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RESEARCH ARTICLE

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Efficacy of *Beauveria bassiana* against adults of *Prostephanus truncatus* (Horn), *Sitophilus zeamais* Motschulsky and *Teretrius nigrescens* Lewis in stored maize

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The larger grain borer, *Prostephanus truncatus* (Horn) and the maize weevil, *Sitophilus zeamais* Motschulsky continue to cause tremendous losses to stored maize. Research in the UK has identified *Beauveria bassiana*, IMI 389521 as a suitable control agent for grain storage pests in the UK. The pathogenicity of *B. bassiana*, IMI 389521 was evaluated against adult *P. truncatus*, *S. zeamais* and *Teretrius nigrescens* in Ghana. Fifty adults of each insect species were treated with 0.5 g dry conidia powder of this isolate at 8.65×10^8 conidia/g for 1 minute and mortality recorded daily for 14 days. The results indicated that *B. bassiana*, is pathogenic against *P. truncatus* and *S. zeamais*, inducing over 90% mortality by day 7. *Teretrius nigrescens* was, however less susceptible to the fungus with 30% mortality. To determine the most effective concentration of *B. bassiana* for the control of *P. truncatus*, a laboratory dose response experiment using four concentrations of *B. bassiana* (10^8–10^{11} cfu/kg maize) was also conducted. Maize grains (250 g) in separate jars were treated with the four concentrations of the product. Fifty adults of *P. truncatus* were placed into the jars containing the treated maize and mortality was assessed weekly for 3 weeks. The most effective dose was 10^{10} cfu/kg maize, which resulted in 96% and 100% mortality of *P. truncatus* after 2 and 3 weeks, respectively. This study shows that *B. bassiana* could effectively be integrated into bio-control programme of these two key pests of maize in Ghana after further field trials.

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INTRODUCTION

Maize is the most important staple food in Ghana. Its production, however, is constrained by low yields and high postharvest losses (MiDA 2010). Insect pests contribute about 20–50% of postharvest losses of stored maize in Ghana (Anankware et al. 2012). Several species of insect pests infest stored maize in Ghana. Some of these include the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), the red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), the Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) and the larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) (Obeng-Ofori 2008). The two most destructive species, however, are *S. zeamais* and *P. truncatus* (Vowotor et al. 2005; Obeng-Ofori 2008).

Prior to the introduction of the larger grain borer, *P. truncatus*, *S. zeamais* was the most important pest of maize in Africa (Arbogast and Mullen 1990), causing about 7–20% weight loss of stored maize in Ghana (Hall 1970). The introduction of *P. truncatus* has, however, tremendously increased postharvest losses and has outcompeted the former due to its tolerance to dry conditions and ability to breed in grains with lower moisture content compared to *S. zeamais* (Obeng-Ofori 2008; CABI 2019).

Prostephanus truncatus is an occasional important pest of stored maize in its native regions: Central America, tropical South America and extreme South of USA (Boxall 2002; CABI 2019). This pest was accidentally introduced into Africa, likely through importation of infested maize grains (Harnisch and Krall 1984), and has spread across the continent (Borgemeister et al. 2003; Gueye et al. 2008). Currently, this pest is a collective problem in 20 African countries (CABI 2019) and may establish in all countries in Sub-Saharan Africa if preventive measures are not put in place (Bergvinson and García-Lara 2011). In Tanzania, where this pest was first reported (Borgemeister et al. 2003), on-farm storage losses of up to 34 and 70% over a 3 month and 4 months storage period have been reported for maize and dried cassava chips, respectively (Hodges et al. 1983; Hodges et al. 1985). It is currently the major storage pest of the two crops in Ghana (Obeng-Ofori 2008; Asante 2013) and Sub-Saharan Africa (Holst and Meikle 2003; Phiri and Otieno 2008). Some of the improved varieties of maize widely grown in Ghana including, 'Obatanpa' and 'Okomasa' with greater yields (Abdoulaye et al. 2012) are equally susceptible to this pest (Golob et al. 1999), therefore, suggesting an urgent need to search for an alternative, sustainable and environmentally friendly control measure.

Insect pests of stored produce are currently controlled using chemical insecticides (Obeng-Ofori 2011). In Ghana, broad spectrum organophosphate-pyrethroid insecticides such as

pirimiphos-methyl dust and fenvalerate are usually used as protectant for stored produce (Ogbonna et al. 2014). This practice is no longer desirable due to the high cost of control, development of insecticide resistance and environmental and food safety concerns.

This necessitated the search for a biocontrol agent against this destructive pest. The histerid beetle, *Teretrius nigrescens* Lewis (Coleoptera: Histeridae) obtained from its native Central America, has been identified to be the most effective predator, strongly attracted to aggregation pheromones of male *P. truncatus* (Boye 1988; Rees et al. 1990; Scholz et al. 1998) and reducing the population of its prey under both laboratory and field conditions (Pöschko 1993; Bonu-Ire 2001; Schneider et al. 2004; Bonu-Ire 2015; Muatinte and Cugala 2015). Adults and larvae of this predator feed on eggs, larvae (Pöschko 1993) and occasionally adults (Bonu-Ire 2015) of *P. truncatus*. The wide distribution of *T. nigrescens* in Central America is believed to be responsible for the low pest status of the prey in its native region (Omondi et al. 2011). Accordingly, classical biological control of *P. truncatus* in the field using *T. nigrescens* has been introduced in several African countries particularly, West and East African countries; Beginning with Togo in 1991 (Biliwa et al. 1992), Ghana in 1994 (Compton and Ofosu 1994) and Kenya in 1996 (Giles et al. 1996). Notwithstanding the introduction of this predator, *P. truncatus* is still causing tremendous losses to stored maize in Ghana (Birkinshaw and Hodges 2000; Birkinshaw et al. 2002). *Teretrius nigrescens* is believed to be undergoing difficulty in establishing in Ghana (Boateng 1996). This has been partly attributed to indiscriminate bushfires (Boateng 1996; Asante 2013). *Teretrius nigrescens* is also known to feed on commodities with high starch content, for example wheat, sorghum and dried cassava (Pöschko 1993; Bonu-Ire 2015) hence may not be entirely beneficial (Anankware et al. 2012). Holst and Meikle (2003) in a review of the impact of *T. nigrescens* as biocontrol agent against *P. truncatus* in West Africa concluded that, control using this predator might fail, as a result of the intra-specific density dependence and low population growth rate of *T. nigrescens* in comparison to *P. truncatus*. A more efficacious alternative to *T. nigrescens* is urgently needed. There is, therefore, a growing interest in the use of microbial pest control agents (MPCAs) as alternatives to complement other integrated pest management measures in order to improve food security in Ghana.

One such MPCA, is the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavipitaceae) which is a soil-borne natural enemy, used in the control of important agricultural arthropod pests globally (de Faria and Wraight 2007; van Lenteren et al. 2018; Hatting et al. 2019). Several studies have confirmed the infectivity of strains of this fungus against many storage insect pests as well as the larger grain borer (Bourassa et al. 2001; Cherry et al. 2005; Smith et al. 2006; Dhuyo and Selman 2007; Sedehi et al. 2014; Osipitan et al. 2015; Athanassiou et al. 2017; Batta 2018; Ak 2019). Even though *T. nigrescens* adults are mildly susceptible to *B. bassiana*, they are more resistant compared to *P. truncatus* (Bourassa et al. 2001; Dhuyo and Selman 2007; Nboyine et al. 2015). Notwithstanding, the susceptibility of *T. nigrescens* to *B. bassiana*, Dhuyo and Selman (2007) suggested that both *B. bassiana* and *T. nigrescens* could be used together for greater control of the larger grain borer.

Research in the United Kindgom (UK) has identified *B. bassiana*, IMI 389521 as a suitable control agent for grain storage pests in the UK (Cox et al. 2004; Wakefield et al. 2013). Although, high efficacies of this isolate against storage beetles and others, especially *P. truncatus* and *S. zeamais,* have been reported in the European Union (EU), its pathogenicity against storage pests especially, *P. truncatus* and *S. zeamais* under tropical climatic conditions, such as Ghana, is unknown. Evaluation of the infectivity of *B. bassiana*, IMI 389521 for use on both *P. truncatus* and *T. nigrescens* in Ghana, is also particularly important since *T. nigrescens* is being reared for mass release in fields and storage barns across several regions, where the larger grain borer is still a major problem. Although no follow up study has been done to determine the establishment of *T. nigrescens* in maize storage barns in Ghana, this predator could come into contact with the proposed fungal strain (*B. bassiana* IMI 389521) when used in storage protection of grains. Thus, the susceptibility of *T. nigrescens* to the proposed fungal strain could be detrimental to the biocontrol programme of *P. truncatus* with this predator. This paper therefore determined the pathogenicity of *B. bassiana*, IMI 389521 to *P. truncatus*, *S. zeamais* and *T. nigrescens* in the laboratory. To determine the most effective concentration of *B. bassiana,* IMI 389521 for the control of *P. truncatus* in a semi-field trial, a laboratory dose response experiment using four concentrations of *B. bassiana* product (1×10^8 to 1×10^{11} cfu/kg maize) was also studied.

MATERIALS AND METHODS

Study site

The two studies were conducted in the Entomology laboratory of the Department of Crop Science, University of Ghana, Legon. The temperature and relative humidity (RH) in the laboratory averaged 27 ± 2 °C and 67 ± 5% RH respectively.

Source of insects, maize and fungal isolate for the study

Adult insects for both trials (unsexed *P. truncatus, S. zeamais* and *T. nigrescens*) were obtained from a continuous rearing culture established at the Plant Protection and Regulatory Service Directorate (PPRSD) of the Ministry of Food and Agriculture (MoFA), Accra. Insects were reared at 28.6 °C, 65.6% RH and 12L:12D photoperiod (Bonu-Ire 2001) in the Entomology laboratory of the Bio-control Unit, of PPRSD, MoFA, Accra. *Prostephanus truncatus* and *S. zeamais* were cultured on whole maize grains, while *T. nigrescens* was cultured on a mixture of maize grains, larvae, eggs and adults of *P. truncatus*. Insects used were approximately one week post adult eclosion.

Untreated (insecticide and fungicide-free) healthy grains of maize variety 'Obaatanpa' with an average moisture content of 11% were purchased from farmers during the harvesting period (August–September) in Accra. The moisture content was determined using a digital grain moisture meter (Protimeter Grainmaster I, Merlin Lazer Ltd, UK).

Dry conidia of *B. bassiana*, IMI 389521 (8.7×10^8 conidia /g) and formulated dry conidia powder (1×10^8, 1×10^9, 1×10^{10} and 1×10^{11} cfu/kg maize) were obtained from Agrauxine, France and stored at −4 °C in a refrigerator prior to use. This isolate was originally obtained from an infected *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae) on stored wheat in a UK grain store. Molecular identification of the species of this isolate was provided in Luke (2014). Formulated conidia contained Entostat™ (Exosect®, Winchester, U.K.) and kaolinite. Entostat is an electrostatically charged powder made from Brazilian Carnauba palm, *Copernica martius* (Palmae) which aids in the adhesion and dispersal onto a target surface such as grain or insect cuticle (Wakefield et al. 2010).

Insect removal from maize

Maize grains were stored in a freezer, at −4 °C for 48 hours. The maize was then removed, oven dried for 24 hours at 50 °C and left for a day to cool. This was done to kill residual insects in the grain.

Determination of pathogenicity of B. bassiana*, IMI 389521 against* P. truncatus*,* S. zeamais *and* T. nigrescens

A factorial treatment combinations of dry conidia powder of *B. bassiana*, IMI 389521 and a control of no *B. bassiana* added, and three insect species. (*P. truncatus*, *S. zeamais*, *T. nigrescens*) were used in a completely randomised design. Dry conidia powder of *B. bassiana* IMI 389521 applied at 8.65×10^8 conidia/g was used to determine its pathogenicity against the three insect species.

A total of 500 unsexed adults of *P. truncatus* were introduced into 10 Kilner jars (500 ml each containing 50 insects). Five 500 ml Kilner jars (5 replicates) were each treated with 0.5 g of the conidia powder of *B. bassiana* and 50 adult *P. truncatus* introduced into each jar and left to stand for 1 minute. The contents of the jars were then emptied into sterilised Petri dishes and insects removed and placed into 9 cm diameter plastic Petri dishes containing 7 g of broken maize for feeding and were incubated under room temperature. A control treatment which comprised five clean, sterilised jars containing the same number of insects without treatment with *B. bassiana* conidia powder were handled in a similar manner. This procedure was repeated for *S. zeamais* and *T. nigrescens*. Bioassays for all three insect species were conducted concurrently. Temperature and relative humidity were recorded daily using a data logger (EL-USB-2, Lascar Electronics Ltd UK). Mortality was recorded daily for 14 days and individual insects that remained immobile following probing with a blunt probe were considered dead. To determine whether mycosis was the cause of death, cadavers of the three insect species, including those of the control were surface sterilised in 2% sodium hypochlorite for 1 minute, followed by two rinses in sterile distilled water. Cadavers were then transferred onto Petri dishes with Whatman filter papers moistened with 1 ml sterile water, left at 27 ± 2 °C, 67 ± 5% RH and examined for external growth of fungus (Acheampong et al. 2016).

Determination of concentration of B. bassiana*, IMI 389521 for the protection of stored maize against* P. truncatus

A completely randomised design was used with 5 treatments (*P. truncatus* infested with four concentrations (1×10^8, 1×10^9, 1×10^{10} and 1×10^{11} cfu/kg maize) of formulated *B. bassiana*, IMI 389521 conidia powder and a negative control where no *B. bassiana* was added with five replicates.

Maize (1.25 kg) was treated with 4.5 g of the highest concentration (1×10^{11} cfu/kg maize) of *B. bassiana* and divided into five lots of 250 g each contained in a 1000 ml Kilner jar. For the other concentrations, 1.25 kg of maize each was mixed thoroughly with 3.2 g of *B. bassiana* at 1×10^{10} cfu/kg maize, 3 g of *B. bassiana* at 1×10^8 and quantity 1×10^9 cfu/kg maize and divided into five equal parts (250 g) representing five replications. There was a negative control in which no *B. bassiana* product was added. The grains were left for 24 hours after which 50 unsexed adults of *P. truncatus* were introduced into each jar. The treatments were left at 27 ± 2 °C, 67 ± 5% RH in the laboratory. The temperature and relative humidity in the laboratory was monitored using a data logger (EL-USB-2, Lascar Electronics Ltd, UK). Mortality of *P. truncatus* was recorded at 7-day intervals for three weeks by emptying each jar onto laboratory trays. On each assessment day, the grains, live insects and powder were placed back into their respective jars after inspection. Dead insects, however, were removed and the cause of death was ascertained using the same procedure described above. On day 21, grains were sieved to separate grain powder from the kernel. Maize grains (500) were randomly selected from each jar. The number of damaged (grains with holes) and undamaged grains from this sample were separated, counted and weighed. Subsequently percent weight loss was determined using the count and weigh method (Boxall 1986).

Data analysis

The total number of dead insects in *B. bassiana* and control treatments were compared using student t-test for the pathogenicity test. To compare the mortality of the three insects, percent mortality data was arcsine transformed and subjected to analysis of variance (ANOVA) to give the back transformed means presented in Figure 1. Cumulative percentage mortality data from the dose response bioassay were corrected for the corresponding control mortality using Abbott's formula (Abbott 1925), arcsine transformed, and subjected to ANOVA. The least significant difference ($LSD_{5\%}$) was used to compare means and this was accomplished using Genstat statistical software (12th edition).

RESULTS AND DISCUSSION

Pathogenicity of B. bassiana*, IMI 389521 to* P. truncatus*,* S. zeamais *and* T. nigrescens

The mortality of the treated insects was significantly higher than the untreated insects ($t_{(28)} = 6.33$, $p < 0.001$). Analysis of a 14-day mean (%) mortality data revealed that, main effects as well as associated interaction were significant ($p < 0.05$) (Figure 1). There was a sharp increase in mean percentage mortality of *P. truncatus* and *S. zeamais* treated with *B. bassiana* conidia powder, with over 90% mortality by day 7 (data not shown), reaching 100% in day 14 (Figure 1). The mean percentage mortality of *T. nigrescens*, however, increased slightly to just 30% by the 14th day, when treated with the dry conidia powder of *B. bassiana*. The mean percentage mortality of control *S. zeamais* was slightly higher than the control *P. truncatus*. However, the mortality of treated *P. truncatus* and *S. zeamais* were the same. About 97% mycosis was observed on cadavers of the three insect species treated with *B. bassiana* and none on the controls, confirming fungal mycosis as cause of death. External sporulation on incubated cadavers commenced three days after incubation for all the three insect species and completely covered the cadavers after a week (Plate 1).

The results obtained from the pathogenicity study, indicates that *Beauveria bassiana*, IMI 389521 is pathogenic against adults of *P. truncatus* and *S. zeamais*. This result is consistent with the findings of Karanja et al. (unpubl.) who observed 100% and over 90% mortality of *P. truncatus* and *S. zeamais*, respectively, with over 96% kill of the former after 7 days of exposure to this same isolate in Tanzania. Smith et al. (1998) and Kassa (2002) also showed that isolates of *B. bassiana* that were most virulent to *S. zeamais* were also highly pathogenic to *P. truncatus*, with *P. truncatus* being the most susceptible. Similarly, Adane et al. (1996) demonstrated the virulence of a dry formulation of *B. bassiana* isolate against adults of *S. zeamais* in the laboratory. The most virulent isolate in their study, *B. bassiana*, s.l. (T190-520) at all concentrations tested (3.54×10^8, 1.77×10^9 and 3.54×10^9 conidia/25 g maize seeds) induced 100% mortality within 14

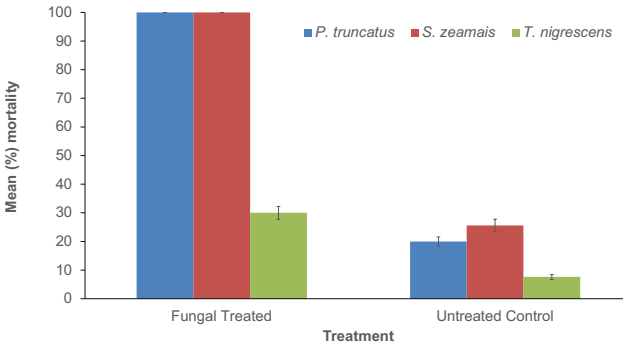

Figure 1. Mean % mortality of *P. truncatus, S. zeamais and T. nigrescens* treated with 0.5 g of dry conidia powder of *B. bassiana,* IMI 389521 and incubated at 27 ± 2 °C and 67.1 ± 5% RH for 14 days

Plate 1. Cadavers of *P. truncatus* (A), *S. zeamais* (B) and *T. nigrescens* (C) infected with *B. bassiana*

days with over 70% mortality by the 5th day. The pathogenicity study also revealed that *T. nigrescens* was rather less susceptible to the fungus. The resistance of adults *T. nigrescens* to *B. bassiana* relative to its prey (*P. truncatus*) has been previously reported (Bourassa et al. 2001; Dhuyo and Selman 2007; Nboyine et al. 2015). Although the susceptibilities of both *P. truncatus* and *T. nigrescens* are dependent on the isolate of *B. bassiana* used, around twofold (Bourassa et al. 2001), fourfolds and up to eightfolds (Nboyine et al. 2015) more *P. truncatus* are killed compared to *T. nigrescens* at the same conidia quantity and concentration. Following the results obtained in this study, Nboyine et al. (2015) assessed the compatibility of *B. bassiana*, IMI 389521 and *T. nigrescens* against *P. truncatus* in stored maize and recommended this isolate at 1×10^9 cfu/kg maize in storage systems where *T. nigrescens* is already well established as mortality of this predator was eightfolds less than its prey at this concentration.

Entomopathogenic fungi (the only exception being Microsporidia) infect their host by contact (Shah and Pell 2003). Infection by these fungi generally involves three steps: (a) adherence of infective propagules (for example conidia) on to host cuticle (b) germination and penetration under suitable temperature and humidity (c) proliferation within the host and sporulation on the surface of cadavers (Shah and Pell 2003; Boomsma et al. 2014). Host insect death generally resulted from massive fungal biomass due to proliferation, extensive tissue damage in addition to toxic metabolites produced by some species, for example *B. bassiana* (Shah and Pell 2003; Boomsma et al. 2014). The adherence and germination of conidia on the insect cuticle, which are key determinants in the initial infection process, were not determined in this study. However, we speculate this as one possible reason for the differences in susceptibilities of the three insect species (particularly *P. truncatus* and *T. nigrescens*) used in the present study. Wakefield (2006), for instance, using dry conidia powder of *B. bassiana*, IMI 389521 found *Oryzaephilus surinamensis* (Linnaeus) (strain Tram) (Coleoptera: Silvanidae) to be the most susceptible, followed by *Sitophilus granarius* (Linnaeus) (strain Windsor) (Coleoptera: Curculionidae) with *Tribolium confusum* Jacquelin du Val (strain W44) (Coleoptera: Tenebrionidae), being the most resistant to this fungus at the same conidial concentration. In the same study and using a scanning electron microscope, quantitative and qualitative differences in conidia adherence and germination on host cuticle were found between species *O. surinamensis* and *T. confusum*. At 24, 48 and 72 hours post treatments, a greater number of conidia, out of which many had germinated were found on the most susceptible species, *O. surinamensis* at all body areas examined, compared to *T. confusum*. The greater number of setae particularly on the ventral abdomen of the most susceptible species, potentially retaining high moisture on the cuticle is believed to have enhanced conidial adherence and germination, hence succumbing to infection (Wakefield 2006). *Prostephanus truncatus* has a heavily tuberculated body surface (prothorax and elytra) (CABI 2019), while *T. nigrescens* has a compact body (Pöschko 1994) with an unusually hard, waxy cuticle (Boateng 1996), which may have prevented conidia adherence and subsequent penetration of its cuticle, consequently making it less susceptible to the dry conidia powder of this fungus. The results from this study suggest that *B. bassiana*, IMI 389521 poses no serious threat to *T. nigrescens* release programme in Ghana.

Response of P. truncatus *to four concentrations (cfu/kg maize) of* B. bassiana, *IMI 389521 in the laboratory*

Table 1 shows the cumulative percentage mortality of *P. truncatus* over a 21 day period. The two higher concentrations showed a good control of *P. truncatus*. Sporulation of *B. bassiana* on cadavers from treatments, excluding the control, confirmed mycosis as the cause of death. Mortality of *P. truncatus* increased with increasing days of exposure for all concentrations (Table 1). Maize grains treated with *B. bassiana*, IMI 389521 at 10^{10} and 10^{11} (cfu/kg maize) resulted in significantly ($p < 0.05$) higher mortality of adult *P. truncatus* compared to 10^8 and 10^9 (cfu/kg maize). For these concentrations (10^{10} and 10^{11}), mortality of *P. truncatus* was 65.3% and 68.9%, respectively, after 7 days of exposure reaching 95.7% and 98.7% by day 14, and finally inducing 100% mortality by day 21 (Table 1). By contrast, mortality of adult *P. truncatus* in *B. bassiana*, IMI 389521 treated grains at 10^8 and 10^9 (cfu/kg maize) were less than 13% after 7 days of exposure and remained less than 20%, for 21 days after exposure. Although, *B. bassiana* at 10^{10} and 10^{11} cfu/kg maize resulted in higher mortality of adult *P. truncatus*, there were no significant differences in mortality between these two concentrations at all days of assessments (Table 1).

These results corroborate studies by Osipitan et al. (2014) who reported 86% mortality of *P. truncatus* in maize grains treated with *B. bassiana* (GHA) after 15 days of exposure. Kassa (2003) also observed the highest mortality of *P. truncatus* (98.8%) at 30 °C and 60–70% RH, 5 days after exposure to *B. bassiana* isolate PPRC-HH, at 3×10^8 conidia/g of maize grains. Bourassa et al. (2001) using conidial suspension of four isolates of *B. bassiana* at 1×10^9 conidia/ml, reported between 90–96% mortality of adult *P. truncatus*, 14 days post exposure at 28 °C and 55% RH, with over 83% mortality at day 6. Nboyine et al. (2015), however, reported a much lower (53%) mortality

Table 1. Cumulative percentage mortality of *P. truncatus* infested with conidia powder of *B. bassiana* (cfu/kg maize) and incubated for 21 days at 25 ± 2 °C and 65 ± 5% RH

Dose of *B. bassiana* (cfu/kg maize)	Corrected cumulative mortality (%) days after treatment		
	7	14	21
1×10^8	5.2^c	5.9^c	7.2^c
1×10^9	12.1^b	15.4^b	17.9^b
1×10^{10}	65.3^a	95.7^a	100.0^a
1×10^{11}	68.9^a	98.7^a	100.0^a
Control	0.00^d	0.0^d	0.0^d

Means within each column with the same lower case letter are not significant at 5% level of probability (LSD test).

of *P. truncatus* on maize grains treated with *B. bassiana*, IMI 389521 at 10^{10} (cfu/kg maize), after 8 weeks of exposure at 25 °C and 35% RH. One possible reason for the observed differences in mortality may be due to the differences in laboratory conditions, especially in relative humidity observed in their study compared to those of the current study (25 ± 2 °C and 65 ± 5% RH).

Effect of B. bassiana *product on grain weight loss and dust*

Table 2 shows the percentage weight loss of maize grains and dust produced by *P. truncatus* 21 days post infection with *B. bassiana* product. Grains treated with the two highest concentrations of *B. bassiana* product (10^{10} and 10^{11} cfu/kg maize) recorded the lowest percentage weight loss of 0.4% and this was significantly lower than the untreated control maize (1.9%). A significantly ($F = 49.7$, $p < 0.001$) higher grain weight loss (1.7% and 1.8%) was, however, recorded on maize grains treated with the two lowest concentrations of *B. bassiana* product (10^{8} and 10^{9} cfu/kg maize), respectively after infestation by *P. truncatus*. The percentage weight loss of grains treated with *B. bassiana* at 10^{8} and 10^{9} (cfu/kg maize) was not significantly different from the untreated control maize. Similarly, the weight of grain dust in maize treated with the two highest concentrations of *B. bassiana* product (10^{10} and 10^{11} cfu/kg maize) was significantly ($p < 0.05$) lower than all other treatments (Table 2).

Maize grains treated with the two highest concentrations of *B. bassiana* product (10^{10} and 10^{11} cfu/kg maize) resulted in lower weight loss of grains compared to *B. bassiana* product at 1×10^{8} and 10^{9} (cfu/kg maize). This was not unexpected since higher mortalities were recorded in the former resulting in reduced grain damage. The higher mortalities recorded in the aforementioned were due to mycosis by *B. bassiana*. The mean temperature and relative humidity of the laboratory during the trials may have supported conidia development and infection of the insects.

Although higher mortalities of LGB were recorded in maize treated with the two lowest concentrations of *B. bassiana* (10^{8} and 10^{9} cfu/kg maize) compared to the control maize, considering that the weight loss recorded in these treatments were not significantly different from the control, the protection of maize against LGB was not adequately achieved with these two lower concentrations.

The present study showed that *B. bassiana,* IMI 389521 is pathogenic against *P. truncatus* and *S. zeamais*. *Teretrius nigrescens*, however, is less susceptible to the fungus and it is not envisaged that *B. bassiana* will have a significant negative effect on populations of *T. nigrescens* currently being used for management of LGB in Ghana. Due to the significant reduction in grain damage demonstrated at 1 x 10^{10} cfu/kg maize, there is no additional benefit of using 10^{11} cfu/kg maize. The minimum effective concentration, for Ghana, is therefore, between 10^{9} and 10^{10} cfu/kg maize. Consequently, the two middle concentrations 10^{9} and 10^{10} (cfu/kg maize) are proposed for further evaluation for the control of LGB under both laboratory and field conditions.

TABLE 2. Mean grain dust and percentage weight loss of maize grains treated with *B. bassiana* (10^{8} to 10^{11} cfu/kg maize) after infestation by *P. truncatus* for 21 days at 25 ± 2 °C and 65 ± 5% RH

Dosage (cfu/kg maize)	Mean grain dust (g) ± SE	% Weight loss of grain ± SE
Control	3.6 ± 0.08[a]	1.9 ± 0.04[a]
1×10^{8}	3.1 ± 0.13[b]	1.8 ± 0.18[a]
1×10^{9}	2.8 ± 0.10[b]	1.7 ± 0.10[a]
1×10^{10}	0.7 ± 0.02[c]	0.4 ± 0.07[b]
1×10^{11}	0.5 ± 0.04[c]	0.4 ± 0.05[b]

Means within each column with the same lower case letter are not significant at 5% level of probability (LSD test).

CONFLICT OF INTEREST

There is no conflict of interest.

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