Research Article

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The effect of synthetic pesticides and sulfur used in conventional and organically grown strawberry and soybean on *Neozygites floridana*, a natural enemy of spider mites

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Abstract

BACKGROUND: The beneficial fungus *Neozygites floridana* kills the two-spotted spider mite *Tetranychus urticae*, which is a serious polyphagous plant pest worldwide. Outbreaks of spider mites in strawberry and soybean have been associated with pesticide applications. Pesticides may affect *N. floridana* and consequently the natural control of *T. urticae*. *N. floridana* is a fungus difficult to grow in artificial media, and for this reason, very few studies have been conducted with this fungus, especially regarding the impact of pesticides. The aim of this study was to conduct a laboratory experiment to evaluate the effect of pesticides used in strawberry and soybean crops on *N. floridana*.

RESULTS: Among the pesticides used in strawberry, the fungicides sulfur and cyprodinil + fludioxonil completely inhibited both the sporulation and conidial germination of *N. floridana*. The fungicide fluazinam affected *N. floridana* drastically. The application of the fungicide tebuconazole and the insecticides fenpropathrin and abamectin resulted in a less pronounced negative effect on *N. floridana*. Except for epoxiconazole and cyproconazole, all tested fungicides used in soybean resulted in a complete inhibition of *N. floridana*. Among the three insecticides used in soybean, lambda-cyhalothrin and deltamethrin resulted in a significant inhibition of *N. floridana*.

CONCLUSION: The insecticides/ acaricides abamectin and lambda-cyhalothrin at half concentrations and fenpropathrin and permethrin and the fungicide tebuconazole at the recommended concentrations resulted in the lowest impact on *N. floridana*. The fungicides with the active ingredients sulfur, cyprodinil + fludioxonil, azoxystrobin, azoxystrobin + cyproconazole, trifloxystrobin + tebuconazole and pyraclostrobin + epoxiconazole negatively affected *N. floridana*.

Keywords: non-target effects; conservation biological control; integrated pest management; *Tetranychus urticae*; organic farming

1 INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), has a broad host plant range and is an important pest of many crops globally, including strawberries and soybeans.[1−3] *T. urticae* lowers crop yields by feeding on mesophyll and parenchyma cells, which results in a reduction in photosynthesis and transpiration.[4,5] Densities higher than five *T. urticae* per leaflet significantly reduce fruit production.[6]

Pesticides are frequently used to control pests and plant pathogenic fungi in strawberry and soybean crops. Acaricides are the main strategy used to control *T. urticae* in field crops; however, the pesticide application rate is often higher than the recommended rate, and pest population levels often peak after pesticide treatments. Some authors have suggested that increases in *T. urticae* populations after pesticide treatment are caused by the reduction in natural predators.[7−9] Klingen and Westrum[10] also report that the reduction in the fungal pathogen *Neozygites*

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floridana (Weiser and Muma) Remaudière and S. Keller (Neozygitomycetes: Neozygitales: Neozygitaceae) is associated with an increase in *T. urticae* populations. In Brazil, the spider mites *T. urticae* and *Mononychellus planki* became important pests in soybean after the introduction of soybean rust disease and as a result of the extensive use of fungicides in this crop.[11]

N. floridana has been observed infecting several species of Tetranychidae mites in various agroecosystems.[12] The genus *Neozygites* has a restricted host range[13–15] and consists of only obligate pathogens that are difficult to maintain in artificial culture media.[16] *N. floridana* is reported to be a major factor causing a decline in populations of *T. urticae* in corn, cotton, peanut and strawberry crops, and infection levels as high as 80–100% have been reported.[17–20] Conservation biological control by preservation of naturally occurring *N. floridana*, along with the judicious use of pesticides, as defined by Eilenberg *et al.*,[21] is a promising strategy to manage *T. urticae*.

N. floridana develops inside *T. urticae* as hyphal bodies, kills its host, penetrates the cuticle and produces primary conidia on conidiophores on the exterior of *T. urticae*. Primary conidia are actively ejected from the swollen brown cadavers, referred to as mummified mites. These conidia germinate to form the infective and more persistent capilliconidia that infect other mites.[22]

Only a few controlled laboratory studies have been conducted on the effect of pesticides to *N. floridana* as a natural enemy of *T. urticae*.[10,23] Some field or semi field studies have been conducted with *T. urticae*, however,[20,24] and a few with another host species, *T. evansi*.[25,26] In this study, we tested sulfur-based pesticides in addition to many other synthetic pesticides. To our knowledge, sulfur have until now not been tested against insect- and mite pathogenic fungi of the Neozygitales. According to Jepsen *et al.*,[27] sulfur is commonly used by grape growers against plant pathogenic fungi both in organic and conventional crop systems because it is effective and inexpensive, and resistance to sulfur by plant pathogenic fungi is not/seldom reported. Furthermore, sulfur is reported to have acaricidal and insecticidal effects,[28] and there is evidence that it may have a negative impact on the fecundity and longevity of *T. urticae*.[29] Nevertheless, sulfur applications are commonly associated with outbreaks of spider mites, apparently because sulfur is deleterious to predatory mites.[30] Another possible explanation is that sulfur is detrimental to *N. floridana*.

Integrated pest management (IPM) is a practice that has been increasingly adopted in various agroecosystems to reduce the use of pesticides, avoid problems such as pest resurgence, resistance evolution and public concern about the negative effects of chemical pesticides on human health and the environment.[31,32] In IPM, natural enemies are one of the tools used for alternative pest control. However, synthetic pesticides are still needed and used in IPM strategies, and it is important to seek the use of pesticides that are efficient against pests but selective to natural enemies.[33]

The purpose of this study was to evaluate the effect of synthetic fungicides, acaricides and insecticides commonly used in strawberry and soybean in Brazil and in strawberry crops in Norway on the production of primary conidia and capilliconidia by the beneficial fungus *N. floridana*. Further, we tested the effect of sulfur-based pesticides, which are widely used against plant pathogenic fungi in both conventional and organic farms. Soybean pesticides used in Brazil were also included owing to the recent outbreaks of spider mites in this crop, most likely associated with the extensive use of fungicides against the soybean rust detected in this country in 2001.

2 MATERIALS AND METHODS

In order to test the effect of several pesticides on the *N. floridana* isolate ESALQ1420, two experiments were performed. In the first experiment, 12 pesticides (two acaricides, nine fungicides and one insecticide) that are commonly used to control pests and diseases in strawberry crops in Brazil and/or Norway were tested. In the second experiment, nine pesticides (three insecticides and six fungicides) used in soybean crops in Brazil were tested (Table 1). The pesticides were applied at two concentrations: (1) the recommended concentration on the product label (RC); (2) half of this concentration (RC/2), to mimic field dilution factors (moisture/dew point). The pesticides were diluted in distilled water with 0.05% Tween 80. Additionally, distilled water plus 0.05% Tween 80 was used as the control treatment, giving a total of 25 treatments for the first experiment and 19 for the second experiment. The first experiment was repeated three times, using a randomised complete block design with occasion as a complete block containing 25 treatments, and as a plot a petri dish with five mummified mites. The second experiment was a completely randomised experiment with 19 treatments and five replicates, and as a plot a petri dish with one mummified mite.

The *N. floridana* isolate ESALQ1420 was collected in Piracicaba, São Paulo, Brazil (22° 42′ 30″ S, 47° 38′ 00″ W), from *T. urticae* on jack bean, *Canavalia ensiformis* (Fabales: Fabaceae). The mummified mites were placed individually on photoetched cover slips (18 × 18 mm) with alphanumeric coded squares (Electron Microscopy Sciences, Hatfield, PA), which were placed on a 9 cm filter paper lining the petri dish.

For the pesticides used in strawberry, a computer-controlled sprayer (Bukard Manufacturing Co. Ltd, Rickmansworth, Herts, UK) was used for pesticide application, and pesticides were applied at a rate of 2.6 mg cm^{-2} at a pressure of 0.5 psi. For the soybean pesticides, a Potter spray tower (Burkard Manufacturing Co. Ltd) was used. The device was adjusted to a pressure of 0.7 psi, with an average of 1.5 mg cm^{-2}. To avoid contamination between pesticide treatments, the sprayers were washed 3 times with water, alcohol, acetone and distilled water, and the first spray of each treatment was discarded. To obtain an RH suitable for *N. floridana* production of primary conidia (sporulation) and capilliconidia (germination), the sprayed mummified cadavers on sprayed coverslips were placed inside closed plastic boxes (17.5 × 11.0 × 4.0 cm) containing filter paper moistened with distilled water. The boxes containing the different treatments were wrapped in aluminium foil for darkness and put into climatic chambers at 25 ± 2 °C and 100% RH for 12 h in total darkness.

In order to determine the effect of pesticides on the sporulation and germination of *N. floridana*, coverslips were observed under a microscope (400×), and the numbers of primary conidia and capilliconidia produced by the mummified cadavers were counted. Germinated primary conidia were defined as capilliconidia. The response variables used were total counts and total proportion of germination of the five mummified cadavers in the first experiment.

2.1 Data analysis

Quasi-Poisson models were fitted to sporulation (the production of primary conidia) data with the effect of block and treatment for tests with strawberry pesticides (first experiment) and the effect of treatment as the linear predictor for soybean pesticides (second experiment). Quasi-binomial models were fitted to the proportion of capilliconidial data with the same linear

Table 1. Specifications of the pesticides (commonly used in strawberry and soybean) applied in the bioassays to determine the effect of pesticides on the production of primary conidia (sporulation) and capilliconidia (germination) of *N. floridana*

Active ingredient	Trade name (manufacturer)	Type of pesticide (chemical group)[a]	Recommended concentration[c] per 100 L
Abamectin[a]	Vertimec 18 EC (Syngenta)	Acaricide/insecticide/nematicide (avermectin)	75 mL
Azoxystrobin[a]	Amistar 250 CS (Syngenta)	Fungicide (strobylurin)	13 g
Azoxystrobin[b]	Priori CS (Syngenta)	Fungicide (strobylurin)	200 mL
Azoxystrobin + cyproconazole[b]	Priori Xtra CS (Syngenta)	Fungicide (strobylurin + triazol)	200 mL
Cyproconazole[b]	Alto 100 CS (Syngenta)	Fungicide (triazol)	200 mL
Cyprodinil + fludioxonil[a]	Switch 62.5 WG (Syngenta)	Fungicide (anilinopyramidine + phenylpyrrole)	50 g
Deltamethrin[b]	Decis EC (Bayer)	Insecticide (pyrethroid)	200 mL
Epoxiconazole[b]	Opus CS (BASF)	Fungicide (triazol)	400 mL
Fenpropatrin[a]	Danimen 300 EC (Sumimoto)	Insecticide/acaricide (pyrethroid)	65 mL
Fluazinam[a]	Shirlan CS (Syngenta)	Fungicide (dinitroanaline)	100 mL
Iprodione[a]	Rovral 75 WG (Bayer)	Fungicide (dicarboximid)	150 g
Lambda-cyhalothrin[b]	Karate Zeon 50 CS (Syngenta)	Insecticide (pyrethroid)	50 mL
Penconazole[a]	Topas 100 EC (Syngenta)	Fungicide (triazol)	25 mL
Permethrin[b]	Talcord 250 EC (BASF)	Insecticide (pyrethroid)	80 mL
Procymidone[a]	Sumilex 500 WP (Sumimoto)	Fungicide (dicarboximid)	100 g
Propargite[a]	Omite 720 EC (Chemtura)	Acaricide (alkylchloride sulfite)	30 mL
Pyraclostrobin + epoxiconazole[b]	Opera EC (BASF)	Fungicide (carbamate + triazol)	400 mL
Sulfur[a]	Thiovit Jet WG (Syngenta)	Fungicide (inorganic)	300 g
Sulfur[a]	Kumulus WG (BASF)	Fungicide (inorganic)	300 g
Tebuconazole[a]	Folicur 200 EC (Bayer)	Fungicide (triazol)	75 mL
Trifloxystrobin + tebuconazole[b]	Nativo CS (Bayer)	Fungicide (strobylurin + triazol)	333 mL

[a] Pesticide used in strawberry.
[b] Pesticide used in soybean.
[c] All recommended concentrations were obtained from the product labels.

predictor.[34] The quasi-likelihood approach was used because the data presented overdispersion, and hence *F*-tests were performed to assess significance of effects. Goodness of fit was assessed using half-normal plots with simulated envelopes.[35] Treatment differences were tested using 95% confidence intervals. All analyses were performed using the R statistical software environment (R Foundation for Statistical Computing, Vienna, Austria).[36]

3 RESULTS

3.1 Effect of pesticides used in strawberry on *N. floridana* sporulation and germination

Among the strawberry pesticides tested, the fungicides sulfur (Kumulus WG) and cyprodinil + fludioxonil completely inhibited the sporulation of *N. floridana*, even at half concentration (RC/2) (Table 2). The fungicide fluazinam also reduced sporulation drastically at half concentration and completely inhibited sporulation at the recommended concentration (RC). Neither the fungicide tebuconazole and the insecticide/acaricide fenpropathrin at both concentrations nor the insecticide/acaricide abamectin at half concentrations significantly affected *N. floridana* sporulation. However, at the recommended concentration, abamectin showed a significant and drastic reduction in the production of primary conidia compared with the control.

All the strawberry pesticides tested affected germination (production of capilliconidia) significantly compared with the control at both concentrations; only tebuconazole at RC/2 showed no difference. The fungicides that resulted in no sporulation (the sulfur product Kumulus WG and cyprodinil + fludioxonil) did not result in any germination (production of capilliconidia). Further, the fungicides fluazinam, iprodione and the sulfur product Thiovit Jet WG and the acaricide propargite at both concentrations resulted in no or almost no germination. Both concentrations of the fungicides penconazole, azoxystrobin and procymidone also affected the formation of capilliconidia significantly. Tebuconazole at the recommended concentration and abamectin and fenpropathrin at both concentrations also reduced the production of capilliconidia significantly, although less than mentioned above.

3.2 Effect of pesticides used in soybean on *N. floridana* sporulation and germination

With the exception of two, all soybean fungicides tested resulted in a complete inhibition of *N. floridana* sporulation and germination (Table 3). The fungicide epoxiconazole did not result in a significant reduction in the production of primary conidia and capilliconidia compared with the control. Production of primary conidia in cadavers sprayed with cyproconazole was halved compared to the control. Among the three insecticides tested, lambda-cyhalothrin resulted in a significant inhibition of sporulation and germination at full concentrations, and deltamethrin resulted in a significant inhibition of germination at both concentrations.

4 DISCUSSION

Our results indicate that all fungicides tested reduced the sporulation and/or germination of the fungal pathogen *N. floridana*, while the acaricides/insecticides tested seemed to be less detrimental to this beneficial fungus. The effect of the different products on *N. floridana* varied considerably. Among all pesticides tested in this study, only cyprodinil + fludioxonil, lambda-cyhalothrin and

Table 2. Effect of pesticides used in strawberry on the sporulation (production of primary conidia ± SE) and germination (production of capilliconidia ± SE) of *N. floridana*. Pesticides are applied at half the recommended concentration (RC/2) and at the recommended concentration (RC)

Active ingredient (trade name)[a]	Mean umber of primary conidia per mummified mite ± SE[b]		Percentage capilliconidial formation ± SE[b]	
	RC/2	RC	RC/2	RC
	$F_{19,36} = 7.05$, $P < 0.01$		$F_{13,22} = 18.61$, $P < 0.01$	
Control	1834 ± 125 a	1834 ± 125 a	85 ± 2 A	85 ± 2 A
Abamectin (Vertimec 18 EC)[A]	1283 ± 169 abc	13 ± 10 f	38 ± 12 BC	17 ± 9 D
Azoxystrobin (Amistar 250 CS)[F]	158 ± 67 ef	350 ± 135 cdef	2 ± 1 D	7 ± 4 D
Cyprodinil + fludioxonil (Switch 62.5 WG)[F]	0	0	0	0
Fenpropathrin (Danimen 300 EC)[A/I]	1057 ± 201 abcd	1129 ± 298 abcd	36 ± 2 C	37 ± 11 C
Fluazinam (Shirlan CS)[F]	80 ±70 f	0	0	0
Iprodione (Rovral 75 WG)[F]	363 ± 92 bcdef	124 ± 41 f	2 ± 0.1 D	0
Penconazole (Topas 100 EC)[F]	413 ± 121 bcdef	615 ± 202 abcdef	10 ± 4 D	6 ± 6 D
Procymidone (Sumilex 500 WP)[F]	163 ± 78 cdef	116 ± 75 f	7 ± 2 D	4 ± 4 D
Propargite (Omite 720 EC)[A]	119 ± 31 f	142 ± 142 ef	0	0
Sulfur (Kumulus WG)[F]	0	0	0	0
Sulfur (Thiovit Jet WG)[F]	162 ± 84 def	177 ± 103 cdef	0	0
Tebuconazole (Folicur 200 EC)[F]	1051 ± 186 abcde	1558 ± 308 ab	65 ± 1 AB	37 ± 11 C

[a] A = acaricide; F = fungicide; I = insecticide.
[b] Different letters between rows denote significant differences using quasi-Poisson models for sporulation (the production of primary conidia) data with the effects of block. Quasi-binomial models were fitted to germination (the production of capilliconidia) data with the same linear predictors ($P \leq 0.01$). Treatments with only zeros were removed from the analysis.

Table 3. Effect of two concentrations of pesticides used in soybean on the sporulation (production of primary conidia) and germination (production of capilliconidia) of *N. floridana*. Pesticides are applied at half the recommended concentration (RC/2) and at the recommended concentration (RC)

Active ingredient (trade name)[a]	Mean umber of primary conidia per mummified mite ± SE[b]		Percentage capilliconidial formation ± SE[b]	
	RC/2	RC	RC /2	RC
	$F_{10,47} = 6.64$, $P < 0.01$		$F_{8,39} = 24.25$, $P < 0.01$	
Control	1598 ± 155 a	1598 ± 155 a	73 ± 3 A	73 ± 3 A
Azoxystrobin (Priori CS)[F]	0	0	0	0
Azoxystrobin + cyproconazole (Priori Xtra CS)[F]	0	0	0	0
Cyproconazole (Alto 100 CS)[F]	609 ± 52 b	605 ± 65 b	32 ± 3 B	0.11 ± 0.04 D
Deltamethrin (Decis 25 EC)[I]	1119 ± 145 ab	899 ± 381 ab	26 ± 4 BC	6 ± 4 C
Epoxiconazole (Opus CS)[F]	821 ± 126 ab	787 ± 159 ab	60 ± 12 A	58 ± 5 A
Lambda-cyhalothrin (Karate Zeon 50 CS)[I]	985±337 ab	39 ± 12 c	83 ± 5 A	0
Permethrin (Talcord 250 EC)[I]	1242 ± 213 ab	1292 ± 335 ab	76 ± 1 A	74 ± 4 A
Pyraclostrobin + epoxiconazole (Opera EC)[F]	0	0	0	0
Trifloxystrobin + tebuconazole (Nativo CS)[F]	0	0	0	0

[a] F = fungicide; I = insecticide.
[b] Different letters between rows denote significant differences using quasi-Poisson models for sporulation (the production of primary conidia) data with the effect of treatment as the linear predictor for soybean. Quasi-binomial models were fitted to germination (the production of capilliconidia) data with the same linear predictors (P ≤ 0.01). Treatments with only zeros were removed from the analysis.

propargite that were previously tested on *N. floridana* originated from *T. evansi* in Brazil[26] and from *T. urticae* in Norway.[10] However, in these studies, different methods of pesticide application were used. Cyprodinil + fludioxonil showed a complete inhibition of sporulation in our study, and in the studies by Klingen and Westrum[10] it reduced both the sporulation and the efficacy of the fungus against *T. urticae* (increased the median lethal time). Wekesa *et al.*[26] studied the effect of lambda-cyhalothrin applied at a concentration of 0.4 mL L^{-1} and observed that capilliconidial formation was reduced by 16.8% compared with the control. In the present study, there was a total inhibition of capilliconidial formation when exposed to this insecticide. This may be due to the fact that we tested a different fungal strain in our experiment that was isolated from *T. urticae*, while Wekesa *et al.*[26] isolated from *T. evansi*. Further, we used a higher concentration (0.5 mL L^{-1}). The acaricide propargite did not inhibit sporulation, but it did affect the germination of primary conidia.

The negative effects of other fungicides on *N. floridana* had also been demonstrated by Boykin *et al.*[17] using benzimidazole (Benomyl) and dithiocarbamate (Mancozeb). They demonstrated that these products reduced the incidence and efficiency of *N. floridana* on *T. urticae* in peanut fields. Brandenburg and Kennedy[24] also reported a lower incidence of *N. floridana* in *T. urticae* after application of the fungicides benzimidazole (Benomyl) and chlorothalonil (Termil) and attributed this effect to the inhibition of capilliconidial formation by these fungicides in cornfields. Klingen and Westrum[10] conducted controlled laboratory studies on the killing capacity of *N. floridana* to *T. urticae* and reported that most of the fungicides they tested may potentially reduce the survival and efficacy of *N. floridana*, while some of the acaricide/insecticide/molluscicide products they tested seemed to have a stimulating effect on this beneficial fungus. The tests were conducted by letting *N. floridana*-infected *T. urticae* feed on strawberry leaves treated with different pesticides (three fungicides and one acaricide). Hence the effect of the pesticides was measured using an indirect method (through feeding/contact with the leaf by the mite) and not directly on the fungus itself, as in the present studies. The methodology in our study simulated a worst-case scenario and investigated the mode of action of pesticides in two parts of the life cycle of the fungus. The studies by Klingen and Westrum[10] further showed that the fungicide tolylfluanid (Euparen M) and the acaricide methiocarb (Mesurol 500 CS) did not reduce the mortality of *N. floridana*-inoculated mites when compared with the control mites. However, tolylfluanid lowered the sporulation of the fungus to 15.5%. Fenhexamid (Teldor 50 WG) also reduced the mortality of fungus-inoculated mites.

Studies conducted by Wekesa *et al.*[26] with pesticides used in tomatoes also showed that the fungicides [Orthocide (Captan) and Dithane (Mancozeb)] tested on *N. floridana* isolates from *T. evansi* (red tomato spider mite) resulted in a high reduction in sporulation and germination. The insecticide methomyl (Lannate) and the acaricide abamectin (Abamex) resulted in less pronounced effects on *N. floridana*. Two different methods were used in their studies: (1) fungus-killed mite cadavers were either immersed or sprayed with the pesticides; (2) the substrates used for sporulation (leaf discs and coverslips) were either immersed or sprayed with the pesticides.

If a pesticide is considered to be compatible with *N. floridana* in laboratory bioassays (maximum exposure), we can assume that it will also be selective in the field. Further, we suggest that the acaricide abamectin and the insecticide lambda-cyhalothrin at half concentrations and the insecticides fenpropathrin and permethrin and the fungicide tebuconazole at the recommended concentrations may be the most suitable for use in *N. floridana* conservation biological control as part of an IPM programme because these pesticides resulted in the lowest impact on this beneficial fungus. In contrast, a pesticide resulting in negative effects on natural enemies in laboratory bioassays may not always necessarily result in negative effects in the field. This can be attributed to the fact that the entomopathogenic fungi may find refuge spaces within the crop, as pesticide applications in the field do not result in full coverage. Therefore, based on the present study, and considering that *N. floridana* is important in the natural population regulation of two-spotted spider mite, we suggest that farmers must apply with caution the pesticides sulfur, cyprodinil + fludioxonil, azoxystrobin, azoxystrobin + cyproconazole, trifloxystrobin + tebuconazole and pyraclostrobin + epoxiconazole until more comprehensive field studies are conducted. The sulfur-based fungicides Thiovit Jet WG and Kumulus WG were detrimental to *N. floridana*. Sulfur-based fungicides are extensively used by organic farmers, and there are few control alternatives against plant pathogenic fungi for organic farmers. The impact of sulfur on this beneficial fungus is reported for the first time here, and this information should be considered in IPM and organic cropping systems.

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