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Laboratory investigation of cauliflower-fungus-insect interactions for cabbage maggot control

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ABSTRACT

The cabbage maggot (also known as cabbage root fly – CRF; *Delia radicum* L.) is a serious pest in brassicas. The pest's soil dwelling larvae are especially damaging to young brassica transplants. In light of toxic soil insecticide phase-out novel biocontrol management solutions are sought for. Our research is focused on the development of a biological control strategy involving cauliflower plantlet inoculation with insect pathogenic fungi. The article presents the results of a laboratory investigation of cauliflower × microbe × CRF interactions. Seven isolates of fungi (entomopathogenic and rhizosphere competent fungi and soil saprotrophs) were tested for their pathogenicity to CRF and their effects on cauliflower plantlets. The laboratory experiments were performed in sterilized substrate. Several strains significantly increased CRF mortality, some at par with a commercial bioinsecticide based on *B. bassiana*. All strains colonized the rhizoplane, however to varying extent. Some isolates were also re-isolated from within healthy plant tissues and thus identified as endophytes. The method of applying conidia had a significant effect on survival and weight of seedlings and rhizoplane and endophytic colonization rates. Two *M. brunneum* Petsch isolates exhibited plant growth promotion effects when ungerminated seeds were coated with conidia. The ecological implications of plant × microbe × pest interactions and options for improving the effectiveness of a fungal-based biological CRF management strategy are discussed.

KEYWORDS

Biological control, crop-arthropod-microbe interactions; entomopathogenic fungi, insect; plant-microbe-insect interactions; low-risk; plant protection; rhizosphere competence; soil pest.

Introduction

The cabbage maggot (*Delia radicum* L.) or the cabbage root fly (CRF) is a significant pest of cabbage crops. Problems are caused after females lay their eggs within 5 cm of the stem base of the host plants (Mukerji 1971). The newly hatched larvae crawl through the soil to find and feed on roots or bore directly into the young stems and this can cause serious economic damage. Most challenging are the early fly generations as they affect early developing, high value crops (Bligaard 1999). Depending on the measures taken and the CRF population pressure, yield losses of up to a 100% are possible (Ferry et al. 2009). Especially problematic are direct damages when parts of plants for consumption (e.g., roots of radish, swedes or turnips) are infested. Indirect damages caused by larvae feeding on non-consumed tissue can be tolerated a to a certain degree (Finch 1993, Herbst et al. 2017, Razinger et al. 2017). Often only the young transplants need chemical protection from CRF in commercial broccoli or cauliflower production (Bligaard 1999).

The assortment of organophosphate and carbamate insecticides, used to manage CRF in the past (Chandler and Davidson 2005), is declining because of environmental concerns. Accordingly, the (European Parliament 2009) promoted implementation of low-risk methods including nettings and fleeces, lime nitrogen, straw mulch, parasitoid/predator release, chemical cues, variety selection, intercropping, and altered planting time and densities (Straub 1988, Städler and Schöni 1990, Dosdall et al. 1996, 2000, Nawrocka 1996, Ferry et al. 2009, Hummel et al. 2010, Reddy 2011, Cotes et al. 2015, Joseph and Zarate 2015, Herbst et al. 2017). In addition, several articles report the possibility to manage CRF using entomopathogenic or insect-associated fungi (EPF) which can infect and kill dipterous insects (Vänninen et al. 1999, Klingen et al. 2002, Bruck et al. 2005, Thomas and Read 2007, Toledo et al. 2007, Razinger et al. 2014a, Razinger et al. 2014b, Cotes et al. 2015, Myrand et al. 2015, Rännbäck et al. 2015). Pest control through EPF could be optimized by selecting fungal

71 species that well-align to the CRF lifecycle or associate cabbage crops as root colonizers and
72 endophytes or soil saprotrophs. There is thus an increasing need to understand interactions
73 between fungi, plants, and pests to increase the efficiency of pest control strategies with EPF.

74 This article presents the results of our ongoing research on evaluating EPF and potentially
75 plant growth promoting fungi as CRF biocontrol agents. Selected fungal species were
76 previously tested already in glasshouse settings and in commercial field cauliflower
77 production settings (Razinger et al. 2014b, Razinger et al. 2017). The present study focused
78 on plant × microbe interactions under controlled laboratory conditions.

79 ## Materials and methods

80 ### Fungal pathogenicity to CRF

81 Flies used in the *in vitro* pathogenicity bioassays were reared as described elsewhere
82 (Razinger et al., 2014b). The fungal strains used were obtained and conidial suspensions
83 prepared as described elsewhere (Razinger et al., 2014a). The tested fungi were *Metarhizium*
84 *brunneum* (strains H.J.S. 1154 and 1868), *Beauveria bassiana* (Bals.-Criv.) Vuill. (H.J.S.
85 1174), *Clonostachys solani* f. *nigrovirens* (J.F.H. Beyma) Schroers (H.J.S. 1828),
86 *Trichoderma atroviride* P. Karst. (H.J.S. 1873), *T. koningiopsis* Samuels, C. Suárez & H.C.
87 Evans (H.J.S. 1874), and *T. gamsii* Samuels & Druzhin. (H.J.S. 1876). The ability of the fungi
88 to infect CRF eggs or larvae and negatively affect their survival was tested. The test vessel
89 was a 100 ml polypropylene plastic pot, into which a 9-cm sterile filter paper, moistened with
90 1.5 ml of sterile demineralized water, and a 5×20×20 mm piece of surface sterilized rutabaga
91 was placed. Five fresh CRF eggs were placed into the test vessel 35 mm from the rutabaga
92 slices. A volume of 50 µl of 1×10^8 viable conidia ml^{-1} was pipetted onto CRF eggs. The test
93 vessels were sealed with parafilm and put into an environmental chamber at 22°C, 77% RH,
94 without illumination. Insecticide Marshal 25 CS (a.i. carbosulfan 24.5%; 0.1% (v/v) dilution;

Maag Agro, Switzerland) was used as a positive control. Bioinsecticide Naturalis (a.i. *Beauveria bassiana* ATCC 74040; 0.1% (v/v) dilution; Andermatt biocontrol AG, Switzerland,) and bioinsecticide Delfin (a.i. *Bacillus thuringiensis* var. *kurstaki*; 0.5% (v/v) dilution; Andermatt biocontrol AG, Switzerland) were used as reference biocontrol agents. Tween 80 was used in the negative control treatment (0.05% (v/v) dilution). Five separate test vessels were made for each treatment and the experiment replicated three times independently (n=15).

Cauliflower-fungus interactions in multi-well plates

Experimental set-up

The multi-well plate experiment was performed under controlled conditions at 77% RH, 18: 6 h at 20: 18 °C day: night regime in sterile 6-well plates. To each chamber of the 6-well plate 2.5 g of twice autoclaved sterile commercial planting substrate and one surface sterilized seed of cauliflower cultivar 'Neckerperle' was placed. The seed surface sterilization was performed by immersing seeds for 3 min in 70% ethanol with mixing. Then, the seeds were rinsed with sterile demineralized water and allowed to dry in a laminar flow chamber. The surface sterilization procedure was tested by placing five surface sterilized seed onto PDA plates. No fungal colonies were observed after one week incubation at 25°C.

Treatments

Fungi were inoculated into the test vessels by placing sporulating agar plugs (MEA for *Trichoderma* spp. and PDA for other strains; diameter of 8 mm) below germinating seeds, or coating surface sterilized seeds with conidia. For seed coating exposure, conidial suspensions of a concentration of 5×10^7 conidia mL^{-1} were prepared in 1% carboxymethyl cellulose (CMC; Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Eighty seeds were vortexed in 50 mL centrifuge tubes containing 10 mL of CMC-conidial suspensions for 30 s, then the

119 excess conidial suspension was removed and the seeds spilled onto a sterile petri dish and
120 allowed to dry in a laminar flow chamber. The amount of conidia attached to the seeds was
121 estimated by washing the conidia off five seeds per fungal treatment in 0.05% Tween 80. The
122 number of conidia was assessed by plating serial dilutions on 1.5% malt extract agar (Sigma–
123 Aldrich Chemie GmbH, Steinheim, Germany). Washing and plating was performed in
124 triplicate. The coating procedure resulted in (means ± SE) $4.5\times10^3 \pm 2.3\times10^3$ viable *M.*
125 *brunneum* 1154 conidia per seed, $3.5\times10^3 \pm 1.0\times10^3$ (*M. brunneum* 1868), $2.7\times10^4 \pm 5.8\times10^3$
126 (*B. bassiana* 1174), $2.0\times10^4 \pm 5.4\times10^3$ (*C. solani* 1828), $1.3\times10^4 \pm 3.5\times10^3$ (*T. atroviride*
127 1873), $6.8\times10^3 \pm 6.9\times10^2$ (*T. koningiopsis* 1874), $4.0\times10^3 \pm 1.2\times10^3$ (*T. gamsii* 1876), and 0.0
128 ± 0.0 (control). The 'plugs' treatment was performed in three wells per fungal strain and the
129 experiment repeated independently twice (n=6). The 'seed coating' treatment was performed
130 in six wells per fungal strain and the experiment repeated independently three times (n=18).
131 The negative control comprised of surface sterilized seeds in the 'plugs' treatment or surface
132 sterilized seeds coated with 1% CMC in the 'seed coating' treatment.

133 **Evaluation**

134 After 28 days parameter plantlet survival was calculated as the quotient between living
135 plantlets and the number of input seeds. Additionally, plants' rhizoplane and endophytic
136 colonization was evaluated as described elsewhere (Razinger et al., 2014b). In brief, five 1-
137 cm-long root pieces were sampled per well to evaluate rhizoplane colonization. The root
138 pieces were washed twice with tap water and five times with sterile demineralized water. The
139 washed root pieces were transferred onto Strasser agar plates (Strasser et al. 1997) for the
140 detection of *M. brunneum* (1154 and 1868), *B. bassiana* 1174 and *C. solani* 1828.
141 *Trichoderma* spp. (1873, 1874 and 1876) treated plant material was transferred onto *T.*
142 *harzianum*-selective medium (THSM; Williams et al. 2003). The Strasser plates were
143 incubated for 14 days, and THSM plates for four days at 22 ± 2 °C. Another collection of five

144 washed root pieces, three stem pieces (one cm in length) or two leaves per well were surface
145 sterilized for evaluating endophytic colonization. Surface sterilization was performed in 25-
146 mL Falcon tubes in 10 mL 70% ethanol for three min. During the three minutes submersion
147 the tubes were vigorously vortexed three times for ten seconds. The pieces were then washed
148 with sterile demineralized water. No fungal colonies were encountered when 100 µl final
149 wash-water was pipetted onto Strasser or THSM plates and incubated for one week at 25°C.

150 ## Pot experiment

151 ### Experimental set-up

152 The pot experiment was performed under controlled conditions at 77% RH, 18: 6 h at 20: 18
153 °C day: night regime in 0.5 L plastic pots. To each pot 0.4 L of twice autoclaved sterile
154 commercial planting substrate was placed. Eight surface sterilized seeds of cauliflower
155 cultivar 'Neckerperle' were placed into each pot. Three pots per treatment were considered
156 replicates; the experiment was repeated twice independently (n=6). The seed surface
157 sterilization was performed as in the multi-well plate experiment described above.

158 ### Treatments

159 The fungi were applied by inoculating the growing substratum via drenching, or by adding
160 conidia-coated seeds, prepared as described in the multi-well plate experiment. The pots were
161 watered with 30 mL of autoclaved demineralized water. In the drenching exposure, 15 mL of
162 water was replaced with conidial suspensions of a concentration of 5.3×10^5 viable conidia
163 mL^{-1}. Three separate negative controls were performed. In 'Zero control' untreated seeds
164 were used. In the drenching application negative control seeds were surface sterilized but
165 drenched only with sterile demineralized water. In the seed coating application negative
166 control seeds were surface sterilized and coated with 1% CMC, without conidia.

Evaluation

After 28 days plantlet survival was evaluated. The plantlets were carefully dug out with the help of tweezers and a spatula. The roots were washed with tap water to remove any adhering test substrate, and blotted on a paper towel to remove any excess tap water. Blotted plantlets were weighed using a high precision laboratory scale (BP 301 S, Sartorius, Germany).

Data analysis

Results from fungal pathogenicity tests were corrected using Abbott's formula that eliminates errors due to deaths in the control sample (Abbott 1925), and their normality of distribution tested by D'Agostino–Pearson omnibus K2 test. Abbott's mortalities were tested by ANOVA and Student's t-tests. The proportion of surviving plantlets and the degree of rhizosphere or endophytic colonization from multi-well plate experiments was arcsine square root transformed and analyzed by 2-way ANOVA with inoculation method and fungal strain as principal factors, and Bonferroni's multiple comparison post-test. Numerical data from pot experiments was analyzed by ANOVA and Student's t-tests (Motulsky 1995). The analyses were carried out with the statistical software GraphPad Prism 5.00 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

Fungal pathogenicity to CRF

Fungal or (bio)insecticide treatments had a significant effect on the number of surviving larvae in the fungal pathogenicity tests ($F_{10,\ 153} = 8.43$; $P < 0.0001$). T-tests showed a significant increase of mortality caused by *M. brunneum* 1154 and 1868, *B. bassiana*, *T. koningiopsis*, bioinsecticide Naturalis and insecticide Marshal. The two most pathogenic agents were *M. brunneum* 1154 (39.9 ± 9.6% mortality) and *B. bassiana* (38.2 ± 6.9% mortality). The reference biocontrol formulations Naturalis and Delfin and the insecticide Marshal caused a mortality of 38.9 ± 6.6, 23.1 ± 9.7 and 100.0 ± 0.0%, respectively (Figure

192 1). Mycelial growth of *M. brunneum* (1154 and 1868) and *B. bassiana* 1174 emerged from
193 eggs and larvae and of Naturalis only from CRF eggs.

194 **Cauliflower-fungus interactions in multi-well plates**

195 The inoculation method (sporulating agar plugs or seed coating) had a significant effect on
196 plantlet survival in multi-well experiments ($F_{1, 176} = 7.35$; $p = 0.0074$). The effect of the
197 individual fungal strain ($F_{7, 176} = 1.49$; $p = 0.174$) or the interaction of inoculation method ×
198 strain ($F_{1, 176} = 0.74$; $p = 0.637$) was not significant. Greatest survival of plantlets was
199 observed in the wells with sporulating agar plugs of *B. bassiana* (100 ± 0%) and the lowest in
200 negative control of 'seed coating' inoculation (surface sterilized seeds coated with CMC; 30.6
201 ± 7.2%; Figure 2).

202 The inoculation method ($F_{1, 57} = 18.01$; $p < 0.0001$) and fungal strain ($F_{7, 57} = 4.72$; $p =$
203 0.0003) had a significant effect on rhizoplane colonization of the plantlets in multi-well
204 experiments. The interaction of inoculation method × strain ($F_{7, 57} = 1.10$; $p = 0.381$) was not
205 significant. Greater rhizoplane colonization was observed when fungi were delivered into test
206 systems as sporulating agar plugs. The roots of plantlets growing above *M. brunneum* 1154
207 sporulating agar plugs were significantly more colonized by the fungus than roots grown from
208 coated seeds (Figure 3).

209 Endophytic tissue colonization was significantly affected by factors inoculation method,
210 fungal taxon and their interaction in all plant organs investigated (Table 1). The highest and
211 most consistent endophytic colonization was observed in the three *Trichoderma* isolates,
212 which were reisolated from all plant organs investigated. Generally, higher endophytic tissue
213 colonization rate in the three *Trichoderma* spp. isolates was obtained when the plantlets were
214 inoculated via sporulating agar plugs. *Metarhizium brunneum* 1154 and 1868 and *C. solani*
215 1828 were reisolated sporadically from stems or leaves, but were not found in root tissue.

Beauveria bassiana 1174 was not reisolated as an endophyte in any plant organ regardless of the inoculation method.

Pot experiment

The factor fungal strain had a significant effect on plantlet survival in pot experiments ($F_{8, 89}$ = 2.19; p = 0.0356); the effect of inoculation method (drenching or seed coating; $F_{1, 89}$ = 0.08; p = 0.7839) or the interaction of strain × inoculation method ($F_{8, 89}$ = 0.72; p = 0.6806) was not significant. Plantlet survival and fresh biomass increased in pots where *M. brunneum* (isolates 1154 and 1868) conidia were coated onto surface sterilized seeds compared with plants that emerged from seeds coated without fungus (Figure 4).

The inoculation method ($F_{1, 87}$ = 12.5; p = 0.0006) had a significant effect on average plantlet weight in pot experiments. The effects of fungal strain ($F_{8, 87}$ = 1.02; p = 0.4298) and the interaction of inoculation method × strain ($F_{8, 87}$ = 0.50; p = 0.8508) had no significant effect on average plantlet weight. Drenching surface sterilized seeds with *T. atroviride* 1873 resulted in significantly higher average plantlet weight as compared to seed coating application. Coating surface sterilized seeds with CMC alone significantly reduced average plantlet weight (Figure 4).

The inoculation method ($F_{1, 88}$ = 4.80; p = 0.0311) had a significant effect on fresh biomass production in pot experiments; the fungal strain ($F_{8, 88}$ = 1.66; p = 0.1188) and the interaction of inoculation method × strain ($F_{8, 88}$ = 0.472; p = 0.8729) was not significant. Surface sterilization and coating seeds with CMC alone significantly reduced fresh biomass production. In contrast, when *M. brunneum* (isolates 1154 and 1868) conidia were coated onto surface sterilized seeds, fresh biomass production was significantly higher than the respective control, and reached levels statistically indistinguishable to zero control treatment (non-

239 coated, non-surface sterilized seeds). Seed surface sterilization did not have a significant
240 effect on plantlet survival, average plantlet weight or fresh biomass production (Figure 4).

241 ## Discussion

242 The entomopathogenic fungi (EPF) and other fungal isolates tested in the present study were
243 pathogenic to CRF eggs or larvae, as demonstrated previously (Razinger et al., 2014a, b).
244 Additionally, the investigated unformulated fungal taxa could form root associations under
245 controlled laboratory conditions in autoclaved substrate, i.e., they were rhizosphere competent
246 to cauliflower roots as per definitions set by Hu and Leger (2002) and Pava-Ripoll et al.
247 (2011), which may make them suitable biological control agents against soil borne pests, if
248 the virulence of the fungi were not reduced by growing on the plant substrate. Colonizing the
249 rhizosphere, they might have multiple roles in protecting plants from pests and diseases and at
250 the same time promoting plant growth (Vega et al. 2009).

251 The EPF regularly produced abundant sporulating mycelial outgrowth on CRF eggs and
252 larvae, whereas in the case of *Clonostachys solani* and *Trichoderma* spp. this was not
253 observed. The best performing EPF isolates caused CRF mortality on par with the reference
254 biocontrol agent Naturalis and in range with several other EPF tested against CRF (Vänninen
255 et al. 1999, Chandler and Davidson 2005, Myrand et al. 2015) or the onion maggot *Delia*
256 *antiqua* (Davidson and Chandler 2005). Additionally, the *M. brunneum* isolates (1154 and
257 1868) exhibited plant growth promoting effects, highlighting their potential as multi-faceted
258 biocontrol agents.

259 The EPF and *C. solani* were only sporadically reisolated from within plant tissue as
260 endophytes. *Trichoderma* spp. isolates on the other hand were continuously reisolated from
261 within healthy plant tissue and were even able to colonize aboveground plant organs after
262 application by drenching or seed coating. While the genera *Beauveria* and *Clonostachys* are

not mentioned as common brassica endophytes, *Metarhizium* and *Trichoderma* are (Card et al. 2015). This may indicate that our *Metarhizium* isolates did not possess the traits needed to form an endophytic relationship with cauliflower plantlets.

A relatively small assortment of fungal isolates exhibited a high variation in rhizosphere competence and endophytism in the laboratory experiments performed with sterilized seeds in sterile substrate. It might be possible that the different tested fungi have a different capacity of cauliflower rhizodeposits utilization and tissue penetration, or that they are differentially susceptible to or able to degrade brassica glucosinolates (Vänninen et al. 1999, Klingen et al. 2002). It would be expected that EPF and other fungi would benefit from rhizosphere exudation, provided they were adapted to soil environments and to the rhizosphere conditions of a specific crop (Pava-Ripoll et al. 2011). Vice versa, the plants would benefit from such a mutualism with rhizosphere competent insect pathogens (Hu and Leger 2002, Vega et al. 2009, Johnson and Rasmann 2015, Steinwender et al. 2015). We suggest that the first phase of a fungus-based biological control strategy against soil pests should focus on obtaining isolates well adapted to the crop's specific soil and rhizosphere conditions, potentially even showing plant growth stimulating effects. Next, the isolates would be evaluated for their virulence and pathogenicity to the target pest. This would improve the success probability of such biocontrol attempts, as well as reduce inoculum used and therefore minimize unwanted environmental and non-target effects.

The evaluation of different fungal application methods revealed that placing sporulating agar plugs below germinating seeds resulted in higher rhizoplane colonization, as well as improved plantlet survival, compared to seed coating procedure. Similarly, a significantly higher endophytic colonization was observed in plantlets growing above *Trichoderma* spp. sporulating agar plugs. Significant effects of different application methods of biologicals on the success of vine weevil biocontrol were published by Ansari and Butt (2012). Successful

seed coating application of *M. robertsii* or *M. brunneum* onto wheat seeds, whose roots were later found to be carrying enough conidia to cause significant mortality of *Tenebrio molitor* larvae were reported by Keyser et al. (2014). Likewise, the coating of *M. pingshaense* conidia onto maize kernels resulted in making maize roots pathogenic to the white grub *Anomala cincta* (Peña-Peña et al. 2015). Indeed, if the fungi were applied as a seed treatment and could infect soil pests, such a biological control strategy would be both easier and more economical than inundative delivery of inoculum (Peña-Peña et al. 2015).

Conclusions

A high variation of rhizosphere competence to cauliflower roots and significant effects of fungal delivery methods was revealed. This indicates several opportunities of future research, namely searching for CRF-pathogenic fungal isolates with a high rhizosphere competence and collaboration with formulation specialists focusing on improving fungal delivery methods which would provide constant colonization pressure and improve persistence of fungal inoculi on plant roots. In addition, fungi other than solely EPF genera might be considered when investigating such biocontrol strategies.

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Figure and table legends

Figure 1: Mortality of CRF treated with various fungi and three commercial products in *in vitro* bioassays. The experiments were evaluated 14 d after infection to determine the number of surviving larvae. Asterisks denote significant difference from the control (P < 0.05). Data presented are means ± standard error. *Naturalis* – commercial product based on *Beauveria bassiana* ATCC 74040; *Delfin* – commercial product based on *Bacillus thuringiensis* var. *kurstaki*; *Marshal* – commercial insecticide based on carbosulfan.

Figure 2: The fraction of surviving plantlets in multi-well plate experiments. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Seed coating). Data presented are means ± SE.

Figure 3: Rhizoplane colonization of the plantlets in multi-well experiments, expressed as the fraction of infected root pieces. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Seed coating). Data presented are means ± SE. Bars marked with different lower-case letters are significantly different at P <0.05.

Figure 4: Effect of fungal strain and inoculation method on plantlet survival, average plantlet weight and fresh biomass production. Inoculation was performed by drenching the growing substratum with conidial suspensions (Drenching) or by coating the conidia onto seeds (Seed coating). Data presented are means ± SE. Bars not sharing the same lower-case letters are significantly different, whereas bars without lower-case letters are statistically indistinguishable. Asterisks (*) denote significant difference from the respective negative controls (P <0.05). Cauliflower seeds used in the Zero control treatment were neither surface sterilized nor drenched, nor coated with conidia.

Table 1: Effect of fungal strain and inoculation method on endophytic tissue colonization in different plant organs. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Coating). Data presented are mean percentages of infected plant tissue pieces ± SE. Values marked with different lower-case letters are significantly different at P < 0.05.

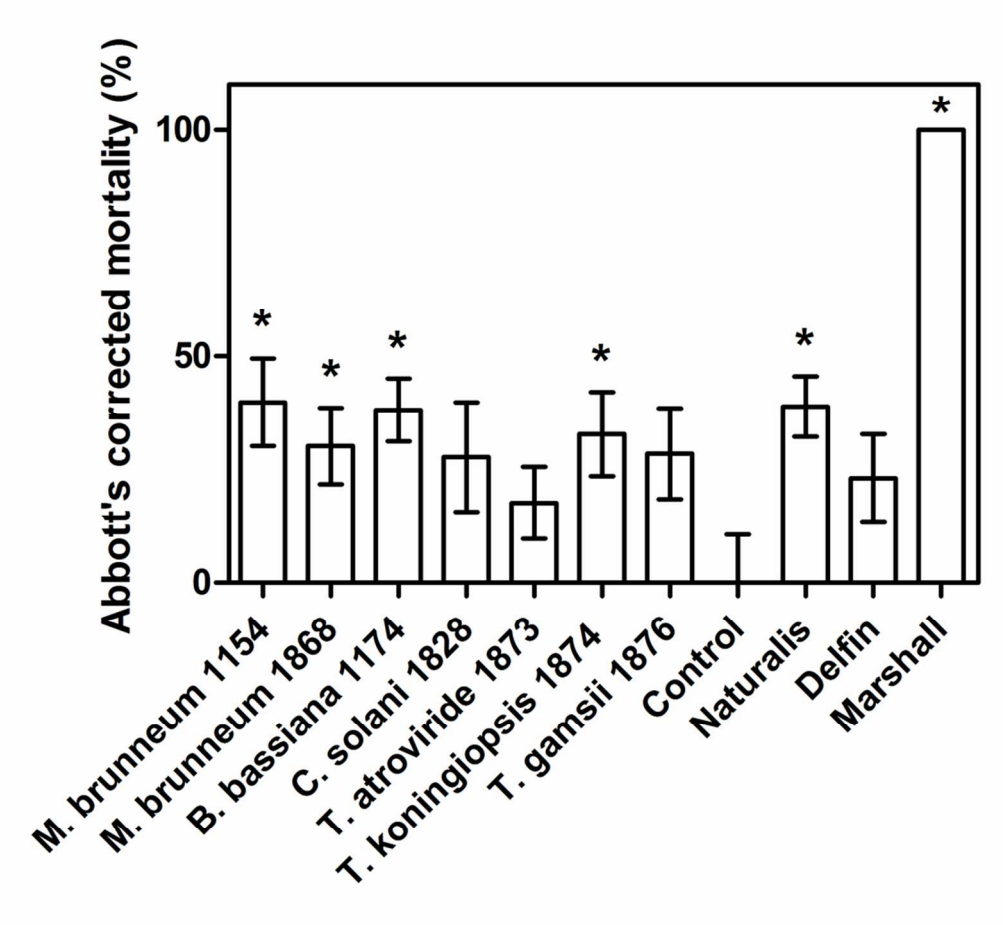

Figure 1: Mortality of CRF treated with various fungi and three commercial products in in vitro bioassays. The experiments were evaluated 14 d after infection to determine the number of surviving larvae. Asterisks denote significant difference from the control ($P < 0.05$). Data presented are means ± standard error. Naturalis – commercial product based on Beauveria bassiana ATCC 74040; Delfin – commercial product based on Bacillus thuringiensis var. kurstaki; Marshal – commercial insecticide based on carbosulfan.

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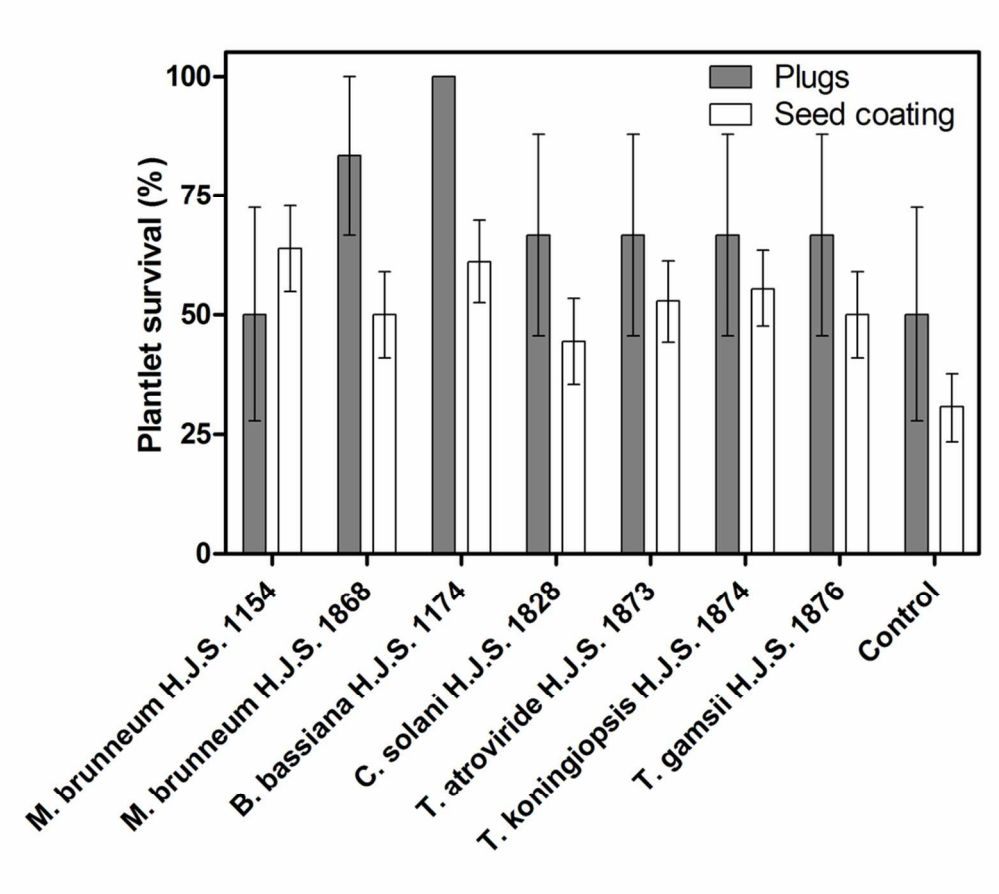


Figure 2: The fraction of surviving plantlets in multi-well plate experiments. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Seed coating). Data presented are means ± SE.

89x80mm (300 x 300 DPI)

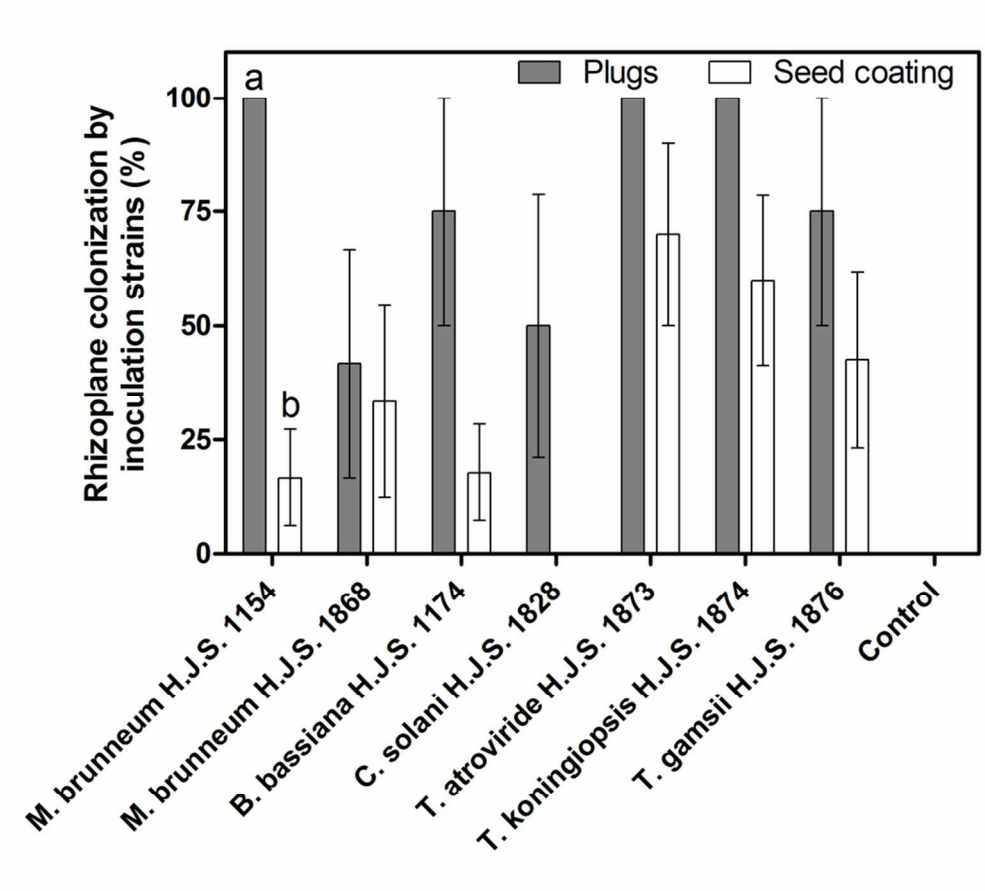
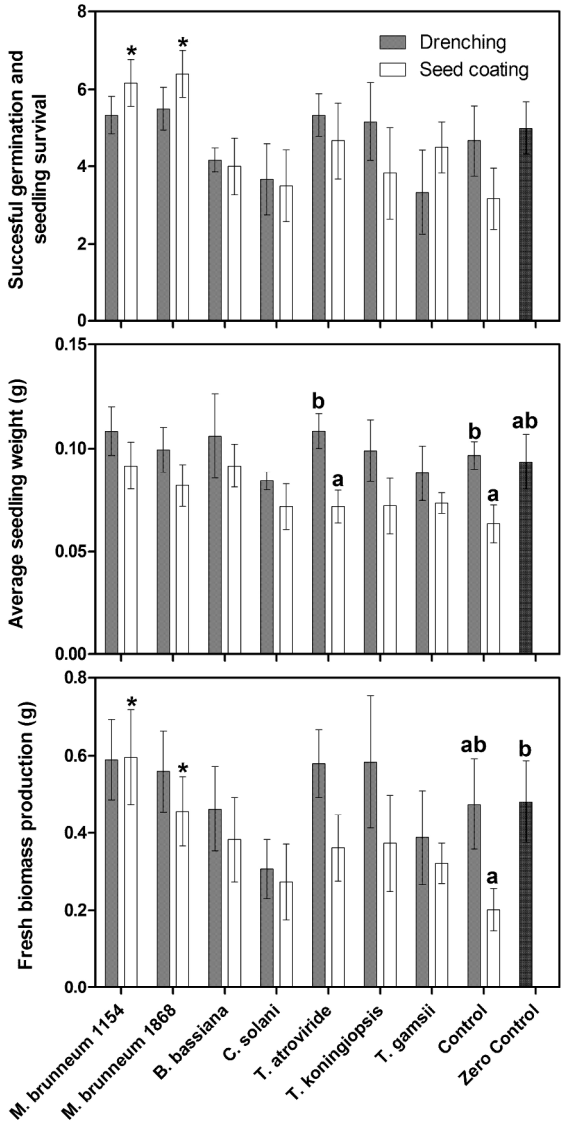


Figure 3: Rhizoplane colonization of the plantlets in multi-well experiments, expressed as the fraction of infected root pieces. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Seed coating). Data presented are means ± SE. Bars marked with different lower-case letters are significantly different at P <0.05.

89x80mm (300 x 300 DPI)

Table 1: Effect of fungal strain and inoculation method on endophytic tissue colonization in different plant organs. Inoculation was performed by placing sporulating agar plugs beneath germinating seeds (Plugs), or by coating the conidia onto surface sterilized seeds (Coating). Data presented are mean percentages of infected plant tissue pieces ± SE. Values marked with different lower-case letters are significantly different at P < 0.05.

Plant organ	**Root**		**Stem**		**Leaf**	
Inoculation method	Plugs	Coating	Plug	Coating	Plug	Coating
***M. brunneum* 1154**	0.0±0.0	0.0±0.0	0.0±0.0	11.1±7.0	0.0±0.0	0.0±0.0
***M. brunneum* 1868**	0.0±0.0	0.0±0.0	12.5±12.5	0.0±0.0	12.5±12.5	5.6±5.6
***B. bassiana* 1174**	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
***C. solani* 1828**	0.0±0.0	0.0±0.0	0.0±0.0	26.7±12.5	0.0±0.0	0.0±0.0
***T. atroviride* 1873**	0.0±0.0	25.0±17.1	100.0±0.0 a	23.6±15.0 b	87.5±12.5 a	27.8±18.1 b
***T. koningiopsis* 1874**	50.0±50.0 a	0.0±0.0 b	50.0±20.4	43.3±19.4	25.0±14.4	30.0±12.2
***T. gamsii* 1876**	83.3±16.7 a	0.0±0.0 b	100.0±0.0 a	25.0±17.1 b	87.5±12.5 a	5.6±5.6 b
Control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Statistical parameters						
Inoculation method	$F_{1,52}$=8.08; p=0.0064		$F_{1,60}$=8.96; p=0.0040		$F_{1,60}$=14.83; p=0.0003	
Fungal strain	$F_{7,52}$=6.43; p<0.0001		$F_{7,60}$=12.31; p<0.0001		$F_{7,60}$=13.17; p<0.0001	
Interaction	$F_{7,52}$=7.11; p<0.0001		$F_{7,60}$=6.43; p<0.0001		$F_{7,60}$=6.76; p<0.0001	



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191x367mm (300 x 300 DPI)