

Comparison of wheat growth-response to endophytic *Beauveria bassiana* (Hypocreales: Cordycipitaceae) derived from an insect versus plant host

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Abstract

Beauveria bassiana (Hypocreales) is a cosmopolitan entomopathogen, infecting >700 insect species. Although traditionally associated with insects, endophytic colonisation of plants is also known. Endophytism may protect plants against insects/diseases and enhance plant growth. Both insect- and plant-derived (endophytic) ‘sources’ of *B. bassiana* may be present in an agroecosystem, both of which may be in contact with plants. Here, growth response, viz., root length, shoot height, fresh root biomass, fresh and dry shoot biomass of wheat, *Triticum aestivum* L. (Poaceae), is reported following inoculation with *B. bassiana* (strain PPRI 7598). The strain was passaged and re-isolated from an insect (IN) versus plant (PL) substrate. When five wheat cultivars were inoculated with either *B. bassiana* PPRI 7598IN or -PL isolates through seed imbibition, a significantly higher level of endophytism (roots, stems and leaves, combined) was recorded with 7598IN (29.74%) compared to 7598PL (26.13%). Cultivar Baviaans responded best to endophytic colonisation (plant parts combined) at 33.54%, followed by Tugela (31.34%), Kariega (27.87%), Gariep (25.67%) and Elands (21.28%). On average, *B. bassiana*-treated plants showed a 71% growth increase over control plants. In topically sprayed bioassays, 7598IN caused 57% mortality to Russian wheat aphid, *Diuraphis noxia*, compared with 50% by 7598PL; also recording a significantly shorter mean time to aphid mortality (4.14 days) versus 7598PL (4.58 days). A significantly higher level of overt mycosis (58.2%) was noted with 7598IN compared with 7598PL (47.9%). Results underscored several positive aspects associated with endophytic *B. bassiana* in wheat, creating new and exciting IPM possibilities.

Keywords: *Beauveria bassiana*; *Diuraphis noxia*; endophyte; plant colonisation; plant growth response; *Triticum aestivum*.

Abbreviations: EPF_Entomopathogenic fungi, IPM_Integrated pest management, PHS_Pre-harvest sprouting, SA_South Africa, ARC_Agricultural Research Council, RWA_Russian wheat aphid, FHB_*Fusarium* head blight, SDAY_Sabouraud dextrose agar with yeast, CBC_Conservation Biological Control, DPI_Days post inoculation, LSD_Least significant differences, HPR_Host plant resistance

Introduction

In South Africa, wheat, *Triticum aestivum* L. (Poaceae), is prone to infestation by at least six cereal aphid (Homoptera: Aphididae) species, of which the Russian wheat aphid (RWA), *Diuraphis noxia*, is considered the most damaging (Prinsloo and Tolmay, 2015). Host plant resistance (HPR) currently forms the backbone of RWA control under dryland conditions in the summer rainfall region. However, since the arrival of the first aphid biotype, RWASA1 in 1978, four subsequent biotypes, RWASA2, RWASA3, RWASA4 and RWASA5 made their appearance in 2005, 2009, 2011 and 2018, respectively (Jankielsohn, 2016, 2019). The development of aphid biotypes is not unexpected, as widespread deployment of HPR implies evolutionary adaptation, fueled by a higher selection pressure (Smith and Chuang, 2014; Yates and Michel, 2018). To counteract such selection, indiscriminate mortality inflicted by natural enemies (predators, parasitoids and pathogens), is seen as a

critical component in a HPR x natural enemy-based control programme (Sunderland et al., 1988; Marasas et al., 1997).

The natural enemy complex associated with RWA in South Africa is diverse, inclusive of both endemic and (classically) introduced species (Hatting, 2002; Prinsloo et al., 2002). The impact of entomopathogenic fungi (EPF) on cereal aphid populations is of particular importance, especially epizootics induced by *Pandora neoaphidis* (Entomophthoromycota: Entomophthorales) (Hatting et al., 2000). Although EPF grow in culture medium, the fastidious nature of the Entomophthoromycota renders fungal spores a difficulty to mass produce/formulate into commercial mycoinsecticides. On the other hand, the highly amenable EPF, *Beauveria bassiana* (Hypocreales: Cordycipitaceae), is also known to naturally infect RWA in South Africa (Hatting et al., 1999), albeit at a low prevalence (Hatting et al., 2000).

Integration of *B. bassiana* (topical applications) against RWA in combination with HPR, was explored by Hatting et al.

(2004); finding around 65% fewer aphids in treated field plots compared with controls. Locally, application of *B. bassiana* on various crop commodities has been based on an augmentative approach (Hatting et al., 2018), similar to that of a chemical paradigm. However, the ubiquitous nature of this EPF within global agro-ecosystems, makes it suitable for exploitation in Conservation Biological Control (CBC) (Fuxa, 1998), as proposed by Meyling and Eilenberg (2007). Therefore, the notion of exploiting *B. bassiana* in a CBC approach carries relevance, not only in terms of insect suppression, but potentially for disease suppression (Busby et al., 2016) and also as a plant growth-promoting factor (Behie and Bidochka, 2014; Sánchez-Rodríguez et al., 2015; Liao et al., 2017; Jaber and Enkerli, 2017; Sánchez-Rodríguez et al., 2018). In nature, insect-derived inoculum is the primary source of *B. bassiana*, while the historical frequency of recycling remains largely unknown. Host plant contact with such inoculum can be via seed/roots in the soil environment [see Zimmerman (2007) for a list of worldwide soil extractions] and/or via above ground phylloplanes (Meyling and Eilenberg, 2006; Howe et al., 2016).

Beauveria bassiana can be linked to three basic associations, namely soil, insects and/or plants (Meyling and Eilenberg, 2007). In the latter association, the fungus may exist as an endophyte (Vega, 2008). In the endophytic state, there is potential to exploit the fungus for induced systemic resistance to biotic and/or abiotic stressors (Rodríguez et al., 2009). Moreover, as a host plant, *T. aestivum* appears endophytic-friendly, both as natural host (Crous et al., 1995; Larran et al., 2002, 2007; Vujanovic et al., 2012; Comby et al., 2016; Grudzinska-Sterno et al., 2016) and when artificially inoculated (Dingle and McGee, 2003; Gurulingappa et al., 2010; Russo et al., 2015; Sánchez-Rodríguez et al., 2015; Sánchez-Rodríguez et al., 2018).

In a recent review on EPF as endophytes in biological control, Vega (2018) found that 40% (34/85) of papers covered examined plant responses to endophytism, covering 20 plant species. In that review, generally, the use of different inoculation techniques to inoculate plants resulted in colonisation of plants. The current study is the first to investigate the growth-response of five South African wheat cultivars to endophytic *B. bassiana* (strain 7598; originally extracted from a soil sample), passaged through an insect versus plant substrate (Russian wheat aphid, *Diuraphis noxia* versus *T. aestivum*, respectively). Three inoculation techniques were employed to simulate potential contact mechanisms possible under field conditions.

Results

Endophytic colonisation of wheat (inoculation of seeds through imbibition)

Beauveria bassiana was never re-isolated from 'control' plants in any experiment. The level of endophytic colonisation varied significantly among cultivars ($F_{4,90;0.00} = 24.81$), between fungal isolates (PPRI 7598IN vs PPRI 7598PL; $F_{1,90;0.00} = 17.48$), among plant parts (root, stem and leaf; $F_{2,90;0.00} = 106.90$) and between evaluation times (7 vs 14 DPI; $F_{1,90;0.00} = 168.15$). The most colonised cultivar (combined plant parts; $n = 96$ per cultivar; $LSD_{0.05} = 4.44$) was Baviaans (33.54%), followed by Tugela (31.34%), Kariega

(27.87%), Gariiep (25.67%) and Elands (21.28%). A significantly higher ($P < 0.05$) level of colonisation (plant parts combined) was recorded with PPRI 7598IN (29.74%; $n = 240$) compared with PPRI 7598PL (26.13%; $n = 240$). The highest level of colonisation (44.15%) was recorded in roots with PPRI 7598IN at 7 DPI, statistically outperforming all other treatments (Fig.1). Generally, leaf colonisation was low, ranging from 12% to 28%. Recovery of *B. bassiana* showed a general decline from 7 - 14 DPI for all treatments.

Effect of three inoculation techniques on plant growth of five wheat cultivars

On average, treated plants showed a 71% growth increase over controls, with mean growth parameter responses ranging from 29% to 104% (Table 2). Although seed imbibition ranked first (mean improvement of 87.8%) among the three inoculation techniques employed (Table 2), leaf spraying resulted in a more consistent, high level of plant response, ranking first for four of the five parameters, viz., fresh shoot biomass, dry shoot biomass, root length and shoot height (Table 2). There were significant differences among the cultivars for three of the five parameters measured, viz., dry shoot biomass ($F_{4,90;0.00} = 3.75$), fresh root biomass ($F_{4,90;0.00} = 68.66$) and root length ($F_{4,90;0.02} = 3.10$), but not for shoot height ($F_{4,90;0.06} = 2.31$) and fresh shoot biomass ($F_{4,90;0.11} = 1.91$). However, when pairwise comparisons were performed among the cultivars for shoot height and fresh shoot biomass, the Fisher's unprotected LSD test (Hsu, 1996) did indicate differences. This variation is further emphasized by the fact that for each growth parameter, a different cultivar ranked first (Table 3). Of particular interest was cultivar Kariega, showing a 260% increase in fresh root biomass (inoculation techniques combined), far exceeding the second-best responder (Gariiep) at 81%. When considering the fresh root biomass-response of cultivar Kariega, by inoculation technique, seed imbibition significantly outperformed leaf spraying and soil drenching at +672%, +54% and +7%, respectively ($LSD_{0.05} = 52.15$).

Virulence of Beauveria bassiana PPRI 7598 to RWA following passage and re-isolation from an insect ('IN') versus plant ('PL') host

The conidial depositions (estimated number of propagules deposited per square millimeter on an agar plate; counted at 40x magnification using the light microscope) for PPRI 7598IN and -PL, were 1820 ± 68 conidia mm^{-2} and 1958 ± 60 conidia mm^{-2} , respectively. During these assays, the insect-derived versus plant-derived isolates caused respective aphid mortalities of 57% versus 50%, 7 days post inoculation; albeit not significantly different at the 5% test level with LSD of 7.56. (Fig. 2). Moreover, the insect-derived isolate recorded a shorter mean time of mortality (4.14 ± 0.04 days) compared to the plant-derived isolate (4.58 ± 0.08 days) ($LSD_{0.05} = 0.25$), while the level of overt mycosis recorded with PPRI 7598IN (58.2%; $n = 60$) was significantly higher ($LSD_{0.05} = 6.49$) compared with PPRI 7598PL (47.9%; $n = 55$) (Fig. 3).

Table 1. Wheat cultivars, their pedigree and general characteristics.

General characteristics	Cultivars				
	Tugela	Elands	Gariiep	Kariega	Baviaans
Breeding history and pedigree origin [‡]	Kavkaz/Jaral	PI137739/*4Molopo (77)	PI137739/*4Molopo (20)	SST44// K4500 /SAPSUCKER"S"	Queen Fan(A50)/4/Jup/Emu"S"// Gjo"S"/3/Kvz/K4500L-6-A-4
Agronomic traits ^{**}	High yield Good straw strength Good kernel attachment Good kernel attachment Susceptible to PHS [§]	140 days to flowering Good straw strength Good kernel attachment Resistant to PHS High yield potential	138 days to anthesis Good straw strength Good kernel attachment Resistant to PHS High yield potential and excellent yield stability	114 days to flowering Good straw strength (similar to Gamtoos) Good kernel attachment Resistant to PHS yield potential similar to Gamtoos	109 days to anthesis Good straw strength (similar to Kariega) Good kernel attachment Resistant to PHS High yield potential similar to Kariega
RWA resistance ^{**}	Susceptible to all SA biotypes	Resistant to RWASA1 [^]	Resistant to RWASA1	Susceptible to all SA biotypes	Susceptible to all SA biotypes
Disease resistance ^{**}	Resistant to stem and leaf rust* (similar to Tugela - DN)	Moderately resistant to stem rust; moderately susceptible to stripe rust and susceptible to leaf rust	Resistant to stem rust; susceptible to stripe rust and leaf rust (at adult stage)	Susceptible to 1 of 5 stem rust races in SA [#] , moderately susceptible to leaf rust and resistant to stripe rust	Susceptible to 1 of 5 stem rust races in SA, moderately susceptible to leaf rust and resistant to stripe rust
Production area [‡]	Winter dryland	Winter dryland	Winter dryland	Irrigation & Spring dryland	Spring dryland & Irrigation
Year released [‡]	1986	1998	1995	1993	2000

N/A = not available; [‡]PHS = Pre-harvest sprouting; [§]SA = South Africa; [^]RWASA1 = Russian wheat aphid South African Biotype 1; Sourced from ARC-Small Grain Production guideline (*1998 (Tugela only); **2018); [#]Sourced from Smit et al. (2010) and Coale (2017)

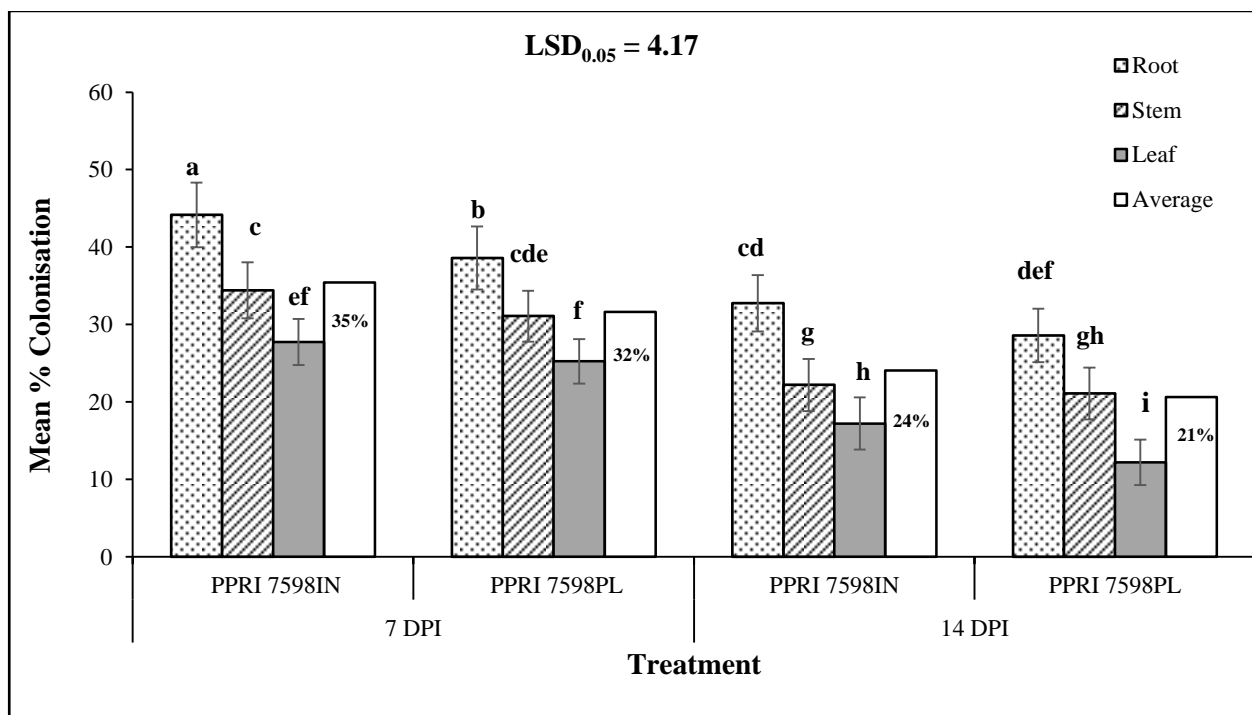


Fig 1. Colonisation of different plant parts by *Beauveria bassiana* PPRI 7598 isolates at 7 and 14 days post inoculation. Bars (Means±SEM) marked with different letters indicate significant differences at P<0.05; LSD = 4.1742.

Table 2. Effect of three inoculation techniques on seedling growth of five wheat cultivars (combined) after 21 days.

Inoculation technique	Fresh shoot biomass (g)	Dry shoot biomass (g)	Fresh root biomass (g)	Root length (cm)	Shoot height (cm)	Grand mean
Seed imbibition	80.03b ¹ (2) ²	45.25b (3)	235.23a (1)	48.85b (2)	29.47ab (2)	87.77±42.22 (1)
Soil drenching	76.96b (3)	75.35b (2)	39.48b (2)	32.20c (3)	25.84b (3)	49.97±12.20 (3)
Leaf spraying	92.96a (1)	152.63a (1)	35.88b (3)	56.30a (1)	32.53a (1)	74.06±25.04 (2)
LSD _{0.05}	9.10	33.95	23.32	6.74	5.99	-
Grand mean	83.32±4.9	91.08±31.98	103.53±65.86	45.78±7.12	29.28±1.93	70.60±15.79

¹ Means±SEM (% increase over controls) within columns followed by the same letter are not significantly different at the 5% test level, ² Performance ranking within a given growth parameter (column) in parenthesis

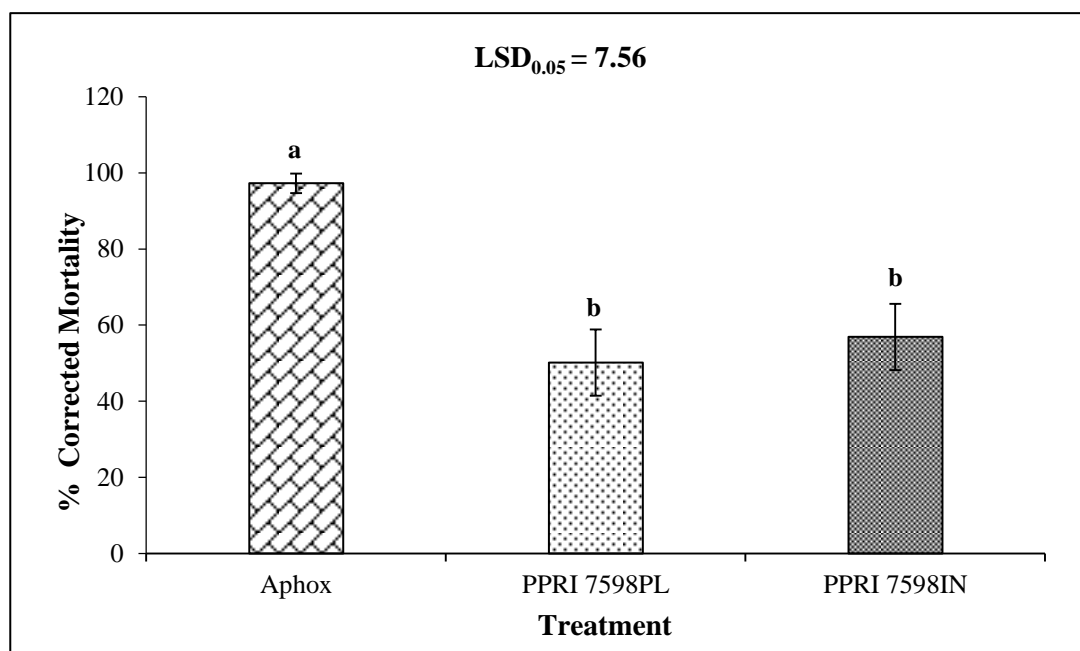


Fig 2. Mean percentage Schneider-Orelli - corrected mortality of the Russian wheat aphid 7 days post inoculation, caused by the chemical standard, Aphox and two PPRI 7598 isolates. Bars (Means±SEM) marked with different letters indicate significant differences at the P value <0.05; LSD = 7.562.

Table 3. Cultivar growth response at 21 days following inoculation (three techniques, combined) with *Beauveria bassiana* PPRI 7598IN.

Cultivar	Fresh shoot biomass (g)	Dry shoot biomass (g)	Fresh root biomass (g)	Root length (cm)	Shoot height (cm)	Grand mean
Tugela	86.84ab ¹ (2) ²	61.98b (5)	70.07bc (3)	50.39a (1)	26.63b (4)	59.18±10.07 (5)
Kariega	77.06b (5)	89.14b (3)	260.37a (1)	47.88a (3)	25.22b (5)	99.93±41.64 (1)
Baviaans	89.44a (1)	93.97b (2)	63.78bc (4)	37.59b (5)	28.87ab (3)	62.73±13.17 (4)
Elands	77.22b (4)	139.75a (1)	42.40c (5)	42.97ab (4)	29.62ab (2)	66.39±19.97 (2)
Gariep	86.03ab (3)	70.56b (4)	81.04b (2)	50.10a (2)	36.08a (1)	64.76±9.46 (3)
LSD _{0.05}	11.75 ³	43.83 ⁴	30.11 ⁴	8.71 ⁴	7.74 ³	-
Grand mean	83.32±2.58	91.08±13.51	103.53±39.71	45.79±2.44	29.28±1.89	70.6±6.91

¹ Means±SEM (% increase over controls) within columns followed by the same letter are not significantly different at the 5% test level

² Performance ranking within a given growth parameter (column) in parenthesis

³ Fisher's unprotected LSD test

⁴ Fisher's protected LSD test

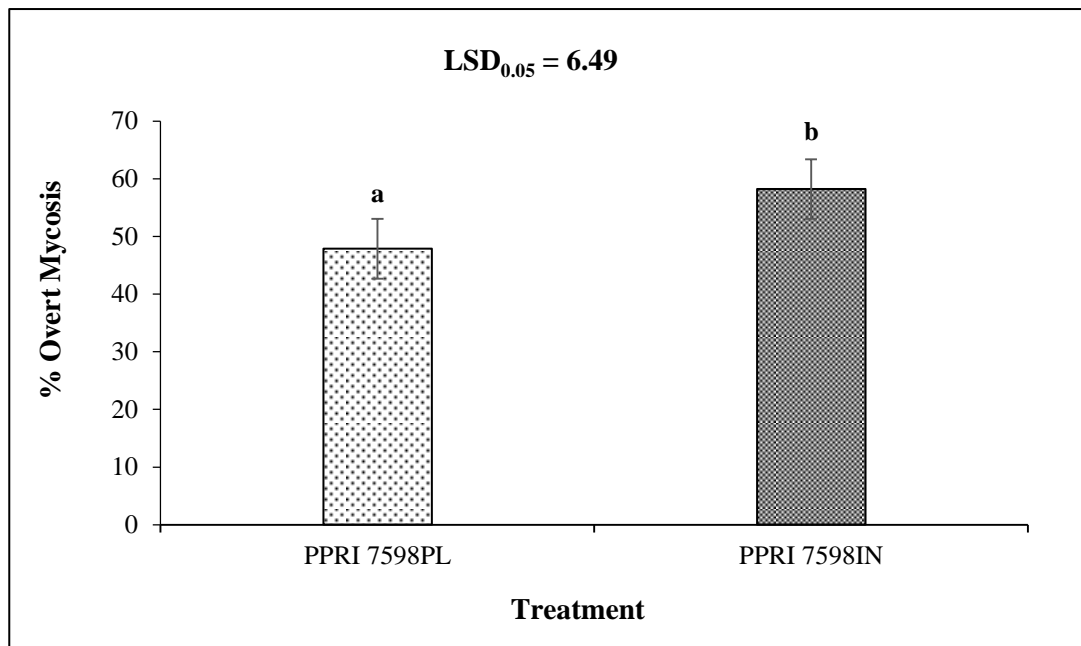


Fig 3. Mean percentage overt mycosis by the two PPRI 7598 backgrounds 7 DPI. Bars (Means±SEM) marked with different letters indicate significant differences at P value <0.05; LSD = 6.488.

Discussion

Successful colonisation of roots, stems and/or leaves, following seed imbibition with *B. bassiana*, has been reported for wheat (Russo et al., 2015; Sánchez-Rodríguez et al., 2015; Sánchez-Rodríguez et al., 2018), broad bean (Jaber and Enkerli, 2016), sorghum (albeit only in sterile soil; Tefera and Vidal, 2009) and also for cotton, tomato and snap bean, following topical seed inoculation with dry conidia (Ownley et al., 2008). Similar results were found in our study, although compared with other plant parts, a significantly higher level of endophytism was observed in roots for PPRI 7598IN and -PL, at both 7 and 14 DPI. Clearly, the uptake and systemic spread of endophytic *B. bassiana* (Behie et al., 2015) via seed treatment, holds significant potential as an economical and practical inoculation method; especially for wheat, where seeding volumes range from 15 – 30 kg per hectare (ARC-Small Grain, 2018). Although the current study did not measure endophytic persistence beyond 14 days, fungal colonisation of host plants at the early developmental stage may provide a crucial competitive advantage to these plants, potentially improving their ability to cope with stress and/or utilise limited resources (Hubbard et al., 2013; Bokati et al., 2016). In South Africa, wheat cultivated under dryland conditions in the summer rainfall region, is typically sown in the winter months of May-July and plants emerge during June-August, just prior to the arrival of spring rains in late September/early October, onwards. During and shortly after seedling emergence, soil moisture levels (residual from the preceding summer season) continue to drop amidst increasing temperatures. Endophytic colonisation of such seedlings, with special emphasis on improved root development (on average, 65% increase in fresh root biomass among the three dryland-production cultivars tested in this study) may hold significant advantages; a notion supported by the findings in our plant-response trials. For all five plant growth parameters measured within 42-45 DPI, fungus-treated plants significantly outperformed

control plants. Similarly, positive growth response with *B. bassiana*-inoculated wheat was reported by Gurulingappa et al. (2010) and more recently by recording an impressive 40% increase in wheat grain yield (Sánchez-Rodríguez et al., 2018). Using only one wheat cultivar (Chinese Spring) and essentially the same techniques and conidial concentrations as in this study, Sánchez-Rodríguez et al. (2018) reported superior colonisation and subsequent growth responses with seed dressing compared with controls. A similar 'cultivar-specific' reaction may be involved with cultivar Kariega, showing a very high affinity towards seed treatment with *B. bassiana* in the current study. An interesting observation from the studies by Quesada-Moraga et al. (2014) and Sánchez-Rodríguez et al. (2018), is vertical transmission of *B. bassiana*. This phenomenon could hold potential for suppression of seed-related diseases like *Fusarium* head blight (FHB), an important wheat disease not only in South Africa, but globally (Dean et al., 2012). According to Rabiey and Shaw (2016), application of the root-colonising endophyte, *Piriformospora indica* (Sebacinaceae) to wheat, reduced FHB disease severity and incidence by 70%, while lowering mycotoxin (DON) concentration of winter and spring wheat samples by 70 and 80%, respectively. The approach also increased aboveground biomass, 1000-kernel weight and total grain weight. Although endophytic *Beauveria* is known to suppress damping-off in cotton (Griffin et al., 2005; Griffin, 2007), bacterial blight in tomato (Ownley et al., 2008), zucchini yellow mosaic virus in squash (Jaber and Salem, 2014) and downy mildew in grapevines (Jaber, 2015), its biocontrol potential against FHB in wheat is yet to be explored. Artificial introduction of *B. bassiana* as an endophyte in wheat or any other host plant implies subjecting the fungus to a different growing environment in terms of nutrients, pH, phytochemicals, etc. Generally, passage of an EPF through an insect host is seen as advantageous, *i.e.* a means of either enhancing or restoring virulence (Song and Feng, 2011). However, the effect on virulence following passaging

through a wheat host plant has remained largely unexplored, with this study being the first to report on such endeavour. Use of plant-passaged conidia caused slightly lower aphid mortality, a significantly longer mean time of mortality as well as a lower level of overt mycosis (sporulation of cadavers), suggesting some level of impediment demonstrated by the plant-derived isolate. Relatively, the insect-derived isolate outcompeted the plant-derived isolate in all aphid mortality parameters measured. Our findings underscored several positive aspects associated with endophytic *B. bassiana* in wheat. Additional to our study, endophytic *B. bassiana* is also being explored locally (in South Africa) in crops like sugarcane (Memela, 2014; Memela et al., 2017) and 'Rooibos' (Hatting, 2017), an indigenous shrub from which tea is made through leaf fermentation. Clearly, expanding the use of *B. bassiana* (and other EPFs) from a topically applied bioinsecticide to systemic bioinsecticide, with potential disease suppressive (Jaber and Ownley, 2018) and plant growth promoting attributes (in addition to wheat, see also Sasan and Bidochka, 2012; Lopez and Sword, 2015; Jaber and Enkerli, 2017), creates new and exciting IPM possibilities.

Materials and Methods

Plant materials

Seeds of three dryland-production bread wheat cultivars (Tugela, Elands and Gariep) and two irrigated-production cultivars (Kariega and Bavians), all developed by ARC-Small Grain, Bethlehem, South Africa, were used in this study (Table 1). Seed surface sterilisation based on the Akello and Sikora (2012) procedure was performed prior to seed treatment. Following sterilisation, seeds were dried in the laminar flow hood for 30 minutes and later immersed in a *B. bassiana* fungal suspension at the concentration of 1×10^8 conidia ml^{-1} for 18-24hrs (Dhingra and Sinclair, 1995). Each cultivar was treated separately, with the "IN-" and "PL-reisolated" *B. bassiana* strain PPRI 7598. Control seeds were soaked in sterile distilled water with 0.01% Break Thru[®] surfactant for 18-24 hours. During the soaking period, seeds were maintained on an orbital shaker at room temperature (Dhingra and Sinclair, 1995). Inoculated and control seeds were later dried on sterile paper towels in a laminar flow hood for three hours prior to planting. Inoculated and control seeds were planted in sterilised 295 ml plastic pots containing sterile soil (pH_{KCl} = 4.8, P = 5.0 mg/kg, K = 125.0 mg/kg, Ca = 600 mg/kg, Mg = 203 mg/kg, Acid saturation = 4.2%), heat-treated at $91 \pm 1^\circ\text{C}$ for 4 hrs. Plants were grown and maintained under glasshouse conditions of $22 \pm 3^\circ\text{C}$, 70 \pm 4% relative humidity (RH) and natural light.

Insect material

Colonies of *D. noxia* biotype RWASA1 were reared in the ARC-Small Grain insectary unit. An aphid colony was established by infesting clean wheat seedlings (cultivar Tugela) at four-leaf growth stage (Hatting and Wraight, 2007); the populations were then reared in gauze cages in a glasshouse at $22 \pm 3^\circ\text{C}$, 40 \pm 4% RH and natural light. Aphids were monitored until they reached the adult stage prior to use in bioassays.

Fungal isolates

The *B. bassiana* fungal isolate, PPRI 7598, was cultured on *B. bassiana* selective medium amended with 0.55g/L dodine (guanidine) and 0.005g/L chlortetracycline (Sigma – Aldrich, Germany) (Chase et al., 1986). Fungal cultures were incubated in full darkness at $25 \pm 1^\circ\text{C}$ and 60 \pm 10% RH. Conidia were harvested from 14 day-old cultures using a sterile scalpel and suspended in a sterile aliquot of distilled water with 0.01% Break Thru[®] surfactant (Polyether-polymethylsiloxane-copolymer surfactant; Goldschmidt Chemical Corporation, USA). Suspension concentrations were adjusted to 1×10^8 conidia ml^{-1} using a Nikon Optiphot light microscope (Nikon, Japan) and improved Neubauer Haemocytometer (Neubauer, Germany).

Fungal strain inoculation and re-isolation from insects and plants

The *B. bassiana* fungal strain PPRI 7598 was inoculated (passaged) through the target insect (*D. noxia*, via topical inoculation; Inglis et al., 2012) and wheat (*T. aestivum*, cultivar Tugela) via seed imbibition with a conidial suspension containing 1×10^8 conidia ml^{-1} using the protocol of Akello and Sikora (2012). The fungus was re-isolated from the two hosts and designated as insect- and plant-derived isolates, "IN" and "PL", respectively.

Treatments

Endophytic establishment through seed treatment with "IN" versus "PL" recovered isolates in five South African wheat cultivars

The purpose of this experiment was to establish *B. bassiana* isolates as endophytes in South African wheat cultivars, thereby comparing endophytic potential of the insect-versus plant-derived isolates. The experiment was arranged as a completely randomised design with five replicates. The treatment design was a split-split plot. The main plot treatments were the cultivars and first subplot was the fungal strains (from the insect and plant derived sources). The second subplot was plant parts (root, stem or leaf). Segments from plant parts (roots, stems and leaves) were excised and surface sterilised according to the method of Bills (1996) and plated on *B. bassiana*-selective medium based on Sabouraud dextrose agar amended with 1 % yeast extract (SDAY) (SDA Biolab, Merck, Longmeadow, Modderfontein, South Africa), containing 0.55 g/L dodine (guanidine) and 5 mg/L chlortetracycline antibiotic (Chase et al., 1986). A total of 270 treated plant sections (9 segments from 3 plant parts x 5 cultivars x 2 observation times) were included in the trial. Four independent trials were conducted, at least seven days apart (total of 13 500 plants). Plates with surface sterilised plant sections were incubated at $25 \pm 2^\circ\text{C}$ for 7-10 days and evaluated in comparison with the controls.

Effect of three inoculation techniques on five plant growth parameters

Wheat cultivars were inoculated with *B. bassiana* best performing isolate (insect-derived isolate) from the previous

assay, employing three different inoculation techniques, viz. seed imbibition, soil drenching and leaf spraying. A volume of 5 ml of a 1×10^8 conidia ml^{-1} suspension was administered in each pot for two inoculation techniques (except for seed imbibition). Seed imbibition was performed as in the previous assay on 1125 treated plants (75 pots x 3 plants per pot x 5 cultivars) and 1125 control plants (imbibition of water plus 0.01% Break-Thru[®], only). Another batch of 150 pots (75 treatment + 75 control) was also included in the soil drenching treatment. The desired conidia ml^{-1} suspension was spread directly onto the soil around the stem-base of three 14-day old seedlings (approximately 1.7 ml per seedling) soaking the roots in each pot, using a 5 ml stepper syringe (Socorex[™] 411 Stepper). For leaf spraying, leaves of 1125 plants (14-day old) were sprayed to a point of run-off (Rondot and Reineke, 2018) with the same suspension concentration as above using a hand-held atomizer. Control plants (1125) were sprayed with water plus 0.01% Break-thru[®], only. Prior to spraying, stem bases and the soil surfaces of treated pots were covered with aluminum foil to prevent inoculation of these areas (Posada et al., 2007). Plants were maintained under glasshouse conditions at 25 ± 2 °C, $40 \pm 5\%$ RH and natural light for 42-45 days. All plants were evaluated for five plant growth parameters: shoot height, root length, fresh shoot biomass, fresh root biomass and dry shoot biomass. Three independent trials were conducted at least seven days apart. The experimental design was organized as completely randomized with five replicates, while the treatment design was a split-plot with a two factorial design as the main plot. The two factors were cultivars with five levels and inoculation techniques with three levels. The subplot factor was fungal strains with two levels (fungus-treated and control).

Virulence of *Beauveria bassiana* PPRI 7598 to RWA following passage and re-isolation from an insect ("IN") versus plant ("PL") host

Bioassays were performed with *B. bassiana* 7598IN and -PL according to the assay methodology of Hatting and Wraight (2007). Treatments included the two *B. bassiana* isolates (at 1×10^8 conidia ml^{-1}) and a chemical standard, Aphox[®] WG (active: pirimicarb), at a concentration of 0.5 g/L. Age-related adult apterae aphids (biotype RWASA1) were sourced from the ARC-Small Grain insectary with 5 replicate groups of 20 aphids each (100 aphids) allocated to each treatment (x3) and control (total 400 aphids). Control aphids were sprayed with sterile water containing 0.01% Break-Thru[®] surfactant, only. All treatments and control were sprayed with 5 ml aliquots inside a Burgerjon precision spray tower (Burgerjon, 1956). During spraying, a Petri dish containing 1.5% water agar was placed adjacent to the aphids on the same radial dimension to quantify the actual number of conidia deposited per mm^2 . As per protocol, inoculated aphids were maintained on wheat seedlings (cultivar Tugela; 4-leaf stage) under glasshouse conditions at 25 ± 2 °C, $40 \pm 5\%$ RH and natural light for 7 days post-inoculation.

Mortality was assessed daily for the duration of the assay and all dead aphids collected and placed on 1.5% water agar and incubated at 22 ± 3 °C in total darkness to facilitate the development of mycosis (external sporulation on cadavers). Three independent series of assays were conducted seven

days apart.

Statistical analyses

Mortality data were corrected according to the Schneider Orelli formula. The homogeneity of four trial variances were verified by Levene's test (Levene, 1960). The normality of the standardized residuals was confirmed using Shapiro-Wilk test (Shapiro and Wilk, 1965). The data of the combined trials were subjected to analysis of variance (ANOVA) using General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Observations over days were combined in a split-plot analysis of variance with day as sub-plot factor (Little and Hills, 1978). A combined ANOVA for the cumulative mortality over 7 days was performed. Fisher's protected least significant difference (LSD) was calculated at the 5% level to compare treatment means (Ott and Longnecker, 2001). A probability level of 5% was considered significant for all tests.

Conclusion

All five South African wheat cultivars tested were amenable to *B. bassiana* endophytic establishment, with levels ranging from 21% (cultivar Elands) to 34% (cultivar Bavians). Similar to other published reports, endophytic *B. bassiana* also promoted overall plant growth in our study. Considering five growth parameters, *B. bassiana*-treated plants outperformed control plants by 71%, on average. Pathogenicity of *B. bassiana* to RWA was retained following passaging and re-isolation from a wheat host plant, supporting the notion of incorporating *B. bassiana*-endophytism as IPM component. Further studies on the interaction of host plant resistance and *B. bassiana*-endophytism are warranted, especially in light of new RWA biotype development. Whether naturally present or artificially induced, *B. bassiana*-endophytism appears beneficial within a wheat cropping system.

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