Comparative growth and efficacy of Trinidadian strains of *Isaria fumosorosea* blastospores for controlling *Trialeurodes vaporariorum* on bean plants

Pasco B. Avery, Monique S. J. Simmonds and Jane Faull

ABSTRACT

The comparative growth and efficacy of three strains of Trinidadian *Isaria fumosorosea* Wize (T, T10 and T11) blastospores were assessed as spray suspensions for controlling greenhouse whitefly, *Trialeurodes vaporariorum* Westwood. Percent germination of all the three strains were unaffected by the surfactants Triton X -100 or Tween 80 compared to the control (water). Radial growth of fungal strain T11 was faster than either T or T10 which were similar in their growth patterns. Virulence of blastospores of three strains were tested against pharate adult greenhouse whiteflies on bean plants (*Phaseolus vulgaris* L.). The corrected mortality of whitefly for strains T, T10 and T11 was 56, 68 and 98% at 10^7 blastospores mL^{-1} and the median lethal concentration observed was 2.1 x 10^7, 1.3 x 10^7 and 2.5 x 10^6 blastospores mL^{-1}, respectively. Strain T11 was the most virulent (range: 97-98% mortality) and had the lowest LC_{50} (range: 1.1-2.5 x 10^6 viable blastospores mL^{-1}) value of the Trinidadian blastospore treatments tested against greenhouse whitefly on *P. vulgaris*.
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INTRODUCTION

The greenhouse whitefly (GW), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) is known to infest and damage bean plants (*Phaseolus vulgaris* L.) both in the field and greenhouse (Webb *et al.,* 1974; Collman and All, 1980; Omer *et al.*, 1992; Cardona *et al.*, 1993; Antonious and Snyder, 1995; Orozco *et al.*, 1995; Manzano *et al.*, 2000). Lloyd (1922), first reported economic damage related to populations of GW on tomatoes (*Solanum lycopersicum* L.), potatoes (*Solanum tuberosum* L.) and bean plants (*P. vulgaris*). In addition, the GW has been reported infesting a number of different crops mostly belonging to families Cruciferae, Leguminosae, Malvaceae and Solanaceae (Gerling, 1983; Byrne *et al.*, 1990). Saldarriaga *et al.* (1988) reported that in Colombia, where potatoes and dry beans are grown together as a mixed crop, GW was a major pest responsible for transmitting the potato yellow vein virus to the host crops. The GW is now an emerging threat responsible for transmitting criniviruses to several vegetables and fruits grown in North America (Wintermantel, 2004).

Use of chemical insecticides is considered the primary mode of controlling GW; however, there are several reports suggesting genetic resistance has developed in populations of GW with many of the chemicals being used both in the greenhouses and in the fields (Elhag and Horn, 1984; Omer *et al.*, 1992; Sanderson and Rousch, 1992; Gorman *et al.*, 2002; Karatolos *et al.*, 2010, 2012). In order to forestall or delay the development of resistance in GW against a specific chemical, it is important to find an alternate approach which is effective and environmentally compatible. In the past, various studies have demonstrated the effectiveness of fungal entomopathogens including *Isaria fumosorosea* Wize as major components of integrated pest management (IPM) programs for controlling GW and other whiteflies (Hall, 1982, 1984; Landa, 1984; Ramakers and Samson, 1984; Samson and Rombach, 1985; Fang *et al.*, 1986; Fransen, 1990, 1993; Fransen and van Lenteren, 1993; van der

Schaaf *et al.*, 1991; Alma *et al.*, 2007; Hamdi *et al.*, 2011; Jandricic *et al*., 2014). Hypocrealean fungi have been isolated and are known to be effective pathogens for controlling Aleyrodidae pests since they possess the ability to penetrate the cuticle of these plant sucking insects (Fransen, 1990; Gökce and Er, 2005; Scorsetti *et al*., 2008; Huang *et al*., 2010).

The entomopathogenic fungus, *I. fumosorosea* offers great potential for use in biological control (Lacey *et al*., 1995). This fungus is geographically widespread and various strains have been found that are aggressive pathogens of a variety of pestiferous insects (Fang *et al*., 1986; Inch *et al*., 1986; Smith, 1993; Lacey *et al.*, 1995; Bolckmans *et al.*, 1995; Wraight *et al*., 1998; de Faria and Wraight, 2007; Shapiro-Ilan *et al*., 2008; Zimmermann, 2008). However, the feasibility of using this fungus as a biocontrol agent against the greenhouse whitefly is dependent upon several biological constraints, including the ability to produce high concentrations of stable propagules in a short time and at a reasonable cost (Jaronski, 1986; Latgé *et al.*, 1986; Wraight and Carruthers, 1999; Slininger *et al*., 2003; Jackson *et al*., 2010).

Fungal spores can be produced in bulk using either semi-solid or submerged culture. However, commercial production of fungal spores using semi-solid substrates is difficult due to problems associated with substrate sterilization, gas exchange, temperature and light control, maintenance of pure culture, and product recovery from the substratum. Time for sporulation on solid substrates generally requires weeks rather than days as in submerged culture, thereby increasing production costs (de la Torre and Cardenas-Cota, 1996; Jackson, 1997). When *I. fumosorosea* is grown on solid media, it forms chains of conidia from phialides (Inch *et al*., 1986). These conidiospores can then be used as an inoculum for the production of blastospores formed in submerged, shake-flask culture. Blastospores are formed by budding from hyphae and /or other blastospores, and they vary in shape from yeast-like cells to elongated structures resembling hyphal fragments. Inch *et al*. (1986) first reported that blastospores of *I. farinosa* and *I. fumosorosea* isolates could be produced in high numbers (1.19 x 10^8 blastospores ml^{-1}) in 89 hours. Following this report, production of blastospores and submerged conidia of *I. fumosorosea* has been accomplished by various workers (Shimizu and Aizawa, 1988; de la Torre and Cardenas-Cota, 1996; Jackson *et al*., 1997; Lozano-Contreras *et al*., 2007; Asaff *et al*., 2009; Kim *et al*., 2013).

Blastospores, typically larger than aerial conidia and with thin cell walls are not amenable to simple drying techniques and tend to perish more rapidly during storage than conidia (Inch *et al.*, 1986; Bidochka *et al.*, 1987; Lane *et al.*, 1991; Hegedus *et al*., 1992). However, after much research, desiccation-tolerant blastospores of *I. fumosorosea* were produced (Eyal *et al.*, 1994). In 1997, Jackson *et al.* reported the production of a high concentration of desiccation-tolerant blastospores in liquid culture medium which contained the basal components of 80 g of glucose and 13.2 g of casamino acids. Smith (1996) tested the Trinidadian conidial strains of *I. fumosorosea* against *Bemisia tabaci* (Gennadius) and found they were efficacious. Later, Vega *et al.* (1999) showed that *I. fumosorosea in vitro* blastospores germinated faster than conidia on the cuticle and were more virulent against the *Bemisia* species under laboratory conditions. The pathogenicity and virulence of all these Trinidadian fungal strains as *in vitro* produced blastospores had not been tested or compared against the GW previously. Therefore, after obtaining the Trinidadian fungal strains, T, T10 and T11, *in vitro* blastospores were produced (Avery, 2002, 2010) and tested against the GW due to its ease and increased virulence potential compared to conidia.

When determining the potential of an entomopathogenic fungal strain for use in controlling an insect pest, such as the GW, the important parameters such as the viability and growth rate of the fungus must be known. These specific parameters can be ascertained simply by finding the percent germination and the mean radial growth rate on a solid media. Overall, the radial growth on solid media can help indicate which fungal strain has the potential to germinate faster on the insect cuticle, contact and subsequently infect the insect host at a given temperature (Taylor and Khan, 2010). This information can then be used to help determine the pathogenicity and virulence of a specific fungal strain or isolate against a target arthropod pest prior to and after spray application under greenhouse or field conditions. Therefore, the

present studies were two-fold: 1) to evaluate and compare the germination time and radial growth rates of three different Trinidadian strains of *I. fumosorosea in vitro* blastospores when grown on potato dextrose agar (PDA) plates, and 2) to determine and compare their pathogenicity and virulence against fourth-instar nymphs (pharate adults; Gelman and Gerling, 2003) of GW on *P. vulgaris* plants.

MATERIALS AND METHODS
Fungal strains
Isaria fumosorosea (*Ifr*) strains T, T10 and T11 used in this study, were originally collected in Trinidad and maintained at CABI Bioscience, formerly known as the International Mycological Institute, Egham, UK on PDA slants. Strains T and T11 were both originally isolated from *B. tabaci* (in 1990 and 1991, respectively) and strain T10 from an unknown aphid species. Smith (1996) determined the genetic fingerprints from a RAPD-PCR analysis and found that both T10 and T11 were very similar in their DNA profiles, but strain T contained a singular band not present in either of the other strains tested. These strains were maintained on PDA (Oxoid™: Oxoid Limited, Hampshire, UK) slants at Birkbeck, University of London and were stored at $4.0 \pm 1.0°C$. All strains were cultured at $25 \pm 0.5°C$ on PDA.

Culture conditions and conidial suspensions
In preliminary studies (Avery, 2002), it was determined that 25°C and constant white light were the optimum conditions for conidial production for all *Ifr* strains on PDA solid media. Therefore, these optimum conditions were used for the following experiments for culturing *in vitro* blastospores of *Ifr* strains. The three strains of *Ifr* were cultured on PDA slants in glass screw top tubes at constant $25 \pm 0.5°C$, 24:0 LD photoperiod under a fluorescent lamp for 14 days. Conidial suspensions for inoculating the submerged cultures were prepared by flooding each PDA slant with 10 mL sterile, distilled water. Sterile glass Ballotini beads (20-25 beads; 1 mm in diameter) were then added, and the tube suspension was agitated using a vortex mixer. The concentration of the inoculum for all submerged cultures as determined by hemocytometer was (Mean ± S.E.) $2.2 \pm 0.2 \times 10^6$ conidia mL^{-1}. Each *I. fumosorosea* inoculum

suspension (10 mL) was then carefully poured into the separate flasks containing the liquid culture medium prepared as described in Avery *et al.* (2010). Liquid cultures of each strain (100 mL in 250 mL Erlenmeyer flasks) were incubated at 25°C on an orbital shaker (Gallenkamp™) at 140 rpm for 4 days. Duplicate or triplicate flasks were used for all treatments, and all experiments were repeated at least twice using subcultures of the original culture.

Blastospore germination studies
Blastospore germination and the possible inhibitory effects of surfactants on germination was determined using the following technique. Treatments were: 1) filtered *in vitro* blastospore suspensions of *Ifr* strains only, 2) blastospore suspensions plus 0.01% of Triton X-100 (v/v) and 3) blastospore suspensions plus 0.01% Tween 80 (v/v) added. A 5 µL droplet of a treatment was placed in the center of a PDA plate and immediately spread using a sterile glass spreader. Plates were sealed using Parafilm® and incubated at $25 \pm 0.5°C$ under constant fluorescent light for 6-10 hours. Four drops of lactophenol carmine stain (50:50 mL; v/v) were placed randomly on each replicate plate and a total of 100 blastospores (25 per stained/area) were observed using a light microscope (400X) on each plate. A blastospore was considered to have germinated when it had formed a germ tube. This procedure was repeated in triplicate for each strain per treatment.

Colonial radial growth measurements
To determine the growth rate of fungal strain, the following technique was employed. A sterile needle tip was dipped into the filtered blastospore suspension of the *Ifr* strains and then inserted into the center of an inverted PDA plate. A dot marked on the outside of the dish bottom indicated the location of the point and served as the central point for measuring the radial growth. Every 3 days a new line was traced along the boundary of the outer hyphal outline and the radial growth was determined by measuring the distance between the growth rings. The radial growth (mm) was determined using the mean of four separate measurements taken between the growth rings at 90°s to each other from three replicate plates per strain. Significance of the average radial growth for the fungal strains over the observation period was determined using a one-

factor repeated measures ANOVA ($\alpha = 0.05$). To determine the average number of blastospores present on the needle tip, ten needle tip points were inserted into a separate PDA Petri dish plate in a circular fashion as described above, sealed using Parafilm® and incubated at 25 ± 0.5°C under constant fluorescent light for 6-10 hours. After incubation, plates were unsealed and the number of blastospores were counted using a light microscope (400X).

Plants and test insects

Two replicate experiments were conducted approximately two months apart. Sixty five (Experiment 1) and 23 (Experiment 2) Dwarf French bean plants, *P. vulgaris* variety 'Prince', were grown in plastic pots (7.5 cm diameter) filled with John Innes soil type 3 in greenhouses at the University of London, Birkbeck College. GW adults obtained from Royal Botanic Gardens, Kew reared on *Abutilon* sp. (house lime) plants for unknown generations were used to infest *P. vulgaris* plants for one or two generations prior to the tests. *P. vulgaris* seedlings with two primary leaves were then infested by transferring adult GWs (male:female ratio unknown) from previously infested *P. vulgaris* plants. The average number of adult GW present on the leaves after 72 hours were 65 (Experiment 1) and 52 (Experiment 2). After 72 hours the adults were removed and the GW eggs were counted. Thirty plants with an average of 287 eggs per leaf (Experiment 1) and 21 plants with 172 eggs per leaf (Experiment 2) were selected and placed in a growth chamber for approximately 17 days at 23 ± 2.0 °C, 16:8 LD to develop into the fourth-instar (pharate adult) stage. Preliminary experiments had shown that at this temperature the time required for the egg to develop to the pharate adult was approximately 17 days.

Fungal spray protocol

Each of the *Ifr* strains were assayed in Experiment 1; however, based on the pathogenicity and radial growth, only strain T11 was selected as the best candidate and used in Experiment 2. For both Experiments 1 and 2, data collected from the two leaves of each plant were averaged, and each plant was considered as a single replicate. Blastospores of *Ifr* Trinidadian strains T, T10 and T11 were sprayed on the pharate adults growing on the two primary leaves of each plant. Aqueous suspensions of *Ifr* blastospores in the liquid culture media with 0.01% Triton X-100 (v/v) were sprayed to the point of runoff on each leaf using an air brush sprayer (Badger™) at 1.73 bars while constantly agitating the spore suspension. The concentrations of the spores in the suspensions were determined using a standard hemocytometer and then adjusted to produce low, medium and high doses of 5×10^5, 5×10^6 and 5×10^7 blastospores mL^{-1}, respectively. The three doses of blastospores for each fungal strain were sprayed in ascending order. Distilled water containing only liquid culture media with 0.01% Triton X-100 (v/v) was sprayed as a control.

For each application, each leaf was excised from a plant and placed on a holding platform made from the lid of a standard 10 cm diameter plastic Petri dish containing tissue paper. Each platform was held approximately 0.5 m from the sprayer at a 45° angle and sprayed to the point of runoff. All spray treatments were applied to 3 plants per strain per dosage. Each leaf contained approximately 200 (Experiment 1) or 100 (Experiment 2) GW nymphs. Immediately after spraying, each leaf was placed in a re-sealable plastic bag and transferred to a growth chamber for 48 h at 23 ± 2 °C under a 16:8 LD photoperiod. After this time, each leaf was removed from the bag and placed in a ventilated chamber for an additional 5 days at 23 ± 2 °C. The chamber consisted of a plastic box (20 x 12 x 8 cm) with a lid containing 2 holes (1.5 cm in diameter) covered with plastic screening (1 mm mesh openings). Leaves were transferred to dry tissue paper (folded several times) and then placed on top of moistened tissue paper located in the bottom of the chamber. This was used to keep the leaves moist. The relative humidity (RH) in the growth chamber was maintained at $74 \pm 1.0\%$ for the duration of these studies, however the RH inside the holding boxes chambers was not measured. Treatments were assessed for percent mortality 7 days post-treatment for all doses.

Between each application of a different *Ifr* strain, the sprayer was cleaned with 70 % alcohol for 15-20 seconds and allowed to dry for 2 minutes. After drying, a 0.01% Triton X-100 (v/v) solution was sprayed for approximately 20 seconds prior to the next application. Tissue paper in the holding platform was replaced after each application.

Table 1. Percent germination of *I. fumosorosea* blastospore strains T, T10 and T11 with or without surfactants Triton X -100 and Tween 80 grown on triplicate PDA plates under constant light after 6-8 hours at $25 \pm 0.5°C$.

Strain	Percent blastospore germination / treatment[a]		
	Distilled Water	Triton X-100	Tween 80
T	98 ± 0.7	98 ± 0.9	99 ± 0.5
T10	99 ± 0.6	100 ± 0.0	99 ± 0.7
T11	99 ± 0.5	100 ± 0.0	100 ± 0.0

[a]Mean percentage germination ± S.E. (*n* = 12 samples; 3 replicate plates with 4 samples per plate). Treatment surfactants were not significantly different from the control (ANOVA, *P* > 0.05).

Viable blastospores and efficacy

Spore suspensions of each *Ifr* strain were sprayed onto two PDA plates, sealed and incubated for 6-8 hours at 23 ± 2.0 °C to determine germination as described above. The number of viable spores per strain was determined by using the following formula: mean % germination x the number of spores mL^{-1}. Percent mortality of the GW was determined using a dissecting microscope (10X) and counting the number dead or surviving (eclosed from pupal casing). Each leaf blade was subdivided into 4 sections defined by the midrib and the secondary veins. A total of 100 (Experiment 1) and 50 (Experiment 2) insects (either infected or eclosed) were counted in each section beginning at the leaf margin and counting towards the center.

Statistical analysis

Mean germination percentages with and without surfactants added for each fungal *Ifr* strain were arcsine transformed prior to being statistically analyzed using a one-way ANOVA (α = 0.05). Significance of the average radial growth for the fungal strains over the specified observation periods was determined using a one-factor repeated measures ANOVA (α = 0.05). Percent mortality data were corrected using Abbott's formula (Abbott, 1925) and then arcsine transformed to normalize the data prior to being statistically analyzed using a one-way ANOVA and then means were separated statistically using the Sheffe's *F*-test (α = 0.05). A regression line was produced to determine the median lethal concentration (LC_{50}) value in viable blastospores mL^{-1} for each *Ifr* strain (POLO – PC, LeOra Software).

**RESULTS**

Blastospore germination studies

The effects of different surfactants on the germination of blastospores when grown on PDA plates are compared in Table 1. The percent germination of the blastospores for all *Ifr* strains tested was high (98-100) and was not affected (*P* > 0.05) by the surfactant Triton X -100 (0.01% v/v) or Tween 80 (0.01% v/v) when compared to the distilled water control.
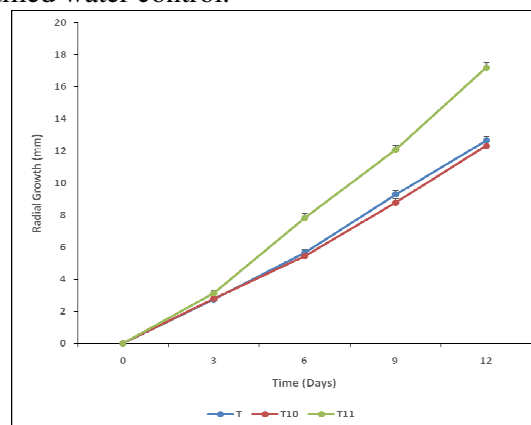
Fig. 1. Radial growth (Mean ± S.E.) of colonies of three Trinidadian (T) strains of *Isaria fumosorosea* over 12 days on triplicate PDA plates at $25 \pm 0.5°C$ under constant light. Bars represent the standard error (S.E.) for radial growth.

Colonial radial growth measurements

Colonial radial growth for the 12-day experiment and 24-day experiment are shown in Figures 1 and 2, respectively. The radial growth rates for all fungal strains were similar for the first 3 days of the
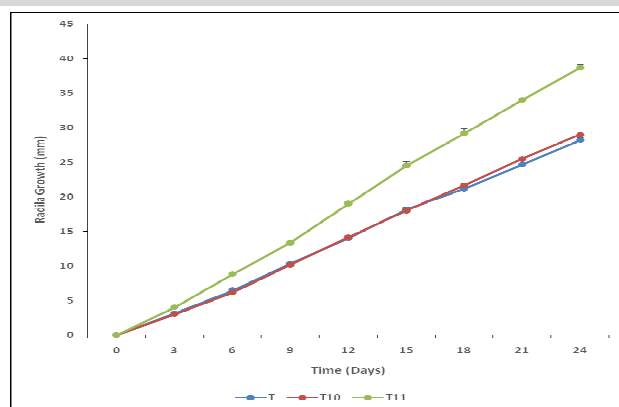
Fig. 2. Radial growth (Mean ± S.E.) of colonies of three Trinidadian (T) strains of *I. fumosorosea* over 24 days on triplicate PDA plates at 25 ± 0.5°C under constant light. Bars represent the standard error (S.E.) for radial growth.

12-day experiment on PDA plates at 25 ± 0.5 °C under a 24:0 LD photoperiod analyzed using a repeated measures ANOVA (RMANOVA) ($F_{2, 33} =$ 2.1, $P = 0.14$). However, in the 24-day experiment, radial growth for strain T11 was significantly faster (RMANOVA: $F_{2, 15} = 31$, $P < 0.001$) than the other two strains after 3 days. Overall, *Ifr* strain T11 grew significantly faster than either T or T10 over either the 12-day (RMANOVA: $F_{2, 33} = 63.5$, $P < 0.001$) or 24-day (RMANOVA: $F_{2, 15} = 427$, $P < 0.001$) growth period. Both *Ifr* strains T and T10 were similar in their growth patterns over both observation periods.

Bioassays
Viability of blastospores was 96-98% for all fungal strains used during Experiments 1 and 2. Natural percent mortality of the fourth-instar nymphs in the control treatments was very low in Experiments 1 (6 ± 1.1%) and 2 (2 ± 0.9%) even though the leaves did desiccate at the later part of these experiments.
Experiment 1
The highest (mean ± S.E.) percent mortality of the whitefly nymphs was obtained by spraying fungal strain T11 at the highest concentration (98.1% at 5 x 10^7 blastospores mL^{-1}) compared to T and T10 (55.9%; 67.8%), respectively (Table 2); strain T10 was also significantly more virulent against the GW than strain T at the highest concentration ($F_{2, 6} =$ 16.2; $P = 0.004$). The percent mortality at the intermediate concentration of 5 x 10^6 blastospores mL^{-1} was higher for fungal strain T11 compared to

Table 2. Mortality of *T. vaporariorum* pharate adults after being sprayed with *I. fumosorosea* Trinidadian strains T , T10 and T11 blastospores on *P. vulgaris* plants at three concentrations for Experiment 1.

Fungal Strain	Mean % corrected mortality of *T. vaporariorum* nymphs / concentration[ab]		
	5 x 10^5	5 x 10^6	5 x 10^7
T	24.9 ± 6.2 a	47.7 ± 3.3 a	55.9 ± 8.0 a
T10	33.5 ± 8.3 a	40.9 ± 8.4 a	67.8 ± 9.0 b
T11	30.7 6.0 a	56.7 ±13.6a	98.1 ± 0.6 c

[a]Mean values ± S.E. ($n = 3$ plants per concentration) were arcsine transformed and then analysed using ANOVA. Percent mortality values within a column followed by a different letter are significantly different (ANOVA + Sheffe's *F*-test, $P < 0.05$)
[b]All concentrations are in blastospores mL^{-1}

the other strains; however, the differences were not significant ($F_{2, 6} = 0.73$; $P = 0.52$). All strains were similar in virulence against the GW at the lowest dosage ($F_{2, 6} = 0.42$; $P = 0.68$). No significant differences were determined between replicate trials per experiment by a two-way ANOVA and a regression line was produced by pooling the mean mortality data from both trials to determine the LC$_{50}$ value for each *Ifr* strain (Table 3).

Table 3. Two-way ANOVA comparing experiment and dosage effects of *I. fumosorosea* strains on the mortality of *T. vaporariorum* pharate adults in Experiment 1.

Source	*df*	SS	MS	*F*-test	*P*
Experiment (A)	1	2.72E-4	2.72E-4	0.005	0.9454
Dose (B)	2	2.015	1.0081	7.661	0.0001
A x B	2	0.002	0.001	0.014	0.9861
Error		30	1.712		0.057

The regression line slope for *Ifr* strain T11 was significantly different ($X_{2[2]} = 216.1$, $P < 0.001$) with a lower LC$_{50}$ value compared to the other strains (Table 4). Both *Ifr* strains T and T10 were similar in their probit slopes and LC$_{50}$ values. The mean corrected mortality at the greatest dose was highest for fungal stain T11 ($F_{[2]} = 16.3$, < 0.001) compared to the other strains; however, T10 was significantly higher than strain T.

Table 4. Probit analysis of *I. fumosorosea* Trinidadian isolates T, T10 and T11 against *T. vaporariorum* pharate adults on *P. vulgaris* plants in a laboratory bioassay conducted at 25°C for Experiment 1.

Fungal strain	No. of insects per 3 plants	Probit slope[a]	Mean corrected mortality at highest dose[b]	LC_{50} (viable blastospores mL^{-1}) (95 % Cl) x 10^6
T	1200	0.482a	55.9 ± 0.08a	20.9 (7.7-81.6)
T10	1200	0.477a	67.8 ± 0.09b	12.9 (4.5-41.2)
T11	1200	1.110b	98.1 ± 0.01c	2.5 (0.8-5.4)

[a]Regression line slopes followed by the same letter are not significantly different as determined by probit analysis ($X_{2[2]}$= 216.1, $P < 0.001$); [b]Means followed by the same letter are not significantly different :Arcsine transformation plus ANOVA ($F_{[2]}$= 16.3, $P < 0.001$).

Experiment 2
Fungal strain T11, which performed the best in Experiment 1, was selected as the best candidate to be assessed in Experiment 2. The mean corrected mortality of the pharate adult GW for strain T11 was 97.2% at the highest dose (5 x 10^7 blastospores mL^{-1}). In addition, the regression line slope was 1.156 ($X_{2[2]}$ = 396.2, $P < 0.001$) with a very low LC_{50} value of 1.10 x 10^6 viable blastospores mL^{-1}.

DISCUSSION
No inhibitory effect in germination of the *Ifr* blastospores due to the presence of the surfactant Triton X-100 or Tween 80 was observed. In a preliminary spray test, it was observed that without adding Triton X-100 to the suspension, the blastospores would be deposited in clumps and not be evenly dispersed on the leaf surface. Hall (1984) found that Triton X-100 at 0.5% inhibited sporulation of *Lecanicillium lecanii* (Zimmerm.) Zare and Gams, on infected cadavers. However, Triton X-100 at a concentration of 0.01% in this study was shown not to inhibit the growth and germination of the blastospores, and it was observed that all the *Ifr* strains were able to sporulate readily (Avery, 2002; Avery *et al*., 2004). Therefore, the surfactant Triton X-100 was used in each spray treatment as a dispersant for the blastospores.

Colonial radial growth is a unique biotic parameter that can be measured in fungi due to its spiralling growth pattern spreading out from a point of inoculum (Robertson, 1968). In addition to spore germination and other biotic factors, this parameter can also provide vital information concerning the potential of each fungal strain being tested when selecting the best candidate for biocontrol of a pest insect (Taylor and Khan, 2010). In this study, the colonial radial growth of fungal strain T11 blastospores was faster than either T or T10. This faster fungal growth rate may allow the hyphae to contact the insect host more quickly, and increase the potential for infection. Taylor and Khan (2010) supported this hypothesis when evaluating another fungal entomopathogen, *Metarhizium anisopliae* (Metch.) and concluded that the isolates which performed the best, e.g. fastest germination rate, fastest radial growth rate, etc. also had the greatest potential for the management of *B. tabaci* in the field. In another study using a glass-slide bioassay, strain T11 was faster at infecting the pharate adults, compared to the other Trinidadian strains (Avery, 2002; Avery *et al.,* 2004). Therefore, strain T11 should develop more quickly on the host cuticle or if applied on the leaf surface, grow, contact and infect the host faster than either strains T or T10.

All the Trinidadian *Ifr* fungal strains demonstrated a range of efficacy against the GW pharate adults, but strain T11 was the most virulent at the highest dosage and had the lowest estimated LC_{50} value. However, at the lowest (10^5 blastospores mL^{-1}) and middle (10^6 blastospores mL^{-1}) dosage, the strains were equally effective. This variability in pathogenicity between strains from a similar geographic location has been noted by other authors

(Vidal *et al.*, 1997; Wraight *et al.*, 1998). The percent mortality of GW after spraying fungal strain T11 blastospores at the highest dose was comparable to other *Ifr* strains and entomopathogenic fungi for controlling whiteflies on other crops using either blastospores or conidia (Kanagaratnam *et al.*, 1982; Fang *et al.*, 1986; Bolckmans *et al.*, 1995; Lacey *et al.,* 1995; Vidal *et al.*, 1998; Wraight *et al.*, 2000). Kanagaratnam *et al.* (1982) indicated that heavy infestations of GW nymphs on glasshouse cucumber plants were kept below the economic crop damage level by using fortnightly and monthly sprays of *L. lecanii* spores. In addition, they found that blastospores and conidia were equally effective in controlling the GW nymphs when sprayed to runoff at concentrations near 10^7 spores mL^{-1} on to the underside of the leaves. Experiments carried out in China gave 71-100% control of GW after spraying *Ifr* var. *beijingensis* conidia on cucumbers in the greenhouse at a mean temperature of 15.6-21.3 °C and 79-93% RH (Fang *et al.*, 1986). Bolckmans *et al.* (1995) found the microbial insecticide blastospore formulation PreFeRal™ WP (*Ifr* strain Apopka 97) provided excellent control of GW on greenhouse vegetables at a dosage of 1g L^{-1} (10^9 colony forming units per gram L^{-1}). The nymphal mortality was between 92-97% when assessed two weeks after being sprayed at this high dosage on cucumber plants. In 2005, Gökçe and Er found that the most virulent isolates of *Ifr* conidia provided 70% mortality against 2nd instars of the GW on tomato leaflets. Nymphs of whiteflies in the greenhouse and field have been effectively controlled with both blastospores and conidia of various *Ifr* strains (Lacey *et al.*, 1995; Vidal *et al.*, 1998; Lacey *et al.*, 1999; Wraight *et al.*, 2000; Kim *et al.*, 2008; Huang *et al.*, 2010; Kim *et al.*, 2013).

The dosage infectivity and percent mortality of the GW by the various *Ifr* strains may also be attributed to genetic characteristics which includes the ability of the fungus to compete on the leaf phylloplane and infect the insect host. Avery *et al.* (2010) found that under optimum conditions using a novel leaf model bioassay, that *Ifr* fungal hyphae protruding from an infected GW pharate adult can grow horizontally across a simulated leaf phylloplane and colonize another susceptible host located 21 mm away. However, from earlier studies conducted by Smith (1996) using the *Ifr* Trinidadian strains and running

a RAPD-PCR analysis, no clear correlation was revealed between their DNA profiles and virulence against the GW. Even though Trinidadian strains T and T11 were both originally isolated from *B. tabaci* (in 1990 and 1991, respectively), this study demonstrated that only T11 was the most virulent against GW.

Based on these studies, *in vitro* blastospores of *Ifr* strain T11 has been shown to be the most virulent of the Trinidadian strains tested against fourth-instar GW nymphs on *P. vulgaris*. The liquid media used in these experiments provided a high number of desiccation-tolerant blastospores of *Ifr*, that when applied against the GW pharate adults on bean plants were efficacious, especially, strain T11. However, these efficacy studies were conducted in a growth chamber and the results only simulate high GW mortality under optimum growing conditions for the blastospores. Therefore, the desiccation-tolerant blastospores of this fungal strain should be tested in a greenhouse or under field conditions to determine and evaluate its efficacy under more sub-optimum growing conditions.

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