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ScienceDirect
Biological Control 43 (2007) 222–230
Biological Control
www.elsevier.com/locate/ybcon

The effect of pesticides used in strawberries on the phytophagous mite *Tetranychus urticae* (Acari: Tetranychidae) and its fungal natural enemy *Neozygites floridana* (Zygomycetes: Entomophthorales)

Ingeborg Klingen *, Karin Westrum

Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Department of Entomology and Nematology, Høgskoleveien 7, N-1432 Ås, Norway

Received 20 March 2007; accepted 20 July 2007
Available online 15 August 2007

Abstract

Neozygites floridana is an important natural enemy of the two-spotted spider mite, *Tetranychus urticae*. Pesticides used in strawberries that might affect the conservation and enhancement of this beneficial fungus were therefore studied. This was done in a laboratory study by letting non-inoculated (healthy) mites and mites inoculated with *N. floridana* feed on strawberry leaf disks treated with one of the following pesticides: the fungicides tolylfluanid, fenhexamid or cyprodinil + fludioxonil or the acaricide/insecticide/molluscicide methiocarb. The effect of these pesticides on mortality and egg production of *T. urticae* and on the killing capacity and sporulation of *N. floridana* were determined. Tolylfluanid increased the mortality of non-inoculated mites (75.3%) compared to the non-inoculated control (27.5%). Methiocarb also killed non-inoculated mites. Fenhexamid did not have any effect on the mortality of non-inoculated mites (19.2%), neither had cyprodinil + fludioxonil (19.1%). Tolylfluanid did not reduce the mortality of mites inoculated with *N. floridana* (89.3%) compared to the inoculated control (80.0%). Neither did methiocarb, it rather increased the mortality of inoculated mites (93.2%). Fenhexamid did, however, reduce the mortality of inoculated mites (66.7%). The same was true for cyprodinil + fludioxonil (48.7%). In addition, cyprodinil + fludioxonil increased the time to death of inoculated mites (6.69 days) compared to the control (6.10 days), and inhibited sporulation of *N. floridana* (7.9% sporulation) compared to the control (42.4% sporulation). Tolylfluanid also reduced sporulation of *N. floridana* (15.5% sporulation). Results from this study indicate that the use of the fungicides tested will potentially reduce the survival and efficacy of the natural enemy *N. floridana* in the field.

Keywords: Pesticides; Side effects; Natural enemy; Conservation biological control; *Neozygites floridana*; *Tetranychus urticae*

1. Introduction

The two-spotted spidermite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an annual pest of many cultivated crops, and it often reaches pest population levels following pesticide treatments. Many authors suggest that increases in *T. urticae* populations after pesticide treatment is caused by the destruction of predator natural enemies (Hoyt et al., 1978; Bentley et al., 1987; Shanks et al., 1992). Several also seem to agree with the view of Huffaker et al. (1970) that "much evidence exists to the effect that other factors than the destruction of enemies may have profound effects on or be a principal cause of mite outbreaks in some situations", and pesticide effects on spider mite dispersal, reproduction, development rate, diapause and resistance are mentioned (Dittrich et al., 1974; Boykin and Campbell, 1982; Penman and Chapman, 1988; Trichilo and Wilson, 1993; Ayyappath et al., 1997; James and Price, 2002; Merabet et al., 2002; Ako et al., 2004; Alston and Thomson, 2004). Other biological factors are also reported to affect *T. urticae* populations, the presence of plant pathogenic fungi being one (Reding et al., 2001; Duso et al.,

* Corresponding author. Fax: +47 64 94 61 10.
E-mail address: ingeborg.klingen@bioforsk.no (I. Klingen).

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doi:10.1016/j.biocontrol.2007.07.013

2003, 2005; Meszka and Labanowska, 2006). None of these studies discuss the possible effect of pesticides to microbial natural enemies such as the mite pathogenic fungus *Neozygites floridana* (Weiser and Muma) Remaudière and S. Keller (Zygomycetes: Entomopthorales). We suggest, however, that it is important to study both predators and pathogens as natural enemies when considering control of *T. urticae*.

Conservation biological control as defined by Eilenberg et al. (2001) may be one way of controlling *T. urticae* by *N. floridana*. According to van der Geest et al. (2000), it is worthwhile to investigate the efficiency of *N. floridana* with respect to conservation biological control strategies. This may be done by the judicious use of pesticides to avoid harm to *N. floridana*. Attempts have been made in soybean in USA to prevent pesticide treatment of mite populations that have already been suppressed by fungal epizootics (Klubertanz et al., 1991). A similar approach is seen for the related insect pathogenic fungus, *Neozygites fresenii* (Nowakowski), infecting cotton aphid, *Aphis gossypii* Glover. Growers withhold insecticide application for aphids when they expect *N. fresenii* epizootics to control the cotton aphid (Hollingsworth et al., 1995). Predictions of potential suppression of mite populations by fungal pathogens should be made only based on solid knowledge about the complex of factors affecting pathogen activity and mite population growth (Klubertanz et al., 1991). The importance and interaction of these factors are not yet well known in most systems.

Neozygites floridana develops inside *T. urticae* as hyphal bodies, kills its host, penetrates the cuticle and produces spores (primary conidia) on conidiophores on the outside of *T. urticae*. Primary conidia are actively ejected from swollen brown cadavers, referred to as mummies. These conidia germinate to form the infective and more persistent capilliconidia that infects new mites (Carner, 1976; Elliot, 1998; Delalibera et al., 2006). *Neozygites* sp. are reported to be a major factor causing decline in populations of *T. urticae* under climatic conditions that are favourable for the fungus (moist periods during night) in corn, soybean, cotton and peanut fields, and infection levels as high as 80–100% are reported (Boykin et al., 1984; Carner and Canerday, 1970; Smitley et al., 1986; Klubertanz et al., 1991; Dick and Buschman, 1995). Other studies also report *N. floridana* infections in *T. urticae* populations in strawberries, and infection levels up to 90% are found (Mietkiewski et al., 1993,2000; Nordengen and Klingen, 2006; Klingen et al., 2007). Macroclimatic conditions that seem to be to dry for mite- and insect pathogenic fungi might still provide the fungus with a microclimatic humidity which is high enough for the establishment of the fungus in a host mite- or insect population. This is elegantly shown by Fargues et al. (2003). Further, *Neozygites tanajoa* Delalibera, Humber and Hajek has been documented to cause epizootics in the cassava green mite, *Mononychellus tanajoa* (Bondar) in dry regions in Brazil (Delalibera et al., 2006).

Some field studies have looked at the effect of pesticides on field prevalence of *N. floridana*, and both reduced and non-reduced prevalence were reported (see Table 1). To our knowledge only one laboratory study has been conducted on the effect of one fungicide (benomyl) to *N. floridana* (Brandenburg and Kennedy, 1983). To be able to conserve *N. floridana* in an agro-ecosystem, it is important to conduct laboratory studies that reveal specific effects of pesticides on this beneficial fungus. Laboratory studies offers a greater level of control than field experiments, and therefore provide a baseline for studying the effect of pesticides on fungal efficacy under the more complex and variable environmental conditions found in the field.

Tetranychus urticae is known to be a serious pest of strawberries and it is an increasing problem in strawberries grown in tunnels. Preliminary studies conducted in Norwegian strawberry fields have shown that *N. floridana* is commonly found as a natural enemy of *T. urticae* (Klingen et al., 2007). The aim of the current study was therefore to assess the potential effects pesticides used in strawberries have on *T. urticae* and its natural enemy *N. floridana*. These effects were accessed through detailed laboratory experiments on *T. urticae* egg production, *T. urticae* mortality, the killing capacity of *N. floridana* to *T. urticae*, days to death of fungal infected *T. urticae* and sporulation of the fungus on its host.

Fungicides are widely used in strawberries, and since they are known to be more detrimental to insect- and mite pathogenic fungi than acaricides, insecticides and herbicides (Klingen and Haukeland, 2006) we focused the study on the effect of the most commonly used fungicides in strawberries, namely tolylfluanid, fenhexamid and cyprodinil + fludioxonil. All three fungicides tested are reported to have different biochemical profiles in plant pathogenic fungi (Table 2). Based on biochemistry, it was therefore difficult to know what effects to expect. It is reported, however, that tolylfluanid and fenhexamid + fludioxinil are used against a wide range of plant pathogenic fungi (Table 2), and it was therefore possible that they would affect *N. floridana* negatively. On the other hand, *N. floridana* is in a different subdivision (Zygomycotina) than plant pathogenic fungi controlled by tolylfluanid and cyprodinil + fludioxonil, and may therefore respond differently to these fungicides. Fenhexamid affects a narrower host range of plant pathogenic fungi, and might be expected to be less detrimental to *N. floridana* than the other two fungicides. We also wanted to determine the effect of a commonly used acaricide/insecticide, and since methiocarb is widely used against the strawberry mite *Phytonemus pallidus* (Banks), it was included in the study. All pesticides used in the present study, except from fenhexamid, have been tested in field studies for their effect on *T. urticae* (Gould and Jessop, 1981; Merabet et al., 2002; Meszka and Labanowska, 2006), but only tolylfluanid have been tested in detailed laboratory studies. To our knowledge, none of these pesticides have been tested for their effect on *N. floridana*.

Table 1
Pesticides tested for their effect on *T. urticae* and its natural enemies, *N. floridana* and predators

Active ingredient (common name)	Type of pesticide Biochemistry Mode of action Use Class[a]	Effect on *N. floridana*	Effect on predators	Effect on *T. urtice*
Ammonical copper	Fungicide Thiol reactant Foliar, protective Wide range Inorganic	*Peanut, field study:* No effect on *N. floridana* infection level (Boykin et al., 1984)	*Peanut, field study:* No effect on predator population (Arachnidae and Insecta) (Boykin et al., 1984)	*Peanut leaves, laboratory study:* Suppression, lower r_m (the maximum rate of potential population increase) than control (Boykin and Campbell, 1982)
Benomyl	Fungicide Tubulin reactant Systemic, protective and curative Wide range Benzimidazole	*Peanut, field study:* Reduced *N. floridana* infection level (Boykin et al., 1984). *Lima bean, fields and laboratory studies:* Suppressed *N. floridana* (Brandenburg and Kennedy 1983). *Corn, field study:* Reduced *N. floridana* infection level (Brandenburg and Kennedy, 1982)	*Peanut, field study:* No effect on predator population (Arachnidae and Insecta) (Boykin et al., 1984)	*Peanut leaves, laboratory study:* Suppression, lower r_m than control (Boykin and Campbell, 1982). *Corn, field study:* No effect (Brandenburg and Kennedy, 1982)
Chlorothalonil	Fungicide Thiol reactant Non-systemic, protective Wide range Chloronitrile	*Corn, field study:* Reduced *N. floridana* infection level (Brandenburg and Kennedy, 1982)		*Corn, field study:* No effect (Brandenburg and Kennedy, 1982)
Fenthin hydroxide	Fungicide Inhibit respiration, attack membrane Non-systemic, protective and curative Wide range Organotin	*Peanut, field study:* No effect on *N. floridana* infection level (Boykin et al., 1984)	*Peanut, field study:* No effect on predator population (Arachnidae and Insecta) (Boykin et al., 1984)	*Peanut leaves, laboratory study:* Supression, lower r_m than control (Boykin and Campbell, 1982)
Mancozeb	Fungicide, acaricide Thiol reactant Non-systemic, protective Wide range Dithiocarbamate	*Peanut, field study:* Reduced *N. floridana* infection level (Boykin et al., 1984)	*Peanut, field study:* No effect on predator population (Arachnidae and Insecta) (Boykin et al., 1984)	*Peanut leaves, laboratory study:* Stimulation, higher r_m than control (Boykin and Campbell, 1982)

Maneb	Fungicide Thiol reactant Non-systemic, protective Wide range Dithiocarbamate	*Corn, filed study:* Reduced *N. floridana* infection level (Smitley et al., 1986)		*Corn, filed study:* Limited effect (Smitley et al., 1986)
Carbaryl	Insecticide Cholinesterase inhibitor Contact and stomach act. Wide range Carbamate	*Peanut, field study:* No effect on *N. floridana* infection level (Boykin et al., 1984)	*Peanut, field study:* No effect on predator population (Arachnidae and Insecta) (Boykin et al., 1984). Corn, field study: Reduced predator population when sprayed with frequent intervals (every week) (Smitley et al., 1986)	*Kidney beans, laboratory study:* Enhanced egg production (Dittrich et al., 1974). *Peanut leaves, laboratory study:* Stimulation, higher r_m than control (Boykin and Campbell, 1982)

[a] Parry (1990), Tomlin (2000), and Anonymous (2007).

2. Materials and methods

2.1. T. urticae stock culture

Tetranychus urticae was collected in a commercial strawberry field (cultivar Zephyr) at Ås, in southeastern Norway (59°42′N, 10°44′E) in 2003. The stock culture was maintained on strawberry plants, *Fragaria* × *ananassa* (Duch), at 22 ± 1 °C, 50–70% RH and L16:D8.

2.2. N. floridana isolate

Strawberry leaves with *T. urticae* cadavers infected with *N. floridana* were collected in 2004 in the same field as *T. urticae*. Even though *in vitro* culture of *N. floridana* is possible (Leite et al., 2000; Delalibera et al., 2003), it is still on an experimental scale and we therefore established an *in vivo* laboratory culture of the isolate NCRI 271/04 used in the bioassay. This was done by placing collected cadavers in humid and dark conditions for sporulation and infection of *T. urticae*. The *in vivo* culture was kept on *T. urticae* on strawberry (cultivar Corona) at room temperature (21–24 °C), ambient light conditions or darkness during sporulation, and varying RH depending on the life cycle stage of the fungus. For detailed description of the *in vivo* production of *N. floridana* on *T. urticae*, see below.

2.3. Bioassay, effect of pesticides on N. floridana and T. urticae

The effect of pesticides on *T. urticae* egg production, *T. urticae* mortality, the killing capacity of *N. floridana* to *T. urticae*, days to death of fungal infected *T. urticae* and sporulation of the fungus on this host were tested in this bioassay. The experiment was set up with both inoculated and non-inoculated *T. urticae*.

To ensure that the pesticide was in mites at the start of the experiment, they were fed on treated strawberry leaves prior to the start of the experiment. This was done by dipping two fully expanded trifoliate strawberry leaves (cultivar Corona) in each of the pesticides at labelled recommended concentrations given in Table 2 or in water (control). To prevent trifoliate leaves from wilting, the petioles of the two leaves were stuck through the lid of a vial (100 ml) containing water. Vials with leaves of each treatment were placed separately inside a plastic box (31.0 × 22.5 × 12.5 cm) covered with a lid to provide a humidity that was high enough (between 50% and 65% RH) to keep the leaves from wilting. At least 100 young adult *T. urticae* females were then transferred with a paint brush to trifoliate leaves treated with fenhexamid or cyprodinil + fludioxonil or water to feed for 48 h. Old dark females were not used. A higher number of mites were transferred to trifoliate leaves treated with tolylfluanid (200 adult females) or methiocarb (150 adult females), since significant percentages, about 50% for tolylfluanid and 30% for methiocarb, died prior to the inoculation

Table 2
Pesticides tested in the bioassay

Active ingredient	Trade name (Manufacturer)	Type of pesticide Biochemistry Mode of action Use Class[a]	Concentration (% active ingredient)
Tolylfluanid	Euparen M (Bayer CropScience)	Fungicide (acaricide) Thiol reactant Foliar, protective Controls a wide range of pathogens, side effect on mites Sulphamide	0.5 g in 0.1 l water (50.5%)
Fenhexamid	Teldor WG 50 (Bayer CropScience)	Fungicide Sterol C-4 demethylase inhibitor Non-systemic, foliar, protective Controls *Botytis cinerea*, *Monilia* spp. Hydroxyanilide	0.15 g in 0.1 l water (50.0%)
Cyprodinil + fludioxonil	Switch 62.5 WG (Syngenta)	*Cyprodinil:* Fungicide Methionine reactant Systemic, foliar Controls a wide range of pathogens Alininopyrimidine *Fludioxonil:* Fungicide Glucose phosphorylation inhibitor Non-systemic, foliar and seed treatment Controls a wide range of pathogens Phenylpyrrole	0.05 g in 0.1 l water (Cyprodinil = 37.5%, fludioxonil = 25.0%)
Methiocarb	Mesurol 500 SC (Bayer CropScience)	Acaricide/insecticide/molluscicide Cholinesterase inhibitor Non-systemic, contact and stomach action Controls a wide range of pests Carbamate	0.2 ml in 0.1 l water (50.0%)

[a] Parry (1990), Tomlin (2000) and Anonymous (2007).

with *N. floridana* because these pesticides also have an acaricidal effect.

After feeding on treated leaves for 48 h, half of the mites were inoculated with *N. floridana* capilliconidia by using a method modified after Delalibera and Hajek (2004): Three cadavers of *T. urticae* from a *N. floridana in vivo* laboratory culture were placed in the bottom of a Cryo tube lid (2.0 ml NUNC No. 343958) inside a Petri-dish (9 cm diameter and 2.5 cm high). Water covered the bottom of the Petri-dish to provide high humidity. The Petri-dish was placed in a plastic box ($22.0 \times 17.0 \times 7.0$ cm) covered with a lid, wrapped in aluminium foil for darkness, and kept at room temperature (21–24 °C) for 48 h to stimulate sporulation and production of capilliconidia. A large headed (3 mm diameter) pin was pierced into the bottom of the Cryo tube lid, and the lid was placed on a strawberry leaf with the under side up on moist cotton in a plastic box ($17.5 \times 11.0 \times 4.0$ cm). To prevent disturbance of capilliconidia in the bottom of the lid, mites from treated leaves were transferred to the pinhead by a paint brush. The mites then moved from the pinhead, down the needle and onto the bottom of the Cryo tube lid where capilliconidia were present. Capilliconidia could then attach to the legs and the body of *T. urticae*. *T. urticae* stayed in the Cryo tube lid or on the strawberry leaf for 24 h (21–24 °C and 100% RH) for germination and infection of the conidia. The Cryo tube lid with capilliconidia and mites was then tilted on the strawberry leaf and mites crawled out of the lid and onto the leaf. The mites moved better when the RH was low, hence, the plastic box lid was removed so that mites would move more readily. Thirty mites fed on treated trifoliate leaves and inoculated with *N. floridana* were then transferred individually to treated strawberry leaf disks (15 mm in diameter) placed with the underside up on 1.5% water agar (10 ml) in 30 ml vials with lids. Six holes were made in the lid of the vials with insect pin no. 0 for aeration. The other half of the mites that had been feeding on pesticide treated leaves were not inoculated with *N. floridana*, but otherwise treated as described above. There were 30 individual mites in each treatment, except from the methiocarb treatment where a number of mites were killed by feeding on treated leaves prior to the start of the experiment. The experiment was repeated three times.

Vials with mites on leaf disks were placed at 25 ± 1 °C and L16:D8. The RH inside vials, were considered to be 100% because of condensation on the walls of the vials.

Starting 3 days after transfer to individual leaf disks, mites were checked for mortality and sporulation using compound microscope (40–80×) daily for 5 days (8 days post-inoculation). The first day of observation (4 days post-inoculation), leaf disks with mites were also checked for numbers of *T. urticae* eggs.

2.4. Statistical analysis

Standard methods for χ^2 tests (PROC FREQ, SAS Institute Inc., 1989) were used to compare mortality of *T. urticae* inoculated with *N. floridana* and non-inoculated *T. urticae* when treated with different pesticides. Standard methods for χ^2 tests were also used to compare sporulation of *N. floridana* infected *T. urticae* cadavers when treated with different pesticides. Data from 7 days post-inoculation, when most infected mites had been killed by the fungus (see Table 4), were used since non-inoculated control mortality rose by 6.6 % the last day of the experiment (day 8 post-inoculation).

The LSD test (PROC GLM, SAS Institute Inc., 1989) was used to compare number of eggs produced by *T. urticae* inoculated with *N. floridana* and non-inoculated *T. urticae* when treated with different pesticides. The LSD test was also used to compare average number of days to death for *N. floridana* infected *T. urticae* when treated with different pesticides.

3. Results

3.1. Mortality of non- inoculated T. urticae

Tolylfluanid increased mortality of non-inoculated *T. urticae* ($\chi^2 = 36.83$, $df = 1$, $P < 0.0001$) compared to the control. Fenhexamid did not increase mortality of non-inoculated *T. urticae* ($\chi^2 = 1.51$, $df = 1$, $P = 0.2198$), neither did cyprodinil + fludioxonil ($\chi^2 = 1.64$, $df = 1$, $P = 0.1997$). Methiocarb caused about 30% mortality of non- inoculated *T. urticae* during the first 48 h of feeding on treated leaves prior to the start of the experiment. The resulting 70% of *T. urticae* that survived these first 48 h of feeding on metiocarb-treated leaves were used in the experiment, and 28.0% were killed. This was not significantly different from the control ($\chi^2 = 0.03$, $df = 1$, $P = 0.8647$) (Table 3).

3.2. Mortality of N. floridana inoculated T. urticae

Tolylfluanid did not reduce the mortality of mites inoculated with *N. floridana* compared to the inoculated control ($\chi^2 = 2.80$, $df = 1$, $P = 0.0944$). Fenhexamid reduced the mortality of mites inoculated with *N. floridana* ($\chi^2 = 3.90$, $df = 1$, $P = 0.0482$). The same applied for cyprodinil + fludioxonil ($\chi^2 = 17.35$, $df = 1$, $P < 0.0001$). Methiocarb increased the mortality of mites inoculated with *N. floridana* compared to the control ($\chi^2 = 4.89$, $df = 1$, $P = 0.0271$) (Table 3).

Table 3
Mortality of non-inoculated and Neozygites floridana inoculated Tetranychus urticae females feeding on leaves treated with different pesticides[a]

Treatment	Non-inoculated *T. urticae*	*N. floridana* inoculated *T. urticae*
Water only	27.5b	80.0b
Tolylfluanid	75.3a	89.3ab
Fenhexamid	19.2b	66.7c
Cyprodinil + fludioxonil	19.1b	48.7d
Mesurol	28.8b	93.2a

[a] Different letters between rows denote significant differences using χ^2 tests ($P \leqslant 0.05$).

3.3. Days to death and sporulation of N. floridana infected T. urticae

Cyprodinil + fludioxonil increased number of days to death for *N. floridana* infected *T. urticae* ($T = 1.97$, $df = 4$, $P = 0.05$) while methiocarb reduced the life span of *T. urticae* infected with *N. floridana* ($T = 1.97$, $df = 4$, $P = 0.05$) compared to the control. Tolylfluanid ($\chi^2 = 14.82$, $df = 1$, $P = 0.0001$) and cyprodinil + fludioxonil ($\chi^2 = 24.71$, $df = 1$, $P < 0.0001$) reduced sporulation of *N. floridana* compared to the control, while fenhexamid increased it ($\chi^2 = 3.93$, $df = 1$, $P = 0.05$) (Table 4).

3.4. Egg production of T. urticae

Fewer eggs were produced by mites inoculated with *N. floridana* than by non-inoculated mites ($T = 1.97$, $df = 1$, $P < 0.0001$) (Table 5).

Tolylfluanid resulted in reduced egg production for non-inoculated mites compared to the non-inoculated control ($T = 1.97$, $df = 4$, $P \leqslant 0.050$). This was also the case for methiocarb and cyprodinil + fludioxonil ($T = 1.97$, $df = 4$, $P \leqslant 0.050$). Fenhexamid did not have any significant effect on numbers of eggs produced by non-inoculated mites ($T = 1.97$, $df = 4$, $P \leqslant 0.050$).

Tolylfluanid resulted in reduced egg production for *N. floridana* inoculated mites compared to the inoculated control ($T = 1.97$, $df = 4$, $P \leqslant 0.050$). Methiocarb also reduced

Table 4
Effect of pesticides on days to death and percent sporulation for *T. urticae* inoculated with *N. floridana*

Treatment	Mean days from inoculation to death (±SE)[a]	Percent sporulating cadavers 7 days post-inoculation[b]
Water only	6.10 (±0.13)b	42.4b
Tolylfluanid	5.96 (±0.23)b	15.5c
Fenhexamid	5.98 (±0.10)b	57.5a
Cyprodinil + fludioxonil	6.69 (±0.22)a	7.9c
Methiocarb	5.28 (±0.15)c	52.7

[a] Different letters in the column denote significant differences using multiple *t*-tests and $P \leqslant 0.05$.

[b] Different letters in the column denote significant differences using χ^2 tests at $P \leqslant 0.05$. For Methiocarb, 25% of the cells had expected counts less than 5. Hence χ^2 was not a valid test and no test was conducted.

Table 5
Number of eggs produced in one day by non-inoculated and *Neozygites floridana* inoculated *Tetranychus urticae* females feeding on leaves treated with different pesticides[a]

Treatment	Non-inoculated *T. urticae* (±SE)	*N. floridana* inoculated *T. urticae* (±SE)
Water only	27.8 (±1.3)a	15.3 (±1.4)b
Tolylfluanid	8.5 (±0.8)c	6.5 (±0.8)c
Fenhexamid	25.8 (±1.4)ab	12.8 (±1.1)b
Cyprodinil + fludioxonil	23.7 (±1.2)b	18.8 (±1.4)a
Mesurol	8.6 (±0.8)c	3.8 (±0.6)c

[a] Different letters between rows denote significant differences using multiple *t*-tests ($P \leqslant 0.05$).

egg production ($T = 1.97$, $df = 4$, $P \leqslant 0.050$). Fenhexamid did not have any significant effect on numbers of eggs produced by inoculated mites ($T = 1.97$, $df = 4$, $P \leqslant 0.050$), but cyprodinil + fludioxonil enhanced egg production for inoculated mites compared to controls ($T = 1.97$, $df = 4$, $P \leqslant 0.050$).

4. Discussion

Our results indicate that all fungicides tested may potentially reduce the survival and efficacy of the natural enemy *N. floridana*, while the acaricide/insecticide/molluscicide tested seemed to have a stimulating effect on this beneficial fungus. The negative effect of the different fungicides on *N. floridana* as well as their effect on mortality and egg production of *T. urticae* varied.

Even though all three fungicides can be considered to be harmful to *N. floridana*, tolulfluanid (chemical group: sulphamide) might be the one causing least harm, since it did not significantly reduce the mortality of mites inoculated with *N. floridana*. However, tolulfluanid did reduce sporulation of *N. floridana*, and might therefore indirectly be harmful since reduced sporulation will subsequently inhibit establishment of *N. floridana* in *T. urticae* populations. On the other hand, tolulfluanid is known to have an acaricidal effect on *T. urticae* (Merabet et al., 2002). This was also confirmed in our experiments since both increased mortality and reduced *T. urticae* egg production were observed for mites treated with tolulfluanid. On the negative side, tolulfluanid is also known to have an acaricidal effect on predatory mites (Raudonis et al., 2004). Merabet et al. (2002) report, however, only a limited negative effect of tolulflyanid to the predatory mite *Phytoseiulus persimilis* used in *Rubus* and *Ribes* species. De Maeyer et al. (1993) suggested that a long established balance between the predator mite *Typholodromus pyri* and the spider mite *Panonychus ulmi* in apple orchards is not disturbed by multiple tolulfluanid applications.

Other fungicides are also known to affect mites, and Alston and Thomson (2004) found, in laboratory studies, that benomyl (benzimidazole) increased mortality and fecundity of both *T. urticae* and the predatory mite *Galendromus occidentalis* (Nesbitt). Boykin et al. (1984) reported that the fungicides ammonial copper (inorganic), mancozeb (dithiocarbamate), and fentin hydroxide (organotin) did not reduce predators (mites or insects) of *T. urticae* in peanuts. Furthermore, they suggested that predators were not responsible for regulating *T. urticae* populations in peanuts but that *N. floridana* was. It is also suggested that predators (insects) are not a major naturally occurring mortality factor of *T. urticae* in strawberries (Cross et al., 2001). Links between the use of pesticides and the effect on *N. floridana* as a natural control factor are often not considered. One example is Shanks et al. (1992) who observed that numbers of fungicide applications in raspberries correlated with increased *T. urticae* numbers. Their explanation is that growers who use fungicides liberally also use insecticides liberally, and that *T. urticae* numbers increase because *T. urticae* predators are killed by insecticides. One of the fungicides they used (benomyl) is, however, known to suppress *N. floridana* (Table 1). We therefore suggest that a better explanation of the results obtained by Shanks et al. (1992) should include the negative effect of fungicides to *N. floridana*.

Our laboratory results suggest that fenhexamid (hydroxyanlilide) and cyprodinil (anlinpyrimidine) + fludioxinil (penylpyrrole) are probably not good choices for the conservation biological control of *T. urticae* by *N. floridana*, since they reduced the mortality of mites inoculated with *N. floridana*. We also showed that cyprodinil + fludioxinil reduced sporulation of *N. floridana* and increased number of days to death for infected mites. This further emphasizes the inhibitive effect of cyprodinil + fludioxinil to *N. floridana*. Fenhexamid did not extend the number of days to death and it increased sporulation. These results suggest that fenhexamid is less inhibitive to *N. floridana* than cyprodinil + fludioxinil. The fungicides benomyl (benzimidazole), maneb (dithiocarbamate) and mancozeb (dithiocarbamate) are also known to suppress *N. floridana* (see Table 1).

Our results showed that fenhexamid and cyprodinil + fludioxinil did not have an acaricidal effects to *T. urticae*. Alston and Thomson (2004) also report that the fungicides triflumizole (DMI-imidazole), myclobutanil (DMI-triazole) and trifloxystrobin (strobilurin) do not have acaricidal effects to *T. urticae*. Even though cyprodinil + fludioxinil did not kill *T. urticae*, this fungicide reduced the egg production in our laboratory experiment. Reduced egg production may result in reduced *T. urticae* populations in the field. Meszka and Labanowska (2006) indicated that treating strawberry plants with cyprodinil + fludioxinil for the control of grey mold (*Botrytis cinera*) resulted in reduced two-spotted spider mite populations. One explanation of their results could be that reduced grey mold attack, due to the effectiveness of cyprodinil + fludioxinil, also results in reduced *T. urticae* densities. This hypothesis is supported by Reding et al. (2001) who found evidence that the presence of plant pathogens, specifically powdery mildew, stimulated densities, incidence and reproduction of *T. urticae*. On the other

hand, predatory mites are known to feed on plant pathogenic fungi and Duso et al. (2003, 2005) report that the predatory mites *Amblyceius andersoni* (Chant), *Typholodromus pyri* Scheuten and *Tydeus caudatus* Dugès seems to be enhanced by the presence of downy mildew, *Plasmopara viticola* (Berk. and Curtis ex de Bary) Berlese and De Toni, in vineyards. No relationship was found, however, between downy mildew spread and spider mite, *Panonychus ulmi* (Koch) abundance (Duso et al., 2003). Even though cyprodinil + fludioxinil reduced the egg production of non-inoculated *T. urticae*, it increased the egg production of mites inoculated with *N. floridana*. This is probably because the inhibitive effect to *N. floridana* outweighs the effect cyprodinil + fludioxinil has on reducing the egg production of *T. urticae*.

Some insecticides and acaricides are known to inhibit deuteromycetous insect pathogenic fungi (Klingen and Haukeland, 2006), but this was not the case with methiocarb (carbamate) in our experiment. Our results rather suggest a stimulation of *N. floridana*. To our knowledge, only one other study on the effect of an insecticide or acaricide to *N. floridana* has been conducted (Boykin et al., 1984). Aslo in their study the insecticide carbaryl, which is in the same chemical group as methiocarb, showed no effect on *N. floridana* infection level in *T. urticae* populations (see Table 1). Even though methiocarb is used mainly against the strawberry mite *P. pallidus* in strawberries, it is also known to have an additional effect to *T. urticae* (Gould and Jessop, 1981). This was also the case in our study, if we look at mites killed prior to experimental start. Numbers of mites killed during the experiment were not different from the control, however. This may suggest that the laboratory population of *T. urticae* tested in our study had a tolerance to methiocarb.

In conservation biological control of *T. urticae* both predators and the mite pathogenic fungus *N. floridana* needs to be taken into consideration. In IPM systems knowledge about pesticides affecting both these groups of natural enemies is important. This study suggest that the effects of fungicides to *N. floridana* might be just as relevant as the effects of insecticides/acaricides to predatory mites when considering conservation biological control of *T. urticae*. The interactions between *T. urticae*, their natural enemies, plant pathogenic fungi, pest management systems, climatic conditions and crop are numerous and complex, and therefore often studied separately. To further understand how we can develop good conservation biological control system of *T. urticae*, including both groups of natural enemies, a system approach where the importance and interaction of these factors are studied in detail is needed.

Acknowledgments

We thank Dr. Italo Delalibera Jr. for discussions and inputs during the planning of the experiment. Thanks to Dr. Richard Meadow and Dr. Arne Stensvand who peer reviewed this paper. The Norwegian Research Council supported the work financially.

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