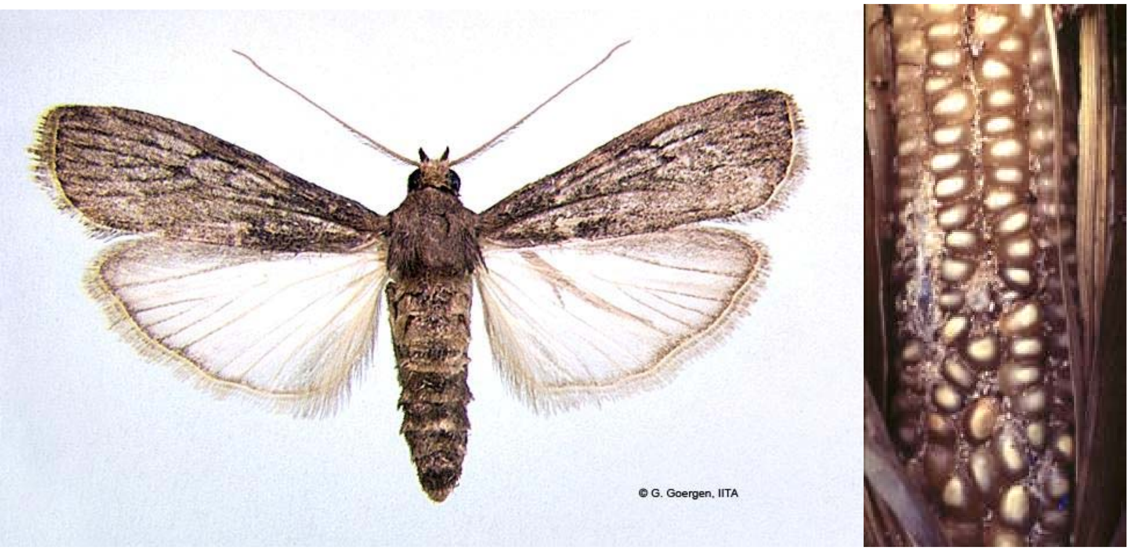
Developing and testing plant health management options against the maize cob borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) in West Africa



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AGBOKA Komi
Born in Gati-Soun, Rep. Togo

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D7

Referee:
Prof. Dr. Stefan Vidal
Department of Crop Sciences,
Agricultural Entomology,
Georg-August University, Goettingen, Germany

Co-Referee:
Prof. Dr. Teja Tscharntke
Department of Crop Sciences,
Agroecology
Georg-August University, Goettingen, Germany

Examiner:
Prof. Dr. Stefan Schütz
Department of Forest zoology and Forest Conservation
Georg-August University, Goettingen, Germany

Place and date of Disputation: Goettingen, 11th November 2009…

Dedicated to my late Mother

TOUGLO Dadakoedji

SUMMARY

The present research project aimed at developing and testing different IPM components focusing on i) habitat management particularly maize-legume intercropping and trap crops, ii) botanical formulations with special emphasis on neem and *Jatropha curcas* and iii) biological control using redistribution or new association approach for sustainably controlling the maize cob borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) in field and in storage systems. To this end, field and lab experiments were conducted mainly in different locations in Benin.

Field experiments conducted in four different locations in Benin using four by two pattern of maize-legumes or cassava planting indicated that intercrops could reduce the number of eggs (>25%) and larvae (17.9-53%) of *M. nigrivenella* compared to the monocrop. Maize-*Canavalia ensiformis* and maize-*Tephrosia vogelii* proved to be the most effective combinations for reducing *M. nigrivenella* populations in the different locations.

The effect of two leguminous cover crops, *C. ensiformis* and *Sesbania rostrata* (which varied in the onset and duration of their fruiting period, and cowpea planted as border rows on infestations of maize by the pyralid *M. nigrivenella* and of other cob-boring lepidopteran pests was studied in two field trials. Towards harvest of both the main and minor season trials, *M. nigrivenella* densities were higher in the maize alone than the legume treatments, though the effect depended on the timing of planting of the cover crop in relation to that of maize. However, pest loads expressed as cumulative number of feeding-days varied with treatment during the minor season only, and they were lower on maize with *C. ensiformis* planted 4 weeks before maize and maize surrounded by *S. rostrata* than in the maize alone treatment. There were no discernable trends for other borers such as the noctuid *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), the pyralid *Eldana saccharina* Walker (Lepidoptera: Pyralidae), and the tortricid *Thaumatotibia leuctotreta* Meyrick (Lepidoptera: Tortricidae). Furthermore, *M. nigrivenella* pest loads were considerably higher on *C. ensiformis* than maize. Thus, the lack of significant differences between or the lower pest loads in some of the treatments as compared to maize alone suggests that there was no movement of *M. nigrivenella* from the legumes to maize. Thus, the presence of alternative host plant species in the vicinity of maize fields does not increase *M. nigrivenella* attack on maize.

The results of laboratory and field experiments using two concentrations of aqueous extracts of *Tephrosia vogelii* and *Hyptis suaveolens*, and of oils of *Azadirachta indica* and *Jatropha curcas*, as well as the pesticide Furadan 5G showed that oil emulsions of *A. indica* and *J. curcas* oils act not only as oviposition deterrent but also as ovicides. Overall, treated plants had a strong deterrent effect on ovipositing *M nigrivenella*. In addition, egg hatch was adversely affected by neem and *Jatropha* oils; it decreased with an increase in concentrations of oil emulsions and varied from 3-25.5% for neem and from 6-16% for *J. curcas*. By contrast, larval survival was not affected by the treatment. In the field, Furadan, neem and *J. curcas* oils significantly reduced the number of *M. nigrivenella* larvae by 16-49.2%, while aqueous extracts of *T. vogelii* and *H. suaveolens* were similar to the control consisting of emulsified water.

Although *M. nigrivenella* is mostly described as a field pest, it can be found feeding on stored maize up to the 4th month. Survey conducted in Benin in 2006 to assess *M. nigrivenella* infestations in different maize storage systems in the Southern (SGS) and Northern Guinea Savanna (SGS) showed that in SGS and NGS the percentage of infested stores decreased from 86.7% to 26.7% and from 51.4% to 14.3%, respectively, during the first 28 weeks of storage. During the same time, mean numbers of *M. nigrivenella* per cob decreased from 0.36 to 0.04 across both zones. All larval stages, but mostly 3rd to 5th instars, were frequently found even after more than 12 weeks, showing that *M. nigrivenella* could reproduce in storage. Highest *M. nigrivenella* incidence of 16.8% and 14.4% were found in the “Ava” and crib stores, respectively. Infestations were highest in “Ava” and lowest in maize grain stored in polyethylene bags or in mud silos. In a laboratory experiment, presence of post-harvest beetles negatively affected the bionomics of the cob borer indicating strong interspecific competition.

Surveys for natural conducted in Malaysia pointed out the presence of three genera parasitizing *Parkia speciosa* pod borer: *Bracon* spp. (Hymenoptera: Braconidae) accounted for 64%, *Eurytoma* sp. (Hymenoptera: Eurytomidae) (32%) and *Sphaeripalpus* sp. (Hymenoptera: Pteromalidae) (4%). Overall, mortality caused by parasitoids could reach <40% hence they were considered a key mortality factor in the population dynamics of the *Mussidia* spp./or pyralid species in Malaysia. These results coupled with parasitoids found in Kenya on *Mussidia* spp. open the ways for the redistribution and new association in *M. nigrivenella* bio-control in West Africa.

Contents

Abbreviations

ANOVA	Analysis of Variance
BC	Biological Control
CID	Cumulative Insect Day
DAP	Day After Planting
DAS	Day After Sowing
DF	Degree of Freedom
DMR	Downy Mildew Resistant
F	Statistical F-value
FRIM	Forest Research Institute Malaysia
GLM	General Linear Model
HM	Habitat Management
HPR	Host Plant Resistance
ID	Insect Day
IITA	International Institute of Tropical Agriculture
IPM	Integrated Pest Management
LSM	Least Square Mean
m.c	Moisture content
NGS	Northern Guinea Savannah
P	P-value (statistical significance level)
QPM	Quality Protein Maize
R^2	Coefficient of determination in regression
r.h	Relative Humidity
SAS	Statistical Analysis System
SD	Standard Deviation
SE	Standard Error of the mean
SGS	Southern Guinea Savannah
SNK	Student Newman Keuls
SSA	Sub-Saharan Africa

CHAPTER 1

General Introduction

Maize is of increasing importance in western Africa, where around 7.5 million ha of the crop are grown (CIMMYT, 2001). It is grown in all major ecologies from the humid forest to the Sudan savannah, and from sea level to over 2000m altitude. Maize has diversified uses, including food, animal feed and industrial uses, but over 70% of the crop is grown for human consumption, and in some regions, maize is also becoming a cash crop; in such areas, farmers may keep maize for extended period in store in order to sell the commodity when market prices are at their peak (Compton et al., 1998). In Benin, maize constitutes the principal staple for the majority of the population (Miracle, 1966; CIMMYT, 1988) and it is grown both as subsistence and as a commercial crop. Food security and human nutritional status of small-scale and resource-poor farmers are directly impacted by losses in quantity and quality of the harvested crop. Average yields are around 1.2 t ha^{-1} which is far below the 4.3 t ha^{-1} world average or the 6.1 t ha^{-1} obtained in trials in Ghana (CIMMYT, 2001). The low productivity is attributed to various factors such as climate, poor soil fertility, inadequate farming practices, pests and diseases, and varieties susceptible to those biotic constraints, and socio-economic factors such as availability of labour, lack of access to credit facilities to purchase inputs, poor road infrastructure etc. (Mokwunye & Vlek, 1985; Stoorvogel et al., 1993; Smaling et al., 1993; McHugh & Kikafunda-Twine, 1995; Weber et al., 1996). Africa-wide, the most cited biotic constraints to yield and stability of maize production are lepidopterous stem- and cob-borers (van Rensburg 1988, Bosque-Pérez and Mareck, 1991, Gounou et al. 1994, Polaszek 1998). The problem is particularly acute in the small-scale, resource-poor systems under which maize is typically produced, and in areas with two cropping seasons. In some cases, losses due to both pre- and post-harvest pests and diseases far outweigh any reasonable hope for increases in productivity through improved germplasm and pre-harvest management. The most damaging field pests of maize in sub-Saharan Africa (SSA) are lepidopterous stem and cob borers belonging to the families Noctuidae, Pyralidae and Crambidae (see overview by Polaszek 1998). Stem borers such as *Sesamia calamistis* Hampson, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Eldana* *saccharina* (Walker), are indigenous to Africa and have moved on to maize after having evolved with native grasses and sedges or cereals such as sorghum and millet, and other host plant species (Bowden, 1976; Conlong, 1990; Schulthess et al., 1997; Polaszek, 1998). The only exception is the cob-boring *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) for which maize is the only gramineous host; it is both a field and storage pest and only important in western Africa (Sétamou et al., 2000).

Mussidia nigrivenella *distribution and host plants*

The distribution of the genus *Mussidia* is mostly limited to Africa. Of eight known species, five are native to Africa, two to Réunion and Madagascar Island in the Indian Ocean, and one to the Himalayan region (Janse, 1941). Although described for the first time in 1888 by Ragonot (Moyal, 1988) from "Baie de Lagoa", *M. nigrivenella* is a poorly known species. Recent taxonomic studies on *Mussidia* spp. undertaken in Kenya indicated that this genus is complex and that several *Mussidia* spp. exist in eastern Africa; however they can not ascertain the presence of *M. nigrivenella* in eastern and southern Africa (Muli et al., 2009). Most of the earlier reports on *M. nigrivenella* are based on scattered observations of the borer in stored commodities and mainly cacao. *Mussidia nigrivenella* has been reported from different parts of the African continent (Janse, 1941; Le Pelley, 1959; Whitney, 1970; Staeubli, 1977; Bordat and Renand, 1987; Moyal, 1988) (Fig. 1.1.), but the borer is particularly abundant in West Africa, where it has been recognized as an economically important pest of maize (Whitney, 1970; Atachi, 1985; Bosque-Pérez and Mareck, 1990; Moyal and Tran 1991; Shanower et al., 1991; Silvie, 1993).

Mussidia nigrivenella appears to be highly polyphagous (Moyal, 1988; Silvie, 1993; Sétamou, 1996) feeding on various cultivated and wild plants. In addition to maize, the borer attacks the maturing structures (cobs, seed pods and fruits) of a great variety of plants, including cotton (*Gossypium hirsutum* L. (Malvacae)), cocoa (*Theobroma cacao* L.), lima bean (*Phaseolus lunatus* L.), jackbean (*Canavalia ensiformis* (L.) DC.), velvetbeans (*Mucuna pruriens* DC.), the néré-tree (*Parkia biglobosa* (Jacq.) Benth.), and the shea butter-tree (*Butyrospermum parkii* (G. Don) Kotschy) (Moyal, 1988; Silvie, 1993; Sétamou, 1996). Surveys in agro-ecological zones of Benin, conducted between

1993 and 1997, revealed about 20 plant species from11 plant families hosting the borer, but only 13 host plants enable the borer to develop to the pupal stage (Sétamou et al., 2000). Whereas a maize crop usually supports one generation per season, several generations of *M. nigrivenella* were recorded on *P. biglobosa* and *Gardenia* spp. (Sétamou et al., 2000). Incidence of the borer in maize varied between the different agro-ecological zones, with a higher prevalence of *M. nigrivenella* in the Savanna zones of West Africa (Moyal, 1988; Gounou et al., 1994; Sétamou, 1996). The high agro-ecological variation in the availability and abundance of wild host plants coupled with their overlapping fruiting periods explained the high *M. nigrivenella* densities on maize.

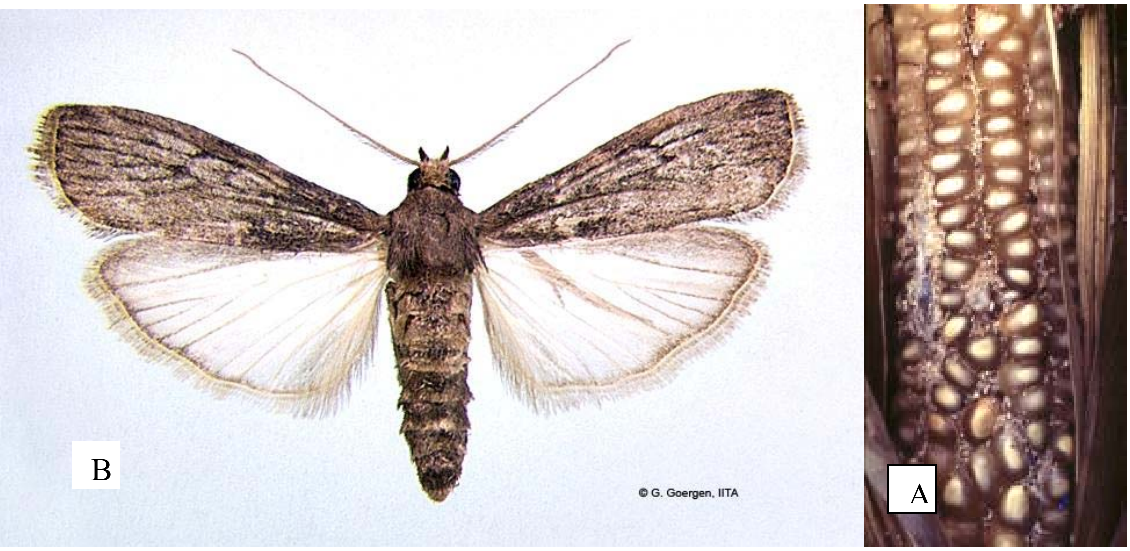

Fig 1.1. Damage caused by larvae to maize ear (A) and adult of *Mussidia nigrivenella* (B)

Biology and damages

On maize, *M. nigrivenella* lays its eggs on the silks and the husk of the cob (Moyal, 1988; Moyal and Tran, 1989; Bosque-Pérez and Mareck, 1990). In the field, egg laying starts 60 days after the emergence of the maize plants. Soon after emergence, neonate larvae enter the cob and feed cryptically in the grains, often causing extensive damage in maize (Fig 1.1). Prior to pupation, the last instar larvae leave the grains and pupae are formed in a tough cocoon near the exit holes. The pupae develop in 10-12 days. No diapause has been observed (Moyal and Tran, 1991). Adults (Fig 1.1) mate the same day of emergence, and no preoviposition period have been observed (Bolaji and Bosque-Pérez, 1998; Sétamou et al., 1999b). Oviposition lasts for 5-7 days. On maize, the life cycle is roughly 38 days (Bordat and Renand, 1987; Moyal and Tran, 1991b; Bolaji and Bosque-Pérez, 1998; Sétamou et al., 1999b).

Mussidia nigrivenella damage is identified according to its characteristic feeding damage as described by Sétamou et al. (1998). *M. nigrivenella* starts feeding from the tip of the cob and its larvae produce conspicuous amounts of silky frass, which is easily detected as the larvae bore into the grains (Sétamou, 1996). The cob borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) is one of the key borers attacking maize in West Africa (Bosque-Pérez and Mareck, 1990; Shanower et al., 1991, Moyal & Tran, 1991a). Because of its feeding behaviour, i.e., attacking the maize grain, *M. nigrivenella* constitutes a major limiting factor for maize production. Typically, more than half of the cobs in the field are infested by the borer (Whitney, 1970; Sétamou, 1996). Yield loss estimates in field grown maize are 5-15% (Moyal and Tran, 1991b; Sétamou et al., 1999a) but *M. nigrivenella* also persists in the store, where it may cause an additional 10-15% loss of grain (Sétamou, 1996; Sétamou et al., 2000a). Moreover the percentage of grains attacked per cob and therefore worthless for sowing is high (15-20%) at five insects per cob (Moyal and Tran, 1991). Furthermore, grain damage by lepidopterous borers also predisposes maize to pre- and post-harvest infestations by storage beetles, infections by *Aspergillus flavus* and *Fusarium verticillioides* and subsequent contamination with mycotoxins such as aflatoxin and fumonisin (Sétamou et al., 1998; Cardwell et al., 2000; Hell et al., 2000; Schulthess et al., 2002; Ngoko et al., 2002). Hence, both the quantity and the quality of the maize are affected by *M. nigrivenella*.

Control strategies

The search for control of stem- and cob-borers has been a prime concern of agricultural researchers in Africa since the 1950s (see overview by Polaszek, 1998; Kfir et al., 2001). Currently, no technologies are available to provide a satisfactory control of *M. nigrivenella*.

Chemical control, using contact insecticides is particular difficult probably because of the cryptic feeding behaviour of the larvae (Moyal, 1988; Sétamou et al., 1995; Ndemah and Schulthess, 2002). Pesticides have to be directed against the ovipositing females and the earlier instars before they penetrate the cob, which requires monitoring by the farmers. Thus, the use of oviposition-deterrent pesticides or ovicides timely applied can play an important role in the borer control. Furthermore timely applied fast acting insecticides can be a convenient solution but such ingredients should meet basic prerequisites: complete environmental degradability, low human toxicity, easy and cheap to produce as well as partial selectivity to various beneficial organisms and low risk of selecting pest biotypes. Given these attributes the so-called “green” insecticides or bio-pesticides are suitable candidates for sound IPM tactics. Therefore, bio-pesticides such as neem products extracted from seeds of the neem tree, *Azadirachta indica* Juss (Meliacae) are of special interest. In Africa, apart from neem products, other indigenous plants derived extracts such as the ones from *Hyptis suaveolens* L. (Lamiacae), *Tephrosia vogelii* (Fish bean) Hook F. (Leguminosae) and *Jatropha curcas* (physic nut) L. (Euphorbiacae) recently gained more attention with regard to their insect pests control potential.

Maize varieties resistant to borers have been suggested as one of the most promising means of control (Bowden, 1976; Girling, 1980; Ajala et al. 2002). Thus, the first approach to borer problems was host plant resistance (HPR). However, no resistant varieties were developed against *M. nigrivenella*. Moreover, strong antibiosis is often achieved at the cost of yield. Thus a holistic breeding strategy that aimed at developing varieties with acceptable agronomic characteristics and yield, and resistance to the major diseases yielded moderate resistance to borers only (Bosque-Pérez et al., 1997; Schulthess and Ajala, 1999, 2002). It was recognized that HPR alone would not solve the problem, thus in the 90ies, alternative solutions such as habitat management (HM) techniques and forms of biological control (BC) were sought, which would complement HPR.

Habitat management, i.e. the manipulation of the cultivated and natural environment to preserve the floral and faunal biodiversity is also another promising technique for the management of stem and cob borers in SSA. This approach includes the use of trap plants, mixed cropping and management of soil nutrients (Sétamou et al., 1995, Schulthess et al., 2004, Chabi-Olaye et al. 2005a&b, Agboka et al., 2006).

Small-scale farmers traditionally intercrop maize with vegetables, legumes, cassava or other cereals in order to obtain a greater total land productivity and insurance against the failure or unsure market value of a single crop, and in many cases pest densities are decreased in diversified systems (see overview by Risch et al., 1983; Vandermeer, 1989; van den Berg et al., 1998; Thies and Tscharntke, 1999; Kruess and Tscharntke, 2000). Consequently, any attempt to control those pests must take into consideration the close link between the ecology and biology of the pest and that of its natural habitats which include alternate hosts and associated crops in the cropping system, which can be hosts or non-hosts, as well as soil physical and chemical properties, which affect the bionomics of pests and thereby also that of their natural enemies (Bowden 1976; Schulthess et al., 1997; Khan et al., 1997a, b; Ndemah, 1999; Ndemah et al., 2001a&b, 2002, 2003). There are number of studies that showed a reduction in stem and cob borer densities when intercropped with legumes or other non-host plants (Adesiyun, 1983; Dissemond and Hindorf, 1990). This may be due to various mechanisms such as the non-host acting as trap plants (Ampong-Nyarko, 1995), increased parasitism (Sokovgard and Pats, 1996) as a result of volatiles produced by non host (Khan et al., 1997), or increased mortality due to starvation and/or predation (e.g. by ground beetles or other predators) of migrating borer larvae from non- hosts (Ndemah et al. 2003; Schulthess et al. 2004; Chabi-Olaye et al. 2005b; Wale et al. 2007; Songa et al. 2007). Many studies in tropical as well as temperate zones reported low pest densities in diversified systems (Altieri and Letourneau, 1982; Risch et al., 1983; Thies and Tscharntke, 1999; Kruess and Tscharntke, 2000). In Africa, such techniques include pest diversion or trap cropping (Khan et al., 1997; Ndemah et al., 2002) and mixed cropping (Litsinger and Moody, 1976; Okigbo and Greenland, 1976; Baliddawa, 1985; Schulthess et al., 2004). There are a number of studies in Africa that have shown a reduction in stem borer densities when maize was intercropped with non-hosts such as cassava or legumes. However, most of them were carried out in eastern Africa and dealt with the invasive crambid stem borer *Chilo partellus* (Swinhoe) (van den Berg et al., 1998; Songa et al., 2007; Wale et al., 2007). Recent work in western and central Africa showed that maize intercropped with cassava or grain legumes considerably reduced the amount of eggs of the noctuids

Sesamia calamistis Hampson (Schulthess et al., 2004) and *B. fusca* (Chabi-Olaye et al., 2005a), as a result of reduced host finding by the ovipositing adult moths.

Biological control is an important component of IPM. At present, however, efficient bio-control strategies for *M. nigrivenella* are not available. Surveys on wild and cultivated host plants of *M. nigrivenella* in West Africa, yielded a paucity of natural enemy species and low parasitism; from most host plants no parasitoids were obtained and they appear to play no role in the population dynamics of the pest (Sétamou et al. 2002). *M. nigrivenella* is only known as a crop pest from western Africa and it was hypothesized that in eastern Africa it is under natural control on wild host plants, which opens possibilities for the redistribution or new association approach (Sétamou et al, 2001; Ndemah et al., 2001c). Tritrophic level studies were proposed in regions where *M. nigrivenella* is not a crop pest such as East Africa and in Asian regions where other *Mussidia* species or sympatric species may occur, in order to identify promising bio-control candidates to be introduced. However, recent studies by Muli et al. (2009) showed that in Kenya parasitoids of *Mussidia* spp., were even scarcer than in West Africa.

Major objectives of the study

i) Establish whether intercropping maize with grain legumes, cover crops, and cassava would reduce the infestation of *M. nigrivenella* and its damages in the maize cropping system in different ecological zones in Benin (Chapter 2)

ii) Evaluate the potential of cover crops such as *Canavalia ensiformis* L. and *Sesbania rostrata* Brem. & Oberm, and cowpea planted as border rows in affecting the infestations of cob borers and their damage in maize field (Chapter 3).

iii) Evaluate the effectiveness of extracts of four indigenous plants to control *M. nigrivenella* infestation, their effects on the borer oviposition behavior as well as their ovicide properties (Chapter 4).

iv) Understand the population dynamic of *M. nigrivenella* in stored maize and study the influence of different storage structures on the borer infestation (Chapter 5).

v) Conduct thorough surveys of *Mussidia* or sympatric species and their parasitoids in Malaysia to identify potential new association parasitoids for introduction into West Africa (Chapter 6).

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CHAPTER 2

The role of maize-legumes-cassava intercropping in the management of maize ear borers with special reference to *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae)

Agboka K.[1,2], Gounou S.[1], Tamo M.[1]

[1] International Institute of Tropical Agriculture 08 BP 0932 Tripostal, Cotonou Republic of Benin 08 BP 0932 Tripostal, Cotonou Republic of Benin. [2] Georg-August University, Goettingen, Agricultural Entomology, Grisebachstrasse 6, 37077 Goettingen Germany Email: k.agboka@cgiar.org

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Abstract:

Effects of intercropping maize with cowpea, lima bean, soybean, three leguminous cover crops (*Tephrosia vogelii*, *Canavalia ensiformis*, *Sesbania rostrata*) and cassava on the infestation of *Mussidia nigrivenella* and other lepidopteran ear borers were studied. Field experiments were conducted in four different locations in Benin using four by two pattern of maize-legumes or cassava planting. Intercrops reduced the number of eggs (>25%) and larvae (17.9-53%) of *M. nigrivenella* compared with the monocrop. Maize-*C. ensiformis* and maize-*T. vogelii* proved to be the most effective combinations for reducing *M. nigrivenella* populations in the different locations. Grain loss and ear damage, which were significantly correlated with number of insects in the ear, were significantly affected by the intercrops, with losses abated by < 46.8%. No parasitized larvae were found in any of the locations.

Key words: Maize, intercropping, *Canavalia ensiformis*, *Tephrosia vogelii*, *Mussidia nigrivenella*.

Introduction

Mussidia nigrivenella Ragonot 1888 (Lepidoptera: Pyralidae) is one of the key pests attacking maize ears in West Africa (Bosque-Perez and Mareck, 1990; Shanower *et al*., 1991; Moyal and Tran, 1991a&b). It is a commonly occurring pest, which causes serious damage to maize grain in the field and store (Moyal, 1988; Moyal and Tran, 1991ab; Silvie, 1993).

Management practices have relied on early harvesting (Sétamou, *personal communication*), drying of the ears after harvest. Trials on the use of chemicals such as deltamethrine did not give any significant effect on the ear borer (Moyal, 1988). Research on the natural enemies of *M. nigrivenella* in West Africa indicated that they are very rare and not efficient (Sétamou *et al*., 2002).

In Benin maize is traditionally intercropped with other crops, some of them are non-hosts of *M. nigrivenella*, which may reduce pest incidence on the crops (Dissemond and Hindorf 1990, Ayisi *et al*., 2001). The only available information on the use of intercropping maize to reduce the infestation by *M. nigrivenella* was the work done with peanut by Moyal (1993 a &b) without any measurable effect, although cases of success have been reported on maize stem borers (Omolo, 1986; Oloo and Ogeda, 1990; Skovgard and Paets, 1996; Paets *et al*., 1997; Schulthess *et al*. 2004).

The contribution of cover crops to the sustainability of agriculture is becoming increasingly evident in many regions of the world. Because of great interest of West African farmers in cover crops such as *Canavalia ensiformis* L., *Tephrosia vogelii* Hook. F. and *Sesbania rostrata* Brem. & Oberm., it is expected that they will become key components of farming systems. However the selection of a cover crop should be based not only on its efficiency in restoring soil fertility but also on its reactions vis-à-vis pests and natural enemies.

This study is to establish whether intercropping maize with grain legumes, cover crops and cassava would reduce the infestation of *M. nigrivenella* and its damages in the maize cropping system in different ecological zones in Benin.

Materials and Methods

The trials were set up during the long rainy season of 2004 in collaboration with farmers, in four locations representing three different ecological zones: the International Institute of Tropical Agriculture (IITA)-Benin located in Abomey-Calavi (latitude 6^{o}24' N longitude 2^{o}24'E in Costal Savanna with 210 days rainfall distributed over two cropping seasons; Cana (latitude 7^{o}13'N longitude 2^{o}07'E) and Djidja (latitude 7^{o}33'N, longitude 1^{o}93'E) in the Southern Guinea Savanna with 181 days rainfall and two cropping seasons; Bantè (latitude 8^{o}42'N, longitude 1^{o}83'E), in the Northern Guinea Savanna with <150 days of rainfall and one cropping season. The following treatments were considered in each location: sole maize, maize-cowpea (*Vigna unguiculata* L. var. KVx erected variety), maize- lima bean (*Phaseolus lunatus* L.), maize-soybeans (*Glycine max* L.), maize-cassava (*Manihot exculenta* Krantz), a common practice in Benin, maize-jackbean (*Canavalia ensiformis* L.), maize-fish bean (*Tephrosia vogelii* Hook. F.) and maize-*Sesbania rostata* Brem. & Oberm. (Leguminosae). All leguminous plants were recorded as host plants of *Mussidia nigrivenella* (Sétamou et al, 2000a). *Canavalia*, *Tephrosia* and *Sesbania* are also used as cover crops in Benin (Carsky *et al*. 2003). Planting pattern in the intercrops was 4 rows of maize and 2 rows of legumes or cassava with 0.4 m within row and 0.75m between rows. Maize, legumes and cassava were sown simultaneously in a complete randomized block design with plots size of 10m x 12.75m; 1m between plots and 2m between blocks. The eight treatments were repeated three times. No insecticide was applied throughout the study period. Fertilizer 15-15-15 (NPK) was applied two weeks after sowing and urea 45 days after sowing. The maize variety QPM (Quality Protein Maize, 110-120 days) was used.

Data collection

From soft dough stage (approximately 70 days after sowing) to harvest, three destructive samples of ten plants per plot were randomly taken at two week interval. Ears were thoroughly examined, dissected and the numbers of *Mussidia* eggs, different stages of larvae and pupae as well as ear damage were assessed. Other insects found in the maize ear such as *Eldana saccharina* Walker 1865 (Lepidoptera: Pyralidae), *Sesamia Calamistis* Hampson 1910 (Lepidoptera: Noctuidae) and *Thaumatotibia* (*Cryptophlebia*) *leucotreta* Meyrick 1913 (Lepidoptera: Tortricidae) were also recorded. The damages caused by the ear borers were calculated as the percentage of grains consumed and contaminated by fungi. At harvest, the percent grain loss was estimated by the following formula: grain loss (%) = 100*(Pi – Pf)/Pi, where Pi is the initial weight of the cob and Pf is the weight of the cob after the damaged grains were removed. The loss (g) per cob is the difference between Pi and Pf. The damage by the ear borers predisposes the ears to pre- and post-harvest infestations by storage beetles, infections by fungi such as *Aspergillus flavus* and *Fusarium verticillioides* and subsequent contamination with mycotoxins. Therefore, both quality and quantity of the grains are seriously affected and the damaged cobs cannot be sold nor used as food. Thus, these damaged grains were removed and considered as actual ear weight loss.

Mussidia nigrivenella larvae or pupae collected were maintained on *Canavalia* pods to record larval or pupal parasitism. Moreover ten ears were also selected randomly from each plot and weighted to determine the effects of each treatment on the ear weight. Additionally fifty pods of cowpea and *Canavalia* were also randomly harvested for *Mussidia* eggs and larvae; the other leguminous were not yet at the fruiting stage.

Statistical analysis

Analysis of variance in the mixed model in repeated measures over sampling dates (SAS, 1997) was used to compare counts of immature pest stages according to borer species, plant damage and grain losses. Variables were compared between cropping systems with location, cropping system and their interaction as fixed effects. The random effects were sampling date, block (or replication), and plant. Plants were nested within treatments, treatments within block, block within location and location within sampling dates. Counts were log (x+1) and percentages arcsine transformed before analyses in order to stabilize variances. However, non-transformed means are reported. Means were separated with Student-Newman-Keuls (SNK) at P =0.05.

Pearson correlation analysis was used to examine whether pest numbers and damage affected crop yield and yield losses. Simple regression analyses were used to assess to which extent numbers of each pest species accounted to ear damage and ear losses.

Results

Ear borers' population densities in different intercrops

The results showed that intercropping maize with legumes or cassava has a significant effect on the infestation of *M. nigrivenella* (F = 3.4, d.f. = (7; 712) $P \leq 0.015$) and other ear borers included *T. leucotreta* (F = 2.5, d.f. = (7; 712) $P < 0.05$) but the performance of each treatment differed from one location to another. Significant interactions between cropping system and locations ($P \leq 0.02$) showed that these factors jointly influenced *Mussidia* population. In Bantè, the intercrops reduced significantly the number of *M. nigrivenella* eggs and larvae and *E. saccharina* immature compared to the monocrop (Table 2.1) while only soybean, *Canavalia* and *Tephrosia* as companion crops showed significant effects on *Sesamia*. Overall, ear borers recorded, numbers of larvae on maize cropped with *Sesbania* were similar to those in sole maize, and they were both significantly different from the rest of the treatments. In Cana similar trend was observed on the number of *M. nigrivenella* eggs. The treatments were not as effective as in Bantè in reducing *M. nigrivenella* larvae, except for maize intercropped with soybean and *Tephrosia* (Table 2.2). The treatments reducing *M. nigrivenella* infestation were not always effective against other insects found in the ear; e.g., numbers of *E. saccharina* on maize intercropped with cowpea and *Phaseolus* were similar to those of the control. Overall, only maize intercropped with cassava, soybean, *Canavalia* and *Tephrosia* gave a significant reduction in pest populations. In Djidja eggs were rarely found in any of the treatments. The numbers of *M. nigrivenella* larvae in the maize-*Canavalia* and *Tephrosia* intercropping were significantly different from maize intercropped with cowpea, *Phaseolus*, and *Sesbania*, which were similar to those in the monocrop (Table 2.3). No difference was found in the infestation of *Eldana*, *Sesamia* and other insects found in the ear between mono- and intercrops ($P \geq 0.05$). Overall, insect numbers in the ear were significantly affected by the intercrops ($P = 0.05$). At IITA, eggs of *Mussidia* were found in the mono- but rarely in the intercrops. Maize intercropped with soybean, *Canavalia* and *Tephrosia* reduced significantly the

Table 2.1. Effect of cropping systems on per ear number of *M. nigrivenella* and other maize ear borers' infestations, ear weight, ear damages in Bantè

Intercrops	No of *M. nigrivenella* eggs	No of *M. nigrivenella* larvae+pupae	No of *E. saccharina*	No of *S. calamistis*	No of Other insects*	Total ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% Loss
Maize mono	0.08±0.06b	1.09±0.20b	0.29±0.14c	0.08±0.03b	0.49±0.12b	1.54±0.23b	92.7±7.8a	17.3±3.1b	10.2±2.0b	12.1±1.8b
Maize-Cass.	0.01±0.01a	0.56±0.11a	0.02±0.02a	0.06±0.02ab	0.31±0.07a	0.95±0.15a	114.8±6.5a	7.0±2.2a	6.7±1.0ab	6.4±1.6a
Maize-Cowp	0.01±0.01a	0.61±0.14a	0.04±0.03a	0.07±0.03b	0.40±0.09b	1.12±0.16a	103.6±8.4a	9.6±2.1a	7.5±1.9ab	7.7±1.7a
Maize-Phaseo.	0.03±0.03a	0.75±0.15a	0.08±0.05b	0.08±0.03b	0.48±0.11b	1.17±0.18a	102.6±9.6a	10.4±2.0a	8.2±1.5ab	9.1±1.8ab
Maize-Soyb.	0.01±0.01a	0.57±0.12a	0.02±0.02a	0.04±0.02a	0.37±0.10b	1.00±0.21a	109.8±9.6a	9.3±2.0a	7.1±1.9ab	7.7±1.6a
Maize-Canav.	0.01±0.01a	0.52±0.09a	0.01±0.01a	0.03±0.02a	0.30±0.06a	0.86±0.16a	113.0±7.0a	7.7±1.8a	6.7±1.4ab	5.9±0.9a
Maize-Sesb.	0.06±0.03b	0.68±0.13a	0.08±0.03b	0.07±0.03b	0.47±0.09b	1.30±0.20b	98.8±7.6a	11.4±2.5ab	10.0±1.1b	9.4±1.9ab
Maize-Tephr.	0.01±0.01a	0.51±0.10a	0.00±0.00a	0.02±0.02a	0.27±0.06a	0.80±0.12a	118.86±7.00a	6.73±0.97a	4.6±1.2a	5.4±1.7a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 230)	(7; 232)	(7; 230)	(7; 230)
P	0.020	0.048	0.006	0.004	0.028	0.049	0.290	0.006	0.016	0.024

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais*, *Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Tephr: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

Table 2.2: Effect of cropping systems on *M. nigrivenella* and other maize ear borers' infestations, ear weight, ear damages in Cana

Intercrops	No of *M. nigrivenella* eggs	No of *M. nigrivenella* larvae+pupae	No of *E. saccharina*	No of *S. calamistis*	No of Other insects*	Total ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% Loss
Maize mono	0.21±0.21b	0.28±0.07b	0.24±0.06b	0.32±0.07 b	1.60±0.61 b	2.45±0.53b	33.4±2.5a	26.7±6.5b	4.3±1.1b	14.0±4.8b
Maize-Cass.	0.00±0.00a	0.21±0.05b	0.12±0.04a	0.23±0.07ab	0.92±0.27ab	1.47±0.29a	40.9±6.2a	17.6±4.8ab	3.1±0.7ab	8.7±1.6ab
Maize-Cowp	0.00±0.00a	0.22±0.06b	0.19±0.05b	0.28±0.07 b	1.36±0.43 b	2.01±0.48b	35.3±3.5a	19.2±5.2ab	3.6±1.6a	10.2±3.8a
Maize-Phaseo.	0.01±0.01a	0.23±0.05b	0.22±0.04b	0.32±0.18 b	1.55±0.58 b	2.17±0.65b	31.7±2.5a	20.5±5.7ab	3.7±1.7ab	11.1±3.7ab
Maize-Soyb.	0.00±0.00a	0.15±0.04a	0.11±0.04a	0.18±0.04 a	0.90±0.23ab	1.33±0.28a	37.3±4.1a	16.7±3.1a	2.4±0.6a	8.6±2.0a
Maize-Canav.	0.00±0.00a	0.19±0.04ab	0.09±0.04a	0.17±0.04 a	0.68±0.19 a	1.13±0.16a	37.6±5.0a	15.5±4.6a	2.2±0.5a	8.3±2.9a
Maize-Sesb.	0.00±0.00a	0.21±0.05b	0.12±0.04a	0.23±0.05ab	1.54±0.47 b	2.06±0.62b	39.5±2.5a	21.6±5.5ab	3.72±1.3ab	13.9±3.4ab
Maize-Tephr.	0.00±0.00a	0.17±0.04a	0.08±0.03a	0.16±0.04 a	0.32±0.07 a	0.73±0.12a	43.9±7.7a	13.3±4.1a	1.4±0.5a	2.7±2.0a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 222)	(7; 229)	(7; 222)	(7; 220)
P	0.0440	0.0042	0.0450	0.0050	0.0048	0.0362	*0.059*	*0.018*	*0.034*	*0.024*

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais*, *Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Tephr: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

Table 2.3: Effect of cropping systems on *M. nigrivenella* and other maize ear borers' infestations, ear weight, ear damages in Djidja

Intercrops	No of *M. nigrivenella* eggs	No of *M. nigrivenella* larvae+pupae	No of *E. saccharina*	No of *S. calamistis*	No of Other insects*	Total ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% Loss
Maize mono	0.00±0.00	0.27±0.08c	0.01±0.01	0.04±0.02	0.87±0.21	0.98±0.22c	119.1±8.7	7.6±2.13c	7.8±3.0c	6.6±1.9
Maize-Cass.	0.00±0.00	0.10±0.04a	0.00±0.00	0.01±0.01	0.37±0.08	0.48±0.15a	133.1±10.4	2.6±0.8ab	2.8±1.0ab	2.3±0.7
Maize-Cowp	0.00±0.00	0.19±0.08bc	0.01±0.01	0.03±0.02	0.48±0.07	0.71±0.11b	126.8±8.0	3.3±1.5ab	3.3±1.1ab	2.9±1.4
Maize-Phaseo.	0.00±0.00	0.19±0.09bc	0.01±0.01	0.04±0.02	0.50±0.11	0.74±0.15b	125.8±5.8	4.4±1.8ab	3.9±1.8ab	3.5±1.3
Maize-Soyb.	0.00±0.00	0.12±0.05ab	0.01±0.01	0.02±0.02	0.47±0.10	0.62±0.13ab	130.4±11.6	3.2±0.9ab	3.2±0.8ab	2.7\±0.7
Maize-Canav.	0.00±0.00	0.11±0.05a	0.00±0.00	0.02±0.02	0.45±0.12	0.58±0.12ab	135.9±7.5	2.21±0.7ab	2.6±0.8ab	1.9±0.6
Maize-Sesb.	0.00±0.00	0.20±0.09bc	0.01±0.01	0.04±0.02	0.68±0.17	0.84±0.20bc	122.4±9.6	6.7±3.8bc	6.4±3.2bc	5.6±3.1
Maize-Tephr.	0.00±0.00	0.08±0.03a	0.00±0.00	0.00±0.00	0.33±0.08	0.41±0.12a	138.5±9.3	1.2±0.5a	1.4±0.7a	1.0±0.5
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 232)	(7; 232)	(7; 232)	(7; 232)
P	1.00	0.031	0.885	0.470	0.064	0.05	*0.087*	*0.050*	*0.050*	*0.027*

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais*, *Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Tephr: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

number of *Mussidia* larvae compared to maize intercropped with *Phaseolus*, which was similar to that of the monocrop (Table 2.4). Cassava and cowpea had intermediate effects. *Eldana* infestations were also significantly reduced by the intercrops. The total ear borers were significantly reduced by cassava, soybean, *Canavalia*, and *Tephrosia* in the system.

Although all intercrops had an effect on *Mussidia* infestation, the combination of maize-*Canavalia* and maize-*Tephrosia* proved to be the most effective in the different locations

The data across locations showed that *Mussidia* population densities varied significantly with time of sampling (Figure 2.1). More larvae and pupae were found during the last sampling when maize was ready to be harvested.

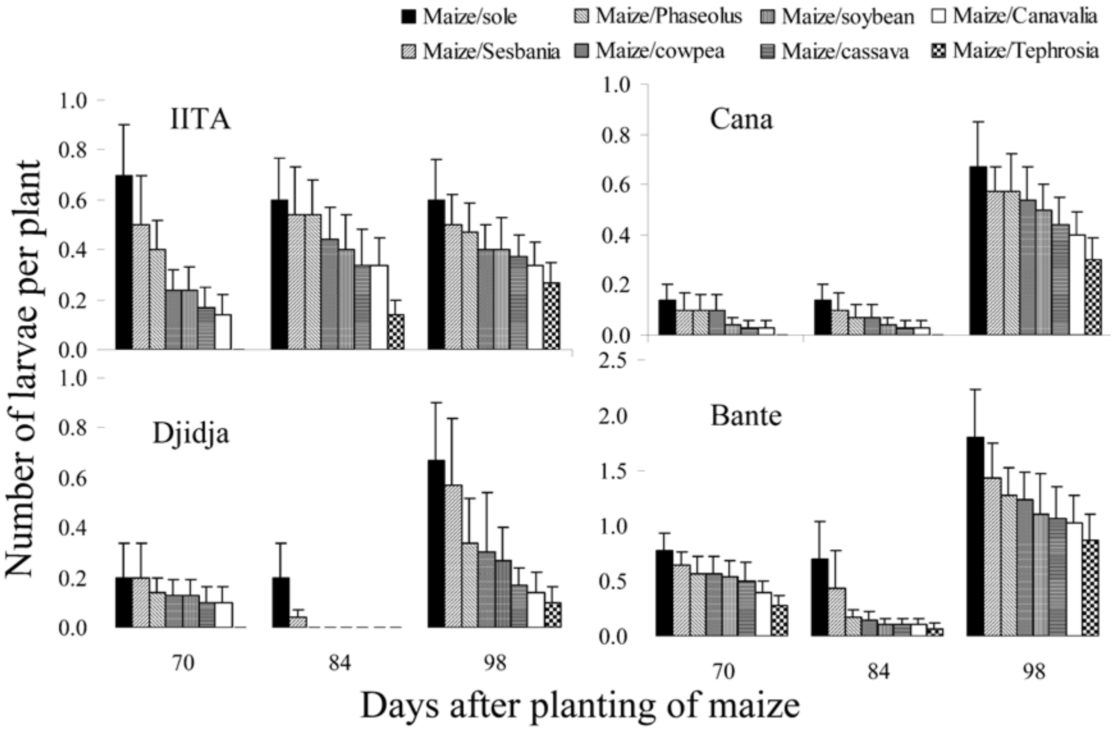

Figure 2.1: Numbers of *Mussidia nigrivenella* per plant collected per location and per planting system during the three sampling dates

Table 2.4: Effect of cropping systems on *M. nigrivenella* and other maize ear borers' infestations, ear weight, ear damages at IITA

Intercrops	No of *M. nigrivenella* eggs	No of *M. nigrivenella* larvae+pupae	No of *E. saccharina*	No of *S. calamistis*	No of Other insects*	Total ear borers	Ear weight (g)	% Ear damage	Ear loss (g)	% Loss
Maize mono	0.10±0.06b	0.54±0.10b	0.16±0.05c	0.30±0.06b	0.75±0.16	1.56±0.21b	119.2±6.4a	13.5±2.5b	13.2±2.3b	11.7±2.2b
Maize-Cass.	0.00±0.00a	0.27±0.06ab	0.06±0.02ab	0.13±0.04a	0.53±0.08	0.95±0.14a	139.1±5.9b	7.3±1.6a	8.8±1.6ab	6.6±1.2ab
Maize-Cowp	0.00±0.00a	0.40±0.07ab	0.10±0.04b	0.18±0.04a	0.66±0.12	1.24±0.20ab	127.6±7.3ab	10.9±2.8ab	9.7±1.4ab	10.4±2.9ab
Maize-Phaseo.	0.00±0.00a	0.41±0.08b	0.12±0.06bc	0.19±0.08a	0.70±0.12	1.32±0.17ab	125.9±8.7ab	11.2±1.8ab	10.3±1.9ab	9.6±1.5ab
Maize-Soyb.	0.00±0.00a	0.39±0.07a	0.09±0.04b	0.16±0.05a	0.57±0.10	1.11±0.14a	133.4±9.6b	10.7±3.9a	8.9±2.4ab	9.3±3.2ab
Maize-Canav.	0.00±0.00a	0.32±0.07a	0.08±0.03ab	0.13±0.04a	0.55±0.09	1.00±0.16a	136.5±6.9b	7.4±1.3a	8.5±1.2ab	6.3±0.9ab
Maize-Sesb.	0.00±0.00a	0.42±0.06a	0.12±0.05bc	0.28±0.12b	0.72±0.16	1.42±0.22b	117.1±6.7a	12.0±3.4b	12.2±2.7b	9.0±2.1b
Maize-Tephr.	0.00±0.00a	0.26±0.05a	0.04±0.02a	0.11±0.03a	0.47±0.11	0.86±0.12a	142.0±8.3b	7.0±1.0a	7.0±1.5a	6.2±1.4a
DF**	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 712)	(7; 230)	(7; 230)	(7; 230)	(7; 230)
P	0.046	0.034	0.002	0.043	0.062	0.045	0.045	0.048	0.043	0.053

Means within columns followed by the same letter are not significantly different (SNK, P=0.05). *Other insects are *Thaumatotibia leucotreta*, beetles (*Sitophilus zeamais*, *Carpophilus* sp. and *Cathartus quadricollis*). Cass: cassava; Cowp: cowpea; Phaseo: *Phaseolus lunatus*; Soyb: soybean; Canav: *Canavalia ensiformis*; Sesb: *Sesbania rostrata*; Tephr: *Tephrosia vogelii*. DF** indicates degree of freedom for both treatment and the experimental error.

No parasitoids were found on *M. nigrivenella* during the experiment in any of the locations.

Influence of intercrops on maize yield and on ear borers damages

Ear damage and grain losses in the different locations were significantly lower in the inter- than in the monocrop except for maize intercropped with *Sesbania*. Ear damages in intercrops were reduced by 34.3-61.1% in Bantè, 19.1-50.2% in Cana, 11.9-84.1% in Djidja and 11-48.2% at IITA. The percent loss was higher in monocrop, maize intercropped with cassava, *Phaseolus*, cowpea and soybean than in maize with *Canavalia* and *Tephrosia*. Ear weight losses were reduced in the intercrops by 22.1-51% in Bantè, 0.9-80.5% in Cana, 13.9-84.1% in Djidja and 11.1-46.8% at IITA. The highest reduction in ear damages and losses were found in maize intercropped with *Tephrosia*. However across locations generally the intercrops have no effect on ear weight ($p > 0.05$). Across the four experimental locations ear weight was negatively correlated with the number of ear borers (Table 2.5) but ear damage increased with the number of the insects found in the ear. Multiple regressions between ear damage and insect variables showed that the numbers of *M. nigrivenella*, *E. saccharina*, *S. calamistis* and other insects including *T. leucotreta* significantly affected the percentage ear damage and ear losses (Table 2.6).

Table 2.5: Pearson correlation coefficients between maize yield, pest and damage variables using data across the four experimental locations.

	1	2	3	4	5	6	7	8	9
1	1.00								
2	-0.31**	1.00							
3	-0.18**	0.92**	1.00						
4	-0.01	0.01	0.01	1.00					
5	-0.13**	0.51**	0.55**	0.03	1.00				
6	-0.12**	0.48**	0.41**	-0.01	0.13**	1.00			
7	-0.22**	0.68**	0.54**	-0.02	0.23**	0.16**	1.00		
8	-014**	0.54**	0.45**	-0.03	0.05*	0.12**	0.17**	1.00	
9	-0.17**	0.78**	0.75**	-0.01	0.60**	0.35**	0.48**	0.74**	1.00

*r values ≥ 0.04 have $P \leq 0.05$ and **r values ≥ 0.10 have $P < 0.01$.

1. Ear weight (g); 2. % Ear damage; 3. % Yield loss; 4. Number of *M. nigrivenella* eggs ; 5. Number of *M. nigrivenella* (larvae + pupae); 6. Number of *E. saccharina* larvae; 7. Number of *Sesamia calamistis,* 8. Number of other insects (*T. leucotreta* + coleoptera larvae) in the ear; 9. Overall larvae in ear

Table 2.6: Multiple regressions between damage and insect variables

Variables	Coefficient ± SE	Partial T-value	Mean ± SE	Partial P
Dependent: arcsin√(% Ear damage)			10.64 ± 0.208	
Independent variables :				
$\log_{10}$ (No of *M. nigrivenella*+1)	25.46 ± 1.43	17.80	0.614 ± 0.013	< 0.0001
$\log_{10}$ (No of *E. saccharina*+1)	30.89 ± 2.70	11.43	0.118 ± 0.006	< 0.0001
$\log_{10}$ (No of *Sesamia sp*+1)	48.21 ± 2.62	18.40	0.192 ± 0.005	< 0.0001
$\log_{10}$ (No of Other insects +1)	24.73 ± 1.07	23.02	1.124 ± 0.036	< 0.0001
Intercept = 2.46 ± 0.39				
N= 954, F= 653.10, P< 0.0001, R^2= 0.73				
Dependent: arcsin√(% yield loss)			7.36 ± 0.139	
Independent variables:				
$\log_{10}$ (No of *M. nigrivenella* +1)	21.76 ± 1.19	18.29	0.614 ± 0.013	< 0.0001
$\log_{10}$ (No of *E. saccharina* +1)	16.46 ± 2.26	7.28	0.118 ± 0.006	< 0.0001
$\log_{10}$ (No of *Sesamia sp* +1)	32.95 ± 2.18	15.13	0.192 ± 0.005	< 0.0001
$\log_{10}$ (No of Other insects +1)	15.14 ± 0.90	16.84	1.124 ± 0.036	< 0.0001
Intercept =3.59 ± 0.33				
N= 942, F= 437.42, P< 0.0001, R^2= 0.65				
Dependent: g Ear loss			5.60 ± 0.099	
Independent variables:				
No of *M. nigrivenella*	1.62 ± 0.22	7.31	0.614 ± 0.013	< 0.0001
No of *E. saccharina*	1.50 ± 0.45	3.30	0.118 ± 0.006	0.0010
No of *Sesamia sp*	7.36 ± 0.65	11.30	0.192 ± 0.005	< 0.0001
No of Borers in stem	1.88 ± 0.71	2.64	0.309 ± 0.009	0.0085
No of Other insects	0.28 ± 0.08	3.47	1.124 ± 0.036	0.0006
Intercept = 2.55 ± 0.29				
N= 942, F= 71.86, P< 0.0001, R^2=0.28				

Discussion

As shown for stemborers by Schulthess *et al*. (2004) and Chabi-Olaye *et al*. (2005) intercropping reduced attacks of maize ears by *M. nigrivenella* and other stemborer species that feed in the ear. Root (1973) and Andow (1992) suggested that the herbivore were likely to find and remain on host plants that occur in large, dense and pure stands due to the resource concentration factor. It has been also suggested that when diverse backgrounds 'disrupt' (Vandermeer, 1989) insects from selecting otherwise-acceptable host plants, the action is mediated through, among other factors, visual camouflage (Smith, 1969) or deterrent or repellent chemicals (Uvah and Coaker, 1984). Ndemah et al, (2003) suggested that the negative relationship between the non-host and plant density and the numbers of larvae were probably due to difficulties encountered by the female moths in finding host plants for oviposition. Vandermeer (1989) listed three possible mechanisms responsible for reducing pest infestation in mixed cropping system: (i) the disruptive-crop hypothesis, in which a second non-host plant species disrupts the ability of the pest to attack its proper host plant species; (ii) the trap crop hypothesis in which a second non host plant species attracts the pest away from its primary host; and (iii) the natural enemy hypothesis, in which the intercropping set up attracts more predators and parasitoids than the monocrop thereby reducing pests on primary host plant. Although each treatment had an effect on borer infestation, the most effective intercrops in the different locations were the treatments where maize was intercropped with *Canavalia* and *Tephrosia*. According to Sétamou *et al*., (1999), jackbean (*C. ensiformis*) was the most suitable host plant for *M. nigrivenella* development. The high suitability of this cover crop for *M. nigrivenella* development and survival compared to maize might have direct effects on the population dynamics of *M. nigrivenella* in maize. In our experiments maize and jackbean seeds were sown simultaneously and both plants reached the suitable stage for *M. nigrivenella* attack at the same time; this could explain why low numbers of *M. nigrivenella* were found in maize-jackbean intercrop. The low number of *M. nigrivenella* observed in Maize-*T. vogelii* intercrop is probably due to the repulsive effect of *T. vogelii*. In semi-field study, oviposition of *Mussidia* was reduced by the leaf extract of *T. vogelii* showing its oviposition deterrent (Agbodzavu, 2005). Moreover these results suggested that attractiveness and deterrence of the legumes intercropped with maize further increase the effectiveness of intercropping in suppressing lepidopterous insects on maize ear. Visual and chemical stimuli from the host and non-host plants might also affect the rate at which insects colonize habitats, and their behavior in those habitats. Moreover in an intercrop, the primary host plant is made less attractive to the herbivore, and this may depend on the kind of cues, either olfactory or tactile perceived by the insect. Volatiles emanating from plant tissues had been reported (Elzen *et al*, 1984; Udayagiri and Jones, 1992) influencing attractiveness of the plant, which may have also played vital role in this experiment.

Although it is stipulated that intercropping may enhance the effectiveness of natural enemies there was no support for this hypothesis in the present study. No parasitoids were found in any of the treatments including the monocrop in our study. According to Sétamou *et al*. (2002), natural enemies of *M. nigrivenella* are rare in cropping system and wild habitats in Benin suggesting that the reduction in pest infestation in this study was not due to parasitism but depended on the performance (attractiveness or repulsiveness) of each intercrop plant or ovipositional preference of the ear borer.

The effectiveness of the treatments differed from one location to another and with borer species. According to Sétamou *et al*., (2000b), the abundance of *Mussidia* is more pronounced in the Northern Guinea Savanna than in the other regions under study due to the abundance of *M. nigrivenella* host plants. The differences observed in the treatments toward the infestation of *M. nigrivenella* and *Eldana* could be explained by the differences in the oviposition behavior of the two borers. *Eldana*, which primarily is a stemborer that later moves into the ear, (Schulthess *et al*., 1997) infests plant at the tasselling stage or later (Kaufman, 1983), whereas the ear borer *M. nigrivenella* oviposits on the silk or husks of young and old ears. Moreover *M. nigrivenella* was recorded on various plants included the legumes tested in this study (Sétamou *et al*. 2000a).

Our study has demonstrated that a change in the vegetation diversity could change the abundance and incidence of maize ear borers. The importance of intercropping as a method of controlling stem borers in sorghum and maize has been reported by Amoako-atta and Omolo (1983), Ampong-Nyarko *et al*. (1994), Skovgard and Paets (1996) and Ayisi *et al*. (2001). It has been successfully used in reducing infestation of maize stem borers especially *Busseola fusca* Fuller 1901 (Lepidoptera: Noctuidae) (Chabi-Olaye et al. 2005), *Chilo partellus* Swinhoe 1885 (Lepidoptera: Pyralidae) (Ampong Nyarko *et al*., 1995, Maluleke *et al*. 2005). Maize-bean intercropping experiments conducted in Ethiopia during the 1992-cropping season showed that sole maize had significantly high incidence of stalk borer and earworms as compared to intercropped treatments (Nigussie and Reddy, 1996). By contrast Schulthess *et al*. (2004) could not show any effect on the ear-boring pests such as *M. nigrivenell*a and *T. leucotreta* by intercropping maize with cassava maybe because they planted maize before cassava.

There was an increase in the incidence of infestation of *M. nigrivenella* at the last sampling date compared to the first one. The low numbers of larvae encountered during the first sampling might have caused by high immature mortality and high numbers of larvae at last sampling due to a cumulating of two generations of *M. nigrivenella* on maize from milk stage till harvest. According to Sétamou *et al*. (1999), the generation time of *M. nigrivenella* on maize is 37.5 days and *M. nigrivenella* continues to infest the ear from milk stage till harvest and even in stores. This oviposition behavior of *M. nigrivenella* in field explained the presence of larvae of all stages and pupae in maize ear during the last sampling in the present study. In contrast to expectation, the high incidence of borer infestation had little effect on the ear weight, an indication that the threshold level of the pest was not reached. But the grain losses were affected by the treatments. This may have great incidence on the aflatoxin content of the maize grains. A study conducted by Hell *et al*. (2003) has shown that association of grain legumes or groundnut with maize would increase aflatoxin in maize. In the present study, the aflatoxin was not measured in maize samples; therefore in future maize legumes intercropping studies, care should be taken to assess the aflatoxin content in each treatment before best crop combinations, which would not only reduce the pest incidence but also aflatoxin contamination, could be selected.

In conclusion, the findings of this study showed that maize-legumes or cassava intercrops could reduce *M. nigrivenella* and other ear borers including *T. leucotreta* infestation compared to the sole maize culture. This study showed that an intercropping system with ‘poor’ hosts of *M. nigrivenella* could be developed, in a ‘push-pull’ strategy for the control of *M. nigrivenella* in small-scale maize farming systems. This strategy will involve *C. ensiformis* as the highly susceptible trap plants (pull) and *Tephrosia vogelii* as repellent intercrop (push). Tests are being conducted to determine the susceptible stages of *C. ensiformis* the most preferred by the ear borer.

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CHAPTER 3

The effect of leguminous cover crops and cowpea planted as border rows on maize ear borers with special reference to *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae)

Komi Agboka[1,2], Fritz Schulthess, Manuele Tamò[1] and Stefan Vidal[2]

[1]International Institute of Tropical Agriculture 08 BP 0932 Tripostal, Cotonou, Republic of Benin. [2]Georg-August-University Goettingen, Agricultural Entomology, Grisebachstrasse 6, 37077 Goettingen, Germany.

Correspondence E-mail: k.agboka@cgiar.org

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Abstract

In southern Benin, the use of cover crops to improve and maintain soil fertility is in the increase. In the present study, the effect of two leguminous cover crops, *Canavalia ensiformis* and *Sesbania rostrata*, which varied in the onset and duration of their fruiting period, and cowpea planted as border rows on infestations of maize by the pyralid *Mussidia nigrivenella* and of other cob-boring lepidopteran pests was studied in two field trials in 2005. Towards harvest of both the main and minor season trial, *M. nigrivenella* densities were higher in the maize alone than the legume treatments, though the effect depended on the timing of planting of the cover crop in relation to that of maize. However, pest loads expressed as cumulative number of feeding-days varied with treatment during the minor season only, and they were lower on maize with *C. ensiformis* planted 4 weeks before maize and maize surrounded by *S. rostrata* than in the maize alone treatment. There were no discernable trends for other borers such as the noctuid *Sesamia calamistis*, the pyralid *Eldana saccharina*, and the tortricid *Thaumatotibia leuctotreta*. Furthermore, *M. nigrivenella* pest loads were considerably higher on *C. ensiformis* than maize. Thus, the lack of significant differences between or the lower pest loads in some of the treatments as compared to maize alone suggests that there was no movement of *M. nigrivenella* from the legumes to maize. Thus, the presence of alternative host plant species in the vicinity of maize fields does not increase *M. nigrivenella* attack on maize.

Per area yields were not different, though in some of the legume treatments, grain damage and grain losses were higher as compared to the maize alone treatment. However, grain damage and loss was correlated with other lepidopteran borers, which attack the stem and ear, rather than *M. nigrivenella*. Thus a control package has to aim at the entire complex of maize pests attacking the ears. This should also include the rotation of cover crops or grain legumes with maize. As shown for other lepidopteran pests in Africa, increasing plant vigour by improving soil fertility not only increases crop yield but also reduces yield loss.

Keywords: *Canavalia ensiformis,* cover crops, damage, infestation, maize, *Mussidia nigrivenella*,

INTRODUCTION

In West Africa, the pyralid *Mussidia nigrivenella* (Ragonot) is a polyphagous pest, which besides maize ears attacks cotton balls, *Phaseolus* beans and the fruiting structures of many economically important trees as well as leguminous cover crops such as *Mucuna pruriens* (L.) DC. and the jackbean, *Canavalia ensiformis* (L.) DC. (Silvie 1990, Sétamou et al. 2000a; Moyal and Tran 1991). In the field, 50% of the plants are usually infested and yield losses range from 5 to 25% (Moyal and Tran 1991; Sétamou et al. 2000b). Furthermore, *M. nigrivenella* continues to feed on maize grains in stores leading to an additional 5% loss. In addition, grain damage by the borer predisposes maize to pre- and post-harvest infestations by storage beetles and infections by mycotoxin-producing moulds (Sétamou et al. 1998; Fandohan et al. 2005).

Several techniques have been tested against *M. nigrivenella* on maize but none achieved satisfactory control. Probably because of the cryptic feeding behaviour of the larvae even systemic insecticides proved to be not very efficient against borers feeding in the ear (Sétamou et al. 1995, Ndemah and Schulthess 2002). Intercropping maize with both host and non-host companion crops or planting border rows with grasses reduced oviposition and larval infestations of stem- and cobborers including *M. nigrivenella*; however, the results were not consistent (Ndemah et al. 2002b; Agboka et al. 2006). Given these results, it became evident that an integrated approach including various control techniques, had to be developed to control this intractable pest.

Leguminous cover crops can contribute to increased and sustainable crop productivity through erosion and weed control, biological nitrogen fixation (Vissoh et al. 1998), and reduction of arthropod pests on the subsequently planted crops (Hokkanen 1991; Chabi et al. 2005a). Because of the high interest of West African farmers in leguminous cover crops such as *C. ensiformis*, *Tephrosia vogelii* Hook. F. *Sesbania rostrata* Brem. & Oberm. and *Mucuna* spp. it is expected that they will become key components of local farming systems (Carsky et al. 2003). The selection of a cover crop by local farmers, however, should be based not only on its efficiency in restoring soil fertility, but also on the effects it has on the population dynamics of pests and natural enemies. As shown by Chabi et al. (2005a) planting leguminous grain and cover crops during the previous season considerably reduced infestations of maize by lepidopterous stemborers during the subsequent cropping season. Planting of cover crops could also divert pests and contribute to the diversity and abundance of natural enemies thereby affecting pest densities in adjacent crop fields (Altieri 1995; Risch 1981). However, the introduction of an additional food source in the maize cropping season of southern Benin, where natural enemies appear to play a minor role in the control of *M. nigrivenella* (Sétamou et al. 2002), bears the potential to also increase infestations of maize by this highly polyphagous pest. In fact, Sétamou et al. (1999) observed high survival and intrinsic rates of increase of *M. nigrivenella* reared on pods of *C. ensiformis* and *M. pruriens*, explaining the high infestations found in the field. However, higher attractiveness of cover crops as compared to maize for ovipositing *M. nigrivenella* females may also play a role. In addition, the two cover crops have a considerably less phasic growth habit than maize. Thus depending on the cultivar, fruiting structures suitable for growth and development of *M. nigrivenella* are available for an extended period and thereby could form a source of infestation of the maize crop. It is therefore suggested that the timing of planting of cover crops should occur in such a way that the emergence of female moths from mature pods does not coincide with the occurrence of maize plants in a suitable development stage for oviposition and development of *M. nigrivenella* in neighboring crop fields.

The present work aimed at evaluating the effect of the presence of *C. ensiformis*, *S. rostrata* and cowpea, *Vigna unguiculata* L., planted in the vicinity of maize fields on the infestation of the crop by pests attacking the ear.

Materials and Method

Location of the experiment

The experiments were conducted in the derived savanna in the south of the Republic of Benin, at the International Institute of Tropical Agriculture (IITA) research farm in Abomey-Calavi (latitude 6°24' N longitude 2°24'E, 12 m above sea level). The site is characterized by a bimodal rainfall distribution, with peaks in June and October. Mean annual precipitation is about 1200 mm and mean temperature 25.5°C. The main rainy season usually lasts from March to July and the minor one from early September to beginning of December. A dry spell of about four weeks may occur in August. The dry season lasts from December to March. Two field trials were carried out, one during the main and another during the minor rainy season of 2005.

Experiment procedures

The maize variety used in both experiments was Quality Protein Maize (QPM) (110-120 days to harvest). Tasseling is between 35 to 40 days after sowing (DAS) while silking starts at 45 DAS. Maize was sown at a density of 0.4 x 0.75 m (within x between rows) with two seeds per hole. To accommodate for a gradient in soil fertility, the experimental design for both trials was a complete randomized block with four replicates. Blocks were separated by 2m and the plots by 1m. Plot size was 12x12m in maize cover crops treatments and 10x10m in the maize alone treatments. Two weeks after sowing, a NPK 15–15–15 composite fertilizer (N, P_2O5 and K_2O) was applied at a rate of 60 kg per ha, and 45 DAS an additional 50 kg of urea per ha. Fields were weeded as needed. No insecticide was applied throughout the study period.

There were five treatments. During the main season, they were (i) maize alone, (ii) maize plots surrounded with *C. ensiformis* sown one week before maize (herewith referred to as Canav1), (iii) maize surrounded with *C. ensiformis* sown three weeks before maize (Canav3), (iv) maize with border row of *S. rostrata* sown three weeks before maize

(Sesbania3) and (v) maize plots surrounded with the erect cowpea cultivar KVx-449 (Cowpea) sown simultaneously. The cover crops species as well as cowpea were sown in two rows at a spacing of 0.5m within row and 1m between rows, 0.5m from the first maize row. In jack bean, flowering starts at around 49 days after sowing (DAS) and first pods appear at 56 DAS (Agboka, unpubl. data). The *C. ensiformis* and *S. rostrata* treatments were modified during the minor season. According to laboratory studies carried out during the main season, rearing of *M. nigrivenella* on fresh green pods with high content of water failed while it succeeded more on mature pods (Agboka, unpubl. data). Therefore, border rows of *Canavalia* were planted four and eight weeks before maize (herewith referred to as Canav4 and Canav8, respectively), so that the majority of the pods encountered by *M. nigrivenella* had low water content. *Sesbania rostrata* was sown 4 weeks before maize (Sesbania4).

Data collection

Four destructive samplings were done at biweekly intervals, i.e. at 74, 88, 102 and 116 DAP. At each sampling, ten plants per maize plot were randomly sampled per sampling occasion. Ears were thoroughly examined, dissected and the numbers of *Mussidia* eggs, larvae and pupae as well as ear damage were assessed according to Sétamou (1996). However, as eggs were rarely obtained – they are very difficult to detect – cumulative numbers of larvae and pupae only are presented in the tables. The damage caused by the ear borers was calculated as the percentage of grains consumed. Numbers of other insects such as *Eldana saccharina* Walker (Lepidoptera, Pyralidae), *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Thaumatotibia* (*Cryptophlebia*) *leucotreta* Meyrick (Lepidoptera: Tortricidae) found in the maize ears were also recorded. In the legume border rows, ten pods of *Canavalia*, 25 pods of *Sesbania*, and 25 pods of cowpea were randomly sampled for assessment of *M. nigrivenella* numbers; for logistic reasons sampling of the border rows were done 3-5 days after sampling maize. At harvest, per area yield of each treatments were estimated using four pre-determined sub-plots of 1.5m x 2m.

For each treatment, insect-days were calculated according to Ruppel (1983) for each sampling date, while a cumulative insect-day (CID) index was calculated as CID = $\Sigma 0.5 (P_a+P_b)D_{a\text{-}b}$, where P_a is the population density (mean insects/maize plant at sampling date a, P_b is the population density at sampling date b, and $D_{a\text{-}b}$ is the number of days between a and b. Feeding days of *M. nigrivenella* per treatment on per plot basis were calculated as:

$CID_x = CID_P * N_x$ a)

$CID_{TA} = (CID_{Legume} * S_{BR} * D_{legume}) + (CID_{maize} * S_{maize} * D_{maize})$ b)

In equation a) CID_x is insect days per legume or maize plant, CID_P is the insect day per pod or maize ear and N_x is the number of pod or ear collected per plant. In equation b), CID_{TA} is the insect day per total area in plots with legume border rows, CID_{Legume} and CID_{maize} represent insect day per legume and maize plant, respectively. S_{BR} and S_{maize} represents area planted to legumes and maize, respectively, while D_{legume} and D_{maize} are legume and maize densities, respectively.

Because *M. nigrivenella* eggs are not easily detectable, pods were stored at 26 ± 1°C and RH of 75 ± 5% for ten days to collect not only the already established larvae in the pods but also additional larvae hatching from non-detected eggs.

Spatial distribution of **M. nigrivenella** *on maize and* **C. ensiformis**

Taylor's (1961) power law was used to describe the dispersion of larvae and pupae of *M. nigrivenella* on maize and *C. ensiformis*. This law postulates a consistent relationship between variance S^2 and mean m:

$$S^2 = am^b \tag{1}$$

where b is a measure of dispersion of the species, with $b > 1$ indicating an aggregated distribution, $b = 1$ randomness, and $b < 1$ a regular distribution, while a is considered a mere scalar factor without biological meaning. These coefficients were computed by regressing the natural logarithm of the between plant variance ($\ln S^2$) against the natural logarithm of mean density (lnm), for each field or sampling occasion. A General Linear Model (proc GLM) (SAS, 1997) was used to compare the b-values.

Wilson and Room (1983) incorporated Taylor's coefficients (a and b) in a model describing the relationship between the proportion of infested plants [P(I)] and a mean density (m) where:

$$P(I) = 1 - e^{-m \ln(a m^{b-1}) / (a m^{b-1} - 1)} \tag{2}$$

(e is the base of natural logarithms). According to Wilson (1982), both a and b are needed to describe the dispersion of a species, i.e., the more aggregated a species, the smaller P(I) for a given mean. The P(I) – m curve can be used for a quick estimate of *M. nigrivenella* densities.

Statistical analysis

Differences in the insect counts per ear among the treatments were analyzed by an analysis of variance (ANOVA), using the mixed model procedure (SAS, 1997) for repeated measures over sampling dates. Treatments were considered as fixed effects, while blocks and plants within replications were considered as random. Wherever the interaction between treatments and sampling dates was significant, treatments were compared at each sampling date by means of ANOVA. An F test was used to test the significance of mean differences and least square mean (LSM) values were computed. The significance level was set at $P \leq 0.05$.

Differences in insect feeding days per plant and per plot basis, and cob damage among cropping patterns were also analyzed by ANOVA, using a GLM procedure. Whenever significant F values were obtained means were separated by the Student Newman Keuls (SNK) at $P \leq 0.05$. Counts were log (x+0.5) and percentages arcsine transformed before analyses. However, non-transformed means are reported in the tables.

Correlation coefficients were calculated using data pooled across treatments and both seasons.

Results

Pest infestations

Mussidia nigrivenella larvae were recovered from maize and *C. ensiformis* but not from *S. rostrata* and cowpea. On cowpea, *Maruca vitrata* Fabricius (Lepidoptera: Crambidae) was the predominant insect pest.

The effects of leguminous border rows on borer densities on maize varied with sampling date and season (Table 3.1). In general, *M. nigrivenella* densities increased towards harvest. During the main season, differences in pest densities varied with sampling date ($F = 75.5$, $P<0.0001$). Around harvest at 116 DAP, they were higher in maize alone than the other treatments. During the minor season from 88 DAP onwards, *M. nigrivenella* densities were always highest in maize alone plots except for 102 DAP, when differences between maize alone and Canav8 were not significantly different.

Table 3.1: Mean (±SE) number of *Mussidia nigrivenella* and total borers per maize cob in the different treatments (maize crops with leguminous border rows and maize alone) and days after planting (DAP) in southern Benin, during the main and minor cropping season of 2005

	Main season							
	M. nigrivenella				Other cob borers			
Treatments	74 DAP	88 DAP	102 DAP	116 DAP	74 DAP	88 DAP	102 DAP	116 DAP
Canav3	0.10±0.04aB	0.00±0.00bA	0.53±0.29B	1.10±0.36cC	1.03±0.25A	1.33±0.42A	0.80±0.18bA	0.33±0.19B
Canav1	0.00±0.00bA	0.10±0.07aB	0.30±0.13B	1.93±0.45bC	0.67±0.17B	1.03±0.21A	1.30±0.29aA	0.63±0.21B
Cowpea	0.17±0.11aA	0.13±0.13aA	0.47±0.18A	1.17±0.31cB	0.60±0.15BC	0.87±0.24B	1.90±0.47aA	0.40±0.21C
Sesbania3	0.20±0.23aB	0.00±0.00bA	0.60±0.40B	1.63±0.49bcC	1.00±0.17B	0.87±0.35C	1.20±0.31aAB	0.77±0.32A
Maize alone	0.23±0.11aA	0.03±0.03aA	0.33±0.13A	2.30±0.32aB	1.03±0.30A	1.27±0.34A	1.23±0.24aA	0.27±0.10B
DF	4,145	4,145	4,145	4,145	4,145	4,145	4,145	4,145
F	2.45	2.48	0.10	3.55	1.0	0.76	2.50	0.94
P-value	0.05	0.05	0.98	0.009	0.41	0.55	0.05	0.44
	Minor season							
Canav8	0.13±0.06aA	0.03±0.03cA	0.63±0.31abB	0.43±0.16bB	0.67±0.21A	0.57±0.11bA	0.40±0.18aA	0.17±0.08B
Canav4	0.03±0.03bA	0.33±0.13bB	0.07±0.05cA	0.13±0.06bA	0.93±0.30A	0.80±0.19bA	0.57±0.12aA	0.20±0.11B
Cowpea	0.00±0.00bA	0.23±0.11bcB	0.40±0.16bB	0.50±0.21bB	0.40±0.20A	0.90±0.23bA	0.50±0.18aA	0.07±0.05B
Sesbania4	0.00±0.00bA	0.23±0.10bcB	0.17±0.07cB	0.20±0.07bB	0.53±0.16A	0.67±0.18bA	0.17±0.07bB	0.13±0.06B
Maize alone	0.03±0.03bA	0.93±0.30aB	0.80±0.21aB	1.13±0.29aB	0.52±0.13B	1.17±0.20aA	0.17±0.01bC	0.13±0.08C
DF	4,145	4,145	4,145	4,145	4,145	4,145	4,145	4,145
F	2.42	3.84	3.69	4.40	0.88	2.43	2.56	0.45
P-value	0.05	0.0054	0.0069	0.0022	0.48	0.050	0.04	0.77

Canav1, 3, 4, 8 = Canavalia border rows sown 1, 3, 4, 8 weeks before maize, and Sesbania 3, 4 = Sesbania sown 3 and 4 weeks before maize. Means within columns and season followed by the same lower case letter(s) and within rows followed by the same uppercase letter(s) are not significantly different at P≤ 0.05 (NSK-test).

Table 3.2: Feeding days of *M. nigrivenella* (mean ±SE) on maize and leguminous border rows

	Per plant		Per plot (Legumes and maize)
Treatments	Legumes	Maize	
		Major season	
Canav3	659.8 ± 88.2aA	15.9 ±10.2B	149247.5 ± 9222.2a
Canav1	838.3 ± 58.5bA	19.1 ± 4.0B	87436.5 ± 4714.8b
Cowpea	-	17.7 ± 8.1	6454.9 ± 2958.1c
Sesbania3	-	21.2 ± 12.5	7728.9 ± 4558.8c
Maize alone	-	22.9 ± 2.4	8323.5 ± 862.0c
DF	1,6	4,145	4,145
F	7.72	0.36	151.4
P-value	0.03	0.83	< 0.0001
		Minor season	
Canav8	3039.9 ± 503.5A	7.4 ± 3.8aB	296674.0 ± 28882.1a
Canav4	2527.0 ± 323.3A	1.4 ± 0.7bB	245055.1 ± 17626.9b
Cowpea	-	6.3 ± 1.7ab	4501.5 ± 84.9c
Sesbania4	-	2.5 ± 0.5b	2548.0 ± 819.1c
Maize alone	-	13.5 ± 0.2a	11805.7 ± 945.8c
DF	1,6	4,145	4,145
F	0.73	4.9	93.1
P-value	0.4	0.019	<0.0001

Means within columns and season followed by the same lower case letter and means per plant within rows and season followed by the same uppercase letter(s) are not significantly different at P≤ 0.05 (SNK-test)

During both seasons *M. nigrivenella* feeding days were considerably higher on *C. ensiformis* than on maize (F= 157.8 and F= 585.6 with P<0.0001 for Canav3 and Canav1 respectively and F= 101.8 and F=183.1 with P<0.0001 for Canav8 and Canav4 respectively, Table 3.2). During the main season, feeding days were lower on early compared to late planted *C. ensiformis* (F = 7.72; P = 0.03), whereas during the minor season there was no difference between the two treatments (F = 0.74; P = 0.4). By contrast, during the main season number of feedings days on maize did not vary with treatment (F = 0.36; P = 0.83), whereas during the minor season, they were higher on maize alone and Canav8 than Canav4 and Sesbania4. (F = 4.9; P = 0.019). During both seasons, cumulative feeding days on a per plot basis were highest on maize surrounded by *C. ensiformis* planted early, followed by *C. ensiformis* planted late, and considerably lower but similar on the remaining treatments.

During both seasons, numbers of feeding days by *S. calamistis* were higher than those of *M. nigrivenella*, while *E. saccharina* and *T. leucotreta* were less common (Table 3.3). During the main season, no differences in *S. calamistis* and *E. saccharina* feeding days were observed between treatments. Feeding-days of *T. leucotreta* were higher on maize alone and

Canava1 than on Canav3 but all three were not different from the other treatments. During the minor season, *S. calamistis* feeding days were higher on Canav8 and maize alone than Cowpea and Sesbania4, while Canav4 was not different from any of these treatments, while *E. saccharina* feeding-days were higher for Canav8 than Sesbania4 and maize alone.

Table 3.3: Feeding days (Means ±SE) of *M. nigrivenella* and of other cob-boring pests on maize

Treatments	*M. nigrivenella*	*S. calamistis*	*E. saccharina*	*T. leucotreta*	F	P
			Main season			
Canav1	19.1±4.0C	29.4±5.0D	9.6±2.1B	2.8±1.2bA	12.4	<0.001
Canav3	15.9±10.2AB	25.0±6.1B	8.4±2.0A	6.1±1.6aA	3.9	0.01
Cowpea	17.7±8.1B	30.1±5.3C	11.9±2.9B	3.7±1.2abA	8.7	<0.001
Sesbania	21.2±12.5B	28.9±7.0B	8.6±2.9A	3.7±0.9ab A	4.9	0.003
Maize	22.9±2.4C	29.6±5.6C	11.7±2.9B	2.8±1.0aA	10.5	<0.001
F	0.36	0.19	0.80	1.44		
P	0.83	0.91	0.53	0.22		
		Minor season				
Canav8	13.3±6.8bBC	16.8±2.7aC	8.9±2.4aB	1.4±0.6A	11.8	<0.001
Canav4	6.8±1.2cA	14.7±3.1abB	6.3±1.9abA	2.1±1.5A	7.1	<0.001
Cowpea	12.4±0.2bB	12.8±3.2bB	4.7±1.7abA	2.1±0.9A	6.5	<0.001
Sesbania	7.0±0.3cB	10.0±2.6bB	2.3±0.9bA	1.6±0.8A	7.2	<0.001
Maize	32.4±2.6aC	16.6±2.6aB	2.3±1.4bA	4.2±1.3A	16.3	<0.001
F	4.31	3.06	2.55	1.51		
P	0.028	0.019	0.042	0.20		

Means within columns and season followed by the same lower case letter and means within rows and season followed by the same upper case letter(s) are not significantly different at P≤ 0.05 (SNK-test)

Damage variables and yield

During the main season, per area yields did not vary between treatments, while ear damage was lower on maize alone, Canav3 and Sesbania3 as compared to Canav1 and Cowpea (Table 3.4). Percent ear damage was lower on Canav1 and Cowpea than the other treatments. During the minor season, per area yields did again not vary between treatments. Ear damage was higher in the Canavalia than the maize alone treatment whereas the cowpea and Sesbania4 treatments were not significantly different from either. There were no significant differences in grain loss between treatments.

Ear damage and grain loss were not correlated with *M. nigrivenella* feeding days (R = 0.16, P = 0.38, and R = 0.26, P = 0.16, respectively) but there was a significant positive relationships with other ear-boring Lepidoptera (R = 0.67, 0.72, respectively, P<0.0001).

Table 3.4: Effect of leguminous border rows on percent plants infested with *M. nigrivenella* [P(I)], on maize yields, damage by ear borers and grain loss (means ± SE)

Treatment	P(I)	Ear damage (%)	Yield (kg/Ha)	Grain loss (%)
			Major season	
Canav3	15.8 ± 6.5	16.2 ± 2.8b	6338.4±844.0	11.2 ± 1.9b
Canav1	23.3 ± 9.2	21.9 ± 2.4a	5267.2±342.3	12.0 ± 1.5b
Cowpea	21.7 ± 6.8	22.9 ± 3.3a	5339.8±894.9	20.8 ± 2.9a
Sesbania3	20.8 ± 6.5	13.1 ± 1.4b	5626.6±561.9	12.0 ± 1.9b
Maize alone	32.5 ±10.1	11.7 ± 1.4b	5964.0±465.5	15.6 ± 1.9ab
DF	4, 145	4, 145	4, 16	4, 145
F	1.52	4.84	0.58	3.60
P-value	0.22	0.0011	0.69	0.0079
			Minor season	
Canav8	11.7 ± 3.9b	10.0 ± 2.4a	5267.4±492.5	5.8 ± 1.8
Canav4	16.7 ± 4.8b	6.5 ± 1.1a	4654.2±1154.8	3.9 ± 0.9
Cowpea	15.8 ± 4.7b	7.4 ± 1.4ab	6259.4±440.3	4.2 ± 1.3
Sesbania4	13.7 ± 4.5b	6.0 ± 1.6ab	5924.2±186.9	3.3 ± 1.4
Maize alone	31.7 ± 4.5a	4.0 ± 1.1b	7450.0±1395.7	3.1 ± 1.1
DF	4, 145	4, 145	4.16	4, 145
F	2.79	2.61	1.49	1.32
P-value	0.039	0.038	0.28	0.27

Canav1, 3, 4, 8 = Canavalia border rows sown 1, 3, 4, 8 weeks before maize, and Sesbania3, 4 = Sesbania sown 3 and 4 weeks before maize. Means within column and season followed by the same lower case letter(s) are not significantly different at P≤ 0.05 (SNK-test).

Spatial distribution of M. nigrivenella

All regression slopes were greater than 1, indicating an aggregated distribution of *M. nigrivenella* on the two host plant species used (Table 3.5). They did not significantly vary with host plant species (F = 0.07, P = 0.81 in main season and F = 0.20, P = 0.68 in minor season), thus a common slope and intercept was used for fitting the P(I)-m curve (Figure 3.1). The curves calculated for *C. ensiformis* and maize fit well the observations and could be used for a quick estimate of the density ranges shown in figure 3.1.

Table 3.5: Taylor's Power coefficients (means ±SE) for *M. nigrivenella* on maize and *Canavalia ensiformis*

Host plant	ln (a)	b	R^2	P
	Main season			
	0.81 ± 0.06a	1.12 ± 1.05a	0.32	0.02
C. ensiformis	0.89 ± 0.14a	1.35 ± 1.13a	0.23	0.0098
	0.82	0.07		
P-value	0.42	0.81		
	Minor season			
Maize	0.84 ± 0.05b	1.21 ± 1.04a	0.39	<0.0001
C. ensiformis	1.79 ± 0.31a	1.57 ± 1.18a	0.26	0.0031
F	27.5	0.20		
P-value	0.006	0.68		

Coefficients within column and season followed by the same letter are not significantly different at P≤ 0.05

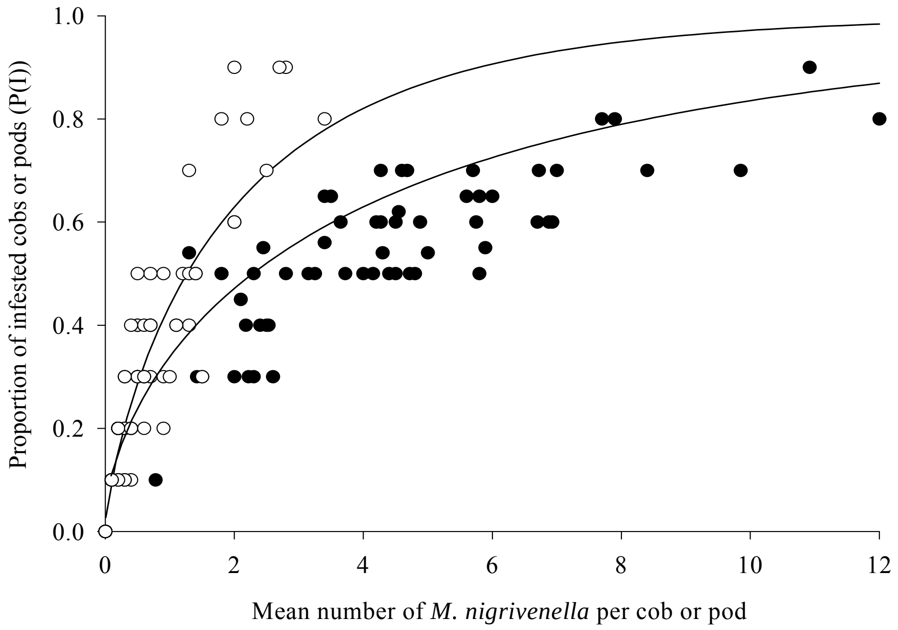

Figure 3.1: Proportion of infested cob or pod (P(I)) as function of mean number of *M. nigrivenella* per cob; observed (open symbols: maize; closed symbols: Canavalia) and calculated via equation 2 (line)

Discussion

The data reported here indicate that the presence of leguminous crops in the vicinity of maize fields do not aggravate *M. nigrivenella* infestations on maize, irrespective of when the legume was planted in relation to maize, and irrespective of the status of the legume species as a host. Thus, although the suitable host *C. ensiformis* had up to 1800 times higher pest loads than maize, there appeared to have been no movement of *M. nigrivenella* to maize. In fact, during the second season, at harvest, all legume treatments had lower *M. nigrivenella* densities on maize than the maize alone treatment. Also, maize surrounded by *C. ensiformis* planted four weeks before, had lower pest loads expressed as cumulative feeding days as compared to maize alone and cowpea. This indicates that *C. ensiformis* was much more attractive to the ovipositing moth than maize. The fact that during the minor season *C. ensiformis* planted 4 weeks before maize had an effect on pest loads on maize while when planted 1 and 3 weeks before in the main season or 8 weeks before in minor season it did not, suggests that the growth stage and/or canopy density of the plants affected the attractiveness of the leguminous crop to the ovipositing moth. As shown by Ritchie (2000) and Haddad et al. (2001), the amount of above-ground plant biomass, which however was not assessed in the present experiment, may have a strong effect on insect abundance. Furthermore the volatile profile of plants changes with the growth stage (Batten et al. 1995; Zhang et al. 2008), which might affect the oviposition behaviour of the moth.

The lack of significant differences in pest loads on maize between most treatments and maize alone also indicates that *C. ensiformis* did not enhance the activity of natural enemies – both predators and parasitoids - as suggested for other systems (Altieri 1995; Risch 1981; Bugg et al. 1991; Tillman et al. 2004). However, parasitoids of *M. nigrivenella* are scarce in Southern Benin and appeared to play no role in the population dynamics of this pest (Sétamou et al. 2002). Similarly, in the present study no parasitoids were obtained during rearing of larvae in the laboratory from *M. nigrivenella* collected from maize or cover crops.

Likewise, planting non-hosts such as cowpea and *S. rostrata* had little effect on infestations of maize by *M. nigrivenella*, though during the minor season maize surrounded by *S. rostrata* had lower pest loads than without. Similarly, Ndemah et al. (2002b) showed that non-hosts such as grasses planted as border rows could reduce densities of cereal stemborers and of *M. nigrivenella* on maize. It was suggested that grasses acted as a barrier to the adult moths in search of a suitable host plant, which would explain the variability of the efficacy of this control technique (Ndemah et al. 2006, 2007; Matama-Kauma et al. 2006) since only well established borders had an effect. We therefore recommend testing perennial non-hosts as barrier crops and achieve a gradient of barrier effect by cutting back the non-host at different times before planting maize.

A more efficient technique for reducing borer densities on maize proved to be the intercropping of non-host species with maize, because the presence of the non-host plants hampers host finding by the ovipositing moth, and thus reducing the number of egg batches deposited on maize (Ndemah et al. 2003; Schulthess et al. 2004; Chabi-Olaye et al. 2005b; Wale et al. 2007; Songa et al. 2007). When diverse backgrounds of plants 'disrupt' insects from selecting otherwise acceptable host plants, the action is mediated through, among other factors, visual camouflage (Smith, 1969) or deterrent/repellent chemicals (Altieri et al. 1978; Uvah and Coaker 1984). Furthermore, both young and old instars of several African stemborer species disperse from the original oviposition site to other plants (Kaufmann 1983; Berger 1989, 1992). Thereby, the chances of encountering a suitable host plant is reduced in mixed cropping systems with non-host plant species leading to high mortality in the dispersing larvae (Ndemah et al. 2003; Schulthess et al. 2004; Chabi-Olaye et al. 2005b; Wale et al. 2007; Songa et al. 2007). Thus, Agboka et al. (2006) studying maize-legumes and cassava mixed systems showed that maize intercropped with *C. ensiformis* and *T. vogelii* reduced *M. nigrivenella* infestation in maize crop, the first acting as a trap crop and the latter as a repellent crop.

Some of the legume treatments used in our experiments also lowered pest loads of *S. calamistis* and *E. saccharina* on maize, while others increased them. The reasons are not obvious because both stemborer species only attack plants of the Poaceae and Cyperacea families (Gounou and Schulthess 2004; Atachi et al. 2005; Le Rü et al. 2006). *Sesamia calamistis* only oviposits on pre-tasseling plants (Kaufmann 1983; Sémeglo 1997) and females avoid plants with egg batches; the resulting distributional pattern is thus regular to random (Sétamou and Schulthess 1995). Thus, in some treatments the border rows might have acted as barrier crops, while in others they might have arrested the ovipositing moth and kept it from moving to other fields. Again, the effects were not consistent and varied with season.

In general, differences between treatments in insect (i.e., borer-days, percentage of infested plants) and damage variables (ear damage, grain loss) were not consistent, and treatments did not have any effect on per area yields. Thus, planting leguminous border rows as a mean of controlling *M. nigrivenella* appears not to be economically meaningful. However, the major finding of the present study is that the presence of leguminous cover crops or grain legumes in the vicinity of maize fields does not increase *M. nigrivenella* densities on maize. Moreover, as shown by Chabi-Olaye et al. (2005a), grain legumes and cover crops enhance yields and reduce yield losses in maize crops subsequently planted in the same field by improving plant vigour. It is obvious that a single control option will not produce satisfactory control of the complex pest problem caused by lepidopteran maize pests, and as proposed by Chabi-Olaye et al. (2006) an IPM package including crop rotation with leguminous cover or grain crops or direct application of synthetic fertilizer, mixed cropping and timely applications of insecticides or botanicals targeting not only *M. nigrivenella* but all stem- and earborers is required.

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CHAPTER 4

Effects of plant extracts and oil emulsions on the maize ear-borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) in laboratory and field experiments

Komi Agboka[1, 2*], Agbodzavu K. Mawufe[3], Manuele Tamò[1] and Stefan Vidal[2]

[1]International Institute of Tropical Agriculture, 08 BP 0932 Tripostal, Cotonou, Republic of Benin. [2]Georg-August-University Goettingen, Agricultural Entomology, Grisebachstrasse 6, 37077 Goettingen Germany. [3]University of Lomé, Ecole Supérieure d'Agronomie BP 1515 Lomé Togo.

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Abstract

Aqueous extracts of *Tephrosia vogelii* and *Hyptis suaveolens*, and of oils of *Azadirachta indica* and *Jatropha curcas*, as well as the pesticide Furadan 5G were evaluated for their insecticidal activity against the maize earborer *Mussidia nigrivenella* in laboratory and field experiments. In general, treated plants had a strong deterrent effect on ovipositing *M nigrivenella*. The ODIs (oviposition deterrence index) were highest with neem oil at both concentrations, *J. curcas* at 5% and *H. suaveolens* at 20%. In addition, egg hatch was adversely affected by neem and *Jatropha* oils; it decreased with an increase in concentrations of oil emulsions and varied between 3-25.5% for neem and 6-16% for *J. curcas*. The LC50 calculated were 1.3% and 0.8% respectively for neem and *J. curcas*. By contrast, larval survival was not affected by the oil treatments. In the field, Furadan, neem and *J. curcas* oils significantly reduced the number of *M. nigrivenella* larvae by 16-49.2%, while aqueous extracts of *T. vogelii* and *H. suaveolens* were similar to the control consisting of emulsified water. The treatments did not significantly influence cob weight, and only neem oil at both concentrations and furadan significantly reduced ear damage and consequently grain losses. These results showed that oil emulsions of *A. indica* and *J.*

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[*] Corresponding Author: Tel: +22921350188, Fax: +22921350556, E-mail: k.agboka@cgiar.org

curcas oils act not only as oviposition deterrent but also as ovicides. The prospects for possible inclusion of botanicals into integrated *M. nigrivenella* control in maize cropping systems are discussed.

Keywords: Aqueous extracts, *Azadirachta indica*, botanicals, integrated pest management, *Jatropha curcas*, oviposition.

Introduction

Mussidia nigrivenella Ragonot 1888 (Lepidoptera: Pyralidae) is one of the key pests attacking maize ears in West Africa (Moyal, 1988; Bosque-Pérez and Mareck, 1990; Shanower et al., 1991; Moyal and Tran 1991 a, b; Silvie, 1993; Sétamou et al., 2000). In field-grown maize, reported yield losses vary between 5 and 25% (Moyal & Tran, 1991b) with additional losses of 10-15% in stores (Sétamou et al., 2000). Moreover, *M. nigrivenella* damage predisposes maize to pre- and post-harvest infestation by coleopteran pests, *Aspergillus flavus* Lk. Fr. (Deuteromycetes: Monoliales) infections and subsequent aflatoxin contamination (Sétamou et al., 1998). Hence both the quantity and quality of maize are affected (Sétamou et al., 1998).

Currently available control options include sun-drying of the cob after harvesting, and insecticides (Sétamou, 1999), but none provides satisfactory control. Probably due to the cryptic nature of the larvae and the timing of application, *M. nigrivenella* is particularly difficult to control with insecticides (Moyal, 1988; Sétamou et al., 1995; Ndemah and Schulthess, 2002). Moreover, insecticides are often not affordable to African peasant farmers. Therefore, biopesticides such as neem products extracted from seeds of the neem tree, *Azadirachta indica* Juss (Meliacae), which are locally available, are of special interest. They are categorized as broad spectrum insecticides and alternatives to synthetics (Isman, 2006, Immaraju, 1998). Juan and Sans (2000) and Carpinella et al. (2002) showed that extracts of neem seeds and fruits had antifeedant activity in larvae of *Sesamia nonagrioides* (Lefebre) or *Spodoptera eridania* (Lepidoptera: Noctuidae). Crude extract of *A. indica* showed growth inhibitory, antifeedant and toxic effects against two noctuids, cabbage looper, *Trichoplusia ni* (auth.) and armyworm *Pseudaletia unipuncta* (Akhtar et al., 2008).

In Africa, apart from neem products, other extracts derived from indigenous plants such as *Hyptis suaveolens* L. (Lamiacae), *Tephrosia vogelii* (Fish bean) Hook F. (Leguminosae) and *Jatropha curcas* (physic nut) L. (Euphorbiacae) recently gained attention with regard to their insect pests control potential. According to Gaskins et al*.*, (1972) leaves of *Tephrosia* species contain at least four compounds that possess insecticidal properties. Likewise, *H. suaveolens* has been recently shown to possess insecticidal properties (Perry, 1980) and, among others, was effective against the cowpea weevil during storage (Fatope et al., 1995). An ovicidal effect of *J. curcas* oil has been demonstrated on *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae) (Shelke et al., 1987). Botanicals have been successful against a number of maize pests in Africa. Mugoya and Chensembu (1995) reported that aqueous fresh-leaf extracts of *T. vogelii* reduced the incidence of the spotted stalk borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) in maize in Zambia. Siddiqui et al, (1990) tested neem products against the spotted stalk borer, and Bruce et al. (2004) against the stemborers *Sesamia calamistis* and *Eldana saccharina*, while Bekele et al. (1997) and Ogendo et al. (2008) used indigenous plant extracts against stored maize pests. The present work aimed at assessing the efficacy of botanicals occurring in West Africa in controlling *M. nigrivenella*.

Materials and methods

Extraction and formulation of **A. indica** *and* **J. curcas** *oils*

Neem oil extraction followed a protocol described by Lale and Abdulrahman (1999). Ripe neem fruits were sun-dried before oven drying at 50°C for 72 hours. Kernels obtained after decortication, using a mortar and pestle, were ground in an electric blender. The neem seed powder obtained was then moistened and kneaded till oil started oozing. The neem oil, which was analyzed by Trifolio-M GmbH, Germany, contained ≈ 0.009% azadirachtin. Neem oil was formulated with a water and emulsifier solution (soap without any detergent) to prepare the different concentrations of neem oil emulsion.

The protocol for the extraction of *J. curcas* oil followed a method developed by Atchall (1999). Shelled seeds of physic nut were treated like the neem seeds and then ground. Five kilograms seed paste were soaked in 37.5 l of water. The mixture, homogenized using an agitator, was then boiled for 5 hours. The volume of the mixture was maintained constant by adding water (approximately 5 l/hour). After two hours, the foaming cream formed on the surface of the mixture was collected periodically. The cream - a mixture of water, oil cake, and oil - was heated for 30 min to retrieve oil floating on the solid residue. The oil thus obtained was oven dried at 105° C for 15 min. and formulated as required for the experiments.

Preparation of aqueous extract of **T. vogelii** *and* **H. suaveolens**

Aqueous extracts at 15 % and 20 % were prepared by soaking 3.75 and 5 kg pounded fresh plant material (leaves and fruits, respectively) in 25 liters of water, leaving it for 12 hours, and then filtering it through muslin cloth. A solution of emulsifier was added to each preparation before field application.

Insect culture

A laboratory culture of *M. nigrivenella* originating from specimens collected from field grown maize in the Southern Guinea savanna of Benin was established at IITA. The insects were continuously reared for two generations on *Canavalia ensiformis* (L) (Fabacae) pods following the protocol developed by Sétamou et al. (1999). Pieces of mature jack bean pods were infested with red eye-spotted eggs (egg stage after five days with a visible red point) collected from the oviposition cage. The pieces were then maintained in 14 cm high and 11 cm diameter plastic containers, and incubated at 26 ± 2°C and a 12:12 (L:D) photoperiod. At pupation the pupae were collected and transferred to oviposition cages.

Oviposition behavior

For the oviposition experiment, maize plants were produced in pots. Silking plants were transferred to metallic cages. 2 m high, 2m long, and 1 m wide cages (blocks) were used for this study and repeated five times. All cages were covered with a netting material to avoid external infestations and to prevent the experimental moths from escaping. At the dough stage, each treatment (except Furadan) was assigned to a pot containing two plants. Approximately eight hours after applying the treatment, five newly-emerged pairs of *M.*

nigrivenella were released in the center of each cage in a Petri dish supported by a cylindrical tube placed in soapy water to keep away predators such as ants. Newly emerged insects were used in the study, as *M. nigrivenella* has no pre-oviposition period and lay the maximum eggs during the first two days (Sétamou et al., 1999). Five days later, cobs were sampled and examined under a binocular microscope. Eggs deposited were counted and recorded. Oviposition deterrence index for the extracts and oils was calculated using the formula: [(C-T)/(C+T)]*100 (Akhtar and Isman, 2003) where C= number of eggs laid on the control and T = number of eggs laid on the treated cobs.

Toxicity to eggs and larval survival

Eggs (2-3 days old) laid on tissue papers were collected from the oviposition cage and adjusted to 50 eggs per tissue paper. These tissues were treated with different concentrations (0, 2.5, 5, 10 and 100%) of neem and physic nut oils and allowed to be air-dried for one hour. The treated tissue papers were then transferred to *C. ensiformis* pods. The pods were introduced into plastic containers (20 cm long, 12 cm diameter, and 10 cm high) and stored in the laboratory at 27±2^{o}C and about 70% RH. After 6 days the numbers of hatched eggs were recorded, and the numbers of larvae still alive were counted after 7 days. The treatments was replicated four times

Topical toxicity of neem and **J. curcas** *to second instar larvae*

Sets of second instars larvae were topically treated with the different concentrations of the oils emulsions and fed *Canavalia* pods by applying the treatment on the anterior pronotum of the second instar larvae using a microliter syringe. The larvae were placed in the *C. ensiformis* pod for feeding. Larval mortality was recorded every week. The number of larvae that reached the pupal stage was also recorded in both experiments. The treatments were replicated four times.

Field trial

The experiment was conducted to evaluate the efficacy of plant extracts and oils in controlling *M. nigrivenella* in field conditions. The study was carried out at the

International Institute of Tropical Agriculture (IITA)-Benin station in Abomey-Calavi (6^{o}24’N, 2^{o}24’E), Republic of Benin, during the first growing season of 2004. The site is located in the derived savanna characterized by a bimodal distribution of rainfall with peaks in June and September, and an annual precipitation of about 1200 mm. During our trials, monthly mean rainfall recorded was 189.2 mm and temperatures ranged from 18.5^{o} C to 32.6°C, with a minimum in September and a maximum in October.

A 4-month maize variety QPM (Quality Protein Maize) was used in this study. The experiment was laid out in a complete randomized block design consisting of ten treatments, each replicated four times. The experimental plots measured 12.5 m × 7 m. Plots were separated by 2 and blocks by 3 m. Alleys were sown with sorghum three weeks before planting maize in order to reduce interaction between treatments. Maize was sown at three seeds in a pocket a spacing of 0.4 m within rows and 0.75 m between rows. Two weeks after sowing, the crop was thinned down to two plants per pocket. NPKSB 14-23-14-5-1 fertilizer was applied 14 and 45 days after sowing at a rate of 60 kg/ha for each application.

The treatments consisted of aqueous extracts of *H. suaveolens* and *T. vogelii* at concentrations of 15 and 20%, oil emulsions of *A. indica* and *J. curcas* at concentrations of 2.5 and 5% and of the synthetic insecticide Furadan 5 G (5% of active ingredient). Emulsified water and an untreated check served as controls. The concentrations of plant extracts used in the present trial are commonly used by local farmers against cowpea pests (Tamo, unpublished data).

The botanical treatments and the control were applied three times, i.e. at the soft dough stage, approximately 70 days after sowing (DAS), and two subsequent applications at two-weekly intervals, according to the infestation pattern of *M. nigrivenella* in the field (Moyal and Tran, 1991a). At each application, the whole ear was sprayed including the silks. Because of its persistence, Furadan was applied only once at the rate of 2g per plant by placing the granules between the stem and the leaf immediately below the ear.

A week after each spray, a sample of ten cobs was randomly taken from each plot. The cobs were taken to the laboratory and checked thoroughly for eggs before being de-husked. However, as eggs were rarely obtained – they are very difficult to detect –

cumulative numbers of larvae and pupae only are presented in the tables.

M. nigrivenella *and other borer's densities*

Different stages of larvae and pupae of *M. nigrivenella* and other borers species found in the maize ear such as *Eldana saccharina* Walker 1865 (Lepidoptera: Pyralidae) and *Sesamia calamistis* Hampson 1910 (Lepidoptera: Noctuidae) were recorded. The density of *M. nigrivenella* at each sampling date was also determined.

Larval survival and parasitism

The data collected here aimed at examining whether the treatments have an effect on larvae and parasitism. All larvae collected were therefore kept in the laboratory at 27 ± 2^{o}C and 70% RH to record larval mortality and parasitism.

Cob weight and damages

The effects of plant extracts and oils on cob weight and damages by cob borers were determined. At harvest, a last sampling was done to assess ear weight, damages and grains losses. The damage caused by the ear borers was calculated as the percentage of grains consumed by the borer and/or contaminated by fungi. The loss (in g) was calculated as the difference between the initial weight of the ear and the weight of the ear after removing the damaged grains.

Statistical analysis

Differences in the insect counts per ear among the treatments in field trial were analyzed by analysis of variance (ANOVA), using the mixed model procedure (SAS, 1997) for repeated measures over sampling dates. Treatments were considered as fixed effects while plants within replications were considered as random. Percentages were arsine and counts were log (x+1) transformed before analyses. Least square means (LSM) of treatments across sampling date and within sampling date and of sampling date within treatment were separated using the t-test. Means of eggs hatching and larval survival in laboratory experiment were separated by Student-Newman and Keuls' test. Significance was set at p

≤ 0.05. Number of eggs deposited on treated and untreated plants in oviposition experiment was analyzed by a non parametric ANOVA using Kruskal Wallis test.

Results

Oviposition behavior

Plant extract and oil emulsion treatments significantly reduced oviposition on the plants offered. More eggs were laid by *M. nigrivenella* on untreated than treated plants (DF = 8, $\chi2$ = 29.3, P = 0.0003; Figure 4.1). The oviposition deterrence index (ODI) was significantly higher with 5% *J. curcas*, both concentrations of neem oil and *H. suaveolens* at 20% compared to the other treatments and control (F = 12.12, P<0.0001) indicating complete oviposition deterrent effects by neem, *J. curcas* oils and *H. suaveolens* extract at their respective concentrations.

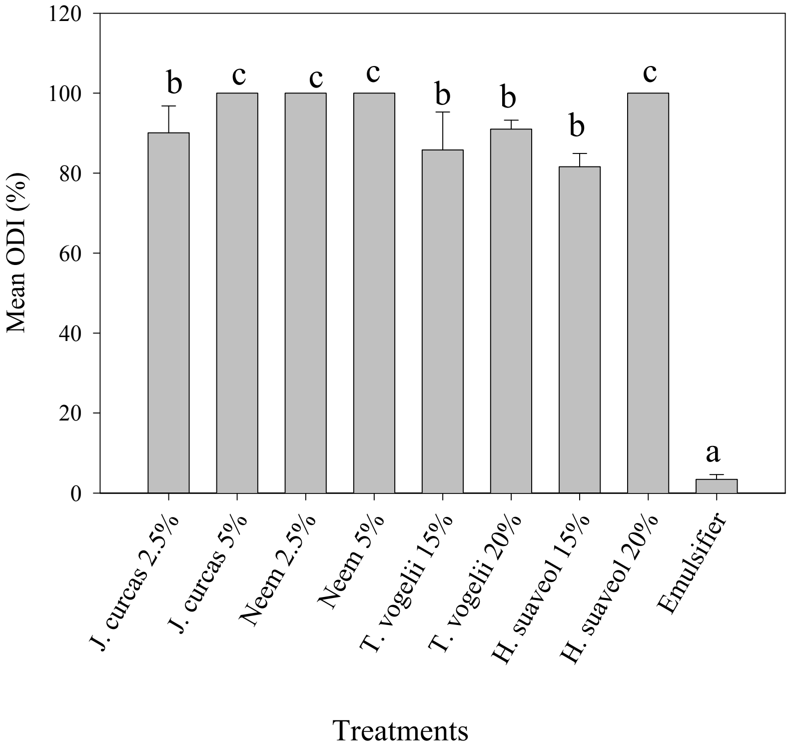

Figure 4.1: Oviposition deterrence index (ODI) of the extracts and oils

Toxicity to eggs and larval survival

Hatching of *M. nigrivenella* eggs was adversely affected by neem and *Jatropha* oils (DF=8, F=14.96, P<0.0001), and it decreased with increasing concentrations of the oils applied (Table 4.1). Only 3 to 6% of eggs hatched in the 100% oils treatments with neem and *Jatropha*, respectively, as compared to 66% in the control treatments. LC50 calculated for neem and *J. curcas* oils were 1.3% and 0.8% respectively. Percentages of larvae developing to the 5th instar and pupae were >75% in all treatments (Table 4.1).

Topical toxicity of neem and **J. curcas** *to second instar larvae*

Topical application of oil emulsion on second instar larvae did not have any significant effect on their survival when compared to the control (DF = 8, F = 1.27, P = 0.32), and pupae formation was >80%. (Table 4.2)

Table 4.1: Mean percentage (± SE) of egg hatching after treated with neem and *J. curcas* oils emulsions and survival of *M. nigrivenella* to pupae

Concentration (%)	% egg hatch		% larval survival	
	Neem	*J. curcas*	neem	*J. curcas*
2.5	25.5 ± 5.3b	16.0 ± 4.9b	96.8 ±1.9	100.0 ± 0.0
5	18.5 ± 4.3bc	11.0 ± 2.1b	100.0 ± 0.0	75.0 ± 15.0
10	11.5 ± 4.1c	8.5 ± 1.3bc	100.0 ± 0.0	100.0 ± 0.0
100	3.5 ± 2.2d	6.0 ± 1.4c	100.0 ± 0.0	83.3 ± 16.7
Control	66.0 ± 9.1a	66.0 ± 9.1a	97.2 ± 1.9	97.2 ± 1.9
LC50	1.3±0.2	0.8±0.1		

Means in a column followed by the same lower case letter(s) are not significantly different (DF= 7, 27; F=14.96, p<0.0001).

Field experiment

M. nigrivenella *and other borer's densities*

Significantly fewer *M. nigrivenella* larvae were recorded on plots treated with Furadan, *A. indica* and *J. curcas* than in the controls (df = 10, F = 7.27, P<0.0001) but there were no significant differences between concentrations of oil emulsions (Table 4.3). *Tephrosia vogelii* and *H. suaveolens* aqueous extracts did not significantly affect *M. nigrivenella* densities, except for the 20% concentration of *H. suaveolens*.

Neem and *Jatropha* oils, at both concentrations, Furadan, and extracts of *T. vogelii* at 20% significantly reduced the numbers of *E. saccharina* larvae but only neem oil and Furadan had an effect on *S. calamistis* (Table 4.3).

Table 4.2: Survival (mean ± SE) to pupa stage of second instar larvae of *M. nigrivenella* topically treated with neem and *J. curcas* oils emulsions

Concentration	% Larval survival*	
(%)	Neem	*J. curcas*
2.5	89.0 ±1.4	88.3 ± 2.9
5	87.0 ± 2.6	87.8 ± 3.9
10	88.4 ± 5.1	86.8 ± 2.2.0
Pure	85.0 ± 5.1	86.5 ± 5.1
Control	90.1 ± 1.0	90.1 ± 1.0

* No differences were found in all treatments (df= 8, F = 1.27, P = 0.32)

Table 4.3: Effects of emulsions of neem and *J. curcas* oils, Furadan, and aqueous plant extracts of *T. vogelii* and *H. suaveolens* on *M. nigrivenella* and other cob borer infestations

	Number of larvae per cob (mean ± SE)		
Treatments	*M. nigrivenella*	*S. calamistis*	*E. saccharina*
J. curcas oil 2,5%	0.29±0.06bc	0.29±0.05a	0.20±0.05b
J. curcas oil 5%	0.14±0.03c	0.30±0.055a	0.20±0.05b
A. indica oil 2,5%	0.14±0.03c	0.22±0.04b	0.11±0.03b
A. indica oil 5%	0.15±0.03c	0.19±0.04b	0.17±0.04b
T. vogelii extract 15%	0.53±0.11a	0.30±0.05a	0.37±0.06a
T. vogelii extract 20%	0.52±0.11a	0.30±0.05a	0.19±0.04b
H. suaveolens extract 15%	0.49±0.09ab	0.37±0.07a	0.41±0.08a
H. suaveolens extract 20%	0.34±0.10b	0.33±0.05a	0.40±0.08a
Furadan	0.13±0.03c	0.19±0.04b	0.10±0.03b
Emulsifier solution	0.53±0.09a	0.38±0.07a	0.44±0.06a
Control (Untreated check)	0.55±0.11a	0.40±0.09a	0.42±0.04a
DF	10, 1716	10, 1716	10, 1716
F	6.11	3.05	4.37
P	< 0.0001	0.001	< 0.0001

Means in column followed by the same letter(s) are not significantly at $P \leq 0.05$ (SNK)

Densities of *M. nigrivenella* varied significantly with sampling date and tended to increase towards harvest (df =3, F=16.4, P<0.0001) (Table 4.4). They were significantly lowest at the second sampling date (P<0.007). In addition, in the oil treated plots, *M. nigrivenella* densities tended not to vary with sampling date (Table 4.4).

Larval survival and parasitism

Survival of *M. nigrivenella* larvae from treated field was not affected by the treatment (DF = 10, F = 1.57, P = 0.12) and mortality varied from zero to 3.2% only.

No natural enemies were recorded on *M. nigrivenella* but *Cotesia* spp. cocoons were obtained from one *S. calamistis* larva.

Table 4.4: Mean ± SE number of *Mussidia nigrivenella* per maize cob in the different botanical and control treatments at different days after sowing (DAS)

	Sampling dates (DAS)			
Treatments	74	88	102	116
J. curcas oil 2.5%	0.33±0.13abAB	0.13±0.08bB	0.25±0.11bcAB	0.45±0.18bA
J. curcas oil 5%	0.15±0.07cA	0.13±0.06bA	0.10±0.05cA	0.18±0.07cA
A. indica oil 2.5%	0.23±0.08bcA	0.08±0.04bA	0.15±0.08cA	0.10±0.05cA
A. indica oil 5%	0.12±0.05cA	0.08±0.04bA	0.23±0.10c A	0.18±0.06cA
T. vogelii extract 15%	0.15±0.07cA	0.08±0.04bA	0.70±0.30aB	1.18±0.30aC
T. vogelii extract 20%	0.40±0.13aA	0.13±0.06bB	0.68±0.27abC	0.88±0.31aC
H. suaveolens extract 15%	0.28±0.11bA	0.10±0.05bA	0.93±0.28aB	0.68±0.19abB
H. suaveolens extract 20%	0.20±0.06bcA	0.13±0.05bA	0.58±0.0.36abB	0.45±0.13bB
Furadan	0.10±0.05cA	0.05±0.03bA	0.08±0.04cA	0.28±0.10bcA
Emulsifier solution	0.51±0.17aA	0.35±0.08aA	0.43±0.13abA	0.83±0.29aB
Control (Untreated check)	0.45±0.12aA	0.30±0.10aA	0.40±0.21bA	1.05±0.37aB
DF	10, 429	10, 429	10, 429	10, 429
F	2.02	1.92	1.97	3.50
P	0.03	0.047	0.04	0.0004

Means within column followed by the same lower case letter(s) and within row followed by uppercase letter(s) are not significantly different at $P \leq 0.05$

Cob weight and damage

The treatments did not significantly influence cob weight (P = 0.45, Table 5), and only neem oil at both concentrations and Furadan significantly reduced ear damage (DF = 10, F = 2.01, P = 0.038) and consequently grain losses (DF = 10, F = 2.25, P = 0.019).

Discussion

The results of field's trials showed that larval population of *M. nigrivenella* can be significantly reduced through the application of oil emulsions of *A. indica* and *J. curcas*, which were as efficient as Furadan 5 G. By contrast, plant extracts of *T. vogelii* and *H. suaveolens* were not efficient. Neem oils and pure compounds of neem have been found to exhibit ovipositional deterrent effects on many crop pests including lepidopteran, homopteran and dipteran species (Singh and Singh, 1998, Schmutterer 1990, Isman 1996, Bruce et al. 2004, Showler et al., 2004). According to Udayagiri and Mason (1995) chemical cues play a major role in host selection. In the ovipositional test, *M. nigrivenella* tended not to oviposit on maize plants treated with the oils indicating a repellent effect. Similar results have been observed by Bruce et al*.*, (2004), who found that application of neem oil at 0.075 ml/plant lead to a reduction in number of egg laid by *S. calamistis* and *E. saccharina* of 88 and 49%, respectively, compared to the control. Oviposition deterrence was also observed in *Liriomyza* spp. (Diptera: Agromyzidae) (Webb et al., 1983, Hossain and Poehling, 2006, Seljåsen and Meadow, 2006) and the beet armyworm (Greenberg et al., 2005). Loery and Isman (1993) suggest that this deterrence results from a variety of compounds working in concert with another, producing different behavioral responses, which vary in magnitude between species. Deterrent and ovicidal effects have also been reported for *J. curcas* (Adebowale and Adedire 2006, Boateng and Kusi 2008).

The reduction of *M. nigrivenella* larvae in the field could also be due to reduced egg viability as indicated by the findings of the laboratory experiment. Although eggs are supposed to be strongly protected by the impermeable chorion, which may inhibit the penetration of neem and *J. curcas* products, egg hatch was adversely affected by both oils. Schmutterer (1990) stated that the ovicides action of neem treatments is common, and the product can obstruct the egg aeropyle, thus impeding the respiratory changes during the embryonic development. Similarly, neem oil also affected the viability of *S. calamistis* and *E. saccharina* (Bruce et al., 2004), and of *Chilo partellus* (Lepidoptera: Pyralidae) (Siddiqui et al. 1990) eggs. Also, Schulz and Schüter (1983) showed changes in the ooplasm and vitelligenesis, which is necessary for oocytes maturity, resulting in egg sterility. This ovicidal effect is advantageous because larvae are killed before they can cause damage.

In this study contact toxicity of the oils on larvae could not be observed as nearly all larvae treated with oils developed to pupae. This indicated that 2nd instars larvae were not susceptible to neem and *J. curcas* oils used in the present experiment. At 100% the larvae demonstrated very low susceptibility to the oils and this result is very relevant, if we consider that in most studies, neem oil has caused insect mortality at doses between 0.1 and 5% (Schmutterer, 1990). The low contact toxicity of neem oil against *M. nigrivenella* larvae could be due to low azadirachtin (0.009%) content of the oil. However, adults of treated larvae were not reared to determine whether the treatments affected fitness. For example, Bruce et al. (2004) showed that neem oil reduced the fecundity of *S. calamistis* and *E. saccharina*. These findings indicate again that the treatments in the fields did not kill the larvae but deterred oviposition and affected the hatching of eggs. By contrast, in other studies neem and *Jatropha* deterred feeding of the larvae, when they were incorporated in the diet (Zabel et al., 1999). In laboratory tests conducted by Ratnadass et al., (1997), artificial diet supplemented with extracts of nuts of *J. curcas* at 0.01% and 1% crude oil concentration, yielded 100% of mortality of *Busseola fusca* Fuller (Lepidoptera: Noctuidae) and *S. calamistis* larvae. However, under natural conditions – except for first instar larvae – the larvae live cryptically inside the ear and may not be affected by the treatment. Thus, the treatments should be targeted towards the first larval instar, before they penetrate the ear.

Table 4.5: Effects of emulsions of neem and *J. curcas* oils, Furadan, and aqueous plant extracts of *T. vogelii* and *H. suaveolens* on ear weight and loss, and damage caused by *M. nigrivenella* and other cob borers (mean ± SE)

Treatments	Ear weight(g)	% ear damage	Ear loss (g)	% grain loss
J. curcas oil 2.5%	99.8±7.8a	13.8±4.0b	10.5±2.9b	12.5±3.2b
J. curcas oil 5%	120.3±1.9a	7.5±2.2ab	7.7±1.5ab	8.4±2.0ab
A. indica oil 2.5%	106.6±10.1a	5.2±1.9a	4.1±1.0a	5.1±1.4a
A. indica oil 5%	116.3±8.2a	6.9±2.2a	6.8±1.71a	7.3±1.8a
T. vogelii extract 15%	130.5±10.8a	15.7±4.1b	15.6±2.2b	14.6±2.6b
T. vogelii extract 20%	112.7±9.7a	11.7±3.5b	9.5±2.4ab	10.5±2.8ab
H. suaveolens extract 15%	126.3±9.4a	14.0±3.3b	12.6±2.6b	10.6±2.3b
H. suaveolens extract 20%	118.7±9.0a	11.6±2.7b	11.9±1.7b	10.9±1.8b
Furadan	124.8±10.7a	8.3±2.3a	7.0±1.6a	6.9±1.7a
Emulsifier solution	121.2±8.1a	12.0±3.1b	11.5±0.5b	10.9±3.2b
Control (untreated check)	117.6±7.7a	11.7±2.3b	12.3±1.9b	12.0±2.0b
DF	10, 429	10, 429	10, 429	10, 429
F	0.98	2.01	2.88	2.25
P	0.45	0.038	0.003	0.019

Ear weight and damage were determined at harvest. Means in a column followed by the same lower case letter(s) are not significantly different at $P \leq 0.05$ (SNK).

Compared to untreated plots, maize treated with oil emulsions of *J. curcas* at 5% and neem at both concentrations were protected from *M. nigrivenella* attack for more than one week indicating a long-lasting effect. Moreover *M. nigrivenella* density in oil treated plots tended to be constant over sampling dates indicating that the oil treatments protect the maize ear from further infestations. In Nigeria, Olaifa and Adenuga (1988) have demonstrated that under field condition during the dry season, neem products prepared from the seeds appeared to lose their efficacy after 11 and 14 days. Likewise, Schmutterer (1990) stated that under field conditions a foliar application of most commercial neem formulations persists for 5 to 7 days. The differential effects of neem and *J. curcas* oils observed on *S. calamistis* and *E. saccharina* might be because the two borers attack maize at different plant growth stages. *Sesamia calamistis* only oviposits on pre-tasseling plants (Kaufmann, 1983; Sémeglo, 1997), while *E. saccharina* infests the plant at or after the tasseling stage, but both species also feed on the ear (Kaufmann, 1983). In the present study, plants were treated at silking, thus *S. calamistis* larvae were already present in the stem and well protected against *J. curcas* oil. However, they might have been affected by the neem oils, which can have systemic properties (Thoeming et al*.*, 2003, Weintraub and Horowitz, 1997). By contrast, at silking both *E. saccharina* oviposition behaviour and its eggs could be affected by the oils as showed by Bruce et al. (2004).

Although *Tephrosia* species and *H. suaveolens* have been reported to be sources of a number of compounds with toxic and deterrent activities toward insects (Simmonds et al., 1990, Machocho et al 1995, Morris 1999) their aqueous extracts did not have any significant effect on *M. nigrivenella* larvae compared to neem and *J. curcas*, even though they reduced oviposition by *M. nigrivenella*. This inefficacy to reduce significantly larval population could be due to their low persistence and characteristics of their active compounds, or they were faster washed-off by rain than oil emulsion. Also, rotenone - one of *T. vogelii* bioactive compounds- is not soluble in water (Anonymous, 1999), which might explain the results obtained. It is therefore recommended to use other solvents (alcohol, acetone…etc.) rather than water or add a good emulsifier or use their essential oils to achieve significant results.

In the present experiment, only neem oils significantly reduced ear damage and grain losses. Neem products have shown to be systemic (Hossain and Poehling, 2006), a property which was not yet demonstrated for *J. curcas* products. Thus neem might also act as feeding inhibitor of larvae present in the ear, though feeding deterrence appears to be more acute in sap sucking insects like the Western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Thoeming et al., 2003).

In conclusion the potential of neem and *J. curcas* in *M. nigrivenella* control has good prospects. However, oils including aqueous extracts of seeds have to be tested in environments with higher infestations as prevalent in the Southern Guinea Savannah (Sétamou, 1996) before being included in an IPM program currently being developed.

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CHAPTER 5

The importance of *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) as a post-harvest pest in different storage structures in Benin

Komi Agboka[a, b], Fritz Schulthess[c], Manuele Tamò[a], Kerstin Hell[a] and Stefan Vidal[b]
[a]International Institute of Tropical Agriculture 08 BP 0932 Tripostal, Cotonou Republic of Benin; [b]Georg-August-University Goettingen, Agricultural Entomology, Grisebachstrasse 6, 37077 Goettingen Germany; [c]Postfach 508-4, 7004 Chur, Switzerland.
Correspondence E-mail: k.agboka@cgiar.org, Tel: +229 21350188, Fax: +229 21 350556

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Abstract

In West Africa, the most damaging lepidopteran pest of maize ears is the pyralid *Mussidia nigrivenella*. Although it is mostly described as a field pest, it can be found feeding on stored maize up to the 4th month. A survey was conducted in Benin in 2006 to assess *M. nigrivenella* infestations in different maize storage systems in the Southern (SGS) and Northern Guinea Savanna (NGS). In SGS and NGS the percentage of infested stores decreased from 86.7% to 26.7% and from 51.4% to 14.3%, respectively, during the first 28 weeks of storage. During the same time, mean numbers of *M. nigrivenella* per cob decreased from 0.36 to 0.04 across both zones. All larval stages, but mostly 3rd to 5th instars, were frequently found even after more than 12 weeks, indicating that *M. nigrivenella* either reproduced in storage or that development was delayed. Highest *M. nigrivenella* incidence of 16.8% and 14.4% were found in the “Ava” and crib stores, respectively. Infestations were highest in “Ava” and lowest in maize grain stored in polyethylene bags or in mud silos. In a laboratory experiment, presence of post-harvest beetles negatively affected the bionomics of the cob borer, indicating strong interspecific competition.

Keywords: Cob borer; infestation; maize; storage time; storage system; beetles

Introduction

Corn (*Zea mays* L.) is an important staple crop in West Africa providing food and income to farmers. In Benin, maize is generally harvested late to facilitate drying. It is stored on the cob with or without the husk cover either in wooden granaries, under the roof or on the floor inside the houses, or as grains in clay containers such as mud silos or in polyethylene bags (Fiagan, 1994; Hell et al., 2000). Stored maize can be infested by a variety of insects, among them the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), which cause losses exceeding 20% (Pantenius, 1988; Borgemeister et al., 1998; Meikle et al., 2002; Schneider et al., 2004).

The maize cob borer *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae), although frequently reported to infest maize in the field (Moyal and Tran 1991a,b, Shanower et al. 1991, Gounou et al, 1994, Sétamou 1996), has also been shown to feed on stored maize (Sétamou 1996). In the field, female moths lay their eggs on the silks, where young larvae feed for a few days before progressing to the grains. In the field, typically more than half of the cobs are infested by the borer (Whitney, 1970, Sétamou, 1996), and reported yield losses measured at harvest vary between 5 and 15% (Moyal and Tran, 1991, Sétamou et al, 2000). In addition, *Mussidia nigrivenella* damage predisposes maize to pre- and post-harvest infestations by storage beetles and fungal pathogens such as the aflatoxin producing *Aspergillus flavus* Lk. Fr. (Sétamou et al, 1998) and *Fusarium* spp. (Ako et al., 2003). Hence, both the quantity and the quality of maize are affected by *M. nigrivenella*.

The egg-to-adult development time of *M. nigrivenella* on maize at 26 ± 2^{o}C and 65±5 relative humidity (rh) is roughly 38 days (Bordat and Renand, 1987; Moyal and Tran, 1991b; Bolaji and Bosque-Pérez, 1998; Sétamou et al., 1999). Thus, the presence of *M. nigrivenella* in maize stores after more than two months might indicate that it also infests stored grain or that the life cycle is extended under storage conditions. In spite of the fact that *M. nigrivenella* could infest stored maize (Ratnadass, 1987; Tran, 1987) and other stored products such as cacao beans (Potter, 1931, Evans, 1952, Varshalovich, 1975), seeds of *Canavalia* sp. and of *Phasesolus* sp. (Buyckx, 1962, Le Pelley, 1959), and soya beans (Moyal, 1988), few studies exist on its importance as a storage pest (Sétamou, 1996). The objective of the present study was to assess the status of *M. nigrivenella* as a pest of stored maize and to evaluate the influence of different storage structures and the presence of storage beetles on the levels of borer infestations.

Material and methods

Surveys

Four surveys for *M. nigrivenella* in stored maize were conducted from July 2005 to February 2006 in the Southern (SGS) and the Northern Guinea Savanna (NGS), which in previous studies showed the highest prevalence of the borer (Sétamou et al., 2000). The SGS is characterized by a bimodal rainfall pattern with precipitations averaging 1100 – 1500 mm, allowing for two maize growing seasons, while the NGS has a monomodal rainfall distribution with less than 1100 mm rainfall and one cropping season only. Three villages were selected in SGS and seven in NGS depending on the availability of stores in each zone. In each village, five stores were randomly selected and sampled four times, once just after harvest (i.e. 0 week after storage), and at 4, 12 and 28 weeks thereafter. (The description of the storage structure is given in the next section). Stores made from plant materials, where maize was stored on the cob, were divided into four sections, and 15 cobs were randomly chosen from each. For mud silos and polyethylene bags, in which maize is stored as grain, approximately 3 kg of grains were taken with a probe. Samples were taken to the laboratory for determination of numbers of all pest species per cob or grain sample. For each sampling period and location, the percentage of infested stores and for each store the percentage of cob or grain sample was calculated.

On-station experiment: effect of storage methods on infestations by **M. nigrivenella**

The trial was conducted in Ouesse (08°29.521 N and 002°26.046 E) to test the effect of four store types on borer infestation over a 28 weeks period. During the study, the monthly ambient temperature varied from 25 to 31° C and the relative humidity from 33 to 76% (Figure 5.1). The improved IITA maize variety DMR-LSR-W (hereafter referred to as DMR) was used. It is a white seeded cultivar that reaches maturity at approximately 110 days. DMR is resistant to downy mildew (*Peronosclerospora sorghi* Weston & Uppal) and to maize streak virus. The treatments consisted of two indigenous and two improved store types with four replicates for each, i.e. 1) The standard storage structure named "Ava", used to store maize as cob with husk; 2) polyethylene bags and 3) mud silos, in which maize grains are stored in bulk; and 4) cribs where maize is stored as cobs without husk. "Ava" is a traditional, cylindrical structure with walls made of woven vegetable material. The cobs with the husks are laid out in a circular fashion to build a hollow cylinder of about 1.5 m in diameter on a 1.2 m platform. The experimental structure was rodent protected by metal sheets (http://www.fao.org/inpho/index-f.htm). In the experimental stores, about 300kg of maize were stored. The mud silo is a hollow cylinder built with a mixture of clay and grasses (mainly sorghum stems or rice straw). The maize crib is an improved narrow structure, in the form of a rectangular basket, 1.2m high, 0.6m wide and about 2.2m long, made of bamboo (http://www.fao.org/inpho/index-f.htm); in the present experiment, maize was stored as de-husked cobs. The top of all structures, except the polyethylene bags, were covered with a thatched roof to ensure protection from rain. The treatments were all arranged in randomized complete blocks with four replications. The blocks consisted of farmers located at about 1km from each other. No insecticides were used and no insects were released into the structures. The changes in damage and insect populations over time were monitored by regular samplings as described above. *Mussidia nigrivenella* damage was identified by its characteristic feeding habit as described by Sétamou et al. (1998); *M. nigrivenella* larvae start feeding from the tip of the cob and produce conspicuous amounts of silky frass. The percent damage (y) was calculated according to Boxall (1986) as y = (B / A) x 100, where A is the number of all grains and B is the number of grains damaged by *M. nigrivenella*. In addition, the percentage of damage caused by beetles was calculated by counting the number of grains damaged over the total number of grains.

The moisture content of the samples from each store type was determined according to the International Organisation for Standardisation (1979) routine method. A sub-sample was ground (Romer Grinding Subsampling Mill®, Union, U.S.A), transferred to a metal container and weighed. The sample was then dried for two hours at 130 °C, and re-weighed in the container. The corn moisture was determined by the following formula $MC = 100\ ((Wi–Wd)/Wi)$, with MC = moisture content, Wi = initial weight and Wd = weight after drying.

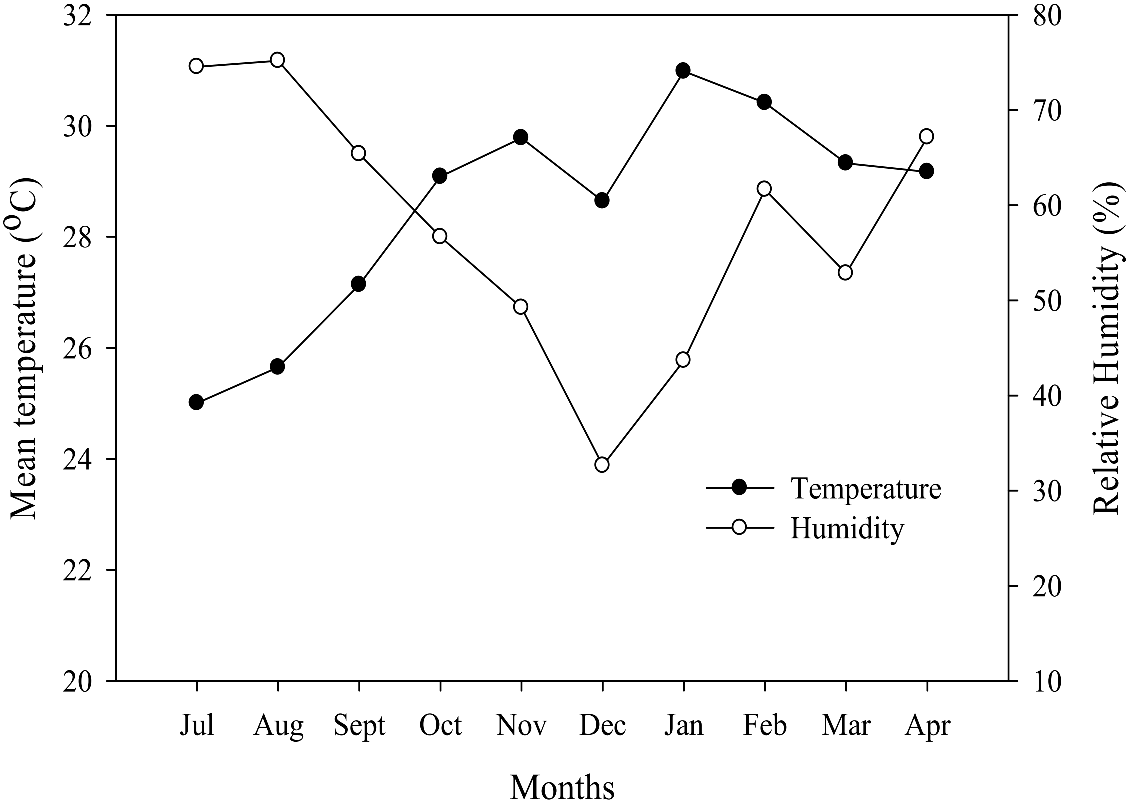

Figure 5.1: Mean monthly temperature and Relative humidity at Ouesse

Laboratory experiment: effect of the presence of storage beetles on the development of M. nigrivenella

Fifty grams of maize grain were placed into 1 liter jars and infested with storage beetles and *M. nigrivenella*. The following treatments were applied: i) *M. nigrivenella* alone, ii) *M. nigrivenella* with *P. truncatus*, iii) *M. nigrivenella* with *S. zeamais* and iv) *M. nigrivenella* with *P. truncatus* and *S. zeamais*. Thereby, 50 eggs of *M. nigrivenella* at the red-spotted eyes stage, 10 pairs of less than 2 weeks old *S. zeamais* or 10 pairs of *P. truncatus* originating from six to eight week old colonies were used. The jars were covered with a mesh lid and incubated at 27 ± 1° C and 75 ± 2% r.h. Each treatment was repeated eleven times in completely randomized blocks. The contents of the jars were monitored every day for 6 weeks for numbers of larva, pupa and moth. At the end of the experiment, the development time of the larvae and pupae, and the number of pupae were determined.

Data analysis

Maximum likelihood analysis using the proc logistic model (SAS Institute, 1997) for categorical variable was applied to survey data to identify the effects of storage period, zone and storage structures on the presence of *M. nigrivenella*. Differences in insect counts per ear/grain sample and in percentages of grain damaged and grain moisture content among the storage systems in the on-station experiment were analyzed by analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of SAS for repeated measures over sampling dates. In the laboratory experiment, developmental time, numbers of pupae formed and pupal weight were analyzed via analysis of variance (ANOVA) using the Generalized Linear Model (GLM). Least square means (LSM) were separated using the t-test. Percentages were arcsin√x- and number of *M. nigrivenella* $\log_{10}(x+1)$ transformed. Correlation coefficients among different insects were calculated using data pooled across zones.

Results

Surveys

Mussidia nigrivenella was found at all sampling dates. Overall, the logistic regression models showed that the store system and storage duration had significant impact on the abundance of *M. nigrivenella* (Table 5.1). The highest odds ratios for *M. nigrivenella* absence were found between 12 and 28 weeks after storage. After 28 weeks of storage, beetles such as *S. zeamais* and *Cathartus quadricollis* Guerin (Coleoptera: Silvanidae) were the most prevalent species followed by *P. truncatus*, while *M. nigrivenella*, *Carpophilus* sp**.** (Coleoptera: Nitidulidae)**,** *Tribolium* sp. (Coleoptera: Tenebrionidae) represented less than 5% of all species collected (Table 5.2). Lepidopteran stem borers such as *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) were collected up to 4 weeks of storage only and they were not included in Table 5.2.

Table 5.1: Logistic regression analysis of the effect of storage duration, storage systems and the ecological zone on the abundance of *Mussidia nigrivenella*

Parameter		DF	Estimate	SE	Wald $\chi 2$	P-value	Odds ratio
Intercept		1	3.73	0.27	190.59	<.0001	
Store	Ava	1	-2.37	0.26	80.34	<.0001	0.09
Store	Cribs	1	-2.05	0.26	61.86	<.0001	0.13
Store	Mud silos	1	-1.13	0.28	16.48	<.0001	0.32
Zone	SGN	1	-0.38	0.14	7.83	0.0051	0.68
Week	0	1	-0.34	0.14	5.45	0.0195	0.71
Week	12	1	0.805	0.18	20.21	<.0001	2.24
Week	28	1	1.68	0.24	50.95	<.0001	5.35

Table 5.2: Densities and relative importance (%) of insect species in stored maize in the Guinea Savannas of Benin after 28 weeks of storage

Insect species	Mean ± SD	% of total insect
Mussidia nigrivenella	0.17±0.0082c	3.92d
Prostephanus truncatus	0.37±0.051c	8.57c
Sitophilus zeamais	1.04±0.058b	24.44b
Cathartus sp.	2.42±0.108a	56.58a
Carpophilus sp.	0.08±0.0059d	1.81e
Tribolium sp.	0.20±0.012c	4.67d

Means in a column followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t- test)

Multiple regressions showed that the numbers of *Catharthus* sp. and *Carpophilus* sp. were positively and the numbers of *P. truncatus* and *S. zeamais* negatively related with *M. nigrivenella* densities (r^2=0.061, P<0.0001; Table 5.3). Percentages of cob or grain samples and of granaries infested, and the number of *M. nigrivenella* per cob/grain sample varied significantly with agro-ecological zone and duration of storage. The percent infested stores (DF = 1, 200; F = 5.3; P = 0.018) and numbers of *M. nigrivenella* per cob (F = 5.6; P = 0.02) was higher in the SGS than in the NGS, but the percentage of infested cobs was higher in the NGS than the SGS (DF = 1, 12000; F = 5.6, P = 0.02, Table 5.4).

Table 5.3: Multiple regression analysis of total *Mussidia nigrivenella* and storage beetles present in maize cobs

Variables	Estimates	Standard Error	P-value
Intercept	0.023	0.0014	< 0.0001
Prostephanus truncatus	-0.021	0.0089	0.018
Sitophilus zeamais	-0.030	0.0058	< 0.0001
Catharthus sp.	0.087	0.0044	< 0.0001
Carpophilus sp.	0.076	0.0150	< 0.0001
Tribolium sp.	-0.018	0.0099	0.064
R^2	0.061		

Prior to analysis, number of insects were $\log_{10}(x + 1)$-transformed

In each zone and across zones the percentage of stores infested by *M. nigrivenella* decreased significantly with sampling date (DF = 3, F = 4.6; P = 0.008; Table 5.5 and 5.6). Cob borer counts decreased from harvest to 28 weeks after storage. This trend was significant in the NGS, while in SGS there was an increase of the *M. nigrivenella* numbers from harvest to 4 weeks after storage and a subsequent decrease thereafter until the 28th week (Table 5.5).

Table 5.4: Store and cob/sample infestation (%), and densities per cob of *Mussidia nigrivenella* in the Southern (SGS) and Northern (NGS) Guinea Savanna in Benin

	zones	
	SGS	NGS
% storage infestation	66.67±9.95a	38.57±6.74b
% cob infestation	6.78±1.24b	8.77±1.59a
Number of *M. nigrivenella*	0.20±0.02a	0.15±0.01b

Means (±SE) within rows followed by the same letter(s) are not significantly different at P≤0.05 (t-test)

Table 5.5: Effect of storage duration on the infestation by *Mussidia nigrivenella* of farmers' stores in Southern (SGS) and Northern Guinea Savanna (NGS)

Zone	Weeks in storage	% store infested	% cob infestation in stores	Number of *M. nigrivenella*
SGS	0	86.67±6.67a	11.2±3.08a	0.23±0.03b
	4	93.33±6.67a	16.0±3.60a	0.41±0.05a
	12	60.0±20.00b	4.53±1.40b	0.08±0.02c
	28	26.67±17.64b	3.33±2.28b	0.06±0.01c
NGS	0	51.43±15.65a	16.06±4.11a	0.41±0.03a
	4	51.43±15.56a	6.29±1.78b	0.12±0.01b
	12	37.14±11.90ab	3.46±1.08c	0.06±0.01c
	28	14.29±5.71b	1.41±0.75c	0.02±0.01c

In each zone, means ±SE within columns followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t- test)

Table 5.6: Effect of storage duration on the infestation of *M. nigrivenella* in farmers' stores across zones (means ±SE)

Weeks after storage	% store infestation	% cob infestation per store	Number of *M. nigrivenella*
0	62.0±12.09a	14.57±3.0a	0.36±0.03a
4	64.0±12.58a	9.20±1.75a	0.21±0.02b
12	44.0±10.24ab	3.78±0.86b	0.07±0.01c
28	18.0±6.29b	2.0±0.87b	0.04±0.01c

Means within columns followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t- test)

On-station experiment: Effect of storage methods on the infestation of **M. nigrivenella**

The storage structures and the duration of storage significantly affected the percentage of cobs infested by *M. nigrivenella* (DF = 3, 960; F=9.2, P<0.0001 and F= 8.3, P= 0.0001, respectively) and the number of larvae (DF = 3, 960; F= 42.9, P<0.0001 and F = 41.9, P<0.0001), respectively). Percentages of cob infested were highest in "Ava" followed by the cribs (Table 5.7). The percentages of grain sample infested and the number of larvae in the mud silo and polyethylene bags were significantly lower than in the other two stores (DF = 3, 960; F=9.6 P<0.0001 and F= 70.1, P<0.0001, respectively). In all structures the percentages of cob infestation and the number of *M. nigrivenella* larvae decreased with storage time (Table 5.7). From harvest throughout storage, a reduction of more than 70% of cob infestations was observed in all structures. All developmental stages but mostly 3rd to 5th instars larvae and/or pupae could be found in “Ava” and cribs after 28 weeks, while lower or no infestations were observed in the mud silo and polyethylene bags.

Table 5.7: Number of and overall means (across sampling dates) of *Mussidia nigrivenella* and mean percentage of cob/grain sample infested in different store types (means ±SE)

	Weeks after storage				
Store types	0	4	12	28	
		Number of *M. nigrivenella*			Overall
“Ava”	0.35±0.05a	0.31±0.04a	0.13±0.03a	0.07±0.02a	0.21±0.02a
Cribs	0.32±0.04a	0.22±0.03b	0.11±0.02a	0.04±0.01b	0.17±0.02b
Mud silos	0.11±0.02b	0.08±0.02c	0.09±9.02a	0.02±0.01b	0.07±0.01c
Polyethylene bags	0.08±0.02b	0.03±0.01c	0.0±0.0b	0.0±0.0c	0.03±0.01d
		Infestation (%)			Overall
“Ava”	26.0±11.14a	25.0±4.20	9.0±3.11	7.0±3.70	16.75±3.66a
Cribs	25.5±2.63a	18.0±8.13	10.5±1.71	3.5±1.71	14.38±2.91a
Mud silos	11.0±6.46b	7.5±4.27	7.5±2.22	1.5±0.96	6.88±2.02b
Polyethylene bags	6.0±1.42b	3.0±1.0	0.0±0.0	0.0±0.0	2.25±0.75c

Means within columns followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t-test)

Similarly, the damage caused by *M. nigrivenella* varied significantly with storage structure and storage time. It was highest in “Ava” and lowest in polyethylene bags (Table 5.8). In all storage structures, percent feeding damage tended to be constant during storage except in “Ava” where it increased slightly from 0 to 4 weeks after storage and then remains stable thereafter. Grain feeding damage by *M. nigrivenella* remained low in the mud silo and polyethylene bags throughout storage. In the present experiment, damage caused by beetles – mostly *P. truncatus* and *S. zeamais* – reached 37.5% in “Ava”, 32.6% in cribs, and 21.5% in polyethylene bags after 12 weeks (data not shown here).

Table 5.8: Percentage grain damaged by *M. nigrivenella* in different stores (means ±SE)

	Weeks after storage			
store type	0	4	12	28
"Ava"	8.0±1.14a	10.3±1.15a	9.55±1.33a	-*
Cribs	7.45±1.93a	6.4±1.27b	5.45±1.0b	-
Mud silos	3.15±1.42b	2.75±1.23c	2.34±1.03c	-
Polyethylene bags	2.45±0.28b	2.07±1.01c	1.97±0.45c	-
F	14.61	22.54	31.61	
P	0.0013	0.0003	<0.0001	

Means within columns followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t-test). * Percentage of grain damaged by *M. nigrivenella* was not measured at 28 weeks after storage because grains were heavily infested with beetles.

The moisture content of the maize grains in all stores decreased from around 18% to <12% after 12 weeks of storage and thereafter slightly increased to 13% (figure 5. 2).There was no difference in moisture content in grain between storage type across sampling date (DF=3, F= 0.63, P=0.53), and neither at 12 (DF=3, F=1.76, P=0.18) and 28 weeks (F=0.98, P=0.14) after storage.

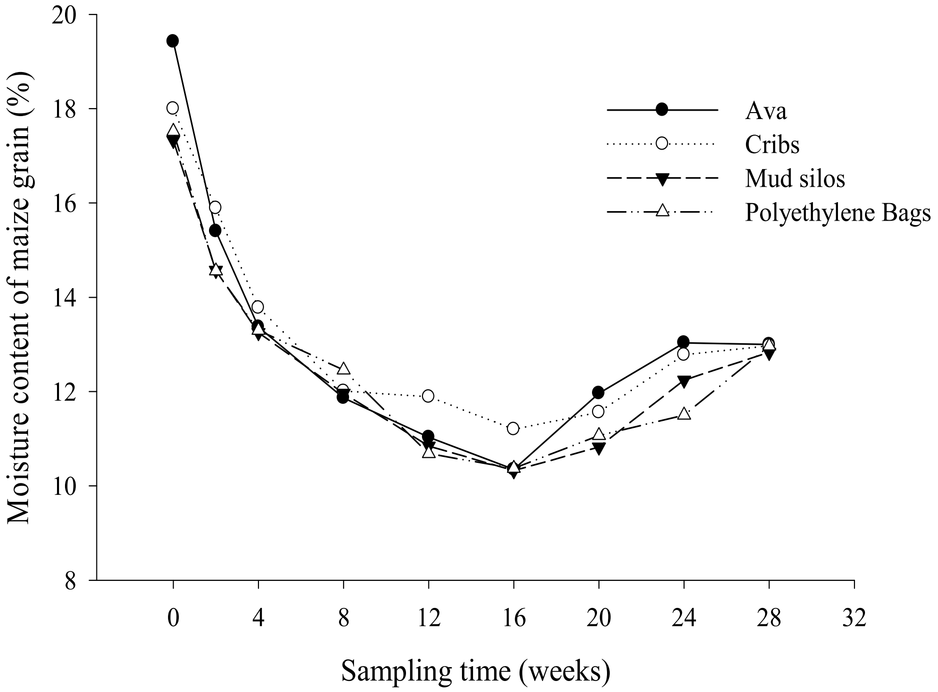

Figure 5.2: Moisture content of maize grain in the different store types

Table 5.9: Effect of mixed infestations with storage beetles on *Mussidia nigrivenella* immature development and survival (means ±SE)

Treatments	Developmental time (days)			Number of pupae	Pupae weight (mg)
	Larvae	Pupae	Total		
M. nigrivenella alone	18.80±0.30c	10.17±0.33a	29.0±0.52b	18.11±0.21a	100.75±1.59a
M. nigrivenella + *P. truncatus*	20.53±0.62ab	10.13±0.22a	30.7±0.70ab	14.34±0.22b	89.13±0.90bc
M. nigrivenella + *S. zeamais*	19.44±0.32bc	10.21±0.25a	29.7±0.43ab	9.29±0.13c	93.00±2.55b
M. nigrivenella + both beetles	21.09±0.16a	10.30±0.12a	31.4±0.24a	7.95±0.25d	87.00±1.09c
F	6.78	0.09	4.33	426.35	13.30
P	0.0009	0.97	0.01	<0.0001	0.0004

Means in column followed by the same letter(s) are not significantly different at $P \leq 0.05$ (t-test)

Laboratory experiment: effect of the presence of storage beetles on the development of **M. nigrivenella**

In the presence of storage beetles, egg to pupae development time and, thus, total development time increased significantly (DF =4, 44; F = 6.8; P = 0.0009 and F = 4.3; P = 0.01) though developmental time of pupae was not affected (DF = 4, 44; F = 0.03; P = 0.98). Likewise, larval survival and numbers of *Mussidia* pupae was considerably reduced in the presence of storage beetles (DF = 4, 44; F = 617.7; P<0.0001; Table 5.9). Moreover pupae were heaviest in the *M. nigrivenella* only treatment (DF =, 4, 44; F=13.30, P=0.0004).

Discussion

Sétamou (1999) found that the pest densities and grain damage increased with delay in harvest indicating that in the field *M. nigrivenella* oviposits and develops on mature maize cobs. In the present study, *M. nigrivenella* was found feeding on maize cobs after more than 6 months of storage. The life cycle of *M. nigrivenella* is around 38 days (Bolaji and Bosque-Pérez, 1998; Sétamou et al, 1999), thus theoretically allowing for several overlapping populations on stored maize. The present experimental set-up, however, does not allow for determining if the presence of *M. nigrivenella* at 6 months after storage was due to re-infestation of stored maize or the result of an extended life cycle under storage conditions; the scarcity of young larval stages indicates the latter. Also, grain damage remained on a similar level up to the 12th week indicating that most of the grain damage occurred at the beginning and that *M. nigrivenella* was not very active during storage. This rather suggests low feeding activity by *M. nigrivenella* later in the storage season indicating a delayed development of the borer under storage condition.

The reduction of *M. nigrivenella* densities with storage time could be due to adverse environmental conditions (i.e. a combination of high temperature and low air and grain moisture content). Most storage insects are able to survive and multiply rapidly on well dried grain. However grain dried to below 12% mc inhibits the development of most species to some extent (FAO, 1994). As shown by Fields and Korunic (1999) the lower moisture content of the grain, the greater mortality. Thus the moisture content below 13% observed in the stores after 12 weeks of storage in this study probably affected the survival of *M. nigrivenella*. However, since moisture contents did not vary between storage types the differences of *M. nigrivenella* infestations were due to other factors, e.g. varying levels of infestations of the grain by storage beetles, which also affected populations growth rates of the borer. In the present surveys, a negative relationship between the numbers of storage beetles such as *P. truncatus*, *S. zeamais*, *Tribolium* sp. and *M. nigrivenella* infestations was observed. Similarly, the laboratory experiment showed that the presence of the beetles increased larval development time and mortality. This suggests a strong interspecific competition between the various grain-feeding insects. Compton and Sherington (1999) found no *M. nigrivenella* larvae in maize cobs heavily colonized by beetles. Storage pests such as the larger grain borer may preferentially attack the germ of the grain, thus removing a large percentage of the protein and vitamin content whereas weevils feed mainly on the endosperm removing carbohydrates mostly (Khare and Mills, 1968; Subramanyam et al., 1987; Demianyk and Sinha, 1988; Vowotor et al., 1998). It is suggested that poor quality grain would affect the bionomics of *M. nigrivenella*. Larsen et al. (2005) describe how resource depletion influences the per capita growth rate of insect species competing for the same resources; for example, the reason responsible for *S. zeamais*'s rapid elimination of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is its superiority in colonizing and monopolizing new patches.

The varying levels of infestation observed in the present study could also be explained by differences in initial egg/larval load between the different forms of stored maize (i.e. with husks vs. dehusked, shelled etc.). In traditional “Ava” stores, in addition to the larvae already present in the cob, eggs laid on the surface of the husk or silks as observed by Sétamou (1999) may still hatch and attack the cobs. Although high mortality of 90% of first two instars larvae are common (Moyal and Tran, 1991b), these additional infestation would augment the number of *M. nigrivenella* present in the ear. By contrast, in the cribs eggs and some of the hatched larvae might have been removed when the ears were dehusked. Furthermore, the husks might form a protection to *M. nigrivenella* from adverse environmental effects and from general predators such as ants, and in addition reduce attacks of the grain by storage beetles (Kossou et al., 1993).

Another important factor influencing population development is the temperature in the granary and the aeration pattern both depending on the storage structures. Contrary to the traditional "Ava" and the cribs, bags and mud silo are tightly closed allowing for little aeration. In addition, in hermetically closed bags and mud silos where air diffusion is prevented or reduced, the respiratory process of the biotic components in the bulk (grains, fungi, insects, etc.) O_2 and produces carbon dioxide CO_2 (Bartosik et al., 2001). This new atmosphere, rich in CO_2 and poor in O_2 might suppress, deactivate, or reduce the reproduction and/or development capacity of lepidopterous insects such as *M. nigrivenella* and other insects like bruchids as observed for hermetic storage of cowpea seeds using triple bagging in Cameroon (http://www.entm.purdue.edu/entomology/research/cowpea/Extension%20bulletins/PDF%20publications/Tripple%20Bagging.pdf). Furthermore, closed stores also form a barrier to invading *M. nigrivenella* moths and storage beetles, which might partly explain the differences in borer infestations between open and closed stores.

Although, *M. nigrivenella* did not cause considerable losses in stored maize, they appear to be one of principal disseminators of fungi such as *A. flavus* and *Fusarium* sp. in granaries. As shown by several authors, insect feeding renders the grain susceptible to *A. flavus* infection, resulting in higher potential aflatoxin levels of grain in both field and during storage (Sétamou et al., 1998; Hell et al., 2000; Fandohan et al., 2005). According to Beti et al. (1995) humidity build-up might occur through convection and metabolic activity of pests thereby increasing moisture levels permitting fungal spores to persist for longer time in the granary and increase the risk of aflatoxin contamination. To overcome this risk, maize should be dried below <15% water content to ensure unfavorable conditions for fungal growth (Hell et al., 2000).

The present findings suggest that storing maize as bulk grains in closed structures or polyethylene bags would reduce additional damage caused by *M. nigrivenella* in storage. As shown for bruchids attacking cowpea seeds (Chauhan et al., 2002), solar heating of the grain in polyethylene bags could also reduce initial infestations by *M.*

nigrivenella and storage beetles. However, additional research is required to assess how hermetically closed structures affect the build-up of aflatoxin producing fungi.

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CHAPTER 6

Surveys for natural enemies of *Mussidia* spp and other pyralids in Malaysia: perspectives of bio-control of the maize cob borer *Mussidia nigrivenella* in West Africa

Komi Agboka[1,2], Schulthess F., Laurence G. Kirton[3], Manuele Tamò[1] and Stefan Vidal[2]

[1]International Institute of Tropical Agriculture 08 BP 0932 Tripostal, Cotonou Republic of Benin 08 BP 0932 Tripostal, Cotonou Republic of Benin. [2]Georg-August-University Goettingen, Agricultural Entomology, Grisebachstrasse 6, 37077 Goettingen Germany. 3 Forestry Research Institute Malaysia (FRIM)

Correspondence E-mail: k.agboka@cgiar.org

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Abstract

In West Africa, the pyralid *M. nigrivenella* is a polyphagous pest, which besides maize ears attacks cotton balls, *Phaseolus* beans, and the fruiting structures of many economically important trees such as shea-butter tree (*Butyrospermum parkii*) and *Parkia biglobosa.* In West Africa, natural enemies appear to play no role in the population dynamics of the pest. It is hypothesized that parasitoids may regulate other *Mussidia* species such as *M. pectinicornella* and other pyralids occurring in Malaysia on *Parkia speciosa*. Surveys were therefore conducted in South-East Asia to assess the pest status of *Mussidia pectinicornella?* or other pyralids on *Parkia* sp. and to identify promising parasitoids to be introduced into West Africa. In Malaysia, *P. speciosa* pods were subjected to serious borer attacks. Though pod infestations could reach 80% the percent seeds attacked within a pod was less than 20%. The most common parasitoids were the braconid *Bracon* spp. (64%), followed by the eurytomid *Eurytoma* sp. (32%) and the pteromalid *Sphaeripalpus* sp. (4%). Overall, mortality caused by parasitoids reached <40% hence they could be considered a key mortality factor in the population dynamics of the *Mussidia* spp in Malaysia.

Keywords: *Bracon* spp., Malaysia, *Mussidia* spp., *Parkia speciosa*

Introduction

In West Africa, the pyralid *M. nigrivenella* (Ragonot) is a polyphagous pest, which besides maize ears attacks cotton balls, *Phaseolus* beans, and the fruiting structures of many economically important trees such as shea butter-tree (*Butyrospermum parkii* (G. Don) Kotschy) (Sapotaceae) and *Parkia biglobosa* (Jacq.) Benth.) (Leguminosae) (Silvie 1990, Moyal and Tran 1991, Sétamou et al., 2000a). Though, pest incidence in maize fields is usually more than 50%, yield losses range from 5 to 25% only (Moyal and Tran 1991, Sétamou et al., 2000b). However, *M. nigrivenella* continues to feed on maize grains in stores leading to an additional 5% loss. In addition, grain damage by the borer predisposes maize to pre- and post-harvest infestations by storage beetles and infections by mycotoxin-producing moulds (Sétamou et al., 1998; Fandohan et al., 2005).

Currently, no technologies are available that provide satisfactory control of *M. nigrivenella*. Pesticides and botanicals are only partly efficient due to the cryptic feeding behavior of lepidopteran larvae in the ear (Sétamou et al. 1995, Ndemah and Schulthess 2002, Agboka et al. submitted). Intercropping maize with both host and non-host companion crops or planting border rows with grasses reduced oviposition and larval infestations of stem- and cobborers including *M. nigrivenella*; however, the results were not consistent (Ndemah et al. 2002a, Agboka et al. 2006, submitted). Surveys on wild and cultivated host plants of *M. nigrivenella* in West Africa and *Mussidia* spp. in Kenya in East Africa, yielded a paucity of natural enemy species and low parasitism; from most host plants no parasitoids were obtained and they appeared to play no role in the population dynamics of the pest (Sétamou et al., 2002; Muli et al., 2008).

According to Janse (1941), five species of *Mussidia* occur in Africa and one species in Asia but their taxonomic status and their distribution are not well known, and they are not always considered as pests in their ecological zones (Muli et al. 2009). In Malaysia and Thailand, *Parkia speciosa* Hassk., commonly called "petai" is an economically important non-wood tree (Wooh and Poh, 1998). Its pods are consumed by the local people. The pod-boring larvae of the pyralid moth *Mussidia pectinicornella* Hamps. and the torticid moth *Argyroploce illepida* Btlr. were frequently reported to infest the ripening seeds of *P. speciosa* (Kalshoven, 1981).

The main objective of the present work was to conduct surveys of *Mussidia* spp. or other pyralids and their parasitoids on *Parkia* spp. in Malaysia to identify potential new association parasitoids for introduction into West Africa.

Materials and Methods

In 2005, from August to September, *Parkia* pods were collected from whole-sell markets in different States of Malaysia. Depending on the market size and the number of pods sellers, 1-5 sellers were chosen and 20-100 pods (five to twenty inflorescences) were randomly selected from each seller.

The samples collected were brought to the laboratory of the Forest Research Institute Malaysia (FRIM) and dissected. All larvae collected were maintained in the seeds attacked from the pod and put in rectangular boxes (10cm x 15 cm x 10 cm). Late instars larvae were maintained on an artificial diet developed for *M. nigrivenella* by Bolaji and Bosque-Pérez, (1999). The larvae were reared to adulthood to record parasitism. The identification of the moths is not yet done however, samples were sent to a pyralid specialist in Germany. Larval parasitoids collected were identified by Dr Georg Goergen at the International of Tropical Agriculture Museum (IITA, Benin). The number of larvae per pod, percent pod infestation in each site surveyed, percent seed infestation in each pod and the number of parasitoid pupae on dead larvae were determined. In each location parasitism was defined as the number of parasitoid pupae collected on dead larvae per total number of larvae collected).

Results

Incidence and level of infestation

Lepidopteran species were found infesting *P. speciosa*. *Parkia* pod infestation ranged from 21.6% to more than 82.2% while seed infestation varied from 1.9% to less than 19.6% (Table 6.1). Two to three larvae could be frequently found in one pod. The mean number of larvae was 1.32 per pod. But each seed bore only one larva.

Table 6.1: Infestation by *Parkia* pod borers and their parasitism in different localities of Peninsular Malaysia and pod originated from Thailand

Pod origin	Seed infestation (%)	Pod infestation (%)	Number of larvae/pod	Parasitism (%)
Pasar Borong	19.6	81.2	1.53	2.2
Thailand	19.5	67.6	1.43	21.3
Tapah	14.9	21.6	2.13	10.2
Gombak	7.3	50.4	1.0	0
Bidor	9.1	39.4	1.03	6.0
Kampung	9.3	47.0	1.39	4.5
Seri Menanti	1.9	28.6	0.29	0
KampungIbol	9.8	37.5	1.08	38.4

All lepidopteran larvae recorded showed the same feeding behaviour: first they feed on the tip of the green kernel beginning to destroy the germ and then the cotyledon leaving frass in the seed (Photo 6.2). Late instars larvae, tunneled through the seed and escaped from leaving an exit hole. In the case of heavy infestation, the whole seed from the inside is consumed. During the survey no eggs and pupae were observed.

Some hymenoptera were also found infesting the pods but their damage was not important and limited to the perisperm.

Photo 6.1: Parkia pod borer

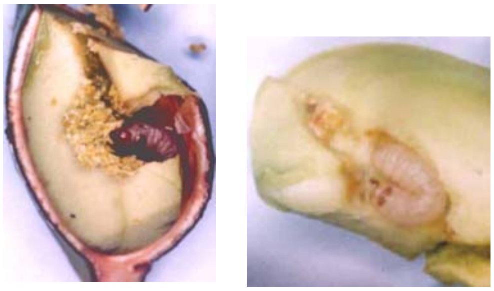

Photo 6.2: Damage by the pod borer

Parasitism

Three larval parasitoids were found parasitizing the 3-4 instars larvae. In Malaysia, parasitism ranged from 2.2 to 38.4%, while in Thailand it was 22% (Table 6.2). Three genera were identified, namely *Bracon* spp. (Hym.: Braconidae) accounted for 64%, *Sphaeripalpus* sp. (Hym.: Pteromalidae (4%) and *Eurytoma* sp. (Hym.: Eurytomidae) (32%). Neither egg nor pupal parasitoids were observed.

Table 6.2: Larval parasitoids recorded on borers infesting *Parkia speciosa* in Malaysia

Parasitoid species	Order: Family
Bracon sp.	Hymenoptera: Braconidae
Sphaeripalpus sp.	Hymenoptera: Pteromalidae
Eurytoma sp	Hymenoptera: Eurytomidae

Discussion

In Malaysia *P. speciosa* is subjected to serious borer attacks. They caused considerable amount of damage in the infested seed, rendering it unfit for consumption. Although the larvae were not yet identified it appear that there might be two different species indicated by the two different colours of the late instar larvae. Reports on pest infestation on *Parkia* spp. in india and Malaysia (Kolshoven, 1981) indicated that the pod-boring larvae of the pyralids moths *M. pectinicornella* Hamps. and the torticid moth, *Argyroploce illepida* Btlr. infest the ripen seeds in the field (http://www.worldagroforestrycentre.org/sea/Products/AFDbases/af/asp/SpeciesInfo.asp?SpID=1258 [Accessed 2009 Jan 14) while the crambid *Cadra cautella* Walker infested the stored seed of *Parkia* spp. (Thanglam et al. 2003). *Mussidia* ssp infesting *P. speciosa* will be confirmed by an accurate identification using molecular tools.

During the three weeks survey, no pupae were found suggesting that the pod borer infests the pods when they matured and ripe and that the survey period was too short to observed all different instars including the pupae. This indicated that larval duration is more than three weeks on Parkia. In the laboratory larvae collected took more than 40 days to become pupae in the seeds as also observed by Thanglam et al. (2003).

Parasitoids, not yet known at species level, were found regulating the borers' damages as indicated by preliminary survey conducted by Jiraporn (2004, personal communication) in Thailand on *Parkia* pod borer. Sétamou et al. (1999) also found *Bracon sesamiae* parasitizing the maize cob borer *M. nigrivenella* in West Africa. Although the parasitism was low in some localities the wasps appeared to have potential to reduce moth populations. But one shortcoming is that to date the genus *Bracon* encompasses 573 described species. This may render the identification to species level very tedious. *Sphaeripalpus* spp. belonging to the Miscogasterinae, which are parasitoids of Diptera burrow in or mining the soft tissues of plants. *Eurytoma* spp. can display phytophagous or parasitic habits. In some instances members of this genus are hyperparasitoids of Braconidae attacking larvae of Lepidoptera. Although these parasitoids are adapted to the climatic condition similar to the one in West Africa giving a prospect for new association for *M. nigrivenella*, an accurate identification of their Phycitinae hosts including *Mussidia* sp. coupled with suitability tests should be done before the use in the bio-control of the maize cob borer.

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CHAPTER 7

General discussion

Maize is an important component of the farming systems and the diet of many people, and is increasing in importance as it expands into the drier savanna zones of West Africa. However, the stability of maize production in West Africa is limited, among others, by cob boring pests. The cob borer, *Mussidia nigrivenella* Ragonot (Lepidoptera: Pyralidae) is one of the key borers attacking directly the maize grain in West Africa (Bosque-Pérez & Mareck 1990; Shanower et al. 1991, Moyal & Tran 1991). Its biology and ecology have been well studied (Sétamou, 1999). However, no detailed control strategies have been developed against the pest. Management approaches developed in the present study focused on botanical formulations with special emphasis on neem and *Jatropha curcas*, habitat management particularly maize-legume intercropping, trap crops and redistribution or new association as biological control for sustainably controlling the maize cob borer *M. nigrivenella*.

As shown for stemborers by Schulthess et al. (2004) and Chabi-Olaye et al. (2005) intercropping reduced attacks of maize ears by *M. nigrivenella* and other stemborer species that feed in the ear. In the present study, the most effective intercrops in reducing *M. nigrivenella* attacks in the different locations were the jack bean *Canavalia* and *Tephrosia*. According to Sétamou et al. (1999), jackbean was the most suitable host plant for *M. nigrivenella* development. The high suitability of this cover crop for *M. nigrivenella* development and survival compared to maize might have direct effects on the population dynamics of *M. nigrivenella* in maize by reducing its density as observed in this study. The cob borer would lay more eggs and survive more on matured pods thus preventing high infestation of maize cobs. The low number of *M. nigrivenella* observed in Maize-*T. vogelii* intercrop is probably due to the repulsive effect of *T. vogelii*. In a semi-field study, oviposition of *Mussidia* was reduced by the leaf extract of *T. vogelii* (Agbodzavu 2005, cf. chapter 4). Moreover these results suggest that the attractiveness or deterrence of the legumes intercropped with maize further increased the effectiveness of intercropping in suppressing lepidopterous insects on maize ear. Visual and chemical stimuli from the host and non-host plants might also affect the rate at which insects colonize habitats, and their behavior in those habitats (Risch 1983, Ndemah et al. 2003). Moreover in an intercrop, the primary host plant is made less attractive to the herbivore, and this may depend on the kind of cues, either olfactory or tactile perceived by the insect. Volatiles emanating from plant tissues had been reported to influence attractiveness of the plant (Elzen et al. 1984, Udayagiri and Jones 1992), which may have also played vital role in this experiment. This study showed that an intercropping system with host plants of *M. nigrivenella* could be developed, in a 'push-pull' strategy (Khan et al. 1997) for the control of *M. nigrivenella* in small-scale maize farming systems. This strategy will involve *C. ensiformis* as the highly susceptible trap plants (pull) and *Tephrosia vogelii* as repellent intercrop (push) (cf. Chapter 3).

The data reported in chapter 3 indicate that the presence of leguminous crops in the vicinity of maize fields do not aggravate *M. nigrivenella* infestations on maize, irrespective of when the legume was planted in relation to maize, and irrespective of the status of the legume species as a host. Thus, although the suitable host *C. ensiformis* had up to 1800 times higher pest loads than maize, there appeared to have been no movement of *M. nigrivenella* to maize. Also, maize surrounded by *C. ensiformis* planted four weeks before, had lower pest loads expressed as cumulative feeding days as compared to the control. This indicates that *C. ensiformis* was much more attractive to the ovipositing moth than maize. The fact that during the minor season *C. ensiformis* planted 4 weeks before maize had an effect on pest loads on maize while when planted 1, 3 in the main season or 8 weeks before in minor season it did not, suggests that the growth stage and/or canopy density of the plants affected the attractiveness of the leguminous crop to the ovipositing moth. As shown by Ritchie (2000) and Haddad et al. (2001), the amount of above-ground plant biomass, which however was not assessed in the present experiment, may have a strong effect on insect abundance. Furthermore the volatile profile of plants changes with the growth stage (Batten et al. 1995; Zhang et al. 2008), which might affect the oviposition behaviour of the moth. A more efficient technique for reducing borer densities on maize proved to be the intercropping of non-host species with maize, because the presence of the non-host plants hampers host finding by the ovipositing moth, and thus reduce the number of egg batches deposited on maize (Ndemah et al.

2003; Schulthess et al. 2004; Chabi-Olaye et al. 2005b; Wale et al. 2007; Songa et al. 2007. The major finding of the present study is that the presence of leguminous cover crops or grain legumes in the vicinity of maize fields did not increase *M. nigrivenella* densities on maize. Moreover, as shown by Chabi-Olaye et al. (2005a), grain legumes and cover crops enhance yields and reduce yield losses in maize crops subsequently planted in the same field by improving plant vigor. It is obvious that a single control option will not produce satisfactory control of the complex pest problem caused by lepidopteran maize pests, and as proposed by Chabi-Olaye et al. (2006) an IPM package including crop rotation with leguminous cover or grain crops or direct application of synthetic fertilizer, mixed cropping and timely applications of insecticides or botanicals targeting not only *M. nigrivenella* but all stem- and earborers is required.

Field and lab experiments showed that oil emulsions of *A. indica* and *J. curcas* oils act not only as oviposition deterrent but also as ovicides (cf. chapter 4). Possible inclusion of botanicals into integrated *M. nigrivenella* control has good prospects. The results of field's trials showed that larval population of *M. nigrivenella* can be significantly reduced through the application of oil emulsions of *A. indica* and *J. curcas*, which were as efficient as Furadan 5 G. Neem oils and pure compounds of neem have been found to exhibit ovipositional deterrent effects on many crops pests including lepidopteran, homopteran and dipteran species (Singh and Singh, 1998, Schmutterer 1990, Isman 1996, Bruce et al. 2004, Showler et al., 2004). According to Udayagiri and Mason (1995) chemical cues play a major role in host selection. In the ovipositional test, *M. nigrivenella* tended not to oviposit on maize plants treated with the oils indicating a repellent effect.

Mussidia nigrivenella was found feeding on maize cobs after more than 6 months of storage, suggesting that the borer not only completes its development but also reproduces under storage conditions (cf. chapter 5). However the densities of *M. nigrivenella* in stores decreased with storage duration. These reductions in pest infestations were due to many factors such adverse environmental conditions (i.e. a combination of high temperature and low air and grain moisture content), the presence of storage beetles mainly *Sitophilus zeamais* and *Prostephanus truncatus*, and the storage systems. Our findings suggest that storing maize as bulk grains in more hermetic structures or polyethylene bags rather than in traditional "ava" or cribs would reduce additional damage caused by *M. nigrivenella* in storage but care should be taken concerning the moisture content of the grains which could increase the fungal infections.

In search for natural enemies to be introduced in West Africa against *M. nigrivenella*, three parasitoids were found parasitizing *M. pectinicornella* and sympatric species infesting *Parkia speciosa* in Malaysia (Chapter 6). Although these parasitoids and the one found in East Africa (Muli et al. 2009) are adapted to the climatic condition similar to the one in West Africa giving a prospect for new association for *M. nigrivenella*, suitability tests should be done before the use in the bio-control of the maize cob borer.

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1) **Agboka**, **K.**, Schulthess, F., Chabi-Olaye, A., Labo, I., Gounou, S. & H. Smith (2002). Self-, intra, and interspecific host discrimination in *Telenomus busseolae* Gahan and *T. isis* Polaszek (Hym.: Scelionidea), sympatric egg parasitoids of the African cereal stem borer *Sesamia calamistis* Hampson (Lep.: Noctuidae). *Journal of Insect Behavior* 15: 1-12).

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CURRICULUM VITAE

Name:	AGBOKA Komi
Date of Birth:	31. 12. 1971
Place of Birth:	Gati-Soun (Republic of Togo, West Africa)
Nationality:	Togolese
Marital situation:	Married with one daughter
Sex:	Male
Address:	International Institute of Tropical Agriculture (IITA)-Benin, 08 BP 0932 Tripostal Cotonou Benin Tel: +229 21 35 01 88/ Fax: +229 21 35 05 56 E-mail: k.agboka@cgiar.org; zagboka@hotmail.com

SUMMARY OF EDUCATION

2006-2009: PhD Student, Georg-August University, Goettingen

2000-2002: M.Sc., in Horticulture, University of Hanover, Institute of Plant diseases and Plant protection (IPP)

1992-1998: Agronomy, Faculty of Agriculture, University of Lomé-Togo.

1983-1991: Secondary School, Tabligbo, Togo

1977-1983: Primary School, Gati-Soun, Togo

WORK EXPERIENCE

Since 2007: Consultant of the International Center of Insect Physiology and Ecology (ICIPE)-Kenya at IITA-Benin.

Sept. 2003 – Sept. 2006: APO, Entomologist/Ecologist at IITA-Benin

June 1998 – August 2000: Research Associate at IITA- Benin

TRAININGS

08 – 21 March 2009: International DAAD Alumni Summer School 2009 in “Post Harvest technologies –High Tec for food security and food safety”, Cologne and Goettingen - Germany

11 August – 01 September 2001: Laboratory training course on vegetable leafhopper and leafhopper egg parasitoids biology, ecology and behavior at Imperial College at Wye (London, UK), Department of Ecology and Entomology.

04 March – 31 March 2001: Training course on formulation and optimization of pesticides at Bayer Ltd. Leverkusen (Germany), Department of Plant Protection.

Declaration

I, Komi, Agboka, hereby declare, that the work presented in this thesis is my own and has not been submitted for a degree in any other University.

K. AGBOKA

I certify that, this thesis has been supervised by:

Prof. Dr. Stefan VIDAL

Dr. Manuele TAMÒ

Dr. Fritz SCHULTHESS