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RESEARCH ARTICLE

Interaction of fungicides with the entomopathogenic fungus *Isaria fumosorosea*

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The effects of eight commonly used fungicides: propamocarb, fenarimol, triadimefon, procimidone, azoxystrobin, carbendazim, cooper oxychloride and *Trichoderma harzianum* on germination, mycelial growth and virulence of *Isaria fumosorosea* (Wize) Brown & Smith (Ascomycota: Hypocreales) was studied. The greatest effect on germination was observed by azoxystrobin, followed by carbendazim, triadimefon and procimidone. Propamocarb, fenarimol, cooper oxychloride and *T. harzianum* did not affect conidial germination with germinations of 95, 93, 79 and 84%, respectively. Virulence was evaluated against early fourth instar nymphs of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). The mix containing fungal conidial suspensions plus cooper oxychloride or fenarimol presented a significant reduction in the mortality in comparison with spore suspension itself. This study suggests that the most appropriate fungicides for use in Integrated Pest Management Programs against *T. vaporariorum* in combination with *I. fumosorosea* are propamocarb and *T. harzianum*.

Keywords: entomopathogenic fungi; *Isaria fumosorosea*; whiteflies; biological control; fungicides

Introduction

Entomopathogenic fungi are important as natural controls of insect pests as well as in Integrated Pest Management (Neves, Hirose, Tchujo, and Moino 2001). Consequently, the use of selective phytosanitary products that are compatible with entomopathogenic fungi should be attempted as much as possible (Storey and Gardner 1986; Moino and Alves 1998; Quintela and McCoy 1998).

Several fungicides have been tested for their effects on entomopathogenic fungal conidial germination, mycelial growth and virulence against insects, under *in vitro* and *in vivo* conditions. The sensitivity of *Beauveria bassiana*, *Metarhizium anisopliae*, *Lecanicillium lecanii* and *Isaria fumosorosea* to several fungicides had been reported (Olmert and Kenneth 1974; Hall 1981; Tedders 1981; Loria, Galaini, and Roberts 1983; Er and Gökce 2004; Saphieha-Waszkiewicz, Marjanska-Cichon, and Mietkiewski 2004). For example, Bayleton (triadimefon), Captan 50WP (captan), Heritage (azoxystrobin), Terraguard 50WP (triflumizole) had significant impact *in vitro* on isolates of *M. anisopliae* (Samuels, Pinnock, and Allsopp 1989; Bruck 2009).

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Faion (2004) determined that sulphur was not found to be compatible with *B. bassiana* and *M. anisopliae*. Sosa-Gómez, Delpin, Moscardi, and Nozaki (2003) found that benomyl, difenoconazole, sulphur and carbendazim affected conidial germination of *Nomuraea rileyi* in laboratory assays. Some laboratory studies have reported no effects of fungicides on entomopathogenic fungi, for example, media amended with guanidine dodine, benomyl, thiabendazole or cupric sulphate resulted in good development of *B. bassiana*, *Paecilomyces lilacinus*, *Metarhizium* spp., *M. anisopliae*, *Evlachovaea* sp. and *Tolypocladium cylindrosporum* (Beilhartz, Parberry, and Swart 1982; Rocha 2006; Luz, Bastos Netto, and Rocha 2007).

Fungicides may affect the natural infection of entomopathogenic fungi in laboratory and field studies, for example reducing infection rates, delaying epizootics and resulting in an increase of population densities (Johnson, Kish, and Allen 1976; Horton, Carner, and Turnipseed 1980; Stansly and Orellana 1990; Smith and Hardee 1996; Sosa-Gomez et al. 2003).

Our goal was to determine the effect of commonly used fungicides by conventional horticultural farmers in La Plata, Buenos Aires province, Argentina, on germination, mycelial growth and virulence of native isolates of the fungus *Isaria fumosorosea* (Wize) Brown & Smith (Ascomycota: Hypocreales) from whiteflies (Hemiptera: Aleyrodidae).

Materials and methods

Fungal cultures

The isolates of *Isaria fumosorosea* (CEP 304 and CEP 315) were isolated from *Trialeurodes vaporariorum* adults from 'tomato' (*Lycopersicon esculentum*) and 'string bean' (*Phaseolus vulgaris*) in La Plata, Buenos Aires, Argentina (S 34° 56' 30.1" W 58° 04' 53.7"). These isolates were selected for their high conidial germination and virulence on whitefly from preliminary laboratory assays. The isolates were cultured on Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY) (Goettel and Inglis 1997) and incubated at 25°C in complete darkness for 15 days prior to use.

Fungicide treatments

The commercial products, active ingredients, concentrations and manufacturers of each of fungicide are presented in Table 1. Triadimefon, cooper oxychloride and *Trichoderma harzianum* were wettable powder formulations and propamocarb, fenarimol, procimidone, azoxystrobin and carbendazim were liquid formulations. All fungicides were dissolved and homogenized in distilled water and the concentration of active ingredients was adjusted to the manufacturers recommended rate.

Conidial germination

Inoculum of *I. fumosorosea* (CEP 304 or CEP 315) was produced on slants containing SDAY. Slants were incubated for 15 days at 25°C and conidia were harvested from the surface by scraping and suspending in 0.01% aqueous Tween 80. One hundred microliters of a conidial suspension (1×10^6 spore/ml) of *I. fumosorosea* conidia were added to thin layers of SDAY culture media on sterile glass microscope slides, which

Table 1. Details of fungicides.

| Active ingredient | Commercial product | Concentration ¹ | Manufacturer |
|----------------------------------|-----------------------|----------------------------|--------------|
| Propamocarb | PREVICUR | 0.15% | Bayer |
| Fenarimol | RUBIGAN | 0.05% | Brometan |
| Triadimefon | BAYLETON | 0.05% | Bayer |
| Procimidone | SUMILEX | 0.1% | Ando |
| Azoxystrobin | AMISTAR | 0.04% | Syngenta |
| Carbendazim | CARBENDAZIM | 0.15% | Reposo |
| Cooper oxychloride | COOPER OXYCHLORIDE | 0.15% | Nufarm |
| <i>Trichoderma harzianum</i> | TRICHO-D | 0.5% | EcoCERT |

¹Concentration (weight/volume) recommended by manufacturers.

were placed in Petri dishes with sterilized discs of moistened filter paper (Lane, Humphreys, Thompson, and Trinci 1988). Three hundred microliters of each fungicide or 300 μ L of sterile distilled water (control) were sprayed onto the surface of the inoculated slides using a glass nozzle of 35 cc capacity. Petri plates were sealed with parafilm and incubated in darkness at 25°C for 24 h. The percentages of germination were assessed by observing 600 conidia under the light microscope. Conidia were considered germinated if their germ tube was twice as long as the spore. Each treatment and the whole assay were replicated three times.

Vegetative growth

Three hundred microliters of each fungicide at the recommended rate were added to the surface of SDAY culture media in 90 mm diameter Petri dishes. A small plug (4 mm diameter and 4 mm deep) of nonsporulated mycelium from an *I. fumosorosea* culture was placed in the center of each fungicide treated Petri dish and incubated in the dark for 10 days at 25°C. The radial growth (beyond the 4 mm diameter of the plug) was measured daily in each cardinal direction on the base of each Petri plate. After 10 days, growth rate was determined as diameter of colony per day (mm/day). Each treatment was replicated three times and the entire assay was performed twice over time under the same conditions as mentioned above.

Bioassays against *Trialeurodes vaporariorum*

The virulence of fungal isolates was tested on *Trialeurodes vaporariorum* nymphs. A laboratory colony of *T. vaporariorum* (Hemiptera: Aleyrodidae) was maintained at CEPAVE using adults field-collected in La Plata, Buenos Aires, Argentina and tomato (*Lycopersicon esculentum*) as host plants. Insects and plants were reared in an incubator at 25 \pm 2°C with a photoperiod of 12 h L:12 h D. Leaves infested with early fourth instar whitefly nymphs were taken from plants and examined under a microscope. The nymphs were removed from the leaf surface and placed on sterile microscope slides (10 nymphs per microscope slide), which were placed into Petri dishes. The laboratory assay was slightly modified from that of Landa, Osborne, Lopez, and Eyal (1994). The treatments assayed were:

1. Sterile distilled water with 0.01% Tween 80 (control)
2. 1×10^7 conidia ml^{-1} suspensions of CEP 304 or CEP 315 isolates of *I. fumosorosea*
3. Fungicide in the recommended concentration (Table 1)
4. 1×10^7 conidia ml^{-1} suspensions of *I. fumosorosea* plus each fungicide (1:1 ratio of spore suspension and fungicide).

The bioassay procedure involved placing a drop (approximately 3 μL) of each treatment on each nymph present on the microscope slide. After the slides were dried under a laminar flow hood, each slide was placed in a Petri dish with sterile discs of moistened filter paper (110 mm diameter). The Petri dishes were maintained in the dark for 7 days at 25°C. Each treatment was assayed by using three Petri dishes (total of 30 nymphs/treatment) and the entire assay was performed three times on different dates. Cumulative mortality (%) was determined by presence of mycelial mass and was recorded daily for 7 days. Fungal infections were confirmed with optical microscopy for all dead insects, and they were mounted in lactophenol/cotton blue to check for fungal infection.

Statistical analysis

Percent germinated conidia, growth rate and percent mortality were analyzed by Statgraphics Centurion 15.2 program (StatPoint, Inc. 2007). An arcsine transformation was performed to stabilize variance of percent germination and mortality. Test of homogeneity of variance was performed to detect variation between each experiment. Then, data were submitted to analysis of variance (ANOVA) and Tukey's multiple range tests ($P < 0.05$). Differences between the two isolates, CEP 304 and CEP 315, were compared with a *t*-test ($P < 0.05$). The lethal times 50 (LT_{50}) were estimated using the MICROPROBIT program (Sparks and Sparks 1987).

Results

Percent conidial germination of isolates CEP 304 and CEP 315 were significantly different among fungicides (Table 2) ($F = 13.60$ and 19.34 for CEP 304 and CEP 315, respectively, $\text{df} = 8$, $P < 0.001$ for both isolates). A *t*-test revealed that for each fungicide, the difference between the mean conidial germination of CEP 314 and CEP 315 was not significantly greater than zero ($P > 0.05$). Propamocarb, fenarimol, triadimefon, procimidone, azoxystrobin, carbendazim, cooper oxchloride and *Trichoderma harzianum* showed a decrease in germination percentage, though none suppressed it completely. The highest effect on percent germination of *I. fumosorosea* was observed with azoxystrobin (20%) followed by carbendazim (49%), triadimefon (54%) and procimidone (75%). However, propamocarb, fenarimol, cooper oxchloride and *Trichoderma harzianum* had no significant impact on conidial germination relative to the control, which was 95, 93, 79 and 84% germination, respectively. Microscopical observation of conidia of *I. fumosorosea* showed a reduction in the length of the germ tube with carbendazim and fenarimol.

The mean vegetative growth of *I. fumosorosea* was significantly different among the isolates ($t = -2.65$, $P = 0.024$), obtaining after 10 days of incubation a diameter of 40 mm for CEP 304 and 49 mm for CEP 315. The growth rates of CEP 304 and

Table 2. Conidial germination and vegetative growth to CEP 304 and CEP 315 isolates of *I. fumosorosea* with fungicides.

| Treatment | CEP 304 | | | CEP 315 | | |
|-----------------------|------------------------------|---------------------------------|-----------------------------------|------------------------------|---------------------------------|-----------------------------------|
| | Germination ¹ (%) | Radial growth ² (mm) | Growth rate ³ (mm/day) | Germination ¹ (%) | Radial growth ² (mm) | Growth Rate ³ (mm/day) |
| Control | 97.60 (0.01) a | 40.0 (3.35) | 3.6 (0.34) a | 98.33 (0.00) a | 49.2 (0.83) | 4.5 (0.08) a |
| Propamocarb | 94.14 (0.02) ab | 36.5 (3.94) | 3.3 (0.39) a | 95.02 (0.01) ab | 48.3 (1.05) | 4.4 (0.11) a |
| Fenarimol | 90.51 (0.03) ab | 32.8 (4.03) | 2.9 (0.40) a | 94.90 (0.01) ab | 44.2 (2.39) | 4.0 (0.24) ab |
| Triadimefon | 58.40 (0.13) cd | 35.0 (4.03) | 3.1 (0.40) a | 49.96 (0.13) d | 48.3 (1.05) | 4.4 (0.11) a |
| Procimidone | 66.41 (0.09) bcd | 35.8 (5.69) | 3.2 (0.57) a | 83.70 (0.03) abc | 46.7 (2.11) | 4.3 (0.21) a |
| Azoxystrobin | 21.19 (0.09) e | 19.5 (2.51) | 1.6 (0.25) a | 18.95 (0.06) e | 38.8 (1.83) | 3.5 (0.18) b |
| Carbendazim | 36.07 (0.13) de | 8.8 (1.13) | 0.5 (0.11) a | 62.48 (0.07) cd | 11.0 (1.32) | 0.7 (0.13) c |
| Cooper oxychloride | 85.82 (0.04) abc | 34.2 (4.41) | 3.0 (0.44) a | 73.27 (0.08) bcd | 46.7 (1.67) | 4.3 (0.17) a |
| <i>T. harzianum</i> | 86.81 (0.05) abc | 36.3 (4.27) | 3.2 (0.43) a | 81.60 (0.05) abc | 45.0 (1.83) | 4.1 (0.18) ab |

¹Mean percentage of conidial germination (standard error) obtained after incubation at 25°C during 24 h.

²Mean radial growth (standard error) measured after incubation at 25°C during 10 days.

³Mean growth rate (standard error) estimated as diameter of colony (mm) at 10 days (beyond the 4 mm diameter of the plug).

Different letters in the same column indicate significant differences among means after Tukey's test ($P < 0.05$).

CEP 315 were 3.6 and 4.5 mm/day, respectively (Table 2). Growth of isolate CEP 304 was not found to be significantly different among fungicides and control treatment ($F=1.55$, $df=8$, $P=0.057$), however, there was a significant reduction of radial growth rate in the presence of carbendazim and azoxystrobin (Table 2). Radial growth of isolate CEP 315 was significantly reduced when exposed to the fungicides compared to the control ($F=53.78$, $df=8$, $P<0.001$) and the growth rate decreased to 0.7 and 3.5 mm/day in presence of carbendazim and azoxystrobin while the control was 4.5 mm/day. The effect of fenarimol and *T. harzianum* was intermediate with growth of 4 mm/day (Table 2). The morphology of *I. fumosorosea* colony not was affected by the fungicides.

The potential impact of fungicides on the virulence of *I. fumosorosea* was performed against early fourth instar nymphs of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae). Propamocarb, fenarimol, cooper oxchloride and *T. harzianum* were selected for the bioassays as they did not affect germination and radial growth of both isolates. These fungicides affected nymph survival causing 1–11% mortality (Table 3). The mix containing fungal conidial suspensions plus one fungicide resulted in a significant reduction in percent mortality of *T. vaporariorum* nymphs as compared to conidia suspension itself ($F=4.96$, $df=4$, $P=0.024$ for CEP 304 and $F=8.35$, $df=4$, $P<0.001$ for CEP 315) (Table 3). A *t*-test revealed that for each treatment, the difference between the mean mortality in CEP 304 and CEP 315 was not significantly greater than zero ($P>0.05$). The mix with cooper oxchloride reduced the mortality to 49% followed by fenarimol (69%), propamocarb (78%) and *T. harzianum* (80%) as compared with the conidia suspension alone (85%).

Table 3. Cumulative mortality and median lethal time (LT₅₀) of early fourth instar *Trialeurodes vaporariorum* nymphs caused by both isolates of *I. fumosorosea* and fungicides.

| Treatment | Mortality ¹ (%) | LT ₅₀ (days) |
|-------------------------------|----------------------------|-------------------------|
| Control | 0.00 (0.00) | ND |
| Propamocarb | 1.11 (0.01) | ND |
| Fenarimol | 6.67 (0.03) | ND |
| Cooper Oxchloride | 11.11 (0.03) | ND |
| <i>T. harzianum</i> | 3.00 (0.03) | ND |
| CEP 304 | 77.78 (0.06) a | 3.94 |
| CEP 304 – Propamocarb | 76.67 (0.07) a | 4.45 |
| CEP 304 – Fenarimol | 67.78 (0.06) ab | 5.53 |
| CEP 304 – Cooper Oxchloride | 44.44 (0.06) b | 8.34 |
| CEP 304 – <i>T. harzianum</i> | 75.56 (0.05) a | 3.49 |
| CEP 315 | 91.11 (0.05) a | 2.9 |
| CEP 315 – Propamocarb | 78.89 (0.05) ab | 3.66 |
| CEP 315 – Fenarimol | 70.00 (0.05) bc | 5.02 |
| CEP 315 – Cooper Oxchloride | 54.44 (0.04) c | 6.98 |
| CEP 315 – <i>T. harzianum</i> | 82.22 (0.05) ab | 3.29 |

¹Mean (standard error) percentage of mortality at 7 days after inoculation. Different letters in the same isolate indicate significant differences among means after Tukey's test ($P<0.05$). ND, data not determined.

Discussion

In the present study, from a total of eight fungicides tested, only carbendazim and azoxystrobin were highly deleterious to conidial germination and mycelial growth of two isolates of *I. fumosorosea*. Shah et al. (2009) demonstrated that azoxystrobin inhibited the germination of *M. anisopliae* and *L. longisporum* and only captan showed a fungistatic effect on *I. fumosorosea*. Sterk (2003) reported that six fungicides (captan, azoxystrobin, kresoxim, methyl sulfur, pyrimethanil and tolyfluanid) influenced conidial germination but not mycelial growth of *I. fumosorosea*. Also, Bruck (2009) found that propamocarb had no significant impact *in vitro* on conidial germination and mycelial growth of *M. anisopliae*. In despite of present results, Sapieha-Waszkiewicz et al. (2004) reported that Rubigan 12 EC (fenarimol) affected the growth and sporulation of *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. Kouassi, Coderre, and Todorova (2003) and Rachappa, Lingappa, and Patil (2007) demonstrated that cooper oxychloride was highly toxic to the development of *M. anisopliae* and *B. bassiana*.

This study also demonstrated that the virulence of *I. fumosorosea* isolates (CEP 304 and CEP 315) was reduced by a mixture of cooper oxychloride and fenarimol while a mixture of propamocarb and *T. harzianum* did not affect virulence. Thus, promamocarb and *T. harzianum* seemed to be compatible with both isolates of *I. fumosorosea*. Moino and Alves (1999) reported that *Trichoderma* sp. did not affect development of *B. bassiana* and *M. anisopliae* when inoculated simultaneously on PDA culture medium. Storey and Gardner (1986) reported that the fungicide mefluidide caused little inhibition of conidial germination and mycelial growth of *B. bassiana* and few deleterious effects on the pathogenicity to *Spodoptera frugiperda* in laboratory bioassays.

In previous studies, it was found that difenoconazole, sulphur and benomyl were among the most active fungicides against *N. rileyi* under *in vitro* assays (Ignoffo, Hostetter, Garcia, and Pinnell 1975) and their effects were enough to inhibit infection of *Trichoplusia ni* larvae. However, it was reported that benomyl was deleterious, while carbendazim (a metabolite of benomyl) did not suppress mycelial growth of *N. rileyi* (Sosa-Gómez et al. 2003). Also, azoxystrobin inhibited the *Galeria melonella* infection by *I. fumosorosea* (Shah et al. 2009).

From results obtained in the present study, we would suggest that the most appropriate fungicides for use in Integrated Pest Management programs against *T. vaporariorum* in combination with *I. fumosorosea* are propamocarb and *T. harzianum*. Further research under field conditions in greenhouses should be done in order to confirm the compatibility of entomopathogenic fungi and fungicides within an Integrated Pest Management strategy.

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