1 Chapter I

General Introduction and Thesis Outline

1.1 The Legume Pod Borer as a Pest Insect

The legume pod borer, *Maruca vitrata* (Fabricius) (Lepidoptera: Crambidae) is a highly migratory species and is distributed throughout the tropics (Sharma et al. 1999; Kawazu et al. 2008). The host plants of *M. vitrata* belong mainly to the Fabaceae plant family (overview by Sharma et al. 1999), including several economically important legume crops. This insect species is a major pest of cowpea (*Vigna uniguiculata*) in sub-Saharan Africa (Sharma et al. 1999) and attacks yard long bean (*Vigna uniguiculata* spp. *sesquipedalis*) in Southeast Asia (Schreinemachers et al. 2014). *M. vitrata* causes severe economic losses in legumes, which are key dietary staples in many developing countries (Fery 2002). Because of the high protein content (Wills et al. 1984), they are considered as the "poor man's meat" (Winch 2007). Legumes are further used as cover crop against soil erosion (Hartwig and Ammon 2002) and as fodder crop for livestock (Sumberg 2002). Since legume plant roots are associated with nitrogen-fixing soil bacteria, plants also serve as green manure (Fujita and Ofosu-Budu 1996) to improve soil fertility (Giller 2001).

Adult *M. vitrata* moths (Fig. 1A) mate during the night (Jackai et al. 1990; Lu et al. 2007 and 2008) and females prefer to oviposit at the flower bud stage of the host plant (Sharma 1998). The eggs are laid singly or in clusters (Jackai et al. 1990; own observation, Fig 1B). An average of 400 eggs per female was recorded in laboratory bioassays revealing the high reproductive potential of this species (Jackai et al. 1990). *M. vitrata* develops through five larval stages (Fig. 1C) and a prepupal stage until pupation (Fig. 1D) (Adati et al. 2004). The shortest developmental period from hatching to pupation (12.2 ±SD 1.0 days) was observed at 29.3°C on a semi-synthetic diet in laboratory bioassays (Adati et al. 2004). Under these conditions, the pupal stage lasted 5.3 ±SD 0.6 days (females) and 5.9 ±SD 0.2 days (males). The larvae feed predominantly inside the reproductive plant organs, such as flowers, flower buds, and pods (Sharma et al. 1999). The larvae are not able to bore into the pods until the third larval stage (Sharma et al. 1999). Hence, the first-instar larvae prefer feeding on flowers, whereas the third- to fifth-instar larvae feed on the pods. In Nigeria, up to 80% of flowers were damaged in untreated cowpea fields (Afun et al. 1991).

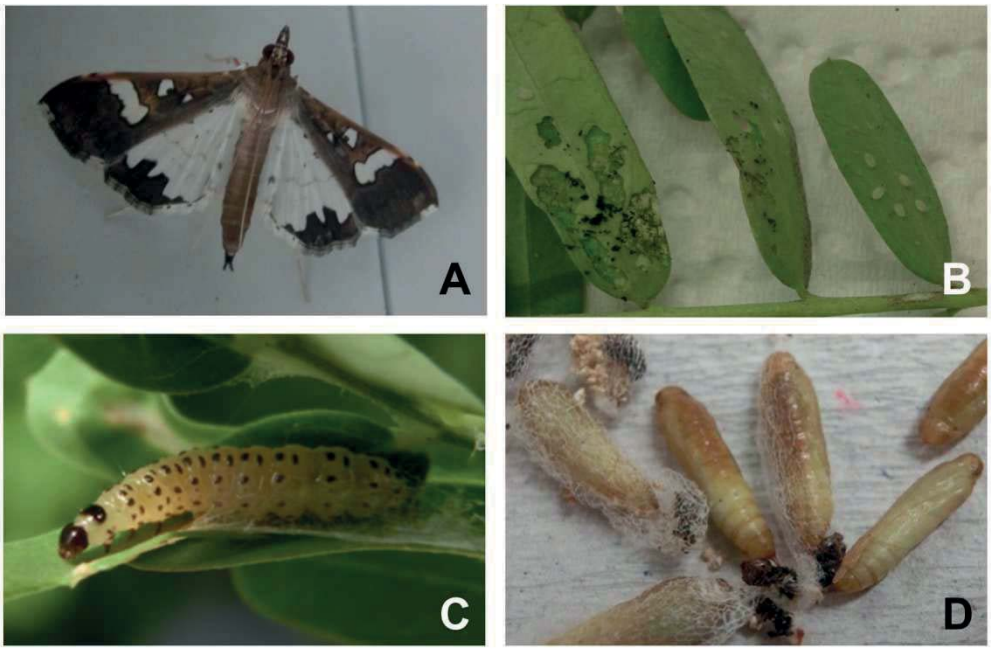

Figure 1 Developmental stages of *M. vitrata*: Male moth (A). Eggs and feeding damages of first-instar larvae on *Sesbania grandiflora* leaves (B). Fifth-instar larva on *S. grandiflora* leaves (C). Pupae (D).

The larvae are mainly controlled by synthetic insecticides (Srinivasan et al. 2013; Schreinemachers et al. 2014), but the results are not satisfactory because it is difficult to determine the most efficient time-point for pesticide application. Larval infestation of flowers shows hardly any external signs of damage (Sharma et al. 1999). Moreover, the larvae are protected from exogenous, adverse effects, e.g. natural enemies and insecticides, because of their internal feeding activity or webbing the inflorescences and leaves (Sharma et al. 1999). Furthermore, the development of pesticide resistances is a serious problem in pest control (Hajek 2004). Cases of insecticide resistance of *M. vitrata* have been reported more than a decade ago in Africa (Ekesi 1999) and in Southeast Asia (Ulrichs et al. 2001). Hence, there is an urgent need for an alternative and sustainable management strategy for *M. vitrata* in legume crops to minimize pesticide use. Successful integrated pest management strategies are often based on the combination of several components. Firstly, to prevent strong pest population growth, appropriate crop cultivation methods need to be established, such as planting of resistant or tolerant crop plants (Jackai et al. 1996; Bottenberg et al. 1998; Adekola and Oluleye 2008), optimal plant spacing (Asiwe et al. 2005), and intercropping (Karel 1993). Secondly, biopesticides and natural enemies can be used for direct control of *M. vitrata* eggs or larvae. Promising results have been reported for the usage of botanicals, such as crude aqueous extracts of black pepper, garlic bulb, and neem seed (Ekesi 2000), and microbial pesticides, such as nucleopolyhedrovirus (Lee et al. 2007), *Bacillus thuringiensis*

(Srinivasan 2008; Yule and Srinivasan 2013), as well as entomopathogenic fungi, for instance *Beauveria bassiana* and *Metarhizium anisopliae* (Ekesi et al. 2002; Mehinto et al. 2014; Tumehaise et al. 2015). Among invertebrates, the braconid wasp *Apanteles taragamae* showed the greatest potential as a biological control agent for *M. vitrata* in Taiwan (Huang et al. 2003), which has resulted in a series of studies to evaluate its introduction to Benin in West Africa (Dannon et al. 2010 a,b; 2012 a,b). Another important tool in integrated pest management is the use of pheromones for pest monitoring or mass trapping.

1.2 Lepidopteran Sex Pheromones and their Application in Pest Management

Pheromones are generally termed as "substances which are secreted to the outside by an individual and received by a second individual of the same species, in which they release a specific reaction" (Karlson and Lüscher 1959). Sex pheromones attract the conspecific opposite sex for mating (Jurenka 2004). Courtship of nocturnal Lepidoptera is mainly mediated by sex pheromones (Howse 1998) which are predominantly produced by females (Rafaeli and Jurenka 2003). In most cases, sex pheromone biosynthesis takes place in glands which are located between the 8^{th} and 9^{th} abdominal segment, as in the case of *Helicoverpa zea* (Raina et al. 2000) and is controlled by the pheromone biosynthesis activating neuropeptide (PBAN) produced in the brain-subesophageal ganglion complexes and released from the *corpora cardiaca* (Rafaeli 2005). For pheromone release, the female extrudes the pheromone gland from the abdomen, the so-called calling behavior (Groot 2014) which occurs for most moth species in the scotophase (Rafaeli and Jurenka 2003). The pheromone compounds are carried along by the wind forming an odor plume (Murlis et al. 1992). The conspecific male perceives the sex pheromones using specific receptors localized in hair-like sensilla on the antennae (Leal 2005) and locks onto the pheromone plume to locate the calling female for mating (Howse 1998). Most moth sex pheromones consist of a precise ratio of several compounds (Jurenka 2004) which are separated into three groups (Ando et al. 2004; Ando and Yamakawa 2011). The major group, Type I pheromones (75%), includes primary alcohols and their derivatives (mainly acetates and aldehydes) with a long straight chain (C10–C18). Type II pheromones (15%) are polyunsaturated hydrocarbons and their epoxy derivatives with a longer straight chain (C17–C23 and, exceptionally, C25 and C27). The remaining identified compounds are secondary alcohols and ketones with a straight chain and esters of a long unbranched-chain acid or methyl-branched compounds. Species specificity of pheromone blends is achieved by the structural diversity of pheromone compounds (polymorphic variation) and shifted pheromone ratios (monomorphic variation), whereas the

latter is more common (Löfstedt 1990). Notably, pheromone blend variation does not only occur between species, but also between geographically different moth populations of the same species (McElfresh and Millar 1999; Gemeno et al. 2000; El-Sayed et al. 2003; Cortés et al. 2010).

Once the sex pheromone composition has been fully elucidated and synthesized, the pheromone blend can be applied on suitable dispensers and used as a species-specific lure in traps. Pheromone lures are mainly used for pest monitoring to determine the appropriate time for control measures (e.g. insecticide treatment) (Jones 1998). Pheromone lures can be also used for direct pest control as in mating disruption. This technique reduces or delays moth reproduction by saturating an area with synthetic pheromone, and thereby preventing odor-based communication and mate-finding (Jones 1998; Witzgall 2001). In pheromone-mediated mass trapping ("lure-and-kill technology"), high numbers of males are attracted and killed in the trap for example by insecticides (Jones 1998; Cork 2004).

For successful application of pheromone traps, the technical set up needs to be optimized for the target pest and adapted to the respective situation in the field. The presence of pheromone blend variation between populations of a pest species needs to be established, males may respond differently to synthetic pheromone lures in different regions, as for example observed in *Choristoneura rosaceana* Harris (Lepidoptera: Tortricidae) (El-Sayed et al. 2003). The chemical stability of the synthetic pheromones needs to be verified for field application as well as for storage conditions because it may have an impact on lure efficiency (Cork 2004). For example, aldehydes are known to react with atmospheric oxygen to form carboxylic acids (Stevens 1998). To prevent oxidation, synthetic pheromone lures are combined with antioxidants in a ratio of 1:1, for example with butylated hydroxytoluene (BHT) (Cork 2004). Pheromone components, which contain conjugated double bonds, are susceptible to photoisomerization induced by sunlight (Cork 2004). To prevent degradation of pheromone components by ultraviolet (UV) light, UV-stabilizers, such as 2-hydroxy-4-methoxybenzophenone (Ideses and Shani 1988), are loaded additionally on pheromone dispensers (Jones 1998).

Additionally, the pheromone dispenser needs to ensure a controlled release of the pheromone compounds in the field for the entire monitoring period (Cork 2004). The optimal trap design facilitates the entry and ensures the retention of the target pest (Cork 2004). Apart from the

pest insect, the trap height depends on growth habit and cultivation method of the crop plant (Cork 2004).

1.3 Sex Pheromone Components and Blends of *M. vitrata*

The sex pheromone of *M. vitrata* has been investigated for more than a decade (Adati and Tatsuki 1999; Downham et al. 2003 and 2004). The first studies focused on insect populations from West Africa. (*E,E*)-10,12-hexadecadienal (*EE*10,12-16:Ald) was identified as the major pheromone component and (*E,E*)-10,12-hexadecadienol (*EE*10,12-16:OH) (Fig. 2) as a minor pheromone compound (at 3-4% of the aldehyde) in female gland extracts from Ghana by gas chromatography – electroantennographic detection (GC–EAD) and gas chromatography – mass spectrometry (GC–MS) (Adati and Tatsuki 1999). Both compounds were confirmed to be present in a mixed *M. vitrata* population from Benin, Nigeria, India, and Taiwan (Downham et al. 2003). Moreover, the authors presumed a monounsaturated hexadecenal in the pheromone blend based on GC–EAD results. Comparing a range of synthetic hexadecenal isomers by electroantennographic responses of *M. virata* males, (*E*)-10-hexadecenal (*E*10-16:Ald) (Fig. 2) elicited the highest response from the antennae. More recently, *E*10-16:Ald was directly detected in two Chinese *M. vitrata* populations (Huazhou and Wuhan) by GC–MS in addition to *EE*10,12-16:Ald, and *EE*10,12-16:OH (Lu et al. 2013). In fact, in females from Wuhan, the proportions of *E*10-16:Ald and *EE*10,12-16:Ald were comparable.

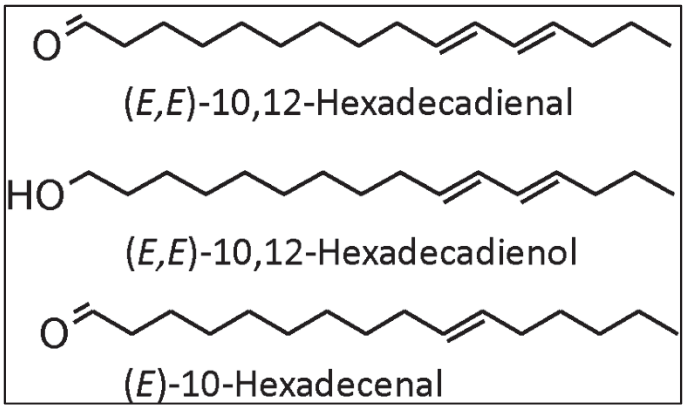

Figure 2 Structures of described *M. vitrata* pheromone components: (*E,E*)-10,12-Hexadecadienal, (*E,E*)-10,12-Hexadecadienol, and (*E*)-10-Hexadecenal.

In field studies, a pheromone ratio of 100:5:5 (*EE*10,12-16:Ald:*EE*10,12-16:OH:*E*10-16:Ald; 100:5:5-blend) was the most attractive blend in cowpea fields in Benin (Downham et al. 2003). Traps baited with this synthetic blend attracted more males than traps baited with two virgin females (Downham et al. 2003). In China, synthetic blends with a ratio of 100:10:80