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ADDITIVE EFFECTS OF THE ENTOMOPATHOGENIC FUNGUS *BEAUVERIA* BASSIANA (BALSAMO) VUILLEMIN (HYPOCREALES: OPHIOCORDYCIPITACEAE) AND *APANTELES TARAGAMAE* VIERECK (HYMENOPTERA: BRACONIDAE), IN CONTROLLING ON THE LEGUME POD BORER MARUCA VITRATA FABRICIUS (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Laboratory and field experiments were carried out to assess the interactions patterns between two biocontrol agents of the legume pod borer *Maruca vitrata*. With the importation of the parasitoid wasp *Apanteles taragamae* from Taiwan to Benin for a classical biological control against *M. vitrata,* it was important to evaluate its non target effects namely its interactions with other biocontrol agents in use such as the fungal entomopathogen *Beauveria bassiana*. In laboratory, a concentration of 10^9conidia/ml of *B. bassiana* isolate Bb115 was applied to *Maruca* larvae prior to their submission to parasitization by *A. taragamae.* In other experiments, *M. vitrata* larvae were parasitized first by females of *A. taragamae* before the fungal suspension application. A lot of *M. vitrata* larvae were considered as control without parasitization and fungal suspension application. The number of dead larvae, *A. taragamae* cocoons, sporulated dead larvae were counted. In field trials, cowpea plants were sprayed with *B. bassiana* suspension at a dose of 75 g of spores powder. Then, 28 m^2 cowpea plots were delimited and covered with net before the release of 5 *A. taragamae* mated females in each delimited cage. Results revealed that *B. bassiana* did not induce significant mortality in the parasitoid and might affect parasitic potential. The overall mortality rate averaged 52.07±0.9% in *B. bassiana* associated with *A. taragamae* treatments against 50.46±1.3% in control treatments. Moreover, additive effect was observed between the two biocontrol agents. Data were discussed with regard to the simultaneous use of the two biocontrol agents for controlling *M. vitrata* in cowpea.

INTRODUCTION

Cowpea, *Vigna unguiculata* L. Walpers (Fabaceae) is one of the grain legumes worldwide produced and consumed particularly in West Africa. According to Atachi *et al*., it ranks first from nutritional, agronomic, economic and socio-cultural point of view inBenin. Cowpea also is a source of vitamins, minerals, fats and oils. Its leaves can be consumed as fresh vegetables, while the plant after harvest is a valuable fodder for cattle.

Among the West African countries, Benin is the fifth producer after Nigeria, Niger, Burkina Faso and Mali. Every year, about 93,488 tons of cowpea are produced in Benin on around 115,000 ha. This production is still low to meed the needs of an increasing population. Indeed, cowpea production is limited by various constraints. Of these, biotic pressure from plant diseases and insect pests remain the major one affecting both yield and grain quality after harvest. Among the insect pests, the legume pod borer *Maruca vitrata* Fabricius (Lepidoptera,

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Crambidae) was reported to cause serious damage to cowpea in tropical and subtropical regions of Asia, Latin America and Africa. Caterpillars induce heavy losses by damaging many plant organs such as flowers, leaves, flower buds, green pods. *Maruca vitrata* can causeup to 80% yield losses in the absence of control measure. It was therefore imperative to apply control measures that could efficiently limit damage from this insect pest. Many control methods have been so far developed. But the most applied was synthetic chemicals application. Chemicals application becomes no more attractive considering the so many side effects including environmental sides effects and human hazards. Moreover, alternative control methods such as host plant resistance and cultural control practices were not always effective enough to keep *M. vitrata* populations below economic thresholds. Therefore, the use of living organisms such as natural enemies and effective microorganisms would be an alternative to the use of synthetic pesticides. Collaborative research studies between scientists in Taiwan and those of the International Institute of Tropical Agriculture (IITA), Benin station, yielded in the identification and importation of promising parasitoids species such as *Apanteles taragamae.* In Taiwan, *A. taragamae* has been reported parasitizing about 60 of the larvae of *M. vitrata* on Sesbania cannabina (Huang *et al*., 2003). Before a large scale release of such biocontrol agent, assessing non-target effects was a key step in the implementation of biological control program. Risk assessment was found to great concern especially with regard to the competitive behavior of introduced species in the presence of native ones. In Benin, the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Ophiocordycipitaceae), isolate Bb 115 has shown its virulence on *M. vitrata* larvae both in laboratory and field trials with mortality rates of more than 85%. The development of a biopesticide made of *B. bassiana* isolate Bb 115 could be applied after the release and establishment of the parasitoid wasp *A. taragamae.* Then, the two biological agent may the same cowpea field. And interactions between the two *M. vitrata* natural enemies could result in additive, synergistic or antagonist effects on the larval population of *M. vitrata*. The current study was designed to address such concern by assessing the interactions between *B. bassiana* and the parasitoid wasp *A. taragamae* in both laboratory and field experiments for the sustainable control of *Maruca vitrata.*

MATERIAL AND METHODS

Experimental sites

The works described below were conducted at the laboratory of insect pathology of the International Institute of Tropical Agriculture(IITA), Benin Station (6°28N and 2°21E, 15 m altitude), near Cotonou, Benin, in climate chamber with a mean temperature of 26 ± 0.50 ºC and a relative humidity of 65.5 ± 5%.

The experiments were conducted also in IITA fields. Sites that hosted the experiments were used to grow crop with different cropping types.

Plant Material

The cowpea variety "Tawa", were used in the different experiments performed at IITA campus. Cowpea seeds were sown at two seeds per sowing hole with 25 cm x 75 cm space (within and between lines). Cowpea plots were separated (alley) with maize plants sown the same day as cowpea. Plots were manually weeded twice. Cowpea plants received the different treatments in study at flowering onset. The cowpea variety "Tawa" is an semi-erect variety with a development cycle lasting 65-70 days.

Rearing of M. vitrata larvae

The mass rearing of *M. vitrata* started with the collection of *M. vitrata* pupae from a stock at the insectarium of IITA-Benin. They were placed in open Petri dishes incubated in wooden cages (44 x 45 x 58 cm) provided with sleeves, sides made of fine mesh and a glass top. Emerged mated females were grouped (4 or 5 individuals per group) in plastic cups (3 cm diameter x 3.5 cm height) for oviposition purposes. Ovipositing females were fed using small pieces of filter paper moistened with 10% glucose solution, which were replaced every 24 h. Caterpillars that emerged from collected eggs were fed using an artificial diet after.Larvae obtained from this mass production were used in the different experiments.

Rearing of Apanteles taragamae

Cocoons of *Apanteles taragamae* were obtained from the stock culture at IITA station in Benin, originally collected from the widely cultivated green manure crops *Sesbania cannabina* (Retz) Pers. at the World Vegetable Center (AVRDC) in Taiwan. Emerged adults were kept in cylindrical plastic cups (4.5 cm diameter x 5 cm height). A hole (2cm diameter) punched in the lid of the cups was covered with fine mesh. Adults of *A. taragamae* were fed with honey streaked on the fine mesh of the lid. Two days old larvae of *M.vitrata* were offered to mated parasitoid females during 24 h for parasitization. They were offered, during 24h, two days old larvae of *M.vitrata* in a small cylindrical cup containing a piece of artificial diet. Parasitized larvae were placed in small cylindrical cups and reared using artificial diet till cocoon stage. New parasitoids adults were obtained from such cocoons. The mass production of wasps took place in a climate chamber with a temperature of de 25.3 ± 0.5 °C and a relative humidity of 78.9±5.6 % (mean ± SD).

Production of the entomopathogenic fungi, Beauveria bassiana

The *B.bassiana* isolate included in the current study was "Bb 115". Colonies of this isolate were mass produced from dried viable conidia (germination rate 95%) kept in fridge at IITA Benin. The mass production was performed in a climate chamber at 26 ± 2°C using Potato Dextrose Agar (PDA) as medium. Conidias collected this fungal culture were used in expriments consisted of fungal application.

Effect of B. bassiana on the parasitization potential of A. taragamae in laboratory

Experiments consisted of inoculating first female parasitoid at a concentration of 10^9 conidia/ml of *B. bassiana* suspension. Then, two inoculated females (2 day old) and one male (2 day old) were transfered into each plastic box containing 30 *M. vitrata* larvae of each of the two larval stages L1 (1 day old) and L2 (3days) of *M. vitrata*. After 24 hours, the parasitoids

were removed and larvae were placed individually intoplastic boxes and reared using artificial diet till pupae stage. The boxes were put in laboratory at 25.5 ± 0.1°C temperature and 81.5 ± 0.7 % relative humidity. The number of parasitoid coccons, *M. vitrata* larvae (dead and alive) or pupae were counted. In parallel, 30 *M. vitrata* larvae were offered to non-inoculated parasitoids females as control. Experiments were repeated 3 times.

Assessment of the effect of the interaction between B. bassianafungus and A. taragamae on the survival of M. vitrata in laboratory

Two experiments were designed to assess the interactions type between *B. bassiana* and *A. taragamae*.

In the first experiment, 1µl of an oily suspension of *B.bassiana* of 10^9conidia/ml concentration was applied topically onto *M. vitrata* larvae. A total thirty larvae were inoculated. Then, three parasitoids (two females and one male) were released in the box containing inoculated larvae and kept for 24 hours before the removal of the parasitoids.

The second experiment consisted to submit *M. vitrata* larvae to parasitization by *A. taragamae* before fungal application. Then, 30 larvae were transfered into box and 3 parasitoids (2 females and one male) were released in the box and kept for 24 hours. After parasitoids removal, parasitized larvae were treated with *B.bassiana* suspension at the same concentration mentioned above. In both experiments, larvae were reared using artificial till pupae stage. The number of dead larvae, *M. vitrata* pupae and parasitoid cocoons were recorded. Dead larvae were kept for checking sporulation.

On-farm evaluation of the interaction between B. bassiana fungus and A. taragamaefor the control of M. vitrata

Field expriments involved three treatments: untreated control, treatments consisted of *A. taragamae* release, treatments consisted of the used of both biological agents *A. taragamae* +*B. bassiana* (isolate Bb 115) and treatments that consisted of *B. bassiana* application only. Each treatment was repeated three times in a complete randomized block design (CRB) with experimental plots of 28 m^2 (7m × 4m). Plants were weeded twice. Inside each plot, the treated cowpea plants were used as feed for the insect. Maize was planted in alleys to avoid bordering effects between treatments. The Biopesticide was applied at a rate of 75g conidia powder (active ingredient)/ha in two litres water. Then, 5 mated females were released in each plot covered by net (cage) for treatments involving the parasitoid. The parasitoids stayed in cages for three days. Cowpea plants were naturally infested (no artificial infestation).The larvae mortality was checked daily and dead larvae were sampled and kept separately in plastic box placed in laboratory for sporulation studies. Sporulation was monitored per treatment. Field experiments were repeated over years.

Statistical analysis

Data on percent *A. taragamae,* mortility rate of *M. vitrata* in the different treatments were analyzed by performing ANOVA uing SAS software followed by the test of Student-Newman-Keuls. Percent data were square root arcsine transformed before being subjected to analysis of variance (ANOVA).

In addition to the statistical analysis, calculations were made to support the results obtained in the interaction studies. The total mortality was calculated using the formula used by Benz, 1971; Brousseau *et al*.,1998 and Lise, 2007.

The following formula was applied to evaluate the theoretical total mortality:

$M_{1+2}=M_1+ M_2 [(1 –M_1)/ 100]$

Where$M_1$is the mortality rate associated to the first biological agent; M_2being one associated to the second agent.

Thus, when the total mortality rate observed in the combination treatment (biological agent 1 + biological agent 2) is equal the expected theoretical rate, then the interaction effect is additive. On the other hand, when the total mortality observed is higher than the expected theoretical one, then the interaction is synergistic. And when the mortalities observed are less than the theoretical one, effects are antagonist.

RESULTS

Effect of B. bassiana on the parasitization potential of A. taragamae in laboratory

Application of *B. bassiana* suspension onto the parasitoid females prior to the submission of larvae to parasitization did not significantly affect the parasitism potential of *A. taragamae* (Table 1). Likewise, no significant difference occurred between the two larval ages tests L1 and L2 (F=1.36, ddl = 2, P=0.8233).

Table 1 Effect of the isolate *B. bassiana* on the parasitism of *M. vitrata* (stages L1 and L2) by *A. taragamae* according to the preferred larval stages of *M. vitrata*

Treatments	Average rates of parasitism (%) of larval stages	
	Stage L1	Stage L2
Control	49.12±0.1 7a	30.88±1.09a
Treated parasitoid	46.67±0.19 a	31.0±2.11a

Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).

Effect of B. bassiana application on the emergence rate of A. taragamae

In treatments including *A. taragamae*, no significant differences were observed between treatments where *A. taragamae* was alone and where it was combined with *B. bassiana* for adult parasitoids emergence, regardless of larval stage (F= 0.08, ddl = 2, P= 0.9466) (Table 2). Application of *B. bassiana* did not significantly affect the parasitism of *M. vitrata* larvae L1 and L2 by *A. taragamae.*

Table 2 Emergence of the adults of *A. taragamae* treated with *B. bassiana* suspension

Treatments	Emergence of the adults of *A. taragamae*	
	L1	L2
T1	0.0 ± 0.0b	0.0 ± 0.0b
T2	0.0 ± 0.0b	0.0 ± 0.0b
T3 (Bb)	0.0 ± 0.0b	0.0 ± 0.0b
T4 (Ap)	31.81 ± 3.03a	18.66 ± 4.17a
T5 (Bb-Ap)	24.70 ± 3.51a	15.40 ± 6.06a
T6 (Ap-Bb)	33.27 ± 5.01a	20.52 ± 4.34a

Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).

T1: Treatment 1, Simple control
T2: Treatment 2, control with oil;
T3 (Bb): Treatment 3, *M. vitrata* treated with *B. bassiana*
T4 (Ap): Treatment 4, *M. vitrata* submitted to *A. taragamae*;
T5 (Bb-Ap): Treatment 5, *M. vitrata* treated with *B. bassiana* then submitted to *A. taragamae.*
T6 (Ap-Bb): Treatment 6, *M. vitrata* submitted to *A. taragamae* and then treated with *B. bassiana*

Interaction pattern in the combination of A. taragamae with B. bassiana

Table 3 give the interaction patterns when *M. vitrata* larvae were treated with *B. bassiana* prior to their submission to parasitism by *A. taragamae* and when larvae were treated after parasitization. The statistical analysis revealed no significant differencebetween both theoretical and observed mortalities, regardless of larval stage (F =7.82, ddl = 6, P = 0.0088).This result suggests an additive effect between the two biological control agents (*A. taragamae* and *B. bassiana*) for the control of *M. vitrata*.

Table 3 Interactions pattern for *B. bassiana* and *A. taragamae* combination on *M. vitrata*

Treatments	Larval stages	M_1 (% ± ES)	M_2 (% ± ES)	Theoretical total mortalityM_{1+2} (%± ES)	Observed total mortality (% ± ES)	Effect
Combination 1 Bb-Ap	L1	43.10 ± 0.2b	12.04 ± 0.5c	50.46± 1.3a	52.07 ± 0.9a	Additive
	L2	39.01 ± 0.3b	9.09 ± 0.6c	44.55 ± 1.5a	48.12 ± 0.7a	Additive
Combination 2 Ap-Bb	L1	12.04 ± 0.5c	43.10 ± 0.2b	50.46 ± 1.3a	54.62 ± 1.1a	Additive
	L2	9.09 ± 0.6c	39.01 ± 0.3b	44.55 ± 1.5a	46.28 ± 0.8a	Additive

Means in the same line followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).
Avec, $\mathbf{M_{1+2}=M_1+ M_2 (1 –M_1/ 100)}$
$\mathbf{M_{1+2}}$: Theoretical total mortality which is equivalent to the expected mortality in the association of the two agents
$\mathbf{M_1}$: Observed mortality associated with the first agent of the association
$\mathbf{M_2}$: Observed mortality associated with the second agent of the association
Total observed mortality that is equivalent to the mortality of *M. vitrata* observed in the laboratory when the larvae are submitted to the association of the two agents

Sporulation

The sporulation was checked in treatments in which larvae were inoculated with conidia of the fungus only T3 (Bb) and the combined treatments such as T5 (Bb-Ap) and T6 (Ap-Bb).

The results revealed the highest sporulationin the T3 (Bb) treatment compared to the other treatments, regardless of larval stage (Table 4). However, no significant differences were observed between treatment consisted of *B. bassiana* application alone and when *B. bassiana* treated larvae where submitted to *A. taragamae*.

Table 4 Sporulation of *M. vitrata* dead larvae in treatments consisted of *B. bassiana* application alone or when combined with *A. taragamae*

Sporulation %	Larval stages	
	L1	L2
T1	0.0 ± 0.0b	0.0 ± 0.0b
T2	0.0 ± 0.0b	0.0 ± 0.0b
T3 (Bb)	25.10 ± 2.03a	16.4 ± 0.2a
T4 (Ap)	0.0 ± 0.0b	0.0 ± 0.0b
T5 (Bb-Ap)	21.11 ± 3.30a	10.6 ± 0.04a
T6 (Ap-Bb)	23.9 ± 0.01a	12.80 ± 0.02a

Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK).
T1: Treatment 1, Simple control
T2: Treatment 2, control with oil;
T3 (Bb): Treatment 3, *M. vitrata* treated with *B. bassiana*
T4 (Ap): Treatment 4, *M. vitrata* submitted to *A. taragamae*;
T5 (Bb-Ap): Treatment 5, *M. vitrata* treated with *B. bassiana* then submitted to *A. taragamae*.
T6 (Ap-Bb): Treatment 6, *M. vitrata* submitted to *A. taragamae* and then treated with *B. bassiana*

Effect of the combination of the parasitoid A. taragamae and B.bassiana on the field larval populations of M. vitrata

During the first year, in the different treated plot both parasitized and dead larvae of *M. vitrata* were observed. When the two biological control agents were present in plot, the percent parasitization rate of *M. vitrata* larvae did not differ significantly (31.7±4.01%) compared that obtained when the parasitoid was alone (33.4±2.65%) (Table 5). However, the mortality rate was significantly higher when the two biological control agents were combined (32.6±3.8) compared to that observed when *A. taragamae* was alone. Similar findings were observed in the second year (F=0.38,ddl = 2, P= 0.9951) (Table 5).

Table 5 Effect of the combination of *B. bassiana* and parasitoid *A. taragamae* on *M. vitrata* larvae during the first and the second year

Years	Treatments	Average rates of mortality of *A. taragamae*	Parasitism average rates	Average rates of mortality of *M. vitrata*
First year	Control	0.0±0.00a	0.0±0.00a	3.1±1.3a
	A. taragamae	0.0±0.00a	33.4±2.65b	16.5±1.02b
	A. taragamae + *B. bassiana*	0.0±0.00a	31.7±4.01b	32.6±3.83b
Second year	Control	0.0±0.00a	0.0±0.00a	2.6±0.03a
	A. taragmae	0.0±0.00a	24.6±3.67b	11.3±0.07b
	A. taragmae+ *B. bassiana*	0.0±0.00a	27.2±5.17b	30.9±2.13b

Means in the same column followed by the same letter are not significantly different at the 5% level (ANOVA followed by SNK)

DISCUSSION

Results obtained in the current study confirmed the induction of larval mortality in *M. vitrata by* the two biological agents *B. bassiana* and *A. taragamae.* When applied alone, *B. bassiana* induced and could be combined for the control of *M. vitrata.* In laboratory, despite the inoculation of the female parasitoids, the percent parasitization rate of *M. vitrata* by the parasitoid *A. taragamae* was not significantly reduced. Thus, application of *B. bassiana* suspension to females before or after parasitization of *M. vitrata* larvae by *A. taragamae* did not significantly affect the parasitism potential of *A. taragamae.* This might be explained partly by the shorter period of time between the fungal application and parasitism by *A. taragamae* (24h). Also, parasitized larvae might pupate before the beginning of fungal disease symptoms in *M. vitrata* larvae so that they escape from the harmful effect of the fungus *B. bassiana.* Moreover, *B. bassiana* induced direct moratility of larvae while *A. taragamae* did so through parasitization. Direct effect of *B. bassiana* was observed in *Helicoverpa armigera* by Gopalakrishan and Narayananwith mortality rates of about 15 to 20 % after *B. bassiana* treatment. Similarly, Douro Kpindou *et al*. showed that pupation and adults emergence in *H. armigera* varied with the fungal suspension concentration used to treat larvae. The fungus actitity may last longer when dead larvae sporulated, spreading out then the fungus disease in *M. vitrata* larvae. Therefore sporulation would be considered as supplemental continuing action for fungal disease spread.

Different interaction patterns have been reported when combined different biological control agents. Such interactions may be antagonist, synergistic or additive. In antagonistic pattern, one biological control agent might negatively affect the second one through intrinsic competition when sharing the same host patches or life stage. Thus, the strategic mechanisms of the highly competitive agent could involve physical attack or physiological suppression of the less competitive species. This

was the case reported in some entomopathogenic virus which induced high mortality and low development in some parasitoids. For instance, Nakai and Kunimi found that the infection of the larvae of the little tea budworm *Adoxophyes paraorana* Byun (Lepidoptera: Tortricidae) by AsGV granulose virus had an antagonist effect on its endoparasitoid *Ascogaster randiculatus* Watanabe (Hymenoptera: Braconidae).

On the other hand, synergistic or additive interactions between the combined biological control agents suggest a complementary actions even sharing the same host patches.In the current study, the combination of the parasitoids *A. taragamae* and the entomopathogenic fungus *B. bassiana* yielded in additive effect on *M. vitrata* larvae. Such findings could be explained when considering the latent period of the fungal disease expression and the number of days required for *A. taragamae* to pupate. Indeed, *A. taragamae* was reported to pupate in five days at 26-28°C. For *B. bassiana*, after conidia germination, the penetration speed of the formed hyphae becomes high after 3 days. Therefore, parasitized larvae could escape the fungal disease induced by *B. bassiana.* Moreover, in field conditions, when the two biological control agents were present, the parasitoid *A. taragamae* might avoid *B. bassiana* contaminated larvae when ovipositing suggesting an efficient host selection for oviposition. Similar results were reported by Raimo *et al*., who found a higher mortality and parasitism rates of larvae of *Lymantria dispar* Linnaeus (Lepidoptera: Noctuidae) treated with an entomopathogenic and submitted to a *Apanteles melanoscelus* Ratzeburg (Hymenoptera: Braconidae). Likewise, additive effects were observed in the mortality of diamondback moth when *B. bassiana* was combined with the parasitoid *Oomyzus sokolowskii* (Kurdjumov).

The emergence rate of males was higher than that of females for both larval stages. This may be related to the size of the host offered to *A. taragamae*. Indeed, female parasitoids were known to be selective for the progeny sex they oviposit in the host. Thus, female offsprings was oviposited in larger hosts while the male one was deposited in the smaller hosts. Host size was found to be related to host age in some insect species such as *M. vitrata*. Laboratory study reveal similar results when 1 to 3 days old *M. vitrata* larvae were submitted to *A. taragamae* for parasitization. Such observations were consistent with the those reported byDannon *et al*., who found the first two *M. vitrata* larval stages (L1 and L2) more suitable to *A. taragamae.*

By assessing the interactions between the parasitoid *A. taragamae* and *B.bassiana* on the larval populations of *M. vitrata,* the current study revealed an additive effect between the two biological control agents. They could be included in efficient and sustainable management strategies of the cowpea pod borer *M. vitrata.*

Disclosure

All authors declare no conflict of interests.

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