Towards developing a sustainable management strategy for legume pod borer, *Maruca vitrata* on yard-long bean in Southeast Asia

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ABSTRACT

Legume pod borer, *Maruca vitrata*, is a serious production constraint on yard-long bean which is grown over 7% of the total vegetable production area in Southeast Asia. It can cause up to 80% yield loss on various host plants, and because it is hidden within the pods, it is difficult to control with chemical pesticides. However, farmers rely heavily on the use of chemical pesticides to control *M. vitrata*. Intensive and indiscriminate use of pesticides leads to the development of resistance in *M. vitrata* to chemical pesticides, degrades the environment, kills natural enemies, and causes resurgence of secondary insect pests. To reduce pesticide residues in vegetable legumes and to promote better human health, an integrated pest management strategy based on sex pheromones, natural enemies, and bio-pesticides is being developed based on applied research. Since phenotypic variations have been observed among populations of *M. vitrata* in Southeast Asia and sub-Saharan Africa, *M. vitrata* populations present in Southeast Asia with reference populations from sub-Saharan Africa have been characterized. The inconsistency in the performance of sex pheromone lures has become an obstacle in implementing trap-based pest monitoring of *M. vitrata*. Appropriate pheromone blends for each geographical region are being developed based on the variations in pheromone production and reception in *M. vitrata*. With a more systematic effort, novel species-specific parasitoids of *M. vitrata* have been identified in Lao PDR, Taiwan, Thailand and Vietnam. Studies have indicated the potential susceptibility of *M. vitrata* to *Bacillus thuringiensis*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Maruca vitrata* multiple nucleopolyhedrovirus, and neem.
Hence, an IPM strategy based on sex pheromones, natural enemies, and biopesticides will soon be validated in pilot project sites in Southeast Asia, especially Thailand and Vietnam to manage *M. vitrata*.

**Keywords**

*Maruca vitrata*, integrated pest management, yard-long bean

**INTRODUCTION**

Vegetable legumes are one of the important crops in Southeast Asia. They are important source of plant proteins in the human diet and considered as the ‘meat of the poor’ (Heiser 1990). They are rich in essential micronutrients, especially iron and folic acid, which are particularly important for women of child-bearing age and other vulnerable groups such as infants and young children, adolescent boys and girls and pregnant women. Leguminous crops fix atmospheric nitrogen in the soil, thus improving soil fertility. Legumes can be used as high quality livestock fodder, and are planted to control soil erosion. Yard-long bean (*Vigna unguiculata sesquipedalis*) is the most popular vegetable legume in Southeast Asia, accounting for almost seven percent of the total vegetable production area in the region (Ali et al. 2002). It is estimated to be cultivated on more than 150,000 ha in total, in Indonesia, Thailand, and Vietnam (Chinh et al. 2000; Sarutayophat et al. 2007). Other grain and vegetable legumes are being cultivated on an area of 1.4 m ha in Southeast Asia.

Vegetable legumes are highly susceptible to insect pests and diseases. Among the documented pests, legume pod borer (LPB), *Maruca vitrata* (F.) (syn. *M. testulalis*) (Lepidoptera: Pyralidae), is considered the most serious pest of yard-long bean, mungbean, and soybean in Southeast Asia (Sharma 1998; Ulrichs et al. 2001a; Soeun 2001). *M. vitrata* larvae feed on flowers and pods (Sharma 1998). First instar larvae prefer to feed on flowers rather than pods or leaves. The mature larvae are capable of damaging pods, and occasionally the peduncle and stems (Taylor 1967). Up to 80% yield losses have been reported in cowpea due to *M. vitrata* damage (Singh et al. 1990). Losses in cowpea pod yield ranged from 17–53% in Taiwan (Liao and Lin 2000). In Bangladesh, the damage to lablab bean was 18%, even in pesticide-sprayed fields, and it was 20–30% pod damage in mungbean (Zahid et al. 2008). About 25% pod damage in yard-long bean was estimated in Indonesia (Hammig et al. 2008).

At present, farmers rely almost exclusively on the application of chemical insecticides to combat *M. vitrata*, but without satisfactory control results. The window for effective pesticide application is very brief, as *M. vitrata* larvae are exposed on leaves only for a short time after hatching. Cambodian farmers sprayed up to 20 times per season with up to five different pesticides mixed together per tank per spray on major vegetables including yard-long bean (Sodavy et al. 2000). In Bangladesh, the country bean is being sprayed at weekly or biweekly intervals—sometimes every day—to control *M. vitrata* (Hoque et al. 2002). The destruction of indigenous biodiversity, development of pesticide resistance, and environmental deterioration are direct consequences of pesticide abuse. Occupational health hazards to farm workers are an additional dimension of pesticide overuse. *M. vitrata* resistance to commonly used pesticides has been documented in Southeast Asia (Ulrichs et al. 2001a). The overuse of pesticide also increases production costs, thus reducing profits for farmers. Pesticide residues hamper the export market potential for vegetable legumes. In a 2004 survey conducted in the United Kingdom, almost 45% of green bean samples, including yard-long bean from Thailand and Bangladesh, had at least one kind of pesticide residue above the legal limit (PAN UK 2009).

Thus, vegetable legume growers in different regions of Asia urgently need alternative management strategies to control *M. vitrata*. Therefore, an IPM strategy must be developed and deployed. AVRDC – The World Vegetable Center has recently initiated a project in collaboration with its research and development partners in Benin, Germany, India, Kenya, Lao PDR, Malaysia, Taiwan, Thailand and Vietnam to develop an IPM strategy for managing *M. vitrata* in Southeast Asia and sub-Saharan Africa. This paper reviews some key findings towards developing a sustainable IPM strategy in this project.
Population composition of *M. vitrata*

The Indo-Malaysian region is considered to be the most probable region of origin for the genus *Maruca*, including *M. vitrata*, which is found throughout the tropics (CABI 2005). Although 11 species have been documented in the genus (ZipcodeZoo.com), only two other species, *M. amboinalis* and *M. nigroapicalis*, have been described; they were observed in the Indo-Malaysian and Tonkin area, and the latter never was found again after the first description (CABI 2005). Kirti and Gill (2005) also confirmed the occurrence of *M. amboinalis*, and differentiated it from *M. vitrata* based on the external genitals. As *M. vitrata* is thought to have evolved in Southeast Asia and possibly spread across Asia and into Africa, local *M. vitrata* populations from the project countries are being characterized to confirm the identity of the pest species that damage food legumes.

The male genitalia of *M. vitrata* has long and strongly curved uncus, slightly dilated at distal end, tegument dome-shaped, valva with a short harpe; female genitalia with corpus bursae dropper shaped, signum missing, ductus bursae membranous, with a collar at distal end. The male genitalia of *M. amboinalis* has uncus with slightly dilated at distal end, tuba analis longer than uncus, valva with costa and harpe marked by a small, weakly curved projection; female genitalia has an oblong corpus bursae with a slight constriction in outer wall in middle, moderately long and distally rounded ductus bursae, ovipositor with rounded and well defined lobes, each lobe densely setose with varying sizes of setae (Kirti and Gill 2005). When the adult specimens of *Maruca* sp. from Benin, Kenya, Lao PDR, Taiwan, Thailand and Vietnam were examined, all had the wing venation and the external genital structures (Figure 1) of *M. vitrata*, and hence the *Maruca* species infesting vegetable legumes could be confirmed as *M. vitrata*.

A total of 41 different larval populations on the host plants *Sesbania cannabina*, *S. grandiflora*, *S. rostrata*, *S. vesicaria*, *Vigna unguiculata*, *V. u. subsp. sesquipedalis*, *V. radiata*, *V. cylindrica*, *Phaseolus* sp., *P. vulgaris*, *Cajanus cajan*, *Psophocarpus tetragonolobus*, *Pterocarpus santalinus*, *Canavalia* sp., *Pueraria phaseoloides*, *Dolichos lablab*, *Lonchocarpus cyanescens* and *Tephrosia bracteola* from Benin, India, Kenya, Lao PDR, Taiwan, Thailand and Vietnam, with Indonesia (as a reference sample) were collected by AVRDC and the collaborating partners. Mitochondrial gene, cytochrome c oxidase I (*coxI*) was used as a candidate gene to compare the larval populations of *M. vitrata*. The results have shown that there is no difference among the *M. vitrata* populations based on the host plants. However, single nucleotide polymorphisms (SNPs) have been observed in the *coxI* sequences of different *M. vitrata* populations, although the functional significance of these SNPs among the *M. vitrata* populations is still unknown.

The nuclear internal transcribed spacer 2 (ITS2) region of rDNA was also used for diversity analysis. Although several pairs of primers have been tried for the PCR, only about 135 bp high quality sequence of ITS2 could be obtained from all *M. vitrata* accessions. Since this short sequence contains several SNPs, it will complement the diversity analysis based on *cox1* gene sequences. As a third candidate gene, *M. vitrata* elongation factor 1α (*EF1α*) was also tried for characterizing the *M. vitrata* populations. The EF1α gene specific primer pairs amplified a PCR product covering 1193 bp of the intron-less EF1α gene for 22 *M. vitrata* samples, but did not reveal any sequence polymorphisms among the *M. vitrata* populations.

Refining the sex pheromone lures

Sex pheromone traps mimicking the pheromone of female insects attract males which are trapped and eventually killed. This reduces mating and production of next generation of insects. The major component of *M. vitrata* sex pheromone was identified as (E,E)-10,12-hexadecadienal (Adati and Tatsuki 1999), whereas (E,E)-10,12-hexadecadienol, and (E)-10-hexadecenal were identified as minor components (Downham et al. 2003). Re-examination of *M. vitrata* sex pheromone revealed the presence of two new components, (E)-10-hexadecenol and (Z,Z,Z,Z,Z)-3,6,9,12,15-tricosapentaene (Hassan 2007). Variations have been observed in the responses of *M. vitrata* males to the synthetic sex pheromone lures over various geographical regions. A synthetic sex pheromone lure consisting of (E,E)-10,12-
hexadecadienal, (E,E)-10,12-hexadecadienol, and (E)-10-hexadecenal was developed and attracted male *M. vitrata* moths in Benin and Ghana, while (E,E)-10,12-hexadecadienal alone was most effective in Burkina Faso (Downham et al. 2004). Neither pheromone was effective in Taiwan and Thailand, while a variant blend was effective in India (Hassan 2007). Hence, a thorough investigation into the sex pheromone components among *M. vitrata* populations from different geographical origins is needed to refine the pheromone based pest management strategy. Collaborating partners in Germany, India and Kenya are currently identifying the major and minor components of sex pheromone among the *M. vitrata* populations from target countries.

Sex pheromone biosynthesis in insects usually is regulated by hormonal and neural factors such as pheromone biosynthesis activating neuropeptide (PBAN) (Iglesias et al. 2002). Several PBAN from different insects have been characterized (Rafaeli 2002). However, the role of PBAN in relation to a specific pheromone blend is not known in *M. vitrata*. Identification of genotypic variation in PBAN among distinct *M. vitrata* populations from different countries is in progress to determine its contribution in sex pheromone polymorphism, if any. Pheromone perception in insects is mediated by pheromone binding proteins (PBPs) present in the insect antennae that bind to the pheromone compounds (Vogt et al. 2002). PBPs have been identified from several insects, and multiple PBPs occur in a single insect species. These PBPs could discriminate different components in an insect pheromone. Lack of a particular PBP in an insect population might make that population non-responsive to a specific pheromone blend. Because different *M. vitrata* populations respond differently to the multi-component pheromone, variations may exist in the PBPs of various *M. vitrata* populations, which is being characterized.

Thus, understanding the pheromone polymorphism, diversity in PBAN and/or PBPs could enable us to develop specific pheromone blends for a particular *M. vitrata* population in a given region to monitor and/or mass-trap the pest population.

**Exploration for species-specific natural enemies of *M. vitrata***

Although a substantial number of parasitoid species have been reported to attack *M. vitrata* in tropical Asia and Africa (Waterhouse and Norris 1987; Sharma 1998; Ulrichs et al. 2001b; Huang et al. 2003; Arodokoun et al. 2006), they have not been exploited successfully in biological control programs. This is largely due to the low level of parasitism observed with all recorded species of parasitoids. However, a few parasitoids have been identified in recent years that could control *M. vitrata*. Huang et al. (2003) found that the parasitism of *M. vitrata* by *Apanteles taragamae*, could reach as high as 63% in Taiwan. *Bassus asper* was the most prevalent parasitoid in the Philippines with parasitism rates up to 17.1% (Ulrichs et al. 2001b). A braconid parasitoid, *Phanerotoma leucobasis*, could inflict about 30% parasitism in Benin (Arodokoun et al. 2006). Although these natural enemies could cause higher parasitism under field conditions, most of them are generalists; the performance of generalist parasitoids in a new habitat is highly variable, as the host range is not definite. Some countries may not allow the introduction of generalist parasitoids for biological control programs. To address these issues, explorations are being made in Lao PDR, Malaysia, Taiwan, Thailand and Vietnam to identify species-specific parasitoids of *M. vitrata*.

Although *A. taragamae* and *Nemorilla maculosa* (a tachinid fly) were reported to be the major parasitoids of *M. vitrata* in Taiwan (Huang et al. 2003; Srinivasan et al. 2009), *Therophilus marucae* (Braconidae: Hymenoptera) (Figure 2), besides *T. javanus* emerged as a major parasitoid in a recent survey during May – June 2011 in Southern Taiwan. It is a larval parasitoid and the field parasitism rate was up to 38%. The same parasitoid is also known to exist predominantly in Lao PDR and Vietnam. However, an egg-larval parasitoid, *Phanerotoma philippinensis* (Braconidae: Hymenoptera) (Figure 3) has been identified as a major parasitoid of *M. vitrata* in Thailand. In a monitoring survey during March – July 2011 at Nakhon Pathom area, the field parasitism ranged between 2 and 21%. Mass-culturing methods currently are being developed for these parasitoids with an objective to explore their use in managing *M. vitrata*.
Identification and/or development of promising bio-pesticides against *M. vitrata*

Different *B. thuringiensis* (Bt) δ-endotoxins have already been evaluated against *M. vitrata* at AVRDC. The results showed that Cry1Ab was the most potent toxin, followed by Cry1Ca (Srinivasan 2008). Hence, commercial Bt formulations containing Cry1Ab and/or Cry1Ca should be effective against *M. vitrata*. The commercial Bt formulations such as Crymax® and Redcat® (*B. t. subsp. kurstaki*) and Xentari® and Zitarback F.C.® (*B. t. subsp. aizawai*) were bioassayed against *M. vitrata* in Taiwan and Thailand. In Taiwan, both Crymax® and Xentari® are equally effective against *M. vitrata*, recording similar LC50 values. However, in Thailand, Redcat® was more effective than Zitarback F.C.® which recorded a three-fold higher LC50. The toxicity of isolates and commercial formulations of entomopathogenic fungi such as *B. bassiana* and *M. anisopliae* is being assayed against *M. vitrata* by our collaborative partners.

Variable results have been obtained with the application of neem-based pesticides against *M. vitrata*. The commercial neem formulation (Biofree–I®) in Taiwan did not cause the mortality of *M. vitrata*. However, a neem formulation in Thailand (Thai neem 111) recorded a median lethal concentration of about 2300 ppm. Currently, we are assessing the oviposition repellence and chronic toxicity of neem formulations.

AVRDC – The World Vegetable Center identified a nuclear polyhedrosis virus (NPV) that infects *M. vitrata* in Taiwan in 2005. It was the world’s first recorded instance of an NPV specifically infecting *M. vitrata*. The NPV was characterized based on ultra-structural morphology, restriction endonuclease cleavage patterns, and sequences of the coding region of polyhedrin gene, and named MaviMNPV (Lee et al. 2007). Laboratory bioassays revealed that the first instar *M. vitrata* larvae were the most susceptible stage to MaviMNPV and the median lethal concentrations (LC50s) increased with increasing larval instars (Lee et al. 2007). Formulations of this NPV have been found to be effective against *M. vitrata* on food legumes either alone or in combination with *B. thuringiensis* and neem under laboratory and field conditions in Taiwan and Benin (Tamo et al. 2007; Srinivasan et al. 2009). The formulations of MaviMNPV were evaluated against *M. vitrata* on hyacinth (lablab) bean. After five rounds of spraying at 15-day intervals, the pod damage was significantly lower in treatments containing MaviMNPV formulations (8.38-9.79%) than that of the control (18.29%). Such a control efficacy was similar with those of Xentari® (8.11%) and carbaryl (9.71%) (Srinivasan et al. 2009). In another field trial at Thailand, Redcat® and Zitarback F.C.® recorded lower damages (3.57 and 3.10%, respectively) which was on par with cypermethrin (3.76%) compared to the untreated check (6.48%). Hence, bio-pesticides have promising potential against *M. vitrata* and thus reducing the damage in vegetable legumes. Currently, efforts are underway in identifying the effectiveness of bio-pesticides either alone or in combinations against *M. vitrata*.

**CONCLUSION**

An integrated pest management (IPM) strategy will be formulated based on the refined sex pheromone lures, species – specific parasitoids and promising biopesticides identified from the on-going research activities. This IPM strategy will be validated in small-scale on-station field trials in Taiwan, Thailand and Vietnam. In addition to the on-station trials, a few on-farm field trials will also be conducted in farmers’ fields in Thailand and Vietnam to validate the strategy. If necessary, the component technologies will be adjusted to suit to the local conditions, and promoted among the vegetable legume growers in Southeast Asia.

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**References**


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Figure 1. Genitalia of *Maruca vitrata*

Figure 2. *Therophilus marucae*, a larval parasitoid of *Maruca vitrata*

Figure 3. *Phanerotoma philippinensis*, an egg–larval parasitoid of *Maruca vitrata*