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The use of *Beauveria bassiana* for the control of the larger grain borer, *Prostephanus truncatus*, in stored maize: Semi-field trials in Ghana

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ABSTRACT

Laboratory research in Ghana demonstrated the effectiveness of an isolate of *Beauveria bassiana* (IMI 389521) from the United Kingdom against the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), a major pest of stored maize. The minimum effective concentration, following artificial infestation trials on maize, was between 10^9 and 10^{10} cfu/kg maize. Before moving out to village-level control, a major requirement was to determine if the product could effect control in artificially infested maize held under real environmental conditions in several locations in Ghana. Therefore, this study investigated the efficacy of formulated conidia of *B. bassiana*, IMI 389521, at two concentrations (1×10^9 and 3.16×10^9 cfu/kg maize) to control *P. truncatus* on stored maize kernels under semi-field conditions in Ghana. Maize ('Obatanpa' cultivar) kernels were treated with the formulated *B. bassiana* product and stored in polypropylene woven bags in cribs in Southern Ghana. After 24 h, one hundred adults of *P. truncatus* were placed into each bag containing the treated maize. Mortality and the percent of weight loss of kernels were assessed every two weeks for three months. The semi-field trials revealed the possibility of successfully controlling adult *P. truncatus* on maize kernels treated with *B. bassiana* at 3.16×10^9 cfu/kg maize. However, due to the minimal protection of kernels after four weeks, re-treating maize kernels after this period is recommended to ensure maximum protection during prolonged storage.

1. Introduction

Complexes of arthropod pests cause significant damage to stored maize in many parts of Africa. Amongst the insects, the larger grain borer, *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), first recorded in Africa in Tanzania in 1981 (Dunstan and Magazini 1981) and later in Ghana in 1989 (Dick and Rees 1989), *Sitophilus zeamais* (Motsch) (Coleoptera: Curculionidae), *Sitatroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), and *Tribolium* spp (Coleoptera: Tenebrionidae) are very commonly found (Markham et al., 1994). In Ghana, *P. truncatus* and *S. zeamais* inflict the most damage on stored maize and dried cassava. *P. truncatus* lays eggs on maize cobs and kernels, with both adults and larvae

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feeding on the endosperm and germ tissues. Their life cycle (egg to adult) takes approximately 25–27 days to complete at optimal temperature and relative humidity (RH) conditions of 25–32 °C and 70–80 %, respectively (Quellhorst et al., 2021). Their feeding activities reduce maize kernels to dust, precipitating a 40 % weight loss over a 3-month storage period (Boxall 2002; Quellhorst et al., 2021).

The emergence of resistance to chemical insecticides, the associated environmental risks, and incidents of human poisoning (Nnamonu and Onekutu 2015) have catalysed investigations into the use of biological pesticides based on entomopathogenic fungi (EPF) for pest control (Nboyine et al., 2015; Acheampong et al., 2016, 2023). EPF have demonstrated great promise as control agents for the above-mentioned major stored product insect pests, amongst others, under both laboratory and field conditions. However, none have yet been commercialised for use against these pests in Africa (Lord 2005, 2009; Smith et al., 2006; Khashaveh et al., 2011; Sedehi et al., 2014; Popoola et al., 2015; Storm et al., 2016; Wakil et al., 2022).

The present investigation follows up on work by Adane et al. (1996), which showed that conidia of *Beauveria bassiana* effectively controlled *S. zeamais*, with grain damage that was not significantly different from a pirimiphos-methyl treatment. These positive results led to a project to investigate the use of EPF to control storage pests in Kenya, which commenced in December 1996. A screening found *B. bassiana* to be widespread in geographic distribution, but very rare, with only 29 insects infected with the fungus from over 95,000 individuals collected from stores (Oduor et al., 2000). Used as a mycoinsecticide, one *B. bassiana* isolate demonstrated high levels of kill against *P. truncatus* (Smith et al., 2006).

The work stimulated interest in the UK, leading to a series of projects that obtained isolates of *B. bassiana* from insects from stored wheat and barley in the UK grain stores, and many trials were carried out to identify the most appropriate isolate and its formulation (Cox et al., 2003; Wakefield et al., 2010; Taylor et al., 2011). This research culminated in an Innovate UK grant to carry out field trials in the UK and to help create an EU regulatory submission dossier so that a product can be registered within Europe.

Due to this UK-based effort, the Bill and Melinda Gates Foundation funded the exploratory phase for work to be carried out in Ghana and Tanzania to determine whether the product of the most promising isolate, *B. bassiana* IMI 389521, could be effective in Africa. The results from pathogenicity and dose–response trials indicated that *B. bassiana* IMI 389521 is pathogenic against *P. truncatus* and *S. zeamais*, with the most effective dose between 10^9 and 10^{10} cfu/kg maize (Acheampong et al., 2016, 2023). A major requirement, before moving out to village-level control, was to determine if the product was capable of effecting control in artificially infested maize held under real environmental conditions in several locations in both countries. The main insect of interest was the larger grain borer. Due to the complexity of the field trials, the work has been separated into two separate papers, beginning with this one on work in Ghana.

2. Materials and methods

2.1. *Experimental site,* B. bassiana *product and insect culture condition*

A general protocol was established, derived from pathogenicity tests and bioassays carried out in laboratories in Ghana (Acheampong et al., 2016, 2023). The trials were conducted at the University of Ghana (UG) farm (5°39'24.9"N 0°11'31.7"W). The cribs at the University of Ghana Farm, Legon, consisted of well-ventilated rectangular wooden structures of 3 × 2.5 m and 1.74 m above the ground with a galvanised steel roof overhanging the crib sides to provide shade. Metal sheets were wrapped around each leg of the crib to deter the entry of rodents. Each crib had 6 compartments, each measuring 1.52 m^2 (Fig. 1).

B. bassiana, isolate number IMI 389521, was isolated from *Sitophilus granarius* (Coleoptera: Curculionidae) found in a UK grain store. The conidia were commercially produced by Agrauxine-France, using a modification of the diphasic system developed by Jenkins et al. (1998). In addition to the conidia, the product contained Entostat (Exosect®, Winchester, UK) and kaolin. Entostat is an electrostatically charged powder based on wax obtained from the Brazilian carnauba palm, *Copernica martius,* and adheres readily to surfaces, including insect cuticle. Consequently, it can deliver active ingredients to a target surface with improved efficiency (Baxter et al., 2008; Wakefield et al., 2010). Kaolin (kaolinite) is a silicate mineral used in this context as a carrier with the potential to damage insect cuticle, thereby causing desiccation (Storm et al., 2016).

P. truncatus were reared either in the Entomology Laboratory of the Biocontrol Unit at the Plant Protection and Regulatory Service Directorate (PPRSD) of the Ministry of Food and Agriculture (MOFA), Accra, Ghana, or at the University of Ghana, Accra, under ambient conditions of 28 ± 2 °C and 65 ± 5 % RH. The two continuous insect-rearing cultures established in both institutions enabled sufficient populations for all experiments. Insects were cultivated on whole maize kernels in 500 ml Kilner jars and used one week post adult eclosion.

2.2. *Pre-trial assessments*

Maize (Obaatanpa cultivar) was purchased in Ghana directly from a farmer at Kpong (Lower Manya Krobo District in Ghana) to try to ensure that the maize was residue free. To kill any insects present in maize, the kernels were placed at −4 °C for 2 days and then dried at room temperature for 24 h. A further pre-trial to assess the presence of chemical pesticide residue was carried out by placing 50 unsexed *P. truncatus* adults into 8 × 250 g aliquots of maize. After seven days at room temperature, mortality was assessed.

Fig. 1. Crib at the UG farm, Accra.

2.3. Experimental design

Each trial included three treatments: A. Product with *B. bassiana* conidia at 3.16 × 10^9 cfu/kg maize + *P. truncatus*; B. Product with *B. bassiana* at 1 × 10^9 cfu/kg maize + *P. truncatus*; C. Untreated control of maize + *P. truncatus*. These concentrations were selected based on previous laboratory studies, which established the minimum effective concentration, following artificial infestation trials on maize to be between 10^9 and 10^{10} cfu/kg maize (Acheampong et al., 2016, 2023). Treatments were sampled for insect mortality at weeks 2, 4, 8, and 12 weeks after insect introduction, and there were 8 replicates per treatment and sampling point (i.e., 96 samples in total).

The maize was prepared by placing it at −4 °C for 2 days and dried at room temperature for a further day, as stated above. Maize was weighed into 96 × 2 kg aliquots, and placed into labelled 45 cm^2 polypropylene woven sacks. The two concentrations of *B. bassiana* product were added to appropriate bags, tied at the neck, and mixed for 1 min by inverting and turning the bag to ensure good coverage of the maize with the product. The inoculated bags were left overnight to allow any dust to settle. Starting with the control treatments, to avoid cross contamination with *B. bassiana* product, 100 unsexed *P. truncatus* adults were added to each of the 96 samples. Once the insects had been added, the bags were tied at the neck and placed into cribs (controls in one crib and treated bags in another crib). The bags were placed so that they did not touch each other to discourage the movement of insects between bags. Data loggers were placed in each crib, and one within a 'dummy' bag of maize only to monitor temperature and humidity. Quality Control was carried out on the unused product to determine percentage viability (Oliveira et al., 2015).

On weeks 2, 4, 8, and 12, *P. truncatus* were assessed for mortality by destructive sampling, with 8 bags from each treatment. Kernels were sieved to separate the powder from the kernel, and the following data was recorded: numbers of dead and alive insects, larvae presence/absence, weight of kernels, and powder. All dead insects were surface sterilised by washing in a 1 % bleach solution for 1 min, followed by 2 washes in sterile water for 1 min each. The washed insects were placed onto damp filter paper and incubated at room temperature for 6 days. After 6 days, the insects were examined for any fungal growth. The fungus was checked by a microscopic examination to determine death by mycosis.

2.4. Statistical analyses

Data relating to the powder weight produced from each treatment were analysed using a one-way ANOVA for each time point separately, and a Tukey HSD post hoc was applied to these results where significant differences were observed. At each time point, there were 8 replicates per treatment, and sampling was destructive; therefore, these data were not considered to be pseudoreplicated between time points. A two-way ANOVA was also initially applied to these results to identify changes in powder weight between each treatment and sampling occasion.

Data relating to the number of live and dead insects were collected for each replicate, in each treatment, at each time point. A vector combining the number of dead and live insects was produced, and these data were analysed for each time point separately using a generalised linear model with quasibinomial errors to account for the nature of these data and over-dispersion in the model. All analyses were done in R version 3.0.2.

3. Results

To ensure that the maize was not contaminated with chemical residues, a pre-trial test was conducted to examine if insects died when in contact with the maize alone. Both pre-trial experiments resulted in no insect death after one week in the maize. We concluded from this that the maize would be suitable for use in the semi-field trials.

For each of the four sampling times 2, 4, 8, and 12 weeks, an assessment of the weight of powder, the weight of kernels, insect numbers (alive and dead) were assessed, and the larvae present or absent were recorded by destructive sampling. The weight of the maize powder in both *B. bassiana* treatments were not significantly different from the controls in weeks 2 ($F = 1.25$, $P = 0.306$) and 8 ($F = 2.32$, $P = 0.122$) (Fig. 2). However, significantly more powder was produced in the control and *B. bassiana* 1 × 10^9 cfu/kg maize treatment compared to the *B. bassiana* 3.16 × 10^9 cfu/kg maize on weeks 4 ($F = 6.114$, $P < 0.01$) and 12 ($F = 8.298$, $P < 0.01$). At week 12, only 65 g powder was produced in the *B. bassiana* 3.16 × 10^9 cfu/kg maize compared to 131 g and 138 g in the *B. bassiana* 1 × 10^9 cfu/kg maize and the control treatments, respectively (Fig. 2).

The weight of the maize kernels in both *B. bassiana* treatments was not significantly different from the controls in weeks 2 ($F = 0.34$, $P = 0.718$) and 4 ($F = 1.880$, $P = 0.177$) (Table 1). At week 8 ($F = 19.530$, $P < .001$), the weight of kernels in the two fungal treatments was not different statistically but was significantly higher than the control. By week 12 ($F = 40.060$, $P < .001$), there was a significantly greater decrease in maize kernel weight with the *B. bassiana* 1 × 10^9 cfu/kg maize treatments and the control compared to *B. bassiana* at 3.16 × 10^9 cfu/kg maize. The mean grain weights for *B. bassiana* treatment at 1 × 10^9 cfu/kg maize were not significantly different from the control treatment (Table 1). This corresponds with the powder weight increasing as the kernel weight decreases.

Mortality of *P. truncatus* in the *B. bassiana* 3.16 × 10^9 cfu/kg maize at week 2 was approximately 89.7 %, raising to 93.4 % at week 4 (Fig. 3). For the *B. bassiana* at 1 × 10^9 cfu/kg maize treatment, mortality was 80.9 % for week 2 and 83.7 % for week 4. Mortality in the control treatments was 9.6 % for week 2 and 34.2 % and 40.5 % by week 4. By week 8, the percentage mortality had decreased to 34.8 % in the higher treatment (3.16 × 10^9 cfu/kg maize), with the controls at 13.3 %. Week 12 showed an increase in percentage mortality, with 57.6 % mortality at the higher concentration and 34.9 % at the lower concentration. The control mortality at week 12 was 35.5 %. Mortality of *P. truncatus* in *B. bassiana* 3.16 × 10^9 cfu/kg maize treatment was significantly higher than its counterpart in the lowest fungal concentration and controls at all weeks of assessment (Week 2: $F = 237.060$, $P < 0.001$; Week 4 $F = 137.210$, $P < 0.001$; Week 8 $F = 21.890$, $P < 0.001$; and Week 12 $F = 12.610$, $P = 0.002$), except for week 8, where no significant difference existed between the two concentrations. Mortality of *P. truncatus* in *B. bassiana* 1 × 10^9 cfu/kg maize was also significantly higher than the controls at all weeks of assessment, except for week 12, where no significant difference existed between this treatment and the control (Fig. 3).

Total insect numbers showed very interesting results, with increases in numbers in all treatments by week 8 (Table 2). Insect numbers rose to 318 (control), 175 (1 × 10^9 cfu/kg maize), and 149 (3.16 × 10^9 cfu/kg maize) by week 8 but decreased slightly to 104 (control), 191 (1 × 10^9 cfu/kg maize), and 143 (3.16 × 10^9 cfu/kg maize) by week 12. In weeks 2 and 4, the greatest number of insects collected was 64 (Table 2). Originally, 100 insects were added,

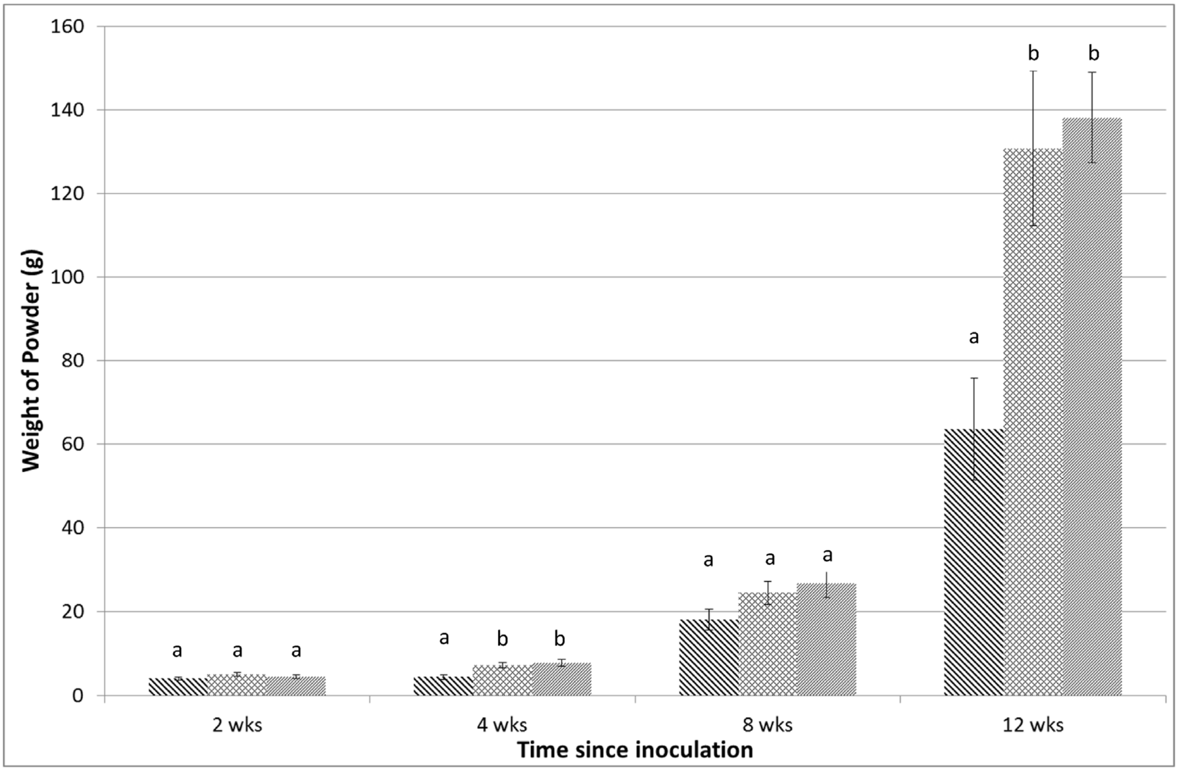

Fig. 2. Weight of powder obtained from maize kernels infected with *P. truncatus* under three different treatments. Key: ▧ *B. bassiana* product at 3.16 × 10^9 cfu/kg maize, ▨ *B. bassiana* product at 1 × 10^9 cfu/kg maize and ▥ control with *P. truncatus* only. Error bars are standard errors. Letters above the bars represent significant differences between treatments for each week.

Table 1
Weight of kernel after being infected with *P. truncatus* for varying levels of time with two treatments of *B. bassiana* and a control treatment containing *P. truncatus* only.

Treatment	Weight of maize kernels (g)			
	Week 2	Week 4	Week 8	Week 12
3.16 × 10^9 cfu/kg maize	1779.20 ± 8.291a	1797.20 ± 10.564a	1806.00 ± 2.758 b	1695.40 ± 22.553 b
1 × 10^9 cfu/kg maize	1785.40 ± 3.915a	1816.70 ± 7.582a	1772.20 ± 9.547 b	1380.30 ± 33.819a
Control	1777.50 ± 8.364a	1793.70 ± 8.748a	1693.80 ± 20.240a	1353.40 ± 32.487a

which suggests that breeding or external invasion occurred in weeks 8 and 12, and not all insects were recovered in weeks 2 and 4. Total insect numbers in the *B. bassiana* 3.16 × 10^9 cfu/kg maize treatments were not significantly different from the controls at all weeks of assessment (Week 2: $F = 3.820$, $P = 0.038$; Week 4 $F = 1.780$, $P = 0.194$; Week 8 $F = 44.150$, $P < 0.001$; and Week 12 $F = 4.720$, $P = 0.020$), except for week 8, where it differed from the control. Total insect numbers in *B. bassiana* 1 × 10^9 cfu/kg maize treatment were also not significantly different from the control at weeks 2 and 4, and from *B. bassiana* at 3.16 × 10^9 cfu/kg maize treatment at each assessment week, except week 2 (Table 2).

An estimate of larvae numbers were determined (Table 3). The results clearly show that by week 12, the control and *B. bassiana* 1 × 10^9 cfu/kg maize treatment had in excess of 100 larvae present, whereas the *B. bassiana* 3.16 × 10^9 cfu/kg maize treatment had less than 20 larvae detected.

4. Discussion

P. truncatus is a major pest of stored maize in Africa, and currently, there are very few alternatives to chemical insecticides. This study demonstrated the potential for a UK isolate of *B. bassiana* to control *P. truncatus* in Ghanaian environmental conditions, resulting in high insect mortality exceeding 90 % by the fourth week. Moreover, kernel weight was significantly greater in the higher concentration fungal treatments compared to the controls for weeks 8 and 12. Conversely, powder weight was significantly reduced in the higher fungal treatment compared to the control for week 12.

B. bassiana significantly affected insect mortality for weeks 2 and 4. However, this impact appeared to decrease after 8 and 12 weeks. Evaluation of the powder data reveals a significant reduction in damage in the *B. bassiana* 3.16 × 10^9 cfu/kg maize treatments. Although the damage remains relatively high in the fungus treatment, it is very significantly reduced compared to the control. These findings corroborate the results of previous studies that have also demonstrated that *B. bassiana* can kill *P. truncatus* (Acheampong et al., 2023; Bourassa et al., 2001; Meikle et al., 2001; Dhuyo and Selman, 2007; Smith et al., 2006; Popoola et al., 2015).

Considerable increases in total numbers were observed amongst treatments. Over 300 insects were detected from 2 kg of maize by week 8 in the trial. The life cycle of *P. truncatus* spans approximately 6–8 weeks depending on temperature and humidity, but it has been recorded under optimum conditions of approximately 30 °C

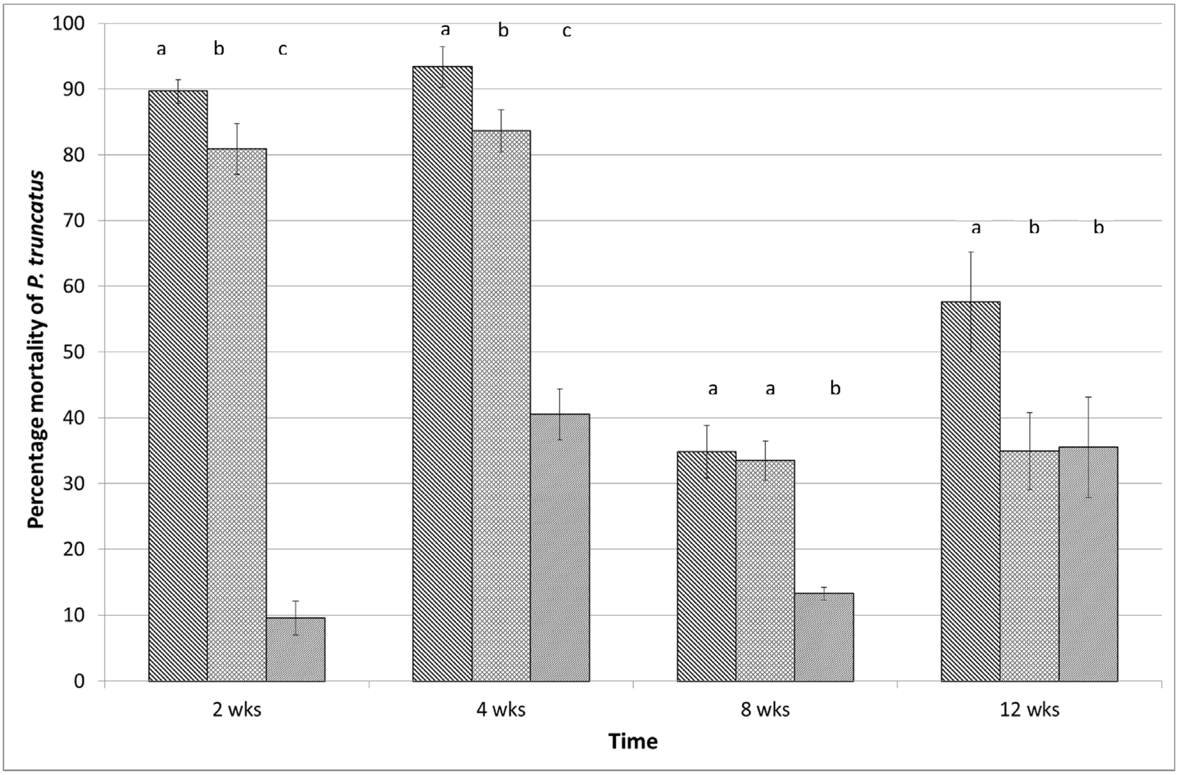

Fig. 3. Percentage mortality of *P. truncatus* over time under three different treatments. Key: ▧ *B. bassiana* product at 3.16 × 10^9 cfu/kg maize, ▨ *B. bassiana* product at 1 × 10^9 cfu/kg maize and ▩ control with *P. truncatus* only. Error bars are standard errors. Letters above the bars represent significant differences between treatments for each week.

Table 2
Total insects recovered from 3 treatments: two treatments of *B. bassiana* and a control treatment containing *P. truncatus* only.

Treatment	Total insect count			
	Week 2	Week 4	Week 8	Week 12
3.16 × 10^9 cfu/kg maize	64.13 ± 2.856 b	54.88 ± 2.755a	148.88 ± 14.529a	142.50 ± 17.135 ab
1 × 10^9 cfu/kg maize	51.63 ± 4.179a	48.25 ± 4.179a	174.88 ± 13.772a	190.50 ± 29.663 b
Control	58.88 ± 2.302 ab	57.38 ± 4.318a	318.38 ± 12.862 b	103.50 ± 5.880a

Table 3
Larvae counted from 3 treatments: two treatments of *B. bassiana* and a control treatment containing *P. truncatus* only.

Treatment	Total larvae count			
	Week 2	Week 4	Week 8	Week 12
3.16 × 10^9 cfu/kg maize	<8	<6	<3	<20
1 × 10^9 cfu/kg maize	<10	<10	<4	>100
Control	<10	>20	<50	>100

and 70 % RH to be as quick as 25 days (Anon 1993; Hodges and Meik 1984). The temperature and humidity in the crib at UG were 25.1 °C and 87.5 % RH. Inside a bag, the temperature was 25.9 °C and 87.1 % RH. Under these conditions, one would expect the life cycle to last slightly longer than 25 days, which aligns with our results as recovered adults of *P. truncatus* surpassed 100 insects by week 8. Adult numbers in the control were 318 after 8 weeks compared to 175 and 149 adults in the *B. bassiana* 1 × 10^9 cfu/kg maize and 3.16 × 10^9 cfu/kg maize, respectively, further demonstrating the impact of *B. bassiana* treatments on population levels.

Tefera et al. (2011) conducted a population density and storage time experiment. They demonstrated that at 28 °C and 65 % RH, 200 g maize infected with 10 (the same ratio used in this study) *P. truncatus* adults had a population density of 842 adults after 3 m. This is higher than seen in this study, where the population density was highest after 2 m, with an average of 318 adult *P. truncatus*. However, the temperature was cooler at the UG trial site. Tefera et al. (2011) concluded that population build-up was fast and thus can cause significant damage and losses. Although an increase in adult insects was detected in all treatments, particularly after 8 weeks, the numbers dropped again by week 12. However, the powder weight indicates that a significantly larger amount of powder was produced in the control compared to *B. bassiana* 3.16 × 10^9 cfu/kg maize at week 12. In addition to population increases, holes were noted in some of the bags from the UG site (Fig. 4). As the holes were few, it is unlikely that this had a major effect on the population levels.

It may appear that the fungus may not have been affecting the egg and larval stages. Given that the adults tend to lay their eggs within the maize kernels at dead-end chambers (Anon, 1993) and the larvae tend to stay inside the kernel (Anon, 1993), the externally applied fungus may not have achieved contact with the eggs or larvae, making the control of these early life stages more challenging. Nevertheless, research carried out at UG has shown that *B. bassiana* can have a significant effect, particularly on eggs (Acheampong et al., 2016). Dhuyo and Sohail (2007) further

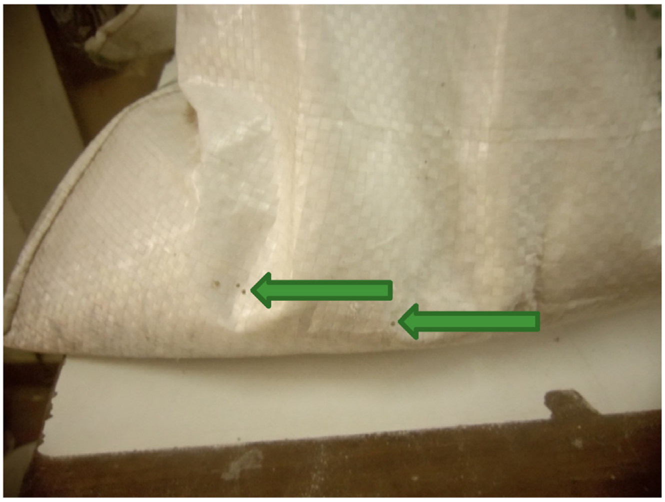

Fig. 4. Arrows show holes where insects have either left or entered the polyproplene bags.

demonstrated that a *B. bassiana* isolate could kill eggs, larvae, and pupae of *P. truncatus*, with the pupal stage being the most susceptible to the fungus.

In conclusion, *B. bassiana* from the UK has been shown to be effective in Ghana under sub-tropical conditions. The fungus displayed very high efficacy in controlling the adult population up to the fourth week, and the powder produced by the adults after 12 weeks was greatly reduced in the higher concentration of *B. bassiana*. Our results suggest a promising alternative to chemical pesticides for controlling *P. truncatus* in maize under tropical conditions.

Declaration of competing interest

The authors declare no conflict of interest.

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