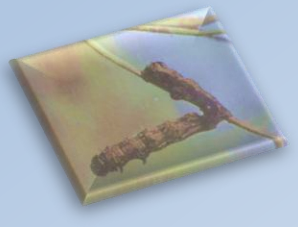



FINAL REPORT
On
BIOLOGICAL CONTROL PROGRAMME

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November 2014


Strengthening a national beet armyworm (*Spodoptera exigua*) management programme in Jamaica

AGP: TCP/JAM/3402

Biological Control Programme

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TCP/JAM/3402 - Strengthening a national beet armyworm (Spodoptera exigua) management Programme in Jamaica

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

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Executive Summary

The beet armyworm (BAW), *Spodoptera exigua* (Hübner), is a polyphagous lepidopteran pest originating from Southeast Asia. Its wide host range includes vegetable, field and flower crops. The BAW has become a major pest of scallion and onion in Jamaica within the past five years. In 2012, the Ministry of Agriculture and Fisheries (MoAF) and the Food and Agriculture Organization (FAO) developed a project under FAO's Technical Cooperation Programme titled "Strengthening a national beet armyworm (*Spodoptera exigua*) management Programme in Jamaica". This Report outlines activities carried out by the Reporting Officer in fulfilment of the Terms of Reference for the Biological Control aspect of the project.

A desk review of the potential natural enemies of the BAW was conducted and a list of natural enemies compiled. It was hoped that more information on BAW natural enemies from the Region would have been forthcoming but it is obvious that (i) there is a lack of adequate information and (ii) the information is not documented or published. It is interesting to note that the information that was obtained for the Region was not recent and was on *Spodoptera* spp., rather than *S. exigua* specifically.

The environment surrounding scallion and onion fields was assessed to determine their capacity as reservoirs for the pest as well as potential biocontrol agents. It was found that milk weed, callaloo and guinea grass (*Panicum maximum*) were suitable plants on which developing larvae could feed but it was not determined if these weeds were able to support the full development of the pest from larva to adult.

An appraisal of the natural enemies of the BAW larvae indicated that the main predators were white egret birds, wasps (*Polistes* spp.), spiders and ants. However, no parasitoids or entomopathogens were determined from field-collected BAW.

Recommendations were made for the management of the environment (which may harbour the pest) surrounding the fields with respect to pest and natural enemy populations. These recommendations included the provision of shelter/nest sites for predators such as spiders and wasps, the planting of flowering plants close to onion and scallion fields to encourage other beneficial insects, such as ladybird beetles and lacewings and the monitoring - implementation of appropriate action/s where necessary - of the surrounding environment/vegetation for signs of the presence of the BAW.

It was anticipated that at least two parasitoids or entomopathogens would have been found, the presence of which could have contributed significantly to the overall management efforts of the BAW. However, it is apparent that the affected farmers' heavy reliance on insecticide control of the BAW has decimated the natural enemy population. Two entomopathogens were identified from the literature and discussions with MoAF Research and Development Division personnel, which show great promise for incorporation in an IPM programme. The protocols for the mass production, and laboratory and field bioassays of the fungus, *Metarhizium anisopliae*, and multiple nuclear polyhedrosis virus are outlined in the document. These entomopathogens can be formulated as spray solutions, which would make them more easily accepted and adopted for use by the farmers.

Introduction

The beet armyworm (BAW), *Spodoptera exigua* (Hübner), is a polyphagous lepidopteran pest originating from Southeast Asia. Its wide host range includes vegetable, field and flower crops such as beet, cabbage, cauliflower, celery, corn, cowpea, lettuce, onion, sweetpotato, tomato, cotton, peanut, sorghum, soybean and callaloo (*Amaranthus* spp.). In Jamaica, the BAW has been reported on legumes since the 1970s, and on scallion and onion in the 1990s (RADA 2009) but it was not considered a major pest until 2009, when there was a major outbreak on scallion in the parish of St Elizabeth. Since then, it has been elevated to 'key pest' status with regular outbreaks during the cropping season. In 2012, the Ministry of Agriculture and Fisheries (MoAF) and the Food and Agriculture Organization (FAO) developed a project under FAO's Technical Cooperation Programme titled "Strengthening a national beet armyworm (*Spodoptera exigua*) management Programme in Jamaica". Under this programme, the National Consultant/Entomologist was recruited in February 2013 to assist in various aspects of the execution of the project.

The Terms of Reference for the Biological Control aspect of the project were as follows:

1. Conduct a desk review of the potential natural enemies of the beet army worm (*Spodoptera exigua)*
2. Assess the environment surrounding the cultivated fields to determine their capacity as reservoirs for the pest as well potential bio-control agents. Provide recommendations for management of these environments with respect to pest/natural enemy population
3. Review field-collected natural enemies (from the Training of Trainers and other collections) and conduct an analysis to prioritize natural enemies for the rearing / mass production programme
4. Based on findings, prepare protocols for at least two biological control agent programme as prioritized with the Ministry, including rearing / mass production and release techniques.

This Report outlines activities carried out in fulfilment of each of the four components of the ToRs.

1. ***Desk review of the potential natural enemies of the beet armyworm (Spodoptera exigua)***

The beet armyworm (BAW) originated in Southeast Asia and is found in many parts of the world, including Jamaica. The first report of the beet armyworm (BAW) in Jamaica was in the 1970s when it was recorded from legumes (RADA 2009). Its first reported outbreak on scallion was in South St Elizabeth in the 1990s. Further severe outbreaks of BAW on scallion and onion fields in South St. Elizabeth occurred between 2009 and 2012, during the May/June and October/November months, which coincided each time with the end of the rainy season (Plant Prot. Unit, 2013).

Classification, Description and Life History of the beet armyworm

The beet armyworm (Lepidoptera: Noctuidae) belongs to the genus *Spodoptera* and species *exigua* Hübner.

The eggs of the BAW are white to white with a green tinge, with a circular cross-section and tapered at the top. They are covered with a layer of whitish scales so they appear fuzzy. Found on underside of leaves. BAW undergoes five larval instars. The first, second and third instars are yellow/pale green and have head capsules measuring 0.25-0.70 mm. The third instar also has pale stripes along its body.

As the larvae mature, they become darker in colour and their head capsules range between 1.12 and 1.80 mm. The fourth instar has a dark lateral stripe, while it is white in the fifth instar. The fifth instar's colour varies is more variable (green to dark green dorsal surface, dark spot/dashes, pink/yellow ventral surface), with white spiracles and narrow black border; a dark lateral spot on the mesothorax may also be present.

The pupae/pharate adults are light brown and measure 15-20 mm in length. BAW adults have wing span of 25-30 mm mottled grey and brown irregular banding pattern with light-coloured bean shaped spot near the centre on their forewings and grey/white hind wings with dark margins. Adults live nine to ten days of emergence (Heppner 1998).

Soon after emergence, adult moths mate. Two to three days later, females deposit egg masses of 50-150 eggs on the upper half of scallion/onion leaves (Figure 1) over a period of three to seven days. One female can oviposit 300-600 eggs during her lifetime.

Figure 1. Beet armyworm egg masses (encircled in yellow) on scallion leaves

Eggs hatch within three days and the first instars spin loose webs around themselves and begin feeding en masse on the remnants of egg masses after which they swarm onto onion/scallion leaves. By the second-third instar stage, larvae enter the scallion/onion leaves, where they remain feeding until they are ready to pupate (Figure 2). Damage from early instars is evident as small holes near the tips of scallion leaves; if larvae are numerous, many small entry holes can be observed. Damage arising from feeding by later instars (3^{rd}-5^{th}) is more obvious, as they become solitary and eat large irregular holes in leaves; their feeding can cause leaves tend to bend over. Mature larvae may eat entire leaves, and burrow down into the bulbs; larvae may even completely defoliate plants. If the preferred host (onion and scallion) is destroyed, they move to other hosts or any suitable plant to feed and complete development.

Figure 2. Third instar beet armyworm found inside scallion leaf

Duration of the instars under warm conditions is generally 2.3, 2.2, 1.8, 1.0, and 3.1 days, respectively (Wilson 1932), and at constant 30 ºC, instar development time was reported by Fye and McAda (1972) to be 2.5, 1.5, 1.2, 1.5, and 3.0 days, respectively. Preliminary laboratory studies carried out at the Bodles Research Station indicate that the duration of the 1st to 5th instars were 7, 4, 2, 6 and 6 days at mean (±SD) temperature of 23.7 (±0.97) °C and mean (±SD) relative humidity of 36.1 (±9.37)% and 1, 4, 1, 6 and 3 days at mean (±SD) temperature of 29.6 (±0.94) °C and mean (±SD) relative humidity of 26.1 (±6.43)%, respectively (W. Diedrick 2013, Min. Agric. & Fisher. Res. & Dev. Div.; pers. comm.).

Fully mature larvae move to the soil where they construct pupal chambers. The pupal stage lasts 6-7 days while adults live 9-10 days. The entire life cycle takes 15-36 days (Karimi-Malati *et al*. 2014). Dry, hot conditions – as are present in South St Elizabeth – are ideally suited to the rapid development of the BAW, while cool conditions – as are present during the December-March winter season in Jamaica – do not favour the development of BAW populations. Indeed, the BAW population tends to fall to low levels at this time (PPU 2013).

The host range of the beet armyworm is quite extensive, from vegetable to field and flower crops to weeds. The vegetable host crops include beans, beet, broccoli, cabbage, cauliflower, celery, callaloo, chickpea, corn, cowpea, eggplant, lettuce, melon, ochro, onion, pea, pepper, potato, sweetpotato, tomato, corn, cotton, peanut, safflower, sorghum, soybean, sugarbeet, and tobacco. Weed hosts include lambsquarters, *Chenopodium album*; mullein, *Verbascum* sp.; pigweed, *Amaranthus* spp.; purslane, *Portulaca* spp.; Russian thistle, *Salsola kali*; parthenium, *Parthenium* sp.; and tidestromia, *Tidestromia* sp. It has been reported that BAW seems to prefer hybrids of scallion to the local, native varieties of the crop (RADA 2009).

The natural enemies of the beet armyworm include predators, parasitoids, entomopathogenic nematodes, fungi and viruses. *Predators* are free-living, actively seeking and killing their prey (both immature and adult stages of the pest) and have a life span longer than their prey. They can be very specialized, feeding on only one pest species or generalist, feeding on a wide range of pests. Predators are able to consume a number of pest individuals per day. *Parasites* live on or within the pest host's body and may or may not kill their host. Parasitic nematodes which parasitize insects are called entomopathogenic nematodes. Insects which parasitize other insect are called *parasitoids*. Parasitoids spend their immature stages in their hosts and always kill their hosts while adult parasitoids are free living. Parasitoids may attack any host stage but the adult stages are the least attacked. *Pathogenic microorganisms* cause diseases which can reduce the host pest's ability to reproduce normally, slow down its growth and development

and/or cause its eventual death. Fungi, protozoans, viruses and bacteria are the major groups of entomopathogens.
The efficacy of natural enemies in suppressing BAW populations ranges widely. Field parasitism levels of 3-90% (Sertakaya *et al.* 2004) and < 1%-67% (Ruberson *et al.* 1994) were recorded from Turkey and USA, respectively. The viruses tend to effect high mortality in larvae (UC 2014; Kaya 1985), while nematodes inflicted mortality of 68-100% in neonate and 3- and 8-day-old larvae exposed to 50 or more nematodes (Kaya 1985). The combination of nematodes and fungi (*Beauveria bassiana*) resulted in higher total mortality of BAW than either nematode alone or fungus alone (Barbercheck and Kaya 1991).

A list of natural enemies of the BAW, which was compiled from information obtained in a literature search, including a search of the UWI Mona Science Library files, is given in Appendix I. It was hoped that more information on BAW natural enemies from the Region would have been forthcoming but it is obvious that (i) there is a lack of adequate information and (ii) the information is not documented or published. The information that was obtained for the Region was not recent and was on *Spodoptera* spp., rather than *S. exigua* specifically.

2. ***Assessment of the environment surrounding the cultivated fields to determine their capacity as reservoirs for the pest as well as potential bio-control agents, and recommendations for management of these environments with respect to pest/natural enemy population.***

Methodology

Field visits were made to scallion and onion farms. The immediate environs of these fields were scouted for BAW larvae. Observations were made on sightings of BAW and any natural enemies observed feeding on the larvae.

Results and Discussion

Five field visits were made to farms in Flagamans, Tad Town, Comma Pen, Pedro Cross and Junction in St Elizabeth and Whitfield Hall in St Andrew in March, April and November 2013, and July 2014.

Egg masses and/or larvae were observed on milk weed, callaloo and guinea grass (*Panicum maximum*). It was hoped that further work on the rearing of BAW on guinea grass could be conducted as it was not determined that the guinea grass supported the development of the pest. However, the time allotted to this activity could not accommodate these studies.

Generalist predators, namely white egret birds, wasps (*Polistes* spp.), spiders and ants (Figures 3 & 4), were recorded preying on BAW larvae observed on farms visited. These observations were also supported by verbal reports obtained from UWI DLS researchers.

Figure 3. White egrets (encircled in yellow) in scallion field searching for beet armyworm larvae

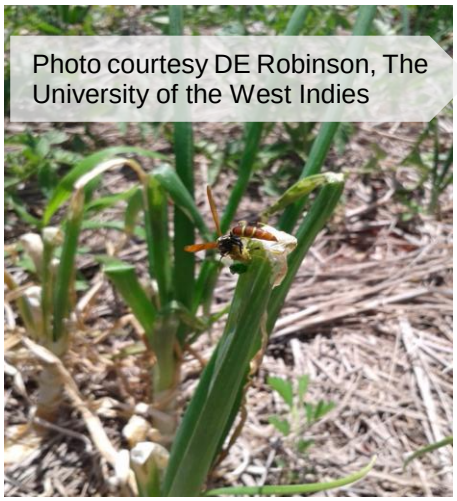

Figure 4. Wasp (*Polistes* sp) removing a beet armyworm larva from a scallion leaf

Recommendations

- Provide shelter for spiders – "trash" (pest-free vegetable debris, mulch, old boards) should be placed at the periphery of fields to provide refuge for spiders, which should be encouraged in the field
- Provide nest sites for wasps – a simple nest shelter, such as a four-sided box with an open front, may be erected about 1-2 m above ground, and attached to a post or tree. The nest shelter should be placed away from human and animal interference, vibrations and disturbances.
- Where feasible/possible, flowering plants should be planted close to onion and scallion fields to encourage other beneficial insects, such as ladybird beetles and lacewings.
- After the onion or scallion crop has been harvested, the surrounding vegetation should be routinely inspected for signs of BAW presence or damage. Once BAW are observed on the vegetation, action (physical removal of BAW developmental stages, spot spraying with a 'soft' insecticide) should be taken to reduce the pest population

3. ***Review of field-collected natural enemies (from the Training of Trainers and other collections) and conduct an analysis to prioritize natural enemies for the rearing/mass production programme.***

Methodology

Field visits were made to scallion and onion farms by the Reporting Officer as well as personnel from the MoAF Research and Development Division (R&D) and the Rural Agricultural Health Authority (RADA). Particular emphasis was placed on searching for larvae which showed signs of infection or parasitism. During the field visits, larvae that were suspected to be parasitized/infected were collected and taken to the laboratory where they were placed in glass jars, covered with cheesecloth and fastened with elastic bands. The larvae were fed on callaloo (*Amaranthus viridis*) leaves and observed for emergence of parasitoids. Verbal reports were also solicited from the University of the West Indies (UWI) Department of Life Sciences (DLS) on sightings of infected or parasitized BAW.

Results and Discussion

None of the field-collected larvae showed outward signs of possible parasitization/infection. More than 100 larvae were collected during ten field trips by MoA R&D to farms in St Elizabeth. Of these, approximately 30% died after pupating and a further 40% did not complete their development to adulthood. No parasitoids emerged from any of the larvae collected. Two larvae, collected by MoAF R&D, which were believed to be infected by pathogens, did not produce any such organisms after being processed by UWI. Additionally, the UWI DLS reported that no possibly parasitized larvae were recorded during observations.

Attempts at finding natural enemies in the past (between 2009 and 2012) have met with little success. Diedrick *et al*. (2011) found a parasitoid pupa in St Elizabeth but it failed to complete its development to adulthood. Two parasitized larvae of the fall armyworm, *Spodoptera frugiperda*, were collected from St Catherine/Clarendon and incubated. The emergent parasitoids belonged to the genus *Euplectrus*; one was positively identified as *Euplectrus plathypenae*. The latter was successfully reared on *S. exigua* in the laboratory but failed to parasitize the BAW in the field. Hence, it should not be totally unexpected that the search for parasitoids during 2013-2014 proved futile. Nonetheless, this aspect of the project was still particularly disappointing, as it was anticipated to contribute significantly to the overall management efforts.

Based on the history and current practices of insecticide overuse on these farms - scallion and onion farmers in St Elizabeth relied heavily on insecticide control of the BAW (Young 2013) - it was not surprising that no parasitoids were recovered from BAW larvae collected from the field. Natural enemies, particularly parasitoids, are usually more susceptible to the fatal effects of insecticides than their host pests and it takes a longer time for their populations to recover after insecticide applications.

While no parasitoids or entomopathogens were determined from BAW larvae, the literature points to fungi and multiple nuclear polyhedrosis viruses (MNPV) as having potential in suppression of BAW populations. Thus, based on this and discussions with MoAF R&D personnel, two pathogens - *Metarhizium anisopliae* and MNPV - show great promise for incorporation in an IPM programme.

4. ***Protocols prepared for at least two biological control agent programme as prioritized with the Ministry, including rearing / mass production and release techniques***.

Protocols for the mass production and laboratory/field bioassays of the two abovementioned entomopathogens that show great promise for incorporation in an IPM programme are described below. It is the RO's opinion that, while the farmers need to change their approach from pest control to pest

management, these entomopathogens are appropriate in the interim since they can be formulated as spray solutions, which would make them more easily accepted and adopted for use by the farmers.

Protocol for Mass Production of *Metarhizium anisopliae*

The following quick method is taken from Tajick Ghanbary *et al*. 2009

Fungal collection and isolation

1. Collect 1-kg soil samples (up to a depth of 20 cm) from agricultural land
2. Store samples in plastic bags at 4°C
3. Make a 1:5000-1:10000 soil suspension using 10 g of each soil sample
4. Transfer one mL of solute to sterilized 9-cm petri dishes and then to the culture medium containing 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 0.5 g peptone, 0.5 g $MgSO_4$, 10 g dextrose, 0.5 g yeast extract, 0.05 g rose Bengal and 0.03 g streptomycin sulphate. *Note*: Add the rose bengal and streptomycin to the culture medium after sterilization and before transferring to petri dishes
5. The isolates can be purified by single spore method (Ho and Ko, 1997) as follows:
    a. Place a 0.1 µL spore suspension on water agar above 50-100 circles (dia. approx.. 3mm) marked on the bottom of a plate
    b. Incubate at 24°C for 12-24 h
    c. Count the number of spores in each circle after 12-24 h
    d. Transfer individually single germinating spores in each circle to agar plates for culturing

Identification and storage

1. Compare all morphological features of isolated *Metarhizium* with valid descriptions of its different species to determine which ones are *M. anisopliae*
2. Transfer colonies of *M. anisopliae* to PDA (potato dextrose agar) slants and store no longer than six months at 4°C

Destruxin production

1. Inoculate the fungus in 50 mL PDB (potato dextrose broth) in 250 mL Erlenmeyer flasks for one week at room temperature
2. Filter (using Whatman filter paper No. 1) the broth from pellets
3. Mix culture filtrate with 10 mL chloroform and shake vigorously for 10 min.
4. After an hour, separate chloroform from broth and completely evaporate
5. Resolve residue in 10 mL distilled water and store at -20°C until application
6. Spore concentration may be determined using a Neaubauer's haemocytometer under a phase contrast microscope after serial dilution for definite concentration of spore mL^{-1}
7. Spore viability may be determined by plating 100 µl of the conidial suspensions on culture medium and counting colonies after 48h. Spore germination should exceed 90%

Laboratory Bioassay of *Metarhizium anisopliae*

The crude *M. anisopliae* extract (from Step 5 above) and its dilutions can be used in laboratory bioassays of 3rd instar *Spodoptera exigua*. Range-finding bioassays may first be conducted, with a few widely-spaced concentrations of the crude extract. The results of the range-finding bioassays can then be used to decide the concentrations to be used in the bioassays to determine the LC_{50} and LC_{95} values. Once the LC_{50} and LC_{95} values are obtained, these concentrations of dilutions of the crude extracts can be used in the field assays.

Field Bioassay of *Metarhizium anisopliae*

The fungal solution (LC_{95} value) maybe assayed using a randomized block design and plot size of 24 m^2 in triplicate in onion and scallion fields. The solutions and control (excluding *M. anisopliae*) may be sprayed directly onto the escallion leaves, as would be done with a conventional insecticide.

Observations of larval mortality and instar should be taken 7, 14, 21 and 28 days after treatment. Climatic variables, such as temperature, precipitation, and relative humidity, should be recorded throughout the study period. First instars should also be collected from the experimental plots one month after treatment, taken back to the laboratory and reared on callaloo (*Amaranthis viridis*) leaves until pupation. Record should be made of the number of larvae that showed symptoms of fungal attack. Initial trials may be done in one area but eventually extend to the main production areas, such as Flagamans, Comma Pen, Junction, etc.

Protocol for Mass Production of NPV

This protocol can be accessed via the following website. It is actually the methodology for the mass production of *S. litura* NPV.
http://agritech.tnau.ac.in/crop_protection/crop_prot_bio_mass_virus.html

1. Rear larvae in diet held in 5 ml glass vials or on stalks of callaloo/potted callaloo plants.
2. Collect 5th instars and transfer them to the virus production facility.
3. Allow the larvae to feed on the semi synthetic diet* coated with a clean inoculum of the NPV that has previously been standardized. This is accomplished by placing aliquots of 10 mL of the viral suspension of concentration 1 x 108 Polyhedral Occlusion Bodies (POB) in the centre over the diet surface in glass vials and spreading the suspension uniformly all over the surface with a polished glass rod.
4. Release larvae singly after 15 min. into each glass vial/cell and incubate at 25°C for 10 days
5. Collect the cadavers (larvae begin to die from 5th day onward) individually.
6. Transfer cadavers to 500 mL plastic containers and freeze immediately until processing.

*The semi-synthetic diet is chick pea based. The ingredients for the diet are given below:-

	Item	Quantity
'A' fraction:	Chickpea flour	105.00 gm
	Methyl para-hydroxt benzoate	2.00 gm
	Sorbic acid	1.00 gm
	Streptomycin sulphate	0.25 gm
	10% formaldehyde solution	2.00 ml
'B' fraction:	Agar-agar	12.75 gm
'C' fraction:	Ascorbic acid	3.25 gm
	Yeast tablets	25 tablets
	Multivitaplex	2 capsules
	Vitamin E	2 capsules
	Distilled water	780.00 ml

Directions: Mix 390 mL of water with fraction 'A' of the diet in the blender and blend for two minutes. Add fraction 'C' to fraction 'A' and blend again for one minute. Boil fraction 'B' in 390 mL water and add to the mixture of A and B, Blend for one minute. Formaldehyde solution is added at the end and the mixture blended again run for one minute.

Processing of NPV

1. Thaw cadavers until they are at normal room temperature
2. Homogenize the cadavers in sterile ice cold distilled water at the ratio 1: 2.5 (w/v) in a blender or precooled, all-glass pestle and mortar
3. Filter the homogenate through double layered muslin
4. Wash repeatedly with distilled water. The ratio of water to be used for this purpose is 1: 7.5-12.5 (w/v) for the original weight of the cadaver processed.

5. Discard the leftover mat on the muslin.
6. Semi-purify the filtrate by differential centrifugation – centrifuge the filtrate for 30-60 sec. at 500 rpm to remove debris, then centrifuge the supernatant for 20 min at 5,000 rpm
7. Suspend the pellet containing the polyhedral occlusion bodies (POB) in sterile distilled water and wash three times by centrifuging the pellet in distilled water at low rpm, followed by centrifugation at high rpm
8. Collect the pellet, suspend in distilled water and make up to a known volume to calculate the strength of the POB in the purified suspension.

Laboratory and Field Bioassays of NPV

Conduct laboratory and field bioassays as described above for *M. anisopliae*. It should be noted that the application of NPV should be carried out in the evenings, as NPV are prone to inactivation by ultraviolet light in sunlight.

Recommendations for further studies / testing

1. Previous laboratory bioassays of *Beauveria bassiana* conducted by the MoAF R&D gave promising results. The bioassays should be repeated to obtain lethal concentration values of the fungus after which the field bioassays should be conducted. Special attention should be paid to prevailing climatic conditions, as the optimal conditions for *B. bassiana* to thrive are temperatures of 24-28°C, relative humidity about 90 % and soil water content above 5%).
2. *Cotesia marginiventris*, a parasitoid of BAW, has been recorded from Jamaica *ex* diamondback moth (Alam 1992). Attempts should be made to collect live specimens or parasitized diamondback moth larvae, from which the parasitoids can emerge. The emerged adults should then be placed on or near healthy BAW larvae to determine if they would oviposit on the larvae. If they do, then they should be reared (for more than one generation) on BAW. Experimental releases can then be made in the field to see if they will parasitize BAW larvae in the field.
3. Entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) have been successfully used as biocontrol agents of Coleoptera and Lepidoptera larvae. In fact, they have been reported to increase the mortality of BAW when combined with *B. bassiana*. Since these nematodes are commercially available, they should be explored as a possible biocontrol option.

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APPENDIX I

NATURAL ENEMIES OF THE BEET ARMYWORM, *SPODOPTERA EXIGUA* (HÜBNER), COMPILED FROM DESK REVIEW OF LITERATURE

Natural Enemy	Order: Family	Scientific Name	BAW stage parasitized	Reference
Parasitoid	Diptera: Tachinidae	*Archytas apicifer* Walker	Larva (1st-5th instar)	Henneberry *et al.* 1991
Parasitoid	Diptera: Tachinidae	*Archytas californiae* (Walker)	Larva (1st-5th instar)	Eveleens *et al.* 1973
Parasitoid	Diptera: Tachinidae	*Archytas marmoratus* (Townsend)	Larva (1st-5th instar)	Cock 1985[1], Ruberson *et al.* 1994
Parasitoid	Diptera: Tachinidae	*Chaetogodia monticola* (Riley)	Larva (1st-5th instar)	Swezey 1935
Parasitoid	Diptera: Tachinidae	*Eucelatoria armigera* (Coquillett)	Larva (1st-5th instar)	van den Bosch and Hagen 1966, Henneberry *et al*. 1991
Parasitoid	Diptera: Tachinidae	*Eucelatoria rubentis* (Coquillett)	Larva (1st-5th instar)	Wilson 1933, Tingle *et al.* 1978
Parasitoid	Diptera: Tachinidae	*Eucelatoria* sp nr *armigera* (Coquillett)	Larva (1st-5th instar)	Henneberry *et al.* 1991
Parasitoid	Diptera: Tachinidae	*Gonia crassicornis* Fabricius	Larva (1st-5th instar)	Wilson 1933
Parasitoid	Diptera: Tachinidae	*Lespesia archippivora* (Riley)	Larva (1st-5th instar)	van den Bosch and Hagen 1966, Eveleens *et al*. 1973, Henneberry *et al.* 1991, Stewart *et al.* 2001
Parasitoid	Diptera: Tachinidae	*Voria ruralis* (Fallén)	Larva (1st-5th instar)	Eveleens *et al.* 1973
Parasitoid	Diptera: Tachinidae	*Winthemia rufopicta* (Bigot)	Larva (1st-5th instar)	Tingle *et al.* 1978
Parasitoid	Diptera: Tachinidae	*Winthemia* sp.	Larva	Cock 1985
Parasitoid	Hymenoptera: Braconidae	*Aleiodes laphygmae*	Larva (1st-3rd instar)	Ruberson *et al.* 1993
Parasitoid	Hymenoptera: Braconidae	*Apanteles ruficrus* (Haliday)	Larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Braconidae	*Apanteles* sp.	Larva	Cock 1985
Parasitoid	Hymenoptera: Braconidae	*Austrozele* sp.	Larva	Cock 1985
Parasitoid	Hymenoptera: Braconidae	*Chelonus insularis* Cresson	Egg, larva (1st- 5th instar)	Wilson 1933, van den Bosch and Hagen 1966, Eveleens *et al.* 1973, Harding 1976, Tingle *et al*. 1978, Soteres *et al.* 1984, Henneberry *et al.* 1991, Ruberson *et al.* 1993

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Parasitoid	Hymenoptera: Braconidae	*Chelonus obscuratus* (Herrich Schäffer)	Egg, larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Braconidae	*Cotesia laeviceps* (Ashmead, 1890)	Larva (1st-4th instar)	Krombein *et al.* 1979
Parasitoid	Hymenoptera: Braconidae	*Cotesia marginiventris* (Cresson)	Larva (1st-4th instar)	Wilson 1933, van den Bosch and Hagen 1966, Tingle *et al.* 1978, Soteres *et al.* 1984, Henneberry *et al.* 1991, Ruberson *et al.* 1993, Stewart *et al*. 2001
Parasitoid	Hymenoptera: Braconidae	*Cotesia militaris*	Larva (1st-4th instar)	Krombein *et al.* 1979
Parasitoid	Hymenoptera: Braconidae	*Cremnops haemotodes*	Larva (1st-3rd instar)	Henneberry *et al.* 1991
Parasitoid	Hymenoptera: Braconidae	*Microplitis rufiventris* Kokujev	Larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Braconidae	*Microplitis tuberculifer* Wesmael	Larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Braconidae	*Meteorus autographae* Muesebeck	Larva (1st-4th instar)	Wilson 1933, Tingle *et al.* 1978
Parasitoid	Hymenoptera: Braconidae	*Meteorus ictericus* Nees	Larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Braconidae	*Meteorus laphygmae*	Larva (1st-4th instar)	Krombein *et al.*, 1979
Parasitoid	Hymenoptera: Braconidae	*Meteorus leviventris* (Wesmael)	Larva (1st-4th instar)	van den Bosch and Hagen 1966, Harding 1976
Parasitoid	Hymenoptera: Braconidae	*Meteorus rubens* (Nees)	Larva (1st-4th instar)	Henneberry *et al.* 1991, Stewart *et al.* 2001
Parasitoid	Hymenoptera: Braconidae	*Zele melea* (Cresson)	Larva (1st-3rd instar)	Soteres *et al.* 1984
Parasitoid	Hymenoptera: Eulophidae	*Euplectrus comstockii* Howard	Larva (4th instar)	Stewart *et al*. 2001
Parasitoid	Hymenoptera: Eulophidae	*Euplectrus platyhypenae* Howard	Larva (3rd – 5th instar)	Wilson 1933, Cock 1985, Stewart *et al.* 2001
Parasitoid	Hymenoptera: Ichneumonidae	*Campoletis argentifrons* (Cresson)	Larva (1st-3rd instar)	van den Bosch and Hagen 1966
Parasitoid	Hymenoptera: Ichneumonidae	*Campoletis flavicincta* (Ashmead, 1890)	Larva (1st-3rd instar)	Ruberson *et al.* 1993
Parasitoid	Hymenoptera: Ichneumonidae	*Campoletis sonorensis* (Cameron)	Larva (1st-3rd instar)	Krombein *et al.* 1979
Parasitoid	Hymenoptera: Ichneumonidae	*Diapetimorpha introita* (Cresson)	Pupa	Jewett and Carpenter, 2001
Parasitoid	Hymenoptera: Ichneumonidae	*Hyposoter annulies* (Cresson 1864)	Larva (1st-3rd instar)	Krombein *et al.* 1979
Parasitoid	Hymenoptera: Ichneumonidae	*Hyposoter didymator* (Thunberg)	Larva	Sertakaya *et al.* 2004

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Parasitoid	Hymenoptera: Ichneumonidae	*Hyposoter exiguae* (Viereck)	Larva (1st-3rd instar)	van den Bosch and Hagen 1966, Eveleens *et al.* 1973, Henneberry *et al.* 1991
Parasitoid	Hymenoptera: Ichneumonidae	*Hyposoter didymator* (Thunberg)	Larva (1st-3rd instar)	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Ichneumonidae	*Nepiera fuscifemora* Graf	Larva (1st-3rd instar)	Krombein *et al.* 1979
Parasitoid	Hymenoptera: Ichneumonidae	*Ophion* sp	Larva (1st-3rd instar)	Ruberson *et al., .,et al.* 1993
Parasitoid	Hymenoptera: Ichneumonidae	*Pristomerus spinator* Fabricius	Larva	Eveleens *et al.* 1973
Parasitoid	Hymenoptera: Ichneumonidae	*Rubicundiella perpturbatrix* Heinrich	Larva (1st-3rd instar)	van den Bosch and Hagen 1966, Krombein *et al.* 1979
Parasitoid	Hymenoptera: Ichneumonidae	*Sinophorus caradrinae* (Viereck, 1912)	Larva (1st-3rd instar)	Krombein *et al.*, 1979
Parasitoid	Hymenoptera: Ichneumonidae	*Sinophorus xanthostomus* Gravenhorst	Larva	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Ichneumonidae	*Temelucha* sp	Larva (1st-3rd instar)	Henneberry *et al.* 1991
Parasitoid	Hymenoptera: Ichneumonidae	*Therion longipes* (Provancher, 1886)	Larva (1st-3rd instar)	van den Bosch and Hagen 1966, Eveleens *et al.* 1973
Parasitoid	Hymenoptera: Trichogrammatidae	*Trichogramma evanescens* (Westwood)	Egg	Sertakaya *et al.* 2004
Parasitoid	Hymenoptera: Trichogrammatidae	*Trichogramma* spp	Egg	van den Bosch and Hagen 1966
Hyper-parasitoid	Hymenoptera: Ichneumonidae	*Mesochorus discitergus* (Say)	Larva	Stewart *et al*. 2001
Hyper-parasitoid	Hymenoptera: Chalcididae	*Spilochalcis hirtifemora* (Ashmead)	Larva	Stewart *et al*. 2001
Predator	Coleoptera: Coccinellidae	*Coccinella septempunctata* Linnaeus	Egg	Ruberson *et al.* 1994
Predator	Coleoptera: Melyridae	*Collops* sp	Egg, larva	Eveleens *et al.* 1973
Predator	Coleoptera: Anthicidae	*Notoxus calcaratus* Horn	Egg, larva	Eveleens *et al.* 1973
Predator	Dermaptera: Labiduridae	*Labidura riparia*	Egg, larva	Ruberson *et al.* 1994
Predator	Hemiptera: Anthocoridae	*Orius insidiosus* (Say)	Egg, larva	Ruberson *et al.*, 1994
Predator	Hemiptera: Anthocoridae	*Orius tristicolor* (White)	Egg, early instar larva	Eveleens *et al*. 1973, Hogg and Gutierrez 1980, Ruberson *et al.* 1994

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Predator	Hemiptera: Lygaeidae	*Geocoris pallens* Stål	Egg, early instar larva	Eveleens *et al*. 1973, Hogg and Gutierrez 1980, Ruberson *et al.* 1994
Predator	Hemiptera: Lygaeidae	*Geocoris punctipes* (Say)	Egg, larva	Ruberson *et al.* 1994
Predator	Hemiptera: Lygaeidae	*Geocoris uliginosus* (Say)	Egg, larva	Ruberson *et al.* 1994
Predator	Hemiptera: Pentatomidae	*Podisus maculiventris* Say	Larva	Wilson 1933, Ruberson *et al.* 1994
Predator				
Predator	Hemiptera: Nabidae	*Nabis americoferus* Carayon	Egg, early instar larva	Eveleens *et al.* 1973, Ruberson *et al.* 1994
Predator	Hemiptera: Nabidae	*Nabis roseipennis* (Reuter)	Larva	Ruberson *et al.*, 1994
Predator	Hemiptera: Reduviidae	*Zelus* spp	Egg, larva	Ruberson *et al.* 1994
Predator	Hemiptera: Reduviidae	*Sinea* spp	Egg, larva	Eveleens *et al.* 1973
Predator	Hymenoptera: Vespidae	*Polistes fuscatus* Fabricius	Larva	Wilson 1933
Predator	Hymenoptera: Vespidae	*Polistes* spp.	Larva	Cock 1985
Predator	Hymenoptera: Formicidae	*Solenopsis invicta* Buren	Egg, larva	Ruberson *et al.* 1994
Predator	Neuroptera: Chrysopidae	*Chrysoperla* (= *Chrysopa*) *carnea* Stephens	Egg, early instar larva	Eveleens *et al.* 1973, Hogg and Gutierrez 1980
Predator	Neuroptera: Chrysopidae	*Chrysoperla refilabris*	Egg, larva	Ruberson *et al.* 1994
Predator	Neuroptera: Hemerobiidae	*Hemerobius* spp	Egg, larva	Ruberson *et al.* 1994
Nematode	Rhabditida: Steinernematidae	*Steinernema.carpocapsae* (Weiser)	Larva (early instars)	Barbercheck and Kaya 1991
Nematode	Rhabditida: Steinernematidae	*Steinernema feltiae*	Larva (early instars)	Kaya 1985
Nematode	Rhabditida: Steinernematidae	*Heterorhabditis bacteriophora* Poinar	Larva (early instars)	Barbercheck and Kaya 1991
Fungal pathogen	Hypocreales: Clavicipitaceae	*Beauveria bassiana*	Larva (5^{th} instar, pupa)	Barbercheck and Kaya 1991, Hung and Boucias, 1992, Studdert and Kaya, 1990, Wraight *et al*. 2010
Fungal pathogen	Zygomycetes: Entomophthorales	*Erynia* sp nr *pieris*	Larva	Ruberson *et al.* 1994
Fungal pathogen	Hypocreales: Clavicipitaceae	*Nomuraea rileyi* (Farlowe)	Larva	Cock 1985, Ruberson *et al.* 1994

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Fungal pathogen	Hypocreales: Clavicipitaceae	*Metarhizium anisopliae*	Larva (3rd instar)	Freed *et al.* 2012
Nuclear polyhedrosis virus	Baculoviridae	Unidentified	Larva	Oatman and Platner 1972, Eveleens *et al.* 1973, Kolodny-Hirsch *et al.* 1993
Multiple nuclear polyhedrosis virus		SeMNPV	Larva	Gelernter and Federici, 1986, Caballero *et al*. 1992, Kolodny-Hirsch *et al*. 1993, Hara *et al*. 1994; Kondo *et al*. 1994, Murillo *et al*. 2001, Takatsuka and Kunimi, 2002, Khattab, 2013 Muñoz *et al.* 1997
		Three strains - SeMNPV-US (Se-US), SeMNPV-SP2 (Se-SP2), and a recombinant virus (SeMNPV-SUR1 [Se-SUR1])		

1. Recorded from the Caribbean from *Spodoptera* spp.

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