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Side-effects of pesticides on the life cycle of the mite pathogenic fungus *Neozygites floridana*

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Abstract The tomato red spider mite, *Tetranychus evansi* Baker and Pritchard, is an invasive species in Africa causing considerable damage to Solanaceous crops. The fungal pathogen *Neozygites floridana* Weiser and Muma from Brazil has been considered a potential candidate for introduction into Africa for the control of *T. evansi*. To be incorporated in the tomato production system, *N. floridana* has to be compatible with the pesticides used for the control of other pests and diseases. Pesticides used in tomatoes that might affect the fungus were therefore studied by the use of different methods. Two insecticides (Lambda-cyhalothrin and Methomyl), two acaricides (Propargite and Abamectin), and two fungicides (Captan and Mancozeb) were tested in two concentrations: the mean commercial rate (CR) and 50% of the mean commercial rate (CR/2). Fungus-killed mite cadavers or the substrates used for sporulation (leaf discs and coverslips) were either immersed or sprayed with the pesticides before testing their effects on sporulation, germination of primary conidia and infectivity of *N. floridana*. Direct immersion of cadavers, coverslips or leaf discs into pesticides affected sporulation and germination stronger than the spray tower method, although infectivity of capilliconidia was neither affected by the method of application nor the concentration of the pesticides. The fungicides Captan and Mancozeb resulted in a high reduction in sporulation and germination at both concentrations. Propargite did not inhibit sporulation but affected germination of primary conidia. Methomyl and Abamectin resulted in less effects on *N. floridana*.

Keywords *Neozygites floridana* · Toxicity · Tomato · *Tetranychus evansi* · Side effects

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Introduction

The tomato red spider mite, *Tetranychus evansi* Baker and Pritchard, is an invasive species in Africa causing considerable damage to solanaceous crops (Saunyama and Knapp 2003). In tomato, *T. evansi* is frequently controlled by intensive application of acaricides (Blair 1989). Lepidopteran insect pests and fungal diseases such as tomato late blight, *Phytophthora infestans* (Mont.), are controlled by the use of insecticides and fungicides, respectively.

In several countries, epizootics of *Neozygites floridana* Weiser and Muma (Zygomycetes: Entomophthorales) have been associated with rapid decline in populations of spider mites (Carner and Canerday 1970; Smith and Furr 1975; Boykin et al. 1984), including *T. evansi* on tomato (Humber et al. 1981). The apparent importance of this fungus as a natural control agent in agro-ecosystems suggests that it can be used in conjunction with other strategies in Integrated Pest Management programs for sustainable pest control. *Neozygites floridana* collected in Brazil has been considered as a potential candidate for classical biological control of *T. evansi* in Africa. To maximize the potential of *N. floridana* in controlling *T. evansi* on tomato, it is necessary to incorporate the use of selective pesticides for management of other tomato pests to reduce the negative effect this might have on *N. floridana*.

The direct impact fungicides have on natural epizootics of entomopathogenic fungi has been demonstrated for different species. For example, application of fungicides has been implicated in the reduction of *Neozygites* spp. incidence in the field resulting in host population increases of mainly aphids and mites (Brandenburg and Kennedy 1983; Boykin et al. 1984; Bower et al. 1995; Klingen and Westrum 2007).

Although it might be desirable to conduct field experiments to determine the effect of pesticides on the disease dynamics of the entomopathogenic fungi, field experiments are expensive, time consuming, and often not appropriate to identify specific factors that affect the entomopathogenic fungi. To identify compatibility of pesticides on entomopathogens, laboratory bioassays are therefore usually the first steps in selecting pesticides for use in integrated pest management programs (Morjan et al. 2002).

Studies conducted to determine the inhibitory effects of pesticides on other species of entomophthoralean fungi usually focus on the impact of pesticides on germination of conidia and hyphal growth in culture media containing each pesticide (Hall and Dunn 1959; Jaques and Patterson 1962; Yendol 1968; Boykin et al. 1984). Studies on the effects of pesticides on *N. floridana* have received little attention, mostly because of difficulties associated with the establishment of in vitro cultures of this pathogen (Morjan et al. 2002).

Neozygites floridana produces three types of spores and has a more complex life cycle than the anamorphs of the Ascomycota within the order Hypocreales ("imperfect fungi" formerly in the Deuteromycota). Primary conidia of *N. floridana* are actively discharged from the conidiophores of the mummified host mites, referred to as cadavers. A primary conidium lands on the leaf surface and germinates to form a secondary type of conidium, the infective capilliconidium (Smitley et al. 1986; Oduor et al. 1996; Delalibera et al. 2006). The host needs to come in contact with the capilliconidium to become infected. *Neozygites floridana* also produces resting spores for long-term survival probably when conditions are unfavorable. Therefore, there are many stages of the life cycle that can be affected by application of pesticides.

The aim of this study was to test the effect of fungicides, acaricides and insecticides used in commercial tomato production on sporulation, germination, infectivity and mortality of

T. evansi by *N. floridana* and to describe laboratory methods which can be used for toxicity tests without necessarily growing the fungus on artificial media.

Materials and methods

Fungal production

The *N. floridana* isolate (LQ2) used in this study was initially collected as mummified *T. evansi* mites at a greenhouse of the University of São Paulo in Piracicaba, São Paulo, Brazil, during an epizootic in September 2004. It was stored for 1 year in vials containing silica gel at −10°C, before use in this study. New cadavers were produced by exposing healthy *T. evansi* females to sporulating cadavers from the stock culture. Sporulation was obtained by keeping cadavers at 25°C in darkness on tomato leaf discs (1.2 cm diameter) placed on top of a moist sponge in closed Petri dishes (9 cm diameter) at 100% RH for 16 h. Mites exposed to the sporulating cadavers were then maintained in an incubator at 25°C and 50% RH under natural light–dark regime (12D:12L) and cadavers were collected 3–7 days later for use in the bioassays. The effects of the pesticides were tested by direct application on only newly formed cadavers (not stored ones) and on conidia discharged from them.

Pesticides used

Information on trade names, active ingredients, and types of pesticide, formulations, chemical groups and recommended concentrations of the pesticides are shown in Table 1. Two insecticides, Karate Zeon® 50 CS (Syngenta) and Lannate® BR (Dupont), two acaricides, Omite® 720 EC (Crompton) and Abamex® (Bequisa), and two fungicides, Orthocide® 500 (Arysta Lifescience) and Dithane NT (Dow Agrosciences), were chosen for their frequent use by farmers in tomato crop. Pesticides were used in two concentrations: commercial rate (CR), which is the mean recommended concentration for application in tomato, and 50% of the mean commercial rate (CR/2). All the pesticides were diluted with distilled water amended with 0.05% Tween 80 as a surfactant.

General experimental set up

The effect of pesticides on *N. floridana* was tested using two contamination methods: dipping and spraying. Cadavers, leaf discs or coverslips used in the bioassays were either dipped into, or sprayed with, the pesticides. Different bioassays were conducted to

Table 1 Pesticides used in selectivity studies with *Neozygites floridana*

Active ingredient	Trade name	Type of pesticide	Formulation	Chemical group	Concentration (CR)
Methomyl	Lannate	Insecticide	SC	Carbamate	100 ml/100 l
Lambda-cyhalothrin	Karate	Insecticide	CS	Pyrethroid	40 ml/100 l
Propargite	Omite	Acaricide	EC	Alkyl sulfate	50 ml/100 l
Abamectin	Abamex	Acaricide	EC	Avermectin	75 ml/100 l
Captan	Orthocide	Fungicide	WP	Dicarboximide	240 g/100 l
Mancozeb	Dithane	Fungicide/Acaricide	WP	Dithiocarbamate	300 g/100 l

CR = Mean recommended concentration of the formulated product for application in 100 l of water per ha, EC = Emusifiable concentrate, WP = Wettable powder, SC = Soluble concentrate, CS = Capsulated suspension

determine the effect of pesticides on sporulation, germination of primary conidia, and development of the fungus inside the mite. All experiments were repeated three times.

Effect of pesticides on sporulation

The effect of pesticides on sporulation was evaluated by measuring direct and indirect effects. The direct effects were measured by immersing cadavers into the pesticides (cadaver treatment). The indirect effect was measured by immersing leaf discs into pesticides (leaf treatment) before transferring the cadavers onto these discs. Similarly, direct and indirect effects were tested by spraying cadavers on leaf discs or spraying leaf discs before transferring the cadavers onto them.

Cadaver treatment

Ten cadavers were introduced into microcentrifuge tubes and then 0.5 ml of each pesticide at either concentration (CR or CR/2) amended with 0.05% Tween 80 was added into each tube and agitated for 2 min. The content of each tube was then poured onto filter papers to drain the excess pesticides. Control cadavers were given the same treatments as described above except that they were introduced into distilled water amended with 0.05% Tween 80. After 2 h, the treated cadavers were individually placed on untreated discs of tomato leaves (1.2 cm in diameter) resting on a wet sponge inside a Petri dish (9 cm diameter). The dishes were closed to reach about 100% RH and incubated at 25°C in darkness for 16 h. The number of conidia discharged per mummy was estimated by observing the leaf disc directly under a compound microscope and scoring conidia numbers according to a categorical scale (0: no sporulation, 1: 1–100, 2: 101–500, and 3: >501 conidia).

In a parallel experiment, ten cadavers were placed on a filter paper and sprayed with 2 ml of each pesticide at either concentration using a Potter Precision Laboratory Spray Tower (Burkard Manufacturing, Rickmansworth, Herts, UK), calibrated at 68.95 kPa with a mean deposition of 1.5 mg of residue/cm^2. Sprayed cadavers were air-dried for 2 h and were then transferred individually onto unsprayed tomato leaf discs and processed as described above.

Leaf treatment

Ten leaf discs were individually dipped in each pesticide at either concentrations (CR and CR/2) for 2 min and were then air-dried for 2 h. Similarly, ten leaf discs were sprayed with 2 ml of each pesticide solution. A cadaver taken from the stock culture was then placed in the center of each disc, transferred onto moist sponge in a closed Petri dish and incubated at 25°C in darkness for 16 h, before sporulation was evaluated as described above. Control leaf discs were dipped in or sprayed with distilled water amended with 0.05% Tween 80 and all other experimental procedures were similar to those described above.

Effect of pesticides on germination of primary conidia

Three square photo-etched coverslips (23 × 23 mm) (Electron Microscopy Sciences, Hatfield, PA, USA) with alphanumeric coded squares were immersed into or sprayed with the mean concentration (CR) of each pesticide and air-dried for 2 h before two cadavers were put at the center of each coverslip. The control slides were immersed into distilled water amended in 0.05% Tween 80. The coverslips with cadavers were then transferred onto a sponge soaked in distilled water in a closed Petri dish at 25°C in darkness for 16 h.

Germination of conidia was observed using a compound microscope and the number of germinated and un-germinated conidia in five arbitrarily selected squares within the field of view was recorded using an enumeration counter. Total conidial germination included conidia that were in the process of forming or had already formed secondary conidia or capilliconidia. Percent germination was computed by dividing the number of germinated conidia with the total number of conidia counted in a specified field and multiplying by 100.

Effect of pesticides on infectivity of capilliconidia

Leaf discs with sporulating cadavers—from the study on the effect of pesticides on sporulation by immersion and spraying—were used to test the infectivity of the produced capilliconidia. Only leaf discs with the highest spore numbers (category 3) were selected for the test. Fifteen *T. evansi* females were introduced onto each of the ten leaf discs containing the spores and placed at 25°C in the incubation chamber. Mortality of these mites was checked daily for 7 days.

Effect of pesticides on mortality of *N. floridana* inoculated *T. evansi*

Only Captan and Methomyl were used in this study because they were the only pesticides that did not kill the mites exposed to leaf discs containing these pesticides. Ten leaf discs were immersed into each pesticide in a single concentration (CR) and allowed to dry for 2 h. Fifteen *T. evansi* females were transferred to the treated leaf discs. After 48 h of feeding on the treated leaf discs, the mites were transferred to new leaf discs each with a sporulating *N. floridana* cadaver. These mites stayed for 24 h on these leaf discs for contamination and were then transferred to new and larger leaf discs and observed daily for infection and mortality for 7 days. Control leaf discs were immersed in distilled water amended with 0.05% Tween 80 and all other experimental procedures were as described for the pesticide treatments. The leaf discs were changed after the fourth day. Dead mites were mounted and observed under the microscope for hyphal bodies to confirm that the cause of death was *N. floridana*.

Data analysis

The effects of pesticides on sporulation, germination and infectivity were compared by using a two-way analysis of variance (ANOVA) with treatment, concentration and application method as factors (PROC GLM, SAS Institute 1998). Percentages germination and mortality were arcsine transformed before analysis to homogenize variances. Means were compared using Duncan Multiple Range Test (DMRT) ($P < 0.05$). A pre-planned comparison between treatments was performed separately for each group of pesticide to determine within group treatment effects.

Results

Effect of pesticides on sporulation of *N. floridana*

Cadaver treatment

The negative effect of pesticides on *N. floridana* sporulation was higher when the cadavers were immersed into pesticides than when sprayed ($F_{35,324} = 11.66$, $P = 0.0001$) (Table 2).

Table 2 Sporulation of *Neozygites floridana* from mummified *Tetranychus evansi* females after immersion or spraying with mean recommended concentration (CR) or half that concentration (CR/2) of pesticides

Pesticide	Immersed cadavers			Sprayed cadavers		
	CR/2	CR	Control	CR/2	CR	Control
Methomyl	1.7 ± 0.2bc	1.4 ± 0.2c	2.3 ± 0.2ab	2.6 ± 0.2a	2.3 ± 0.3ab	2.3 ± 0.1ab
Lambda-cyhalothrin	1.8 ± 0.2bc	1.5 ± 0.2c	2.2 ± 0.2ab	2.3 ± 0.3ab	2.3 ± 0.3ab	2.7 ± 0.2a
Propargite	2.5 ± 0.2b	0.3 ± 0.2d	2.5 ± 0.2ab	1.9 ± 0.4bc	1.9 ± 0.4bc	2.5 ± 0.2ab
Abamectin	3.0 ± 0a	1.3 ± 0.4c	2.8 ± 0.1a	1.8 ± 0.4bc	1.6 ± 0.4bc	2.4 ± 0.2ab
Captan	0.6 ± 0.2c	0.3 ± 0.2cd	2.1 ± 0.2ab	0.5 ± 0.2c	0.4 ± 0.2cd	2.4 ± 0.3a
Mancozeb	0.0 ± 0e	0.0 ± 0.0e	1.6 ± 0.2b	0.3 ± 0.2cd	0.1 ± 0.1cd	2.4 ± 0.2a

Numbers of conidia (Mean ± SE) were estimated based on a categorical scale of 0 = 0, 1 = 1–100, 2 = 101–500, and 3 = >501 conidia/mummified mite

Means within columns and rows followed by the same letters are not significantly different (DMRT, $P > 0.05$)

When cadavers were immersed into the pesticides, Lambda-cyhalothrin, Methomyl, Abamectin and Propargite had no effect on sporulation at CR/2 concentration but sporulation was lower at CR. Captan and Mancozeb significantly reduced sporulation both at CR/2 and CR. When cadavers were sprayed with the pesticides, Methomyl, Lambda-Cyhalothrin, Propargite and Abamectin had no effect on sporulation at neither of the concentrations. Cadavers sprayed with Mancozeb and Captan sporulated less both at CR/2 and CR.

Leaf treatment

Cadavers placed on leaf discs that were immersed into Lambda-Cyhalothrin sporulated and produced as many conidia as the control (Table 3). Methomyl, Propargite and Abamectin at CR/2 did not affect sporulation, but at CR these pesticides affected sporulation. Mancozeb and Captan significantly affected sporulation at both CR/2 and CR. When Propargite was sprayed on leaf discs, sporulation was unaffected at the lower concentration, but at CR sporulation was affected. Leaf discs sprayed with CR/2 or CR of Methomyl, Lambda-Cyhalothrin, Abamectin and Captan did not result in differences in sporulation. Mancozeb, however, inhibited sporulation at both CR/2 and CR when leaf discs were sprayed. In general, sporulation was significantly higher when leaf discs were sprayed than when immersed in both Mancozeb and Captan ($F_{11,108} = 11.78$, $P = 0.0001$).

Effect of pesticides on germination of primary conidia

Germination of primary conidia was significantly affected by the pesticides and also by the method of pesticides application: immersion and spray of coverslips (Table 4). Propargite and Mancozeb totally inhibited germination of conidia after immersion of coverslips. When coverslips were sprayed, germination was totally inhibited by Mancozeb and only 7.0 ± 2.0% of primary conidia germinated when sprayed with Propargite. Lambda-Cyhalothrin and Captan also reduced germination in both application methods. Methomyl was the only pesticide that did not affect germination when coverslips were either immersed or sprayed. Overall, germination of primary conidia was significantly higher on coverslips that were sprayed than immersed ($F_{29,60} = 22.51$, $P = 0.0001$).

Table 3 Sporulation of *Neozygites floridana* from mummified *Tetranychus evansi* females on leaf discs immersed or sprayed with mean recommended concentration (CR) or half the mean recommended concentration (CR/2) of pesticides

Pesticide	Immersed leaf discs			Sprayed leaf discs		
	CR/2	CR	Control	CR/2	CR	Control
Methomyl	2.0 ± 0.2b	1.4 ± 0.2d	2.2 ± 0.2b	2.9 ± 0.1a	2.9 ± 0.1a	2.9 ± 0.1a
Lambda-cyhalothrin	1.9 ± 0.2bc	1.7 ± 0.2bc	2.0 ± 0.3bc	2.5 ± 0.3abc	2.1 ± 0.4bcd	2.7 ± 0.2ab
Propargite	3.0 ± 0.0a	1.8 ± 0.4bc	3.0 ± 0.0a	2.1 ± 0.4bc	1.9 ± 0.3cd	2.7 ± 0.2ab
Abamectin	2.9 ± 0.1a	1.9 ± 0.4bc	3.0 ± 0.0a	2.5 ± 0.3abc	2.3 ± 0.3abc	2.3 ± 0.2abc
Captan	0.7 ± 0.3e	0.4 ± 0.2ef	2.2 ± 0.2b	1.7 ± 0.5abc	1.4 ± 0.4cde	2.5 ± 0.2abc
Mancozeb	0.0 ± 0.0g	0.0 ± 0.0g	1.9 ± 0.2bcd	1.0 ± 0.3de	0.7 ± 0.4de	2.5 ± 0.2abc

Numbers of conidia (Mean ± SE) were estimated based on a categorical scale of 0 = 0, 1 = 1–100, 2 = 101–500, and 3 = >501 conidia/mummified mite

Means within columns and rows followed by same letters are not significantly different (DMRT, $P > 0.05$)

Table 4 Effect of pesticides on germination of *Neozygites floridana* primary conidia when cadavers were placed to sporulate on coverslips immersed into or sprayed with mean recommended concentration (CR) of pesticides

Pesticide	Immersed coverslips		Sprayed coverslips	
	CR	Control	CR	Control
Methomyl	78.2 ± 5.8abc	70.2 ± 5.8abc	84.7 ± 2.0a	78.1 ± 7.6a
Lambda-cyhalothrin	42.8 ± 21.7d	70.2 ± 5.8abc	61.3 ± 4.8bc	78.1 ± 7.6a
Abamectin	54.9 ± 11.2 cd	56.6 ± 9.0 cd	57.1 ± 4.2 cd	82.5 ± 4.0a
Propargite	0.0 ± 0.0e	56.6 ± 9.0 cd	7.0 ± 2.0e	82.5 ± 4.0a
Captan	21.4 ± 1.4e	59.4 ± 9.5bcd	66.8 ± 4.1bc	77.7 ± 0.7a
Mancozeb	0.0 ± 0.0e	59.4 ± 9.5bcd	0.0 ± 0.0e	77.7 ± 0.7a

Data are mean percent germination ± SE

Means within columns and rows followed by the same letters are not significantly different (DMRT, $P > 0.05$)

Effect of pesticides on infectivity of capilliconidia

Infectivity of conidia after exposure to pesticides was measured as a function of mortality of the exposed mites. More than half of the mites transferred to leaf discs treated with Propargite, Abamectin, Mancozeb or Lambda-Cyhalothrin died after 1 day indicating a direct effect of the products on the mites and therefore these products were not used in the infectivity test (Table 5). Methomyl and Captan were the only pesticides used to test infectivity when leaf discs were either immersed or sprayed. Lower mortality of fungus-inoculated mites was observed when leaf discs were immersed rather than sprayed with Methomyl and Captan ($F_{9,39} = 10.22$, $P = 0.0001$) regardless of the concentration used, indicating an effect of the pesticide application method. Neither Methomyl nor Captan affected infectivity when leaf discs were sprayed with the pesticides. However, reduced infectivity was seen for leaf discs immersed into pesticides at CR. When cadavers were either immersed or sprayed, all pesticides except Mancozeb (not tested due to insufficient sporulation) were used to test pathogenicity of the sporulating fungus. None of the pesticides reduced the percent of mites killed by *N. floridana* compared to the controls. However, when cadavers were sprayed, mite mortality was higher than when immersed

Table 5 Effect of pesticides on infectivity of *Neozygites floridana* capilliconidia to *Tetranychus evansi* when leaf discs were immersed or sprayed with mean concentration (CR) or half the mean concentration (CR/2) of pesticides

Pesticide	Immersed leaf discs			Sprayed leaf discs		
	CR/2	CR	Control	CR/2	CR	Control
Methomyl	47.3 ± 7.4cd	41.8 ± 4.4d	61.3 ± 6.1bc	76.9 ± 6.4ab	82.6 ± 1.4a	76.9 ± 2.4ab
Captan	48.2 ± 4.9cd	39.5 ± 3.0d	61.3 ± 6.1bc	77.5 ± 6.7ab	77.1 ± 6.0b	76.9 ± 2.4ab

Data represent the percent mortality (mean ± SE) of *T. evansi* 7 days post-inoculation

Means within columns and rows followed by the same letters are not significantly different (DMRT, $P > 0.05$)

Table 6 Effect of pesticides on infectivity of *Neozygites floridana* capilliconidia to *Tetranychus evansi* when mummifeid mites were immersed in or sprayed with mean recommended concentration (CR) or half the mean recommended concentration (CR/2) of pesticides

Pesticide	Immersed cadavers			Sprayed cadavers		
	CR/2	CR	Control	CR/2	CR	Control
Methomyl	56.0 ± 4.0cdef	53.3 ± 3.4cdef	61.3 ± 6.1bcde	70.5 ± 5.8abcd	82.8 ± 5.1a	72.9 ± 3.4abc
Lambda-cyhalothrin	55.6 ± 4.8cdef	53.0 ± 4.6def	61.3 ± 6.1bcde	73.7 ± 7.1abc	77.7 ± 6.1ab	72.9 ± 3.4abc
Captan	40.4 ± 4.7f	46.7 ± 6.7ef	52.0 ± 5.6def	70.4 ± 4.4abcd	77.8 ± 6.2ab	79.0 ± 4.7ab
Abamectin	54.9 ± 6.7cdef	51.3 ± 5.7def	60.3 ± 3.8bcde	76.0 ± 3.4ab	75.3 ± 6.7ab	78.0 ± 5.4ab
Propargite	63.3 ± 4.9bcde	53.3 ± 3.9def	60.3 ± 3.8bcde	68.6 ± 8.9abcd	75.1 ± 7.2ab	78.0 ± 5.4ab

Data represents the percent mortality (mean ± SE) of *T. evansi* 7 days post-inoculation

Means within columns and rows followed by the same letters are not significantly different (DMRT, $P > 0.05$)

into the pesticides ($F_{25,97} = 4.30$, $P = 0.0001$) (Table 6). For all pesticides, mortality did not differ between concentrations CR/2 and CR when cadavers were immersed ($F_{1,8} = 0.16$, $P = 0.70$) or sprayed ($F_{1,8} = 0.20$, $P = 0.67$). Immersion of cadavers into Methomyl at CR resulted in lower mite mortality than sprayed cadavers ($F_{1,8} = 18.17$, $P = 0.0028$).

Effect of pesticides on mortality of *N. floridana* inoculated *T. evansi*

Neither Methomyl nor Captan affected mortality by *N. floridana* on *T. evansi* ($F_{2,27} = 0.18$, $P = 0.84$). Mean mortality of mites that were placed onto leaf discs contaminated with Methomyl, Captan or water (=control) before transfer to leaf discs with sporulating cadavers of *N. floridana* was 73.2 ± 4.1, 76.5 ± 5.0 and 73.2 ± 4.4%, respectively.

Discussion

The detrimental effects of pesticides used to control insects, mites and fungal diseases in commercial tomato production on sporulation, germination and infectivity of *N. floridana* varied as a function of the methods of contamination, chemical nature and concentration. The fungicides Mancozeb and Captan that resulted in the most negative effects on sporulation and germination of *N. floridana* may reduce disease transmission and development of epizootics. Boykin et al. (1984) demonstrated that application of Benomyl and Mancozeb (Dithane) reduced the incidence and efficiency of *N. floridana* in *Tetranychus urticae* Koch

in peanut fields. Brandenburg and Kennedy (1983) also reported a reduced incidence of *N. floridana* in *T. urticae* after application of the fungicides Benomyl and Chlorothalonil and associated this effect to inhibition of conidial germination by these fungicides.

Acaricides such as Propargite, which do not inhibit sporulation but affect primary conidia germination, may have a moderate effect on the fungus in the field compared to those pesticides that inhibit sporulation because the life span of a primary conidium is much shorter than the life span of a mummified mite. However, any pesticide that inhibits the formation of capilliconidia, the only infective spores of *N. floridana*, may have an impact on the overall mite control. The impact of Propargite on *N. floridana* can only be determined conclusively with results from a field experiment.

No effects on infectivity of the capilliconidia was observed from the pesticides after exposure. Seemingly, some pesticides inhibit sporulation or germination of primary conidia, but the capilliconidia produced under the exposure of these pesticides mantain the potential to infect their hosts. Viability of conidia is very important because the power of the fungus to kill its hosts depends on this factor as only viable conidia have the capacity to germinate and adhere to healthy hosts.

It was expected that once the mites feed on pesticide contaminated leaves, they could ingest and accumulate the pesticides that may inhibit vegetative growth of the fungus and reduce mite mortality due to infection. Since the control mites were not subjected to pesticide contaminated leaf discs, higher mortality due to the fungus was anticipated. However, mortality in treatments with the insecticide Methomyl and the fungicide Captan was similar to the mortality in the controls suggesting that the pesticides did not affect fungal development.

The effect of pesticides on *N. floridana* was higher when immersed than sprayed and this is probably associated with the amount of the product that the fungus is exposed to, despite being of equal concentration. Differences between the controls observed in the germination study were attributed to independent incubation of control lots together with each pesticide group. It is also possible that Tween 80, the surfactant used in the two controls, could have been the cause of differential germination because more of the products could be retained on the coverslips when they were immersed than sprayed.

Although the spray tower method may give comparable results to field application of pesticides, the equipment may not be readily available in many laboratories, as a result, its use in pesticide testing may be limited. However, the effect of direct immersion of leaf discs or cadavers into pesticide solutions is stronger and may not reflect a field situation, but it represents a rapid method to assess both direct and indirect effects of these pesticides on the fungus and may assist in making quick decisions on the pesticides to be applied during pest attack. Also, if a product is considered compatible with the pathogen in this laboratory method (worst scenario) it may warrant selectivity in the field. The same line of thought applies to differences observed between maximum concentration and half the concentrations recommended for field application. A higher concentration in the laboratory that does not affect the fungi has higher chances of being non-toxic in the field than a low concentration that is toxic under laboratory conditions.

An important consideration in the use of laboratory methods is the determination of how accurately they represent field conditions. However, it is unlikely that pesticides which affect the fungus at low concentrations in in vitro tests will fail to produce effects under recommended field concentrations. Given that high toxicity of chemical products in laboratory experiments does not always reveal high toxicity in the field, the laboratory tests are useful and indicate the possibility of the effects that may occur in the field (Alves et al. 1998). Field applications of pesticides usually achieve less-than-perfect coverage, perhaps providing spatial refugia for entomopathogenic fungi. Apart from the presence of refuges,

spatial heterogeneity, photodegradation and removal by rain could be possible reasons for reduced exposure of the fungus to toxic compounds and this could be the reason why in vitro studies and field studies may not always produce similar results (Jaros-Su et al. 1999).

The effect of pesticides on *Neozygites* spp. has been studied mostly in the field (Boykin et al. 1984; Wells et al. 2000). Field studies are usually limited to a small number of products and it takes a long time to reveal any differences in the infection levels or the density of propagules in the soil. For this reason, there is need for the generation of laboratory data on the effect of pesticides on specific aspects of the fungus such as sporulation, germination and viability. However, this has been hampered by lack of a defined protocol to test this fungus without growing it on artificial media. The laboratory tests described here simulate an in vivo situation and allow the flexibility of dosing a pesticide under controlled conditions. These tests also bypass the process of growing *N. floridana* on artificial media. The results obtained using these methods indicate that the insecticide Methomyl, and the acaricide Abamectin produced varied effects on *N. floridana* with both inhibiting sporulation at CR where leaf discs were immersed but not when sprayed. Methomyl also reduces infectivity when leaf discs are immersed and not when sprayed. Thus, these pesticides may not affect the inoculum potential of the *N. floridana* in the field and may be compatible with conservation strategies of pest control. Lambda-Cyhalothrin has a mild effect on conidia germination of *N. floridana* when the coverslips are immersed and this effect substantially reduces when they are sprayed. The acaricide Propargite strongly affects germination just like the fungicides Mancozeb and Captan both of which affect sporulation and may not be compatible with *N. floridana.*

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