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Protection of entomopathogenic conidia against chemical fungicides afforded by an oil-based formulation

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We evaluated the protection afforded by an oil formulation against non-compatible fungicides in mixtures with conidia of the entomopathogenic fungi Metarhizium anisopliae (Ma) and Beauveria bassiana (Bb). Under laboratory conditions, viability of unformulated (aqueous suspensions) Ma conidia was harmed by recommended label doses of carbendazim (not tested for Bb), and both Ma and Bb conidia were affected by triadimefon. On the other hand, effect of fungicides was usually nil or minimal on conidia formulated as oil-containing suspensions (emulsifiable oil + water). Germination rates for unformulated and oil-formulated Ma conidia subjected to carbendazim were reduced by 77.3 and 12.1%, respectively, compared to their fungicide-free counterparts. Germination rates at 16 h post-inoculation for unformulated and oil-formulated Bb conidia subjected to triadimefon were reduced by 20.5 and 5.5%, respectively, compared to their fungicide-free counterparts. No differences were observed at 20 h post inoculation, indicating a fungistatic action of this compound on Bb conidia. Virulence of unformulated conidia amended with fungicides against third instar Diatraea saccharalis larvae was negatively affected compared to their formulated counterparts. These results suggest that oil-formulated conidia can be effectively protected from damage caused by chemicals, which could have applications in tank mixing or alternate applications with shared spraying equipment, being especially relevant for IPM programs in which mycopesticides and chemicals are simultaneously sprayed.

Keywords: biological control; microbial control; mycoinsecticide; Metarhizium anisopliae; Beauveria bassiana; emulsifiable oil

1. Introduction

Several species of entomopathogenic fungi (EF) have been studied as microbial control agents of insects and mites (Faria and Wraight 2007). There is considerable interest in the development of EF as bioinsecticides for use in integrated pest management programs, both in conventional and organic farming. A few EF species have been developed as mycopesticides and are already on the market worldwide (Faria and Wraight 2007; Li et al. 2010). However, factors such as inconsistent results under field conditions have hampered broader commercial uptake of fungus-based products.

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Field efficacy of mycopesticides can be considerably improved with appropriate formulations, leading to enhanced field persistence and insecticidal activity (Burges 1998; Lacey, Frutos, Kaya, and Vail 2001; Jackson, Dunlap, and Jaronski 2010). Oil-based formulations are among the simplest formulation types, in which conidia are mixed with pure (ultra low volume suspensions, ULV, and oil miscible flowable concentrates, OF) or emulsifiable oils (oil dispersions, OD). Fungal conidia in oil-containing formulations have been reported to optimize infection when compared to conventional water-based suspensions (Prior, Jollands, and le Patourel 1988; Bateman, Carey, Moore, and Prior 1993; Inyang et al. 2000; Batta 2003; Polar, Kairo, Moore, Pegram, and John 2005). A wide range of factors may explain the advantage of oil-based suspensions over oil-free preparations, including better adhesion to hydrophobic surfaces and faster insecticidal action (Malsam, Kilian, Oerke, and Dehne 2002), greater rainfastness (Inglis, Ivie, Duke, and Goettel 2000; Wraight and Ramos 2002), protection against imbibitional damage (Faria, Hajek, and Wraight 2009) and improved UV tolerance (Moore, Bridge, Higgins, Bateman, and Prior 1993; Alves, Bateman, Prior, and Leather 1998; Hedimbi et al. 2008). In studies with mycoherbicides, oil-based formulations allowed pathogens to infect and kill weeds at more advanced growth stages, reducing the dew period requirements for maximum weed infection (Greaves, Pring, and Lawrie 2001; Boyette 2006).

The detrimental effect of chemical pesticides on conidial viability or mycelial growth is well known. In vitro studies have shown that several mitosporic fungi (Ascomycota: Hypocreales) can be killed by agrochemicals (Yandoc, Rosskopf, Pitelli, and Charudattan 2006; Luz, Bastos Netto, and Rocha 2007; Bruck 2009). Many pesticides are reported as fungistatic to EF species (Majchrowicz and Poprawski 1993; Venedikian et al. 1999; Kouassi, Coderre, and Todorova 2003), resulting in delayed germination or mycelium growth. For instance, fungicides may influence the persistence of EF in soils and their efficacy for controlling target pests (Mietkiewski, Pell, and Clark 1997; Mochi, Monteiro, Bortoli, Doria, and Barbosa 2006).

The simultaneous application of EF and incompatible pesticides, in tank mixture or as a result of alternate applications with shared equipment, is likely to impair performance of mycopesticides (Kouassi et al. 2003; Gatarayiha, Laing, and Miller 2010a). The present study was undertaken primarily to evaluate the protection afforded by an emulsifiable oil (EO) against the fungicides triadimefon and carbendazim in mixtures with Metarhizium anisopliae (Metsch.) Sorok. (Ascomycota: Clavicipitaceae) and Beauveria bassiana (Bals.) Vuill. (Ascomycota: Cordycipitaceae) conidia. Diatraea saccharalis (Lepidoptera: Crambidae), an important pest in Brazilian sugarcane fields, was used as a model insect in bioassays.

2. Materials and Methods
2.1 Production and formulation of conidia for laboratory studies
Strains CG1027 (= ESALQPL63) of Beauveria bassiana (Bb) and ESALQ1037 of the Metarhizium anisopliae (Ma) complex, preserved at the Invertebrate Fungal Collection at EMBRAPA Genetic Resources and Biotechnology (Brasilia, Brazil) and at the Entomopathogenic Fungal Collection at ESALQ – University of Sao Paulo (Piracicaba, Brazil), respectively, were inoculated on potato dextrose agar
medium (PDA – Difco Laboratories, Detroit, MI, USA) and maintained at 25°C and 12 h photophase. Moisten conidia were scraped from 10- to 12-day-old cultures with a spatula and immediately stored at −12°C until use in germination tests and bioassays. Conidia viability was above 93% for both fungal species by the time they were used. EO-based formulations (OD) were prepared for both strains, adding conidia to a mixture of Soya soybean oil (Bunge Brasil SA, Santa Catarina, Brazil) and a previously selected compatible ethoxylated vegetable oil (5%, v/v) as an emulsifier (proprietary formula; Itaforte BioProdutos Ltda., Sao Paulo, Brazil).

### 2.2 Assessment of protection afforded by oil-formulated Metarhizium anisopliae and Beauveria bassiana conidia against chemical fungicides

Ma and Bb conidia were suspended in distilled water with Tween 80® (0.01%, v/v) and diluted to a final concentration of 6.7 × 10^7 conidia mL⁻¹. EO-containing suspensions with the same conidial concentration were prepared by addition of 2 mL of the oil dispersion (1 × 10⁹ conidia mL⁻¹) to 28 mL of distilled water with Tween 80® at 0.01% (v/v). The systemic fungicide triadimefon (Bayleton BR®; Bayer CropScience Ltda., Sao Paulo, SP, Brazil) was added to the fungal suspensions at a concentration of 100 g commercial product/100 L (equivalent to 25 g active ingredient/100 L). The systemic fungicide carbofuran (Bendazole®; Milênia Agro Science S.A., Londrina, PR, Brazil) was tested only with Ma suspensions at a concentration of 70 mL commercial product/100 L (35 g active ingredient/100 L). Fungicides were selected because of their use for disease control in sugarcane and wheat crops, both susceptible to attacks by Diatraea saccharalis. Concentrations are those recommended by manufacturers (label rates). After vigorous shaking, mixtures containing chemicals and conidia were kept at 25°C for 40 min before viability assessment, since this is a realistic time required for preparation of pesticide mixes and their application on sugarcane fields using tractor-mounted sprayers. Viability was checked through methodology adapted from Oliveira (2009). In short, 1 mL aliquots (top portion of liquid columns for oil-containing suspensions) were transferred to 9 mL plastic tubes with distilled water plus a compatible surfactant (Solub’oil® at 0.01%, v/v), and then vortexed for 30 sec. Resulting suspensions were centrifuged at 6,000 rpm for 5 min, and supernatants were discarded. The washing procedure (WP) with Solub’oil® was performed twice and conidia deposited on the bottom of tubes were re-suspended in Tween 80® at 0.01% (v/v) for germination analyses. For the sake of standardization, the same procedure was also adopted for oil-free suspensions, with or without fungicides. Viability of conidia in oil-free suspensions was also assessed through a conventional protocol (DI = direct inoculation), without the previous washing procedure. Three independent suspensions were used, and for each of them, three 25 μL droplets (replicates) were inoculated onto PDA medium in Petri dishes (9 cm × 1.5 cm) and maintained at 25°C for 20 h for Ma and 16 h for the faster-germinating Bb, according to Faria, Hotchkiss, Hajek, and Wraight (2010). In an attempt to detect the occurrence of conidial debilitation (delayed germination), viabilities for Ma and Bb were also recorded at 25 and 20 h post-inoculation (p.i.), respectively. After the incubation period, germination was assessed microscopically at 400x magnification by examining 300 conidia per replicate. Conidia were considered germinated when germ tubes were longer than conidial length.
2.3 Preliminary bioassays: efficacy of oil-formulated *Metarhizium anisopliae* against *Diatraea saccharalis* larvae under laboratory conditions

An oil-free Ma suspension was prepared by suspending ca. 0.01 g of stored conidia in 10 mL Tween 80® at 0.01% (v/v). After vigorous agitation, the suspension was adjusted to either 1 × 10⁷ or 1 × 10⁸ conidia mL⁻¹. Oil-containing Ma suspensions with the same final concentrations were prepared by adding 1 or 10% (v/v) of EO preparations (OD formulations) containing 1 × 10⁹ conidia mL⁻¹ to Tween 80® at 0.01% (v/v). Third instar *D. saccharalis* larvae reared on artificial diet (Hensley and Hammond 1968) were previously washed in distilled water to remove diet residues. For each treatment, four groups of 12 insects each were directly sprayed with 2 mL of the 1 × 10⁷ conidia mL⁻¹ oil-free or oil-containing suspensions using a Potter tower (Burkard Manufacturing Co Ltd., Rickmansworth, UK) at a pressure of 15 PSI (103 kPa), delivering ca. 5.1 × 10³ conidia/cm². Larvae remained on the treated surface for 2 min following spraying. EO-containing solution without conidia (1%, v/v, OD in Tween 80® at 0.01%) was used as control. After spraying, each group was placed in 115 cm³ rounded plastic receptacles (60 mm in diameter and 40 mm in height) with a filter paper at the bottom and a 1.5 cm³ piece of artificial diet without preservatives (methyl parahydroxybenzoate and formaldehyde), which were used previously during insect rearing. The artificial diet was changed daily until the experiment ended. All receptacles with insects were maintained at 25°C and 12h photophase. Under these conditions, RH inside receptacles was shown in preliminary tests to be > 90%. Mortality was recorded between 4 and 7 days post-application (d.p.a.), and dead insects were transferred to moistened chambers to confirm infection by Ma. The experiment was repeated using the higher concentration (1 × 10⁸ conidia mL⁻¹; 5.1 × 10⁴ conidia/cm²) and control treatment without conidia consisted of 10% (v/v) EO in Tween 80® at 0.01% (v/v).

2.4 Assessment of virulence of oil-formulated *Metarhizium anisopliae* and *Beauveria bassiana* conidia towards *Diatraea saccharalis* larvae following mixture with chemical fungicides

Unformulated and oil-containing suspensions of Ma, adjusted to 6.7 × 10⁷ conidia mL⁻¹, with or without the fungicides triadimefon and carbendazim, were prepared as previously mentioned. For Bb, oil-free and EO-containing suspensions were not tested with carbendazim. Conidial suspensions remained in mixture for 40 min before spraying with a Potter tower. Third instar larvae of the sugarcane borer were rinsed in distilled water, and for each treatment, six groups of 10 insects each were sprayed with 2 mL of suspensions, delivering 3.4 × 10⁴ conidia/cm². Water (Tween 80® at 0.01%, v/v) and oil-containing suspension (6.7% EO in water), without conidia and amended or not with fungicides, were also tested. Plastic receptacles, artificial diet and incubation conditions were the same as previously described. Virulence of oil-formulated Ma and Bb conidia towards *D. saccharalis* larvae following mixture with chemical fungicides was assessed based on insect mortality from 4 to 7 d.p.a. Cadavers were incubated in moistened chambers for confirmation of infection.
2.5 Statistical analyses

Survival analysis was applied to our data in order to estimate the mean and median survival times (ST50) and their 95% fiducial limits (FL95%). The SPSS version 17.0 survival analysis procedure (SPSS 2008) was used to compute nonparametric estimates of the survivor function by the Kaplan–Meier method, and the Log–Rank (Montel–Cox) test with α = 5% probability for pairwise comparisons among treatments. All data sets were tested for normality assumption (normal ‘Gaussian’ distribution) and variance homogeneity by Shapiro–Wilk and Bartlett tests (α = 5%), respectively. Percentages of germinated conidia were submitted to two-way ANOVA using GLM procedure in SAS (SAS Institute 2008), regarding ‘formulation type’ and ‘fungicide presence’ as factors. Means for treatments were organized in descending order and compared according to Tukey Honestly Significant Difference test (referred to here as Tukey’s HSD test) at 5% probability level.

3. Results

3.1 Protection of oil-formulated Metarhizium anisopliae and Beauveria bassiana conidia against chemical fungicides

A significant interaction between formulation type (water or EO-containing formulation) and presence of fungicide in suspension was seen (F = 1275.02; df = 2, 18; P ≤ 0.001). Recommended field doses of triadimefon and carbendazim significantly reduced germination of unformulated Ma conidia after a 20-h incubation period in PDA (F = 2328.08; df = 2, 18; P < 0.001). The effect was more severe for carbendazim, with only 20.9% viability for unformulated Ma conidia (Table 1).

Table 1. Germination (% ± SEM) of unformulated and formulated Metarhizium anisopliae (strain ESALQ1037) and Beauveria bassiana (strain CG1027) following addition of chemical fungicides to conidial suspensions.

<table>
<thead>
<tr>
<th>Fungicides added to conidial suspensions</th>
<th>Triadimefon</th>
<th>Carbendazim</th>
<th>Control (no fungicide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. anisopliae (20 h post-inoculation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unformulated1 (water)</td>
<td>92.4 ± 0.11 bA2</td>
<td>20.9 ± 1.11 cB</td>
<td>98.2 ± 0.24 aA</td>
</tr>
<tr>
<td>Formulated</td>
<td>94.9 ± 0.59 aA</td>
<td>85.4 ± 1.44 bA</td>
<td>97.5 ± 0.55 aA</td>
</tr>
<tr>
<td>B. bassiana (16 h post-inoculation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unformulated (water)</td>
<td>72.9 ± 0.97 bB</td>
<td>–3</td>
<td>93.4 ± 0.59 aA</td>
</tr>
<tr>
<td>Formulated</td>
<td>88.7 ± 0.51 bA</td>
<td>–</td>
<td>93.8 ± 0.11 aA</td>
</tr>
<tr>
<td>B. bassiana (20 h post-inoculation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unformulated (water)</td>
<td>98.6 ± 0.29 aA</td>
<td>–</td>
<td>99.3 ± 0.98 aA</td>
</tr>
<tr>
<td>Formulated</td>
<td>98.8 ± 0.22 aA</td>
<td>–</td>
<td>99.2 ± 0.22 aA</td>
</tr>
</tbody>
</table>

1Unformulated refer to conidia mixed with water plus Tween 80 at 0.01% (v/v), whereas formulated refer to conidia mixed with an Emulsifiable Oil (EO) prior to addition of water (6.7% EO in water).
2Means (±SEM) followed by the same letters in rows or upper case letters in columns (within the same experiment) do not differ significantly (Tukey’s HSD test, P ≤ 0.05).
3Not tested.
Although carbendazim showed some effect on conidia in oil-containing suspensions, viability was still greater than 85%. Germination in oil-containing suspensions amended with triadimefon did not differ from control. Profuse mycelial growth was observed for Ma treatments without fungicides and in EO-containing suspensions with triadimefon at 25h p.i. onto PDA medium. On the other hand, conidial viability in unformulated suspensions with carbendazim was in the 25–36% range at the same time point (data not shown), although the majority of non-germinated conidia treated with carbendazim were swollen or showed germ tubes smaller than their conidial length.

As observed for the Ma strain, the oil-containing suspension also provided significant protection of Bb conidia against triadimefon, and interaction was observed between the two tested factors \((F=153.4; \text{ df } = 1, 8; P < 0.001)\). Bb was affected by triadimefon in oil-free mixtures, since a 21% loss of germination was observed at 16 h p.i. \((F=422.7; \text{ df } = 1, 8; P < 0.001)\) when compared to a similar suspension without this fungicide (Table 1). Although the oil-formulated Bb suspension amended with fungicide was significantly different from the formulated fungicide-free suspension, the difference was less pronounced than in the previous case (21 vs. 5%). For Bb viability determined at 20 h p.i., no interaction \((F=0.31; \text{ df } = 1, 8; P = 0.59)\) and no differences were seen between formulation types, with or without triadimefon \((F=4.5; \text{ df } = 1, 8; P = 0.07)\).

### 3.2 Preliminary bioassays: efficacy of oil-formulated Metarhizium anisopliae against Diatraea saccharalis larvae under laboratory conditions

Third instar sugarcane borer larvae were more susceptible to Ma oil-based suspensions compared to unformulated suspensions with equivalent conidial concentrations \(\chi^2 = 10.62, P = 0.001; \chi^2 = 32.14 P < 0.001 \text{ for } 1 \times 10^7 \text{ and } 1 \times 10^8 \text{ conidia mL}^{-1}, \text{ respectively} \) (Table 2). Insect mortality increased over time, by the end of the experiment reaching 88 and 93.8% for the oil-containing suspensions with \(1 \times 10^7 \text{ and } 1 \times 10^8 \text{ conidia mL}^{-1}, \text{ respectively} \) (data not shown). Maximum mortalities for unformulated suspensions with \(1 \times 10^7 \text{ and } 1 \times 10^8 \text{ conidia mL}^{-1} \) were 38 and 45.8%, respectively, (data not shown). Mean survival times \(\text{ST}_{50}\) varied from 6 (5.6–6.4 for 95% fiducial limits) to 6.3 (6.0–6.7 95% FL) days for unformulated Ma suspensions with \(1 \times 10^7 \text{ and } 1 \times 10^8 \text{ conidia mL}^{-1}, \text{ respectively} \) (data not shown). On the other hand, mean \(\text{ST}_{50}\) ranged from 5.0 (4.8–5.6 95% FL) to 5.2 (4.8–5.6 95% FL) days for oil-containing Ma suspensions with \(1 \times 10^8 \text{ and } 1 \times 10^7 \text{ conidia mL}^{-1}, \text{ respectively} \) (Table 2).

### 3.3 Virulence of oil-formulated Metarhizium anisopliae and Beauveria bassiana conidia towards Diatraea saccharalis larvae following mixture with chemical fungicides

Oil-containing Ma suspensions amended with either carbendazim or triadimefon caused higher larval mortality rates when compared to unformulated suspensions \(\chi^2 = 25.39, P < 0.001 \text{ and } \chi^2 = 21.51, P < 0.001, \text{ respectively} \). Difference in larval mortality was also observed for Bb in oil-containing and unformulated suspensions with triadimefon \(\chi^2 = 94.71; P < 0.001\). Mean survival time \(\text{ST}_{50}\) for third instar sugarcane borer larvae treated with Ma in oil-containing suspensions was 4.4 (4.2–4.5 95% FL) days (Table 3), reaching 98.3% mortality at 7 d.p.a. (data not shown).
On the other hand, when conidia were in oil-free suspension, the ST\textsubscript{50} was 5.2 (4.8–5.5 95% FL) days. In the experiment with carbendazim, which showed a stronger effect on Ma than triadimefon, ST\textsubscript{50}s between 3.8 (3.7–4.0 95% FL) and 4.3 (4.0–4.5 95% FL) days. In the experiment with carbendazim, which showed a stronger effect on Ma than triadimefon, ST\textsubscript{50}s between 3.8 (3.7–4.0 95% FL) and 4.3 (4.0–4.5 95% FL) days.

### Table 2. Mean and median survival times (±SEM) of *Diatraea saccharalis* larvae treated with formulated and unformulated *Metarhizium anisopliae* (strain ESALQ1037) conidia under laboratory conditions. Emulsifiable Oil (EO) tested at either 1 or 10% (v/v).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean (±SEM)</th>
<th>FL (95%)(^3)</th>
<th>Median (±SEM)</th>
<th>FL (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water + EO at 1%, v/v)</td>
<td>&gt; 7.0</td>
<td>ND(^4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unformulated (conidia in water)(^1) ((1 \times 10^7) con mL(^{-1}))</td>
<td>6.0 ± 0.22</td>
<td>5.6–6.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formulated (conidia in EO + water) ((1 \times 10^7) con mL(^{-1}))</td>
<td>5.2 ± 0.20</td>
<td>4.8–5.6</td>
<td>5.0 ± 0.23</td>
<td>4.6–5.4</td>
</tr>
<tr>
<td>Control (water + EO at 10%, v/v)</td>
<td>&gt; 7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unformulated (conidia in water) ((1 \times 10^8) con mL(^{-1}))</td>
<td>6.3 ± 0.17</td>
<td>6.0–6.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formulated (conidia in EO + water) ((1 \times 10^8) con mL(^{-1}))</td>
<td>5.0 ± 0.19</td>
<td>4.6–5.3</td>
<td>5.0 ± 0.22</td>
<td>4.6–5.4</td>
</tr>
</tbody>
</table>

\(^1\)Unformulated refer to conidia mixed with water plus Tween 80 at 0.01% (v/v), whereas formulated refer to conidia mixed with an emulsifiable oil (EO) prior to addition of water (either 1 or 10% EO in water).

\(^2\)Mean and median survival times were determined by the Kaplan–Meier method.

\(^3\)Fiducial limits.

\(^4\)ND, ‘not determined’ (calculation not possible since the horizontal line at 0.5 did not intersect a confidence interval).

On the other hand, when conidia were in oil-free suspension, the ST\textsubscript{50} was 5.2 (4.8–5.5 95% FL) days. In the experiment with carbendazim, which showed a stronger effect on Ma than triadimefon, ST\textsubscript{50}s between 3.8 (3.7–4.0 95% FL) and 4.3 (4.0–4.5 95% FL) days.

### Table 3. Mean and median survival times (±SEM) of *Diatraea saccharalis* larvae treated under laboratory conditions with formulated and unformulated *Metarhizium anisopliae* (strain ESALQ1037; \(6.7 \times 10^7\) conidia mL\(^{-1}\)) mixed with the fungicide triadimefon prior to spraying.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean (±SEM)</th>
<th>FL (95%)(^3)</th>
<th>Median (±SEM)</th>
<th>FL (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>&gt; 7.0</td>
<td>ND(^4)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control (EO + water)</td>
<td>&gt; 7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Water + triadimefon</td>
<td>6.9 ± 0.10</td>
<td>6.7–7.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EO + water + triadimefon</td>
<td>&gt; 7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unformulated (conidia in water)</td>
<td>5.2 ± 0.16</td>
<td>4.8–5.5</td>
<td>5.0 ± 0.17</td>
<td>4.7–5.3</td>
</tr>
<tr>
<td>Unformulated + triadimefon</td>
<td>5.3 ± 0.14</td>
<td>5.0–5.6</td>
<td>5.0 ± 0.15</td>
<td>4.7–5.3</td>
</tr>
<tr>
<td>Formulated (conidia in EO + water)</td>
<td>4.4 ± 0.09</td>
<td>4.2–4.5</td>
<td>4.0 ± 0.10</td>
<td>3.8–4.2</td>
</tr>
<tr>
<td>Formulated + triadimefon</td>
<td>4.5 ± 0.09</td>
<td>4.3–4.6</td>
<td>4.0 ± 0.12</td>
<td>3.8–4.2</td>
</tr>
</tbody>
</table>

\(^1\)Mean and median survival times were determined by the Kaplan–Meier method.

\(^2\)Controls were composed of water plus Tween 80 at 0.01% (v/v) (with or without 6.7% EO), whereas unformulated refer to conidia mixed with water plus Tween 80 at 0.01% (v/v), and formulated refer to conidia mixed with an Emulsifiable Oil (EO) prior to addition of water (6.7% EO in water).

\(^3\)Fiducial limits.

\(^4\)ND, ‘not determined’ (calculation not possible since the horizontal line at 0.5 did not intersect a confidence interval).
95% FL) days were recorded for insects treated with oil-containing suspensions, with or without this fungicide, respectively (Table 4). ST\textsubscript{50} for insects treated with an oil-free conidial suspension was 4.6 days (4.2–4.9 95% FL), with 90% of larval mortality by the end of the experiment (data not shown). However, when carbendazim was added to unformulated suspension before spraying, ST\textsubscript{50} increased to 5.6 (5.3–6.0 95% FL) days. When the experiment was performed with Bb suspensions, with or without triadimefon, mortality rates over 98% for insects treated with oil-containing suspensions were recorded (data not shown). ST\textsubscript{50} for insects treated with an oil-free conidial suspension was 5.2 (4.9–5.5 95% FL) days (Table 5). However, addition of triadimefon to a similar suspension prior to spraying resulted in ST\textsubscript{50} of 6.9 (6.8–7.0 95% FL) days, not differing statistically from water + triadimefon. As in previous experiments, mortality rates for insects treated with solutions without conidia (water or water + emulsifiable oil, with or without fungicides) were always below 2% (data not shown).

4. Discussion

Significant protection afforded by an emulsifiable oil to Ma and Bb conidia against harmful fungicides in mixtures was shown in the present study. Unformulated conidia of Bb, exposed for 40 min to an incompatible fungicide, experienced a pronounced fungistatic effect. Conversely, changes in germination rates for either Ma or Bb conidia encapsulated by oil droplets prior to exposure to fungicides were nil or minimal. In EO-containing suspensions with fungicides, encapsulation of conidia into oil droplets was clearly visible through microscopic observations (400 ×). Bioassays with \textit{D. saccharalis} larvae corroborated these findings. Contrary

<table>
<thead>
<tr>
<th>Treatments\textsuperscript{2}</th>
<th>Mean (±SEM)</th>
<th>FL (95%)\textsuperscript{3}</th>
<th>Median (±SEM)</th>
<th>FL (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>&gt;7.0</td>
<td>ND\textsuperscript{4}</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control (EO + water)</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Water + carbendazim</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EO + water + carbendazim</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unformulated (conidia in water)</td>
<td>4.6±0.17</td>
<td>4.2–4.9</td>
<td>4.0±0.20</td>
<td>3.6–4.4</td>
</tr>
<tr>
<td>Unformulated + carbendazim</td>
<td>5.6±0.18</td>
<td>5.3–6.0</td>
<td>6.0±0.55</td>
<td>4.9–7.1</td>
</tr>
<tr>
<td>Formulated (conidia in EO + water)</td>
<td>3.8±0.07</td>
<td>3.7–4.0</td>
<td>4.0±0.05</td>
<td>3.9–4.1</td>
</tr>
<tr>
<td>Formulated + carbendazim</td>
<td>4.3±0.14</td>
<td>4.0–4.5</td>
<td>4.0±0.07</td>
<td>3.9–4.2</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Mean and median survival times were determined by the Kaplan–Meier method.

\textsuperscript{2}Controls were composed of water plus Tween 80 at 0.01% (v/v) (with or without 6.7% EO), whereas unformulated refer to conidia mixed with water plus Tween 80 at 0.01% (v/v), and formulated refer to conidia mixed with an Emulsifiable Oil (EO) prior to addition of water (6.7% EO in water).

\textsuperscript{3}Fiducial limits.

\textsuperscript{4}ND, ‘not determined’ (calculation not possible since the horizontal line at 0.5 did not intersect a confidence interval).
to most unformulated preparations, formulated conidia mixed with fungicides were usually as effective in controlling this insect as fungicide-free conidial suspensions. These results suggest that tank mix involving EF and incompatible pesticides may be possible for oil-formulated conidia if similar lengths of exposure and fungicide concentrations are used.

The germination protocol based on the surfactant Solub'oil® (WP protocol) was an accurate technique for viability assessment of oil-formulated conidia, as previously shown by Oliveira (2009). Readings using the WP and conventional protocol (DI) were usually in close agreement. A small deviation was observed for unformulated Ma conidia mixed with carbendazim (data not shown). It is very likely that fungicide residues not removed from suspension by the DI protocol remained on the medium surface after inoculation, prolonging exposure of conidia to this pesticide and, therefore, resulting in lower viability. This effect was not observed with triadimefon since this chemical had a considerably milder effect on Ma germination. So, fungal species responded differently to chemical fungicides, even when the same germination protocol was used.

Prolonged exposure of conidia in tank mixtures to incompatible pesticides is likely to compromise the EF potential in controlling insect pests. It is reasonable to assume that intentional (but not usually recommended) mixtures of chemical pesticides and mycopesticides for simultaneous applications, or even unintentional exposure of EF to these substances by shared use of spraying equipment, may kill or debilitate fungal structures, with loss of field efficacy. This may also take place in organic farming, where chemical pesticides are prohibited but several natural products such as plant extracts or inorganic/mineral compounds could be hazardous to EF conidia. For instance, Ma conidia exposed to an incompatible vegetable emulsifiable oil had their germination delayed (Alves et al. 1998). In the present

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean (± SEM)</th>
<th>FL (95%)</th>
<th>Median (± SEM)</th>
<th>FL (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control (EO + water)</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Water + triadimefon</td>
<td>6.9 ± 0.10</td>
<td>6.7–7.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>EO + water + triadimefon</td>
<td>&gt;7.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Unformulated (conidia in water)</td>
<td>5.2 ± 0.15</td>
<td>4.9–5.5</td>
<td>5.0 ± 0.16</td>
<td>4.7–5.3</td>
</tr>
<tr>
<td>Unformulated + triadimefon</td>
<td>6.9 ± 0.05</td>
<td>6.8–7.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Formulated (conidia in EO + water)</td>
<td>4.2 ± 0.07</td>
<td>4.1–4.3</td>
<td>4.0 ± 0.00</td>
<td>ND</td>
</tr>
<tr>
<td>Formulated + triadimefon</td>
<td>4.9 ± 0.12</td>
<td>4.7–5.2</td>
<td>5.0 ± 0.15</td>
<td>4.7–5.3</td>
</tr>
</tbody>
</table>

1Mean and median survival times were determined by the Kaplan–Meier method.
2Controls were composed of water plus Tween 80 at 0.01% (v/v) (with or without 6.7% EO), whereas unformulated refer to conidia mixed with water plus Tween 80 at 0.01% (v/v), and formulated refer to conidia mixed with an Emulsifiable Oil (EO) prior to addition of water (6.7% EO in water).
3Fiducial limits.
4ND, ‘not determined’ (calculation not possible since the horizontal line at 0.5 did not intersect a confidence interval).
study, triadimefon in conidial suspensions also had a marked fungistatic effect on Bb. Viability of unformulated Bb conidia assessed 16 h p.i. was reduced by 20.5% compared to fungicide-free conidial suspensions, whereas at 20 h p.i. germination rates did not differ. The fungistatic effect of triadimefon on several EF species was also reported by Majchrowicz and Poprawski (1993). It is noteworthy that survival times in bioassays with *D. saccharalis* larvae were significantly longer for insects treated with Bb suspension amended with triadimefon than for fungicide-free conidial suspensions. Our data suggest that longer incubation times in viability assessments (20 vs. 16 h) may result in inclusion of Bb conidia with delayed germination in counts, which are thought to be less virulent (Faria et al. 2010).

The impact of 15 chemical fungicides on virulence of four EF species against *Galleria mellonella* showed that only tolylfluanid and azoxystrobin had a negative effect on Ma and *Isaria fumosorosea*, respectively (Shah et al. 2009). On the other hand, none of the tested chemicals influenced the virulence of Bb and *Lecanicillium longisporum* in lab experiments, leading these authors to speculate that certain fungicides would have little or no effect on EF virulence under field conditions. The conflicting results with our study may be attributed to different methodological approaches. For instance, Shah and colleagues added aqueous (0.03% Tween 80) conidial suspensions into a peat-based compost amended with fungicides before introduction of *G. mellonella* larvae, whereas in our work, fungicides (not tested in their study) were added to conidial suspensions (with or without EO) 40 min prior to spraying *D. saccharalis* larvae. In another study, soil samples baited with *G. mellonella* showed a higher percentage of *B. bassiana*-infected larvae than control soil (no pesticide) when coming from field plots previously treated with triadimefon, although *in vitro* studies suggested that radial growth of the fungus is inhibited by this chemical (Mietkiewski et al. 1997). These authors hypothesized that triadimefon either increased susceptibility of *G. mellonella* to Bb or selectively impacted competing soil microorganisms, allowing Bb to cause higher infection rates. Our bioassay results do not hold the first hypothesis, and differences in ST$_{50}$s between unformulated suspensions (with vs. without triadimefon) were not apparent. Therefore, protection against the effects of triadimefon and other non-compatible chemicals is a quite important issue in control of pests sprayed with mycopesticides.

In spite of the advantages of oil-containing formulations (Prior et al. 1988; Bateman et al. 1993; Inglis, Johnson, and Goettel 1996; Inyang et al. 2000; Polar et al. 2005; Gatarayiha, Laing, and Miller 2010b), the most common types of mycopesticides currently available on the world market are technical concentrates in the form of fungus-colonized substrates (29%), most of them sold in Latin America (Faria and Wraith 2007). Wettable powders represent 22% and oil dispersions (conidia + emulsifiable oil) only 18%. A recent survey in Brazil showed that 75% of mycoinsecticides commercially sold in the country were technical concentrates or pure conidia, and only 25% were oil dispersions, the only oil-based formulation type on the Brazilian market (Michereff Filho, Faria, Wraith and Silva 2009). The results shown in our study reinforce the importance of proper formulation for achievement of improved performance.

Oil dispersions and possibly other oil-containing formulations might prevent damage imposed on EF by debilitating water-soluble pesticides. Emulsification results in formation of droplet populations generated by equilibrium between the breakup and coalescence of the oil phase. The surfactant type and its concentration
have a profound influence on droplet size and distribution in oil emulsion (Weiss and Muschiolik 2007; Celis and Garcia-Rubio 2008). Therefore, surfactant selection is an important factor to be considered in development of oil dispersions, in order to guarantee adequate protection (microencapsulation) of fungal conidia against incompatible chemicals. Triadimefon and carbendazim remained active in the water phase of suspensions but had no significant action on conidia encapsulated by oil droplets, allowing for faster germination and higher insect infectivity. This first report on the protective effect of emulsifiable oils provides practical information for mycopesticide users, being suitable for IPM programs in which fungal-based products are used alongside chemicals.

References


Prior, C., Jollands, P., and le Patourel, G. (1988), ‘Infectivity of Oil and Water Formulations of Beauveria bassiana (Deuteromycotina, Hyphomycetes) to the Cocoa Weevil Pest Pantorhytes plutos (Coleoptera: Curculionidae)’, *Journal of Invertebrate Pathology*, 52, 66–72.


SPSS (2008), *SPSS for Windows, Rel. 17.0*, Chicago, IL: SPSS Inc.


