal requirements besides its reproductive behavior would therefore be used to optimize mass production of the host and natural enemies.

6.2 Conclusions

1. Owing to the seasonality of fruit production, it was not clear how the *Mussidia* species survive periods of non-availability of suitable fruiting structures in Kenya.
2. Although the current study indicates that several *Mussidia* spp. exist in Kenya, whether *M. nigrivenella* occurs in Kenya could not be determined in the present study with absolute certainty and molecular tools might be required to separate the different morphospecies into species.
3. The role of parasitoids in the population dynamics of *Mussidia* spp. in Kenya can be regarded as negligible and is unlikely to explain the differences in the pest status of *M. nigrivenella* between the two African regions.
4. Due to the passive role played by parasitoids in the population dynamics of *Mussidia* spp. in Kenya, accidental introduction of the West African *M. nigrivenella* populations into the region could have serious implications for crops such as maize, cotton or *Phaseolus* beans.

6.3 Recommendations

1. Since phycitine pyralids are a taxonomically difficult group, we suggest that molecular (DNA) techniques should be used in addition to detailed morphological examination of *Mussidia* spp.
2. In view of the fact that natural control will not be effective against accidental introduction of the West African *M. nigrivenella* into Kenya region, we suggest

stringent precautions during movement of grains especially maize between the two regions.

6.4 References

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