# Physiological Selectivity of Agrochemicals to Predatory Mites of *Tetranychus urticae* (Acari: Tetranychidae) on Rosebushes Growing in Greenhouse

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***Abstract—*** *The growing of rose (Rosa spp.) in a greenhouse provides favorable conditions for both, the plant and the pest mite Tetranychus urticae Koch (Acari: Tetranychidae), for which chemical control is still used. Consumers' demand has encouraged researches to use less aggressive agricultural practices, making the biological control as a viable option. The objective of the present study was to investigate the physiological selectivity of plant protection products, used on rosebushes for the control of T. urticae and other pests or diseases, to Phytoseiulus macropilis (Banks) and Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) both predatory mites of T. urticae on rosebush growing in a greenhouse. According to IOBC/WPRS, the residual method of spraying on a glass and leaf surface area was used for the physiological selectivity test of plant protection products for the predatory mites. The obtained results shown that with the exception of the acaricides-insecticide chlorfenapyr all other tested products - fungicides, acaricides and acaricides-insecticide - methiram + pyraclostrobin, thiofanate-methyl, boscalid + kresoxim-methyl, chlorothalonil, propargite, mandipropamid, mefenoxam, difenoconazol, bifenthrin and pyriproxifen, were innocuous (class 1) or only slightly harmful (class 2) to both species. Chlorfenapyr was highly toxic only for N. californicus (class 4), however after five days of its application no toxic residue of the product was detected on the glass surface and so the product has been classified as innocuous (class 1) as well for this predatory mite species after this period.*

***Keywords—*** ***Neoseiulus californicus, Phytoseiulus macropilis, Rosa spp.,*** Protected crop, Agricultural acarology.

## I. INTRODUCTION

The flowers trade in Brazil is growing, so that it has been recognized as an emergent business sector of high profitability. In 2014, the sector gains were of approximately R$ 5.7 billion (US$ 1.42 billion, based on the exchange rate as R$4.00/dollar) and an 8% growth is estimated for the year of 2015 [1].

The state of Minas Gerais stands out in this sector, especially, for the production of rosebushes (*Rosa* spp., Rosaceae) and of other conventional cutting flowers. Although there is not available up-to-date data of this activity in Minas Gerais, the flower production in this state occurs especially in the municipalities of Barbacena, Andradas, Araxá, and Munhoz [2].

The rosebush from Asia has developed well in Brazil and is cultivated in various Brazilian regions. A large part of the production occurs in greenhouses. Although this environment offers better conditions for the control of pests and diseases, it is also a more favorable environment to the occurrence of these two issues with a special highlight to the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a pest mite that is one of the main problems that affect rosebushes in a protected cultivation system [3].

Pest control is one of the challenges found in the cultivation of flowers and others ornamental plants, being any damage caused by insects and other arthropods inacceptable by consumers, since it depreciates the final product, the flower and the foliage that will be commercialized [4].

The demand of producers and consumers for the use reduction of agrochemicals is very evident [5]. However, the spraying of plant protection products is still the main tactic used for the control of pests and diseases, being carried out in a preventive way [6] that in many instances causes ecological disequilibrium and environmental contamination [7], especially as a function of the misapplication of these products.

Consumers' concern about human health and environmental preservation has encouraged researchers to investigate the use of less aggressive agricultural practices geared towards sustainability and agroecosystems [6].

The biological control of pest mites has been used in fruit trees, ornamental plants, and other crops for the control of the two-spotted spider mite with the use of predatory mites *Neoseiulus californicus* (McGregor) and *Phytoseiulus macropilis* (Banks) (Phytoseiidae) among others. This biological control method reduces the amount of chemical defensives used in cultivations and holds other benefits such as a lesser exposure of the workers to chemicals and lower amounts of residues [8].

In addition, studies on the selectivity of plant protection products to predatory mites belonging to the Phytoseiidae family are of great importance, since mites of this family have been more widely used for the biological pest control [9] [10].

In a program of integrated pest management - IPM, the use of selective products is highly desirable under certain conditions, as when the pest is at excessively high levels [11].

Therefore, the objective of the present study was to investigate the physiological selectivity of plant protection products, used on rosebushes for the control of *T. urticae*, and other pests or diseases, on *P. macropilis* and *N. californicus* both predatory mites of the *T. urticae* on rosebush growing in greenhouse.

## II. MATERIAL AND METHODS

The present study was conducted in the Acarology Laboratory of the *Empresa de Pesquisa Agropecuária de Minas Gerais - EPAMIG-Sul / Centro de Pesquisa em Manejo Ecológico de Pragas e Doenças de Plantas - EcoCentro*, Lavras, MG, Brazil, under controlled conditions, at temperature of 25 ± 2ºC, RH of 70 ± 10%, and 14 hours of photophase.

The predatory mites, as well as the pest mite, *T. urticae*, were obtained from the *Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas - Campus de Inconfidentes*, Minas Gerais, Brazil.

### 2.1 Lab rearing of the two-spotted spider mite

Uncapped Petri dishes (15 cm in diameter) were used and 1 cm thick foam, which was maintained moist with distilled water, occupying the entire bottom surface of each Petri dish. A Jack-bean leaflet [*Canavalia ensiformis* L. (DC), Fabaceae] was placed on top of the foam and surrounded by strips of hydrophilic cotton that were also in contact with the damp foam in order to prevent the mites from escaping and to better conserve the leaflet. The pest mites, *T. urticae*, were put on top of the Jack-bean leaflets, which were switched out weekly.

### 2.2 Lab rearing of predatory mites

The predatory mite *P. macropilis* was reared in rectangular arenas of black flexible PVC plastic sheets (26 x 22 cm) were used. These were put on Styrofoam™ of equal size, and in turn these were placed in water on plastic trays (32 x 26.5 x 5.5 cm). Cotton was placed around the Styrofoam™ and the arena, and it was in contact with the water from the tray. The cotton was used to prevent the mites from escaping in addition to preserving the Jack-bean leaflets. The leaflets were placed on the arenas with the petiole under the damp cotton and were infested by *T. urticae*, which served as food for predatory mites; as the leaflets withered other new and infested by *T. urticae* were placed on the old leaflets [12].

The predatory mite *N. californicus* was reared in arenas of flexible PVC plastic 3 cm in diameter floating in distilled water in Petri dishes 15 cm in diameter [13] and the predatory mite were fed with pollen of castor bean plant (*Ricinus communis* L., Euphorbiaceae) [14].

### 2.3 Physiological selectivity assays

A survey on listed plant protection products, used by producers for the control of pests and diseases on rosebushes in the region of *Campos das Vertentes* in the state of Minas Gerais, Brazil, was carried out for the physiological selectivity tests, and 11 of these products were tested in this work (Table 1).

**TABLE 1**
**CHARACTERISTICS OF SELECTED PLANT PROTECTION PRODUCTS USED ON ROSEBUSHES AND TESTED ON *NEOSEIULUS CALIFORNICUS* AND *PHYTOSEIULUS MACROPILIS*, UNDER LABORATORY CONDITIONS**

| Active ingredient | Commercial product | Chemical group | Dose per 100 liters of water | Agronomic class | Toxicological Classification[1] |
|---|---|---|---|---|---|
| Methiram + Pyraclostrobin | Cabrio Top® | Dithiocarbamate + strobilurins | 200 g | Fungicide | III |
| Thiofanate-Methyl | Cercobin 700 WP® | Benzimidazole | 70 g | Fungicide | I |
| Boscalid + Kresoxim-methyl | Collis® | Anilide + strobilurins | 50 mL | Fungicide | III |
| Chlorothalonil | Daconil BR® | Isoftalonitrils | 200 g | Fungicide | I |
| Propargite | Omite 720 EC® | Alkyl sulphite | 30 mL | Acaricide | I |
| Chlorfenapyr | Pirate® | Pyrazole analog | 50 mL | Acaricide-insecticide | III |
| Mandipropamid | Revus® | Mandelamide ether | 60 mL | Fungicide | II |
| Mefenoxam (Metalaxyl-M) + Mancozeb | Ridomil Gold MZ® | Acilalaninate + dithiocarbamate | 300 g | Fungicide | III |
| Difenoconazol | Score® | Triazole | 80 mL | Fungicide | I |
| Bifenthrin | Talstar 100 EC® | Pyrethroid | 30 ml | Acaricide-insecticide | III |
| Pyriproxifen | Tiger 100 EC® | Pyridyloxypropil Ether | 75 mL | Insecticide | I |

***[1]Toxicological classification used in Brazil: Class I - Extremely toxic; Class II - Highly toxic; Class III - Average toxicity Class IV - Low toxicity***

The experimental design used was the completely randomized with 12 treatments, being 11 products and one control group sprayed with only distilled water, seven repetitions for *N. californicus* and five repetitions for *P. macropilis*. The spraying of products was carried out in a laboratory Potter tower at pressure of 15 lb/pol$^2$, with an amount of 1.5 ± 0.5 mg/cm$^2$ of surface [15]. The maximum dosages recommended by manufacturers, to use in the control of pests and diseases on rosebushes, were also used in the selectivity assays.

### 2.4 Physiological selectivity assays to *Neoseiulus californicus*

The residual spraying method on glass surface was used for the predatory mite *N. californicus*, recommended method as a standard under laboratory conditions to test adverse or side effects of plant protection products to predatory mites [16]. Glass coverslips for microscopy of 20 x 20 mm, floating in water on an uncapped Petri dish 5 cm in diameter x 2 cm in depth, were used as a surface for the application of the products and as a support for the mites [15]. Under these conditions, the coverslip was relatively in the center of the dish, not touching the edge and making it difficult for mites to scape. After the application of the products the coverslip were set aside for one hour to dry in room temperature. Then, five female of *N. californicus* were transferred, from the lab rearing, with a fine-tipped brush to each of coverslip. Only predatory mite females were used in the assay because this is the stage responsible for the species perpetuation; also because one of the objectives was to evaluate the effect of the products on the predatory mite reproduction. Castor bean pollen was offered as food to the survivor's predatory mites [14].

### 2.5 Physiological selectivity assays to *Phytoseiulus macropilis*

The residual method of spaying on leaf surface was used for the physiological selectivity tests to the predatory mite *P. macropilis*. This method is also recommended as standard to test adverse effects of pesticides to predatory mites under laboratory conditions [16]. Arenas 3 cm in diameter were made up from Jack-bean leaflets and placed on Petri dishes 5 cm in diameter filled with agar-water at 3%. The Petri dishes that contained the leaf discs were sprayed with the products and set aside for one hour to dry in room temperature.

After that, five female of *P. macropilis* were transferred, from de lab rearing, with the use of a fine-tipped brush to each arena. A mixture of all stages of the two-spotted spider mite was offered as food to the survivors. The Petri dishes were sealed with plastic wrap to impede two-spotted spider mites from escaping.

This methodology was not used for *N. californicus* because since they are white mites in color, it would be more difficult to find them and their eggs in the agar-water and on the Petri dish, and also because *T. urticae* was offered as food to the survivor's *P. macropilis* rather than pollen, once the possible survivor's pest mites need plant leaves to feed.

### 2.6 Selectivity assessment

During six days after the application of plant protection products, daily assessments were carried out for the verification of the residual effect of these products on predatory mites, with the aid of a stereomicroscope. The number of predatory mite females alive, of laid eggs, and of hatched larvae were evaluated in order to obtain information on the number and eggs' viability.

The adverse or total effect (E %) was calculated taking into account the corrected mortality in the treatment (Mc) as a function of the control group mortality [17]. The reproduction effect (Er) was calculated based on IOBC/WPRS [16], being E % = 100% - (100% - Mc) x Er.

Daily, during six days, it was counted the number of alive female mites as well as the number of viable eggs (larvae hatched), and removed the dead females. The reproduction effect (Er) was obtained by dividing the female eggs production (R) in the treatment by the production of eggs in the control group ($Er = R_{Treatment} / R_{Control\ group}$).

The average production of eggs for each female (R) was obtained by the relation: R = number of viable eggs/number of alive females. The tests considered valid were the ones in which the mortality of the control group was at the most 20% [16].

Values of the total effects found for each tested product were grouped in classes of 1 through 4, according to the criteria established by IOBC/WPRS in order to categorize agrochemicals based on the adverse effect caused to beneficial organisms in laboratory tests [16] [18], being: class 1 = E < 30 % (innocuous); class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); class 3 = 80 % ≤ E ≤ 99 % (moderately harmful), and class 4 = E > 99 % (harmful).

A new experiment was carried out with the goal of evaluating the duration of the residual effect of products grouped in classes 3 and 4, moderately harmful and harmful, which acted as harmful to predatory mites.

For this new bioassay, approximately 50 glass cover slips will be sprayed with the products, all coverslips in the same day, and experiments with 5 repetitions and 5 female of predatory mite each, were set up daily. The coverslips were evaluated daily, until no more female mortality was found. In the control group, the coverslips were sprayed with distilled water. These coverslips when free of residue, were evaluated during another six days, so the product residual effect could be verified in the reproduction of females; and only at that time a new classification was attributed to the product based on the IOBC/WPRS scale [16].

## III. RESULTS AND DISCUSSION

### 3.1 Physiological selectivity assays

From all the tested products, only the acaricide-insecticide chlorfernapyr (Pirate®) was highly toxic (class 4) and only for *N. californicus* (Table 2). The other tested products were innocuous or slightly harmful for *N. californicus* (Table 2) as well as for *P. macropilis* (Table 3).

In the present study, since only the product chlorfenapyr (Pirate®) was highly toxic to *N. californicus* (Table 2**)**, another experiment was carried out in order to determine the residual effect of this product until all females remained alive.

Based on the results of the new experiment, after five days of evaluation the product residue did not have any more effect on the mortality of *N. californicus* females (Table 4) and after another six days of evaluation to verify the effect on the predatory mite reproduction and the calculation of the total effect, the product that was initially categorized in class 4 (Table 4) was changed to class 1 as innocuous (Table 5).

**TABLE 2**
**EFFECT OF ACARICIDES, INSECTICIDES, AND FUNGICIDES USED ON ROSEBUSHES IN THE REGION OF CAMPO DAS VERTENTES IN THE STATE OF MINAS GERAIS ON ADULT FEMALES OF *NEOSEIULUS CALIFORNICUS*, UNDER LABORATORY CONDITIONS, AT TEMPERATURE OF 25 ± 2ºC, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE**

| Treatment | Number of specimen evaluated | Mortality (%) | Mc (%)[1] | Er[2] | E (%)[3] | Class of toxicity[4] |
|---|---|---|---|---|---|---|
| Control group | 35 | 14.30 | - | - | - | - |
| Bifenthrin | 35 | 22.90 | 10.00 | 0.61 | 44.97 | 2 |
| Boscalid + Kresoxim-methyl | 35 | 25.70 | 13.30 | 1.23 | 0.00 | 1 |
| Chlorfenapyr | 35 | 100.00 | 100.00 | - | 100.00 | 4 |
| Chlorothalonil | 35 | 48.60 | 40.00 | 0.98 | 41.27 | 2 |
| Difenoconazole | 35 | 22.90 | 10.00 | 0.46 | 58.73 | 2 |
| Metalaxyl-M + Mancozeb (Mefenoxam) | 35 | 34.30 | 23.30 | 0.42 | 67.72 | 2 |
| Mandipropamid | 35 | 31.40 | 20.00 | 0.40 | 67.72 | 2 |
| Methiram + Pyraclostrobin | 35 | 20.00 | 6.70 | 0.61 | 42.86 | 2 |
| Propargite | 35 | 17.20 | 3.30 | 0.59 | 42.86 | 2 |
| Pyriproxifen | 35 | 14.30 | 0.00 | 0.34 | 66.14 | 2 |
| Thiofanate-Methyl | 35 | 5.40 | 0.00 | 0.17 | 79.00 | 2 |

***[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful).***

**TABLE 3**
**EFFECT OF ACARICIDES, INSECTICIDES, AND FUNGICIDES USED ON ROSEBUSHES IN THE REGION OF *CAMPO DAS VERTENTES* IN THE STATE OF MINAS GERAIS ON ADULT FEMALES OF *PHYTOSEIULUS MACROPILIS*, UNDER LABORATORY CONDITIONS, AT TEMPERATURE OF 25 ± 2ºC, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE**

| Treatment | Number of specimen evaluated | Mortality (%) | Mc (%)[1] | Er[2] | E (%)[3] | Class of toxicity[4] |
|---|---|---|---|---|---|---|
| Control group | 25 | 4.00 | - | - | - | - |
| Bifenthrin | 25 | 40.00 | 37.50 | 0.72 | 54.88 | 2 |
| Boscalid + Kresoxim-methyl | 25 | 28.00 | 25.00 | 0.62 | 53.56 | 2 |
| Chlorfenapyr | 25 | 72.00 | 70.80 | 0.93 | 72.82 | 2 |
| Chlorothalonil | 25 | 28.00 | 25.00 | 0.98 | 26.65 | 1 |
| Difenoconazole | 25 | 68.00 | 66.70 | 1.29 | 56.99 | 2 |
| Metalaxyl-M + Mancozeb (Mefenoxam) | 25 | 32.00 | 29.20 | 1.04 | 26.65 | 1 |
| Mandipropamid | 25 | 16.00 | 12.50 | 0.57 | 50.13 | 2 |
| Methiram + Pyraclostrobin | 25 | 48.00 | 45.80 | 1.30 | 29.55 | 1 |
| Propargite | 25 | 72.00 | 70.80 | 1.59 | 53.56 | 2 |
| Pyriproxifen | 25 | 40.00 | 37.50 | 1.14 | 29.02 | 1 |
| Thiofanate-Methyl | 25 | 36.00 | 33.30 | 0.47 | 68.87 | 2 |

***[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful)***

**TABLE 4**
**RESIDUAL EFFECT OF THE ACARICIDE - INSECTICIDE CHLORFENAPYR ON ADULT FEMALES OF *NEOSEIULUS CALIFORNICUS* AT 24, 48, 72, 96, AND 120 HOURS AFTER THE APPLICATION OF THE PRODUCT, AT TEMPERATURE OF 25 ± 2ºC, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE**

| Hours after the application | N[1] | Number of mites alive | Percentage of mortality |
|---|---|---|---|
| 24 | 25 | 0 | 100 |
| 48 | 25 | 0 | 100 |
| 72 | 25 | 0 | 100 |
| 96 | 25 | 11 | 44 |
| 120 | 25 | 25 | 0 |

***[1]N = Number of specimens of N. californicus studied***

**TABLE 5**
**EFFECT OF THE ACARICIDE - INSECTICIDE CHLORFENAPYR ON THE MORTALITY AND REPRODUCTION OF FEMALE ADULTS OF *NEOSEIULUS CALIFORNICUS* AFTER 120 HOURS OF THE APPLICATION OF THE PRODUCT, AT TEMPERATURE OF 25 ± 2ºC, UR 70 ± 10% AND 14 HOURS OF PHOTOPHASE**

| Treatment | Number of specimens evaluated | Mortality (%) | Mc (%)[1] | Er[2] | E (%)[3] | Class of toxicity[4] |
|---|---|---|---|---|---|---|
| Control group | 25 | 0 | 0 | - | - | - |
| Chlorfenapyr | 25 | 0 | 0 | 1,51 | 0 | 1 |

***[1]Corrected mortality; [2]Reproduction effect; [3]Total effect; [4]Class of toxicity based on IOBC/WPRS: Class 1 = E < 30 % (innocuous or non-harmful); Class 2 = 30 % ≤ E ≤ 79 % (slightly harmful); Class 3 = 80 % ≤ E ≤ 99 % (moderately harmful); and Class 4 = E > 99 % (harmful).***

The predatory mite *N. californicus* was highly tolerant to the acaricide-insecticide bifenthrin in CL50 equal or higher than the recommended concentration for the use in citrus (*Citrus* spp., Rutaceae) [19].

Regarding the mortality of mites by the product, it was observed in the present study that the CL50 value was also below 50% and bifenthrin was grouped in class 2, slightly harmful for both predatory mites, therefore being considered selective (Table 2 and Table 3).

Other studies that took into account only adult females of *N. californicus* and *P. macropilis*, alike in the present study, have reported that the fungicide boscalid + kresoxim-methyl caused mortality lower than 20% for both mites, being *N. californicus* less susceptive to the product [20]. The same result was observed in the present study and although this product has been considered selective to both predatory mites, the mortality and total effect of the product was lower for the *N. californicus*, which was classified as innocuous (class 1) to this species. The same product was classified as slightly harmful to the predatory mite *P. macropilis* (class 2) (Table 2 and Table 3).

It has been reported that the fungicide chlorothalonil has caused lower mortality than 20% for *N. californicus* and *P. macropilis*; therefore, being selective [20]. In the present study, this fungicide was also considered selective to both predatory mites; however; *P. macropilis* suffered a lesser effect and this product was considered innocuous (class 1) to this species. The effect of this fungicide was slightly greater for *N. californicus* and it was considered slightly harmful (class 2) (Table 2 and Table 3).

The fungicide difenoconazole was slightly harmful (class 2) to predatory mites *N. californicus* and *P. macropilis*, and considered selective (Tables 2 and Table 3). Although studies on the selectivity of this product to predatory mites have not been found in the literature, the difenoconazole was considered innocuous to other group of natural enemies as adults of the *Trichogramma pretiosum* Riley and *Trichogramma atopovirilia* Outman & Platner (Hymenoptera: Trichogrammatidae) in apple tree crops [21] and to adult parasitoids of *T. atopovirilia* found in citrus [22].

In the present study, the fungicide metalaxyl-M + mancozeb was slightly harmful (class 2) to *N. californicus* (Table 2), and innocuous to *P. macropilis* (class 1) (Table 3), as well as to adults of the predator *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) [23].

The fungicide mandipropamid was slightly harmful (class 2) to *N. californicus* and to *P. macropilis* (Tables 2 and 3). Although reports on the selectivity of this fungicide to beneficial organisms have not been found in the literature, this product is widely used for the control of mildew on rosebushes.

In the present study, the fungicide methiram + pyraclostrobin was slightly harmful (class 2) to *N. californicus* (Table 2) and innocuous (class 1) to *P. macropilis* (Table 3). Different results have been reported that *N. californicus* was less susceptive to this product than *P. macropilis*, when taking into account only the female mortality [20]. In the present study, considering only the corrected mortality, it was observed that *N. californicus* was also less affected (Mc = 6.7) than *P. macropilis* (Mc = 45.8). However, regarding also the predatory mite reproduction effect or total effect, it was observed that *N. californicus* was more affected (Table 2) than *P. macropilis* (Table 3); because of that there is a difference when the total effect is examined, although this fungicide is considered equally selective to both predatory mites.

The fungicide thiofanate-methyl in this work was classified as slightly harmful (class 2) to both predatory mites (Tables 2 and Table 3), therefore, it can be considered selective to both; although it has been also classified as innocuous to *P. macropilis* (class 1) [24].

The acaricide propargite was classified as slightly harmful (class 2) to both predatory mites in the present study (Tables 2 and Table 3). These results are different than that found in other studies, in which the propargite was highly toxic to *N. californicus* and *P. macropilis* [25] [24] [19] [26]. However, in those studies only the mortality of the mites by the product was considered, which could have led to the differences between the results of both works. When the total effect on *P. macropilis* was considered, alike in the present study, the propargite was also classified as slightly harmful (class 2) [27].

Still describing the acaricide propargite, this product was classified as innocuous (class 1) to *N. californicus* and it was suggested that the resistance of the predatory mite to this active ingredient was probably selected in the strawberry cropping, in which the mite was collected for these tests, since this acaricide is frequently used in this type of cropping or due to a natural tolerance of the predatory mite [28]. This fact could have also occurred in the present study, in which propargite was considered slightly harmful to the predatory mite *N. californicus*.

The insecticide pyriproxifen was considered selective to both predatory mites, slightly harmful (class 2) to *N. californicus* (Table 2) and innocuous (class 1) to *P. macropilis* (Table 3). However, when only the mortality by the product is observed, *N. californicus* is more resistant than *P. macropilis.* In contrast, although this product has not caused mortality in *N. californicus*, it has reduced the oviposition by the female, which made this insecticide be classified as slightly harmful (class 2). For *P. macropilis* there was mortality of females due to the application of this product; however, the product did not cause decrease in the oviposition of females, which was larger than the oviposition of the control group. Therefore, the product becomes less harmful to the predatory mite because the females will leave their descendants (Table 3).

In the present study, the acaricide-insecticide chlorfenapyr was the only tested product that caused mortality of 100% of the predatory mite *N. californicus* and, therefore, this product was considered harmful (class 4) to this predatory mite (Table 2). Chlorfenapyr was also considered harmful to *N. californicus* in other studies found in the literature [29] [28] [19] as well as to *Iphiseiodes zuluagai* Denmark & Muma and *Euseius alatus* DeLeon (Phytoseiidae) in the tested doses of this product for its use in citrus [20]. Different than that, chlorfenapyr was considered slightly harmful to *P. macropilis*, in the present study (class 2) (Table 3).

Chlorfenapyr did not exhibit a toxic residue to *N. californicus* only after five days of its application (Table 4). After this period, the residue of this acaricide-insecticide in the leaves also stimulated the oviposition of females, and an Er value above 1 was found (Er = 1.51) (Table 5). The phenomenon of hormoligosis has probably occurred, in which sub lethal dose of this product produce a direct stimulus in the reproduction [31] [32].

The results of the residual effect of up to seven days after the application of the chlorfenapyr to the mite *N. californicus* have also been reported in others studies [29] [33]. The high toxicity of chlorfenapyr to *N. californicus* on the strawberry plant could be associated to the fact that this product had not yet been used in the field where the study was carried out. Thus, the mites probably had not yet suffered any pressure of selection to resistance at this product until that moment [29].

Chlorfenapyr or Pirate® is a product widely used by flower growers in the control of *T. urticae* and other pests. If the two-spotted spider mite infestation is very high, the strategy of the integrated management of pest mite might be used, by first apply the product to reduce mite infestation and only after five days can the predatory mite *N. californicus* be released to continue the control.

## IV. CONCLUSIONS

The following products, bifenthrin, boscalid + kresoxim-methyl, chlorotalonil, difenoconazole, metalaxyl-M + mancozeb, mandipropamid, methiram + pyraclostrobin, propargite, pyraclostrobin, and thiofanate-methyl are selective to *N. californicus* and *P. macropilis* and could be used as a strategy in the integrated management of insects, mites and diseases in rosebushes without affecting both predatory mite species.

The acaricide-insecticide chlorfenapyr is non-toxic to the predatory mite *P. macropilis* and, therefore, might be used as a tactic to the integrated management of the two-spotted spider mite in rosebushes. However, this product may not be used together the predatory mite *N. californicus* because this mite is sensitive to the product.

The predatory mite *N. californicus,* in integrated management program using the acaricides-insecticide chlorfenapyr, should only be released after five days of this product application.

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