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Subscriptions: Year 2023 (Volume 63): 450 €
http://www1.montpellier.inra.fr/CBGP/acarologia/subscribe.php
Previous volumes (2010-2021): 250 € / year (4 issues)
Acarologia, CBGP, CS 30016, 34988 MONTFERRIER-sur-LEZ Cedex, France
ISSN 0044-586X (print), ISSN 2107-7207 (electronic)

The digitalization of Acarologia papers prior to 2000 was supported by Agropolis Fondation under the reference ID 1500-024 through the « Investissements d’avenir » programme (Labex Agro: ANR-10-LABX-0001-01)

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Side-effects of a number of insecticides on predatory mites in apple orchards

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Proceedings of the 9\textsuperscript{th} Symposium of the EurAAc, Bari, July, 12\textsuperscript{th}–15\textsuperscript{th} 2022

ABSTRACT

**Background:** *Amblyseius andersoni* is a common predatory mite occurring in fruit orchards located in Europe and North America. Its role in preventing spider mite outbreaks is widely recognized, in particular when selective pesticides are used. The compatibility between plant protection products and predatory mites is crucial to preserve their activity. There is a need to investigate the effects of pesticides on beneficials using multiple approaches. **Objectives:** Field and laboratory experiments were conducted to evaluate the effects of a number of insecticides on *A. andersoni*. **Methods:** The effects of neonicotinoids (i.e., acetamiprid, imidacloprid, thiacloprid, thiamethoxam) were compared with those of pyrethroids (i.e., tau-fluvalinate), well known for their negative impact on predatory mites. Insecticides were applied 1-3 times in an experimental fruit orchard located in Northern Italy. Laboratory trials focused on their effects on the survival and the fecundity of predatory mite females. **Results:** Field experiments showed a decline in predatory mite numbers in plots treated with neonicotinoids or tau-fluvalinate compared to the untreated control. However, predatory mites in neonicotinoid plots reached higher densities compared to those recorded in tau-fluvalinate plots. Spider mite (*Panonychus ulmi*) populations reached moderate to high densities in plots treated with tau-fluvalinate while their densities were negligible in the remaining plots. *Amblyseius andersoni* survival was moderately affected by some neonicotinoids in the laboratory while their densities were negligible in the remaining plots. **Conclusions:** Neonicotinoid applications significantly affected predatory mite densities in field conditions and this phenomenon appeared to be influenced by their impact on female fecundity. Their effects on survival were less severe. Implications of these results for IPM tactics in fruit orchards are discussed.

**Keywords** insecticide side-effects; lethal and sub-lethal effects; predatory mites; Phytoseiidae; Integrated Pest Management

Introduction

Despite progress in reducing pesticide use in fruit growing areas of Europe and North America, insecticides are still requested to control aphids, scales and stink bugs (Ioriatti et al. 2019; Beers et al. 2019). Insecticide side-effects on beneficials can promote outbreaks of secondary pests since of their impact on natural antagonists. Infestations of phytophagous mites in fruit orchards...
are a clear example of this syndrome: mite pests are commonly controlled by predators, mainly by phytophagous mites (Acarina, Phytoseiidae) where pesticide use is minimal or selective pesticides are used (Blommers 1994; Croft 1994; Beers et al. 2016a, b; Schmidt-Jeffris et al. 2019). A number of predatory mite species have been considered non-target organisms in trials aimed at evaluating the effects of pesticides on beneficiaries and various approaches have been adopted in this field of research (Bergeron and Schmidt-Jeffris 2020; Schmidt-Jeffris et al. 2021). The need to conduct field and laboratory trials has been stressed since longtime (e.g., Sterk et al. 1999; Bostanian et al. 2009). The variety of pesticide side-effects, from lethal to sublethal as well from direct to indirect, on beneficials has suggested new experimental models (e.g., Desneux et al. 2007; Stavrinides and Mills 2009; Duso et al. 2020). In addition, it has been recognized that the compatibility of pesticides with conservation biological control tactics based on predatory mites should be developed at a local level (Bostanian et al. 2010; Lefebvre et al. 2011, 2012; Pozzebon et al. 2014).

In the last two decades, reduced risk-insecticides (e.g., neonicotinoids) have been presented as an alternative to broad-spectrum insecticides but their side-effects on beneficials have been matter of discussion (e.g., Calvo-Agudo et al. 2019; Furlan et al. 2021). In this framework the side-effects of neonicotinoids on predatory mites represent an interesting case-study (e.g., Bostanian et al. 2009, 2010; Zanuzo Zanardi et al. 2017). The side-effects of four neonicotinoids on the predatory mite *Amblyseius andersoni* (Chant) have been investigated in field and laboratory conditions. *Amblyseius andersoni* is a common predatory mite occurring in fruit orchards in Europe and North America (Ivancich Gambaro 1975; Genini et al. 1991; Messing and Croft 1991; Blommers 1994; Szabó et al. 2014). Strains resistant to conventional pesticides (organophosphates, carbamates, pyrethroids) in European fruit orchards have been reported for this species since the 1970s (Ivancich Gambaro 1975; Anber and Overmeer 1988; Anber and Oppenoorth 1989; Duso et al. 1992; Bonafos et al. 2007). The replacement of broad-spectrum insecticides by reduced risk-insecticides represents an interesting scenario for studies on the compatibility between pesticides and beneficial organisms.

### Material and methods

#### Field studies

The effects of insecticides on *A. andersoni* populations were evaluated in an apple orchard located at the experimental station of E. Mach Foundation (FEM, S. Michele all’Adige, Trento, Italy) in the 2009 growing season. Five insecticides commonly applied in apple orchards were considered (Table 1). An untreated control was included for comparison.

Insecticides were applied once (12 May), twice (9 June) or three times (8 July) in separate blocks according to codling moth control timing. A completely randomized design was followed with four replicates per treatment; each replicate consisted of 15 plants. Sampling was carried out before and every 5-10 days after insecticide applications (for about one month from the last application). A total of 60 leaves per treatment (15 leaves per replicate) were removed and transferred to the laboratory where predatory and phytophagous mites were

<table>
<thead>
<tr>
<th>IRAC Group</th>
<th>Chemical sub-group</th>
<th>Active ingredient</th>
<th>Trademark</th>
<th>Dose</th>
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<td>Pyrethroids</td>
<td>Tau-fluvalinate</td>
<td>Kliartan® 20EW</td>
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</tbody>
</table>

counted under a dissecting microscope. Phytoseiid specimens were mounted on slides, in Hoyer’s medium, and identified under a phase contrast microscope.

Data were analyzed with a linear mixed repeated measures model with the MIXED procedure of SAS® (ver. 9.4; SAS Institute, 2016). Mite densities were considered as response variables with repeated measures made at different times, i.e., sampling dates. Using an F test ($\alpha = 0.05$) we evaluated the effect of insecticide application, time and their interaction. Degrees of freedom were estimated using the Kenward–Rogers method (Littell et al. 1996). Differences among treatments were evaluated with a $t$-test ($\alpha = 0.05$) to the least-square means with the Tukey’s adjustment. Slice option of the LSMEANS statement was used for the F-test partition of interactions between insecticide application and time. Data were checked for the analysis’ assumptions and square-root transformation was applied.

**Laboratory studies**

Insecticides applied in field trials were tested at the same concentrations in the laboratory. Apple leaves were treated with insecticides using a Potter Burkard tower (1.7±0.1 mg/cm$^2$ of insecticide solution) and then mated $A. andersoni$ females of same age were transferred onto the leaves to expose them to fresh insecticide residues. To prevent mite escape and leaf desiccation, leaves were placed onto wet cotton prior to mite transfer. *Tetranychus urticae* Koch eggs and females were provided every day as food for predatory mites. The experimental units were kept in a climatic chamber at 25±2 °C, 70±10% relative humidity and 16L:8D photoperiod. Effect of insecticides on female survival was evaluated after 2 and 72 h. Surviving females were observed daily for additional 4 days to assess pesticide effects on fecundity. Escaped or drowned females were removed from the initial number. In total we assessed 45–50 females per insecticide.

We analyzed the data with a linear model using the GLM procedure of SAS® (ver. 9.4; SAS Institute, 2016). An F test ($\alpha = 0.05$) was used to evaluate the effect of insecticides on mite survival, fecundity and escaping rate (number of escaped or drowned females/initial number of females). Treatments were compared using Tukey–Kramer test ($\alpha = 0.05$). The Blümel and Hausdorf (2002) formula was used for fecundity calculation. In order to meet the models’ assumptions, data on survival were arcsin-transformed while log $x+1$ transformation was applied to data on fecundity.

**Results**

**Field studies**

In the first experiment (single insecticide application) there were no differences among treatments prior to insecticide applications ($F_{5,202} = 0.67; P = 0.648$). Later, insecticides affected predatory mite populations compared with the control ($F_{5,70.2} = 22.1; P < 0.0001$; Figure 1; Table 2). The effects of time and interaction treatment * time were also significant (respectively: $F_{4,160} = 110.64; P < 0.0001$; $F_{20,172} = 4.01; P < 0.0001$). Predatory mite populations were lower in tau-fluvalinate than in neonicotinoid plots and among the latter in acetamiprid than in thiacloprid plots (Table 2). In this trial *P. ulmi* populations were not detected.

Also in the second experiment (two insecticide applications) there were no differences among treatments prior to the first insecticide application ($F_{5,228} = 0.76; P = 0.578$) while insecticides significantly reduced predatory mite populations compared with the control ($F_{5,72} = 46.99; P < 0.0001$; Figure 2; Table 3). The effects of time and interaction treatment * time were also significant (respectively: $F_{8,204} = 63; P < 0.0001$; $F_{40,209} = 3.17; P < 0.0001$). Predatory mite populations reached the lowest densities in tau-fluvalinate plots. Additional differences emerged among neonicotinoids: predatory mites were less abundant in acetamiprid than in thiamethoxam plots (Table 3). *Panonychus ulmi* populations were detected from...
mid-June onwards. There were more spider mites in tau-fluvalinate than in the remaining plots (F<sub>3, 36.2</sub> = 2.96; P = 0.025; Figure 3; Table 4). The effects of time and interaction treatment * time were also significant (respectively: F<sub>8, 182</sub> = 15.32; P < 0.0001; F<sub>40, 193</sub> = 2.67; P < 0.0001).

In the third experiment (three insecticide applications) there were no differences among treatments prior to the first insecticide application (F<sub>5, 154</sub> = 0.87; P = 0.50) while insecticides significantly reduced predatory mite populations compared with the control (F<sub>5, 49.1</sub> = 48.25; P < 0.0001; Figure 4; Table 5). The effects of time and interaction treatment * time were also significant (respectively: F<sub>12, 132</sub> = 29.34; P < 0.0001; F<sub>60, 120</sub> = 2.49; P < 0.0001). The lowest A. andersoni densities were reached in tau-fluvalinate plots. Moreover, there were less predatory mites in acetamiprid and imidacloprid than in thiacloprid plots (Table 5). Panonychus ulmi numbers increased from mid-June onwards in tau-fluvalinate than in the remaining plots (F<sub>5, 23.7</sub> = 4.65; P = 0.004; Figure 5; Table 6). The effects of time and interaction treatment * time were also significant (respectively: F<sub>12, 133</sub> = 5.98; P < 0.0001; F<sub>60, 119</sub> = 3.1; P < 0.0001).

Table 2 Results of pairwise t-test (α = 0.05) to the least-square means with the Tukey’s adjustment performed on Amblyseius andersoni densities observed in treatments receiving a single insecticide application. Asterisks (*) indicate significant differences between treatments (α = 0.05).
Figure 2  Seasonal abundance of *Amblyseius andersoni* in the second field experiment. Arrows indicate the spraying dates.

**Laboratory experiments**

Insecticides affected *A. andersoni* survival (F$_{5,38}$ = 4.04; P = 0.005; Figure 6), in particular tau-fluvalinate reduced female survival by 75%. Among neonicotinoids, there were no differences between thiamethoxam and the control while the remaining active ingredients were associated to intermediate effects (Figure 6). All insecticides reduced *A. andersoni* fecundity (F$_{5,30}$ = 2.96; P = 0.027) compared to the control (Figure 7). Escaping rate was also influenced by insecticides (F$_{2,38}$ = 3.92; P = 0.006) and the most relevant effects were caused by tau-fluvalinate (Figure 8). Only imidacloprid was not associated to adverse effects in terms of escaping rate.

**Discussion**

Field applications of tau-fluvalinate significantly reduced *A. andersoni* densities especially when repeated two-three times during the growing season. These observations confirm the results obtained on *Kampimodromus aberrans* (Oudemans) in fruit orchards located in the same

**Table 3** Results of pairwise t-test (α = 0.05) to the least-square means with the Tukey’s adjustment performed on *Amblyseius andersoni* densities observed in treatments receiving two insecticide applications. Asterisks (*) indicate significant differences between treatments (α = 0.05).
region (Duso et al. 2014). In the latter investigation, tau-fluvalinate caused 100% mortality on K. aberrans females in the laboratory. In the present study A. andersoni mortality was of about 75% but surviving females did not lay eggs. The detrimental effects of tau-fluvalinate on predatory mites have been recorded even on other species using different experimental procedures (Petit and Karan 1991; Bellows et al. 1992; Grout et al. 1997; Amin et al. 2009). Regarding sublethal effects, tau-fluvalinate significantly increased A. andersoni escaping rate, a phenomenon likely associated to repellency. It should be mentioned that pyrethroid residues can induce adverse effects (increased locomotory activity or escape) also on spider mites (Holland et al. 1994) with implications for mite outbreaks. Field concentrations of pyrethroids can disrupt predator–prey dynamics in apple orchards (Bostanian et al. 1985; Bowie et al., 2001) and this phenomenon was noticed in two of our field trials. Therefore, the use of tau-fluvalinate in fruit orchards requires a careful evaluation.

Most of insecticides tested in the present study belonged to neonicotinoids. Their application reduced A. andersoni densities in field conditions compared to the control plots, but these effects were less severe than those reported for tau-fluvalinate. The reduction in population

![Figure 3](image)

**Figure 3** Seasonal abundance of *Panonychus ulmi* in the second field experiment. Arrows indicate the spraying dates.

| Treatment         | Treatment     | Estimate | Standard Error | DF  | t Value | Pr > |t|
|-------------------|---------------|----------|----------------|-----|---------|------|
| Thiamethoxam      | Thiacloprid   | -0.01361 | 0.05075        | 36.2| -0.27   | 0.7902|
| Thiamethoxam      | Imidacloprid  | 0.000185 | 0.05075        | 36.2| 0       | 0.9971|
| Thiamethoxam      | Acetamiprid   | -0.02515 | 0.05075        | 36.2| -0.5    | 0.6232|
| Thiamethoxam      | Tau-fluvalinate | -0.1572 | 0.05075        | 36.2| -3.1    | 0.0038*
| Thiamethoxam      | Control       | 0.01174  | 0.06216        | 36.2| 0.19    | 0.8512|
| Thiacloprid       | Imidacloprid  | 0.01379  | 0.05075        | 36.2| 0.27    | 0.7874|
| Thiacloprid       | Acetamiprid   | -0.01154 | 0.05075        | 36.2| -0.23   | 0.8214|
| Thiacloprid       | Tau-fluvalinate | -0.1435 | 0.05075        | 36.2| -2.83   | 0.0076*|
| Thiacloprid       | Control       | 0.02535  | 0.06216        | 36.2| 0.41    | 0.6858|
| Imidacloprid      | Acetamiprid   | -0.02533 | 0.05075        | 36.2| -0.5    | 0.6207|
| Imidacloprid      | Tau-fluvalinate | -0.1573 | 0.05075        | 36.2| -3.1    | 0.0037*|
| Imidacloprid      | Control       | 0.01136  | 0.06216        | 36.2| 0.19    | 0.8535|
| Acetamiprid       | Tau-fluvalinate | -0.132  | 0.05075        | 36.2| -2.6    | 0.0134*|
| Acetamiprid       | Control       | 0.03689  | 0.06216        | 36.2| 0.59    | 0.5565|
| Tau-fluvalinate   | Control       | 0.1689   | 0.06216        | 36.2| 2.72    | 0.01*  |

Table 4 Results of pairwise t-test (α = 0.05) to the least-square means with the Tukey’s adjustment performed on *Panonychus ulmi* densities observed in treatments receiving two insecticide applications. Asterisks (*) indicate significant differences between treatments (α = 0.05).
size observed in our trials could be caused by the effect of neonicotinoids on predatory mite fecundity. Similar effects have been reported for other predatory mite species (Castagnoli et al. 2005; Villanueva and Walgenbach 2005; Bostanian et al. 2009). There were some differences between neonicotinoids, in particular acetamiprid proved to be less selective than other neonicotinoids and these effects could be related to a more pronounced reduction in fecundity observed in laboratory trials. Bostanian et al. (2009) reported a high toxicity of acetamiprid and imidacloprid to Galendromus occidentalis (Nesbitt) whereas thiamethoxam and thiacloprid showed slight or negligible effects. An increase in escaping rate was noticed for three out of four neonicotinoids suggesting some alternations in predatory mite behavior. Various sublethal and behavioral effects (included irritancy) have been reported for some neonicotinoids even if their implications for spider mite control are not always clear (e.g., Poletti et al. 2007; Beers and Schmidt-Jeffris 2015; Schmidt-Jeffris et al. 2021). Previous research found that irritability and repellency may favor the escape of phytoseiids from contaminated surfaces and seemed associated with the least selective products (Monteiro et al., 2019). In our case the highest escaping rates were associated with thiamethoxam and thiacloprid; implications of this

| Treatment 1 | Treatment 2 | Estimate | Standard Error | DF | t Value | Pr > |t|
|-------------|-------------|----------|----------------|----|---------|------|
| Thiamethoxam | Thiacloprid | -0.06287 | 0.06371 | 49.1 | -0.99 | 0.3286 |
| Thiamethoxam | Imidacloprid | 0.1025 | 0.06371 | 49.1 | 1.61 | 0.1141 |
| Thiamethoxam | Acetamiprid | 0.06855 | 0.06371 | 49.1 | 1.08 | 0.2872 |
| Thiamethoxam | Tau-fluvalinate | 0.6501 | 0.06371 | 49.1 | 10.2 | <.0001* |
| Thiacloprid | Control | -0.2903 | 0.06371 | 49.1 | -4.56 | <.0001* |
| Thiacloprid | Imidacloprid | 0.1654 | 0.06371 | 49.1 | 2.6 | 0.0124* |
| Thiacloprid | Acetamiprid | 0.1314 | 0.06371 | 49.1 | 2.06 | 0.0445* |
| Thiacloprid | Tau-fluvalinate | 0.713 | 0.06371 | 49.1 | 11.19 | <.0001* |
| Imidacloprid | Control | -0.2274 | 0.06371 | 49.1 | -3.57 | 0.0008* |
| Imidacloprid | Acetamiprid | -0.03395 | 0.06371 | 49.1 | -0.53 | 0.5965 |
| Imidacloprid | Tau-fluvalinate | 0.5476 | 0.06371 | 49.1 | 8.6 | <.0001* |
| Imidacloprid | Control | -0.3928 | 0.06371 | 49.1 | -6.17 | <.0001* |
| Acetamiprid | Tau-fluvalinate | 0.5816 | 0.06371 | 49.1 | 9.13 | <.0001* |
| Acetamiprid | Control | -0.3588 | 0.06371 | 49.1 | -5.63 | <.0001* |
| Tau-fluvalinate | Control | -0.9404 | 0.06371 | 49.1 | -14.76 | <.0001* |

Figure 4. Seasonal abundance of *Amblyseius andersoni* in the third field experiment. Arrows indicate the spraying dates.
phenomenon should be investigated more in depth.

In this study single or multiple applications of neonicotinoids were not associated with spider mite increases in the experimental season. The limited effect of neonicotinoids on *A. andersoni* survival in the laboratory confirms the results of experiments conducted on various predatory mites (James 1997; James and Vogele 2001; Poletti et al. 2007; Lefebvre et al. 2011; Duso et al. 2014). The negative effects of neonicotinoids on *A. andersoni* fecundity represent a serious risk for one of the most important objectives of IPM tactics: to preserve stable populations of predatory mites in fruit orchards. While most of these insecticides have been banned in Europe, their use is still significant in other continents.

It should be stressed that predatory mite species and strains exhibit a variation in their susceptibility to pesticides. In North America, *A. andersoni* proved to be less susceptible to imidacloprid than *Galendromus occidentalis* (Nesbitt) and *Neoseiulus fallacis* (Garman) (James 2003). The use of neonicotinoids could favor the less susceptible species (and strains) in predatory mite communities irrespectively of their adaptation to environmental factors. Climate change is also influencing the composition and structure of predatory mite communities in fruit orchards.

Table 6 Results of pairwise t-test (α = 0.05) to the least-square means with the Tukey’s adjustment performed on *Panonychus ulmi* densities observed in treatments receiving three insecticide applications. Asterisks (*) indicate significant differences between treatments (α = 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>DF</th>
<th>t Value</th>
<th>Pr &gt; [t]</th>
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Figure 5 Seasonal abundance of *Panonychus ulmi* in the third field experiment. Arrows indicate the spraying dates.
perennial crops. Experimental studies should be addressed to evaluate the impact of pesticides in different environmental scenarios.

Acknowledgements

The project was supported by the Grant “CRPV project: SELETTIVITÀ AGROFARMACI VS ORGANISMI UTILI (SAO)” of University of Bologna to G.A., M.B. and V.M; C.D. and A.P. were supported by DOR funds from the University of Padova.

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Figure 8  Escaping rate of Amblyseius andersoni females exposed to fresh residues of a number of insecticides. Different letters indicate significant differences at Tukey–Kramer test ($\alpha = 0.05$).

References


