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## RESEARCH ARTICLES - WASPS

# Selectivity of Old and New Organophosphate Insecticides and Behaviour of Vespidae Predators in Coffee Crop

FL Fernandes, PR da Silva, JER Gorri, LF Pucci, IW da Silva

Universidade Federal de Viçosa, Rio Paranaíba, Minas Gerais, Brazil

**Abstract**

Organophosphates are old agrochemicals that are toxic  for wasps (Vespidae) that are predators of insect pests. However, chlorantraniliprole is the first insecticide produced from the new anthranilic diamide class, which binds to ryanodine receptor modulators. This study uses chlorpyrifos, triazophos, pyridaphenthion and new chlorantraniliprole insecticides that have not yet been extensively tested for effects on non-target organisms. Adults of the predatory wasps were exposed to concentrations of organophosphate insecticides and the most toxic was used for a behavior test. The trial area, located in the Rio Paranaíba, Minas Gerais, received two treatments: with insecticide and without insecticide. Chlorpyrifos, the most toxic insecticide, was used in the dose of 1.5L/ha. In order to evaluate the predation, the number with predation action (injuries made by the wasps) in the leaves were accounted. Behavior activities collect of nine colonies *Protonectarina sylveirae*, *Brachigastra lecheguana*, *Polybia* sp. and *Polistes versicolor* works were quantified. All colonies were in the adult phase. The colonies were observed by 12h, from 6:00 a.m. to 6:00 p.m. The number of wasps that leaved and returned to the nest, type of sources collected (glucidic foods, prey larvae, matherial for construction of the nest). The most toxic insecticide was chlorpyrifos and the most selective was chlorantraniliprole. The species and frequencies of wasps predator were: *P. sylveirae* (71.50%), *B. lecheguana* (12.00%), *Polybia* sp. (7.00%) and *P. versicolor* (1.00%). Chlorpyrifos presented not selective and changes in the behavior to the predatory wasps.

## Introduction

The social wasps collect water, plant fibers and carbohydrates, and hunt arthropod prey or scavenge animal protein (Edwards, 1980). Social wasps are generalist foragers, but individuals are able to learn and may specialize by hunting for prey or collecting other resources at specific locations (Raveret, 1990). The foraging behavior of generalist social insects, which are able to associate the presence and quality of resources with colors, odors, shapes, and features of the environment, has greatly influenced the evolution of floral characteristics (Faegri & Van der Pijl, 1979; Barth, 1985). The lepidopteran are the more attractive prey for natural predators (Osborn & Jaffe, 1998; Marques et al., 2005). In coffee crops, many predators act as biological control agents of *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae). This pest causes severe damage to coffee crops, with losses that

may reach 50% of the total production. In coffee crops, the most common wasp species are the Vespidae *Protonectarina sylveirae* (Saussure), *Polybia* sp. and *Protopolybia exigua* (Saussure) (Parra et al., 1977). Tuelher et al. (2003) reported 90% predation by the action of vespidae predators on *L. coffeella* in coffee trees.

Nowadays, the organophosphate insecticides dissulfotom, etiom, metil parathion and chlorpyrifos are the most used to control this pest. However, the intensive use of them has caused negative impacts to humans, the environment and predatory wasps (Guedes & Fragoso, 1999; Santos et al., 2003; Fernandes et al., 2008a). Among organophosphorus, chlorpyrifos [o,o-diethyl o-(3,5,6-trichloro-2-pyridyl) phosphorothioate] is one of the leading products due to its worldwide use in agriculture and both outside and inside the home environment.

Chlorantraniliprole is the first insecticide produced from the new anthranilic diamide class, which binds to ryanodine

receptor modulators (Whalon et al., 2008). This insecticide was released for use in the control of pests in Brazil in 2009 (MAPA, 2013).

In spite of the importance of *L. coffeella* and of the potential of predatory wasps for biological control, there are few works testing the ecological and physiological selectivity of this new insecticide to these natural enimies. Furthermore, there is a lack of information on the effects of this new insecticide on the behavior of social wasps. This study had two objectives: 1) to evaluate the selectivity of the older organophosphates and of the new group of insecticides chlorantraniliprole (Rynaxypyr) to predatory wasps; and 2) to evaluate the effect of the insecticides on the behavior of social wasps in coffee plantations.

## Material and Methods

### Experiment 1: Study of physiological selectivity

The insecticides used in the bioassays were: triazophos (Hostathion 400 BR, containing 400g/L$^{-1}$, Bayer CropScience, São Paulo, SP), chlorpyrifos (Lorsban 480 BR, containing 480g/L$^{-1}$, Dow Agrosciences, São Paulo, SP), pyridaphenthion (Ofunack 400 EC, containing 400 g/L$^{-1}$, Sipcam UPL, Uberlândia, MG) and chlorantraniliprole (Altacor 350 WG, containing 350g/ L$^{-1}$, Du Pont, Barueri, SP). These insecticides are the most frequently used for *L. coffeella* control in Brazil.

Bioassays were conducted in the laboratory of Integrated Pest Management of UFV- Rio Paranaíba in four replications in a completely randomized design using adults of *B. lecheguana*, *P. sylveirae* and *Protonectarina* spp. Leaves of coffee (*Coffea arabica)* were immersed in insecticide solution or water (control) for ten seconds. Treated leaves were dried at room temperature for 2h and were placed on the bottom of Petri dishes (90 mm × 20 mm). Ten wasps were transferred to each Petri dish using aspirators. The dish was then covered with organza and tied up with a rubber band.

In each experimental unit a honey solution (10%) was added. The Petri dishes with the treated leaves and insects (experimental units) were maintained at 25 ± 0.5ºC and 75 ± 5% of relative humidity. Mortality was recorded 48h after treatment and the mortality was defined as insects that were not able to fly (Sena et al., 2008).

The mortality data was corrected for control using the method of Abbott (1925). The results were transformed to arcscene (x/100)*0.5 in order to reach the assumptions of the analysis of variance (ANOVA) and to compare the averages by the Scott-knott clustering average test at 5% of significance (Scott & Knott, 1974).

### Experiment 2. Toxicity and behavior

The experiment was carried out in Rio Paranaíba, Minas Gerais State (coordinates: 19.21S; 46.14W). The experiment was set up in an area with 5,000 coffee plants (*C. arabica*), vari-

ety "Catuaí amarelo" in production phase. The plants were arranged in a spacing of 3.0m between lines and 1.5m between plants. The experimental site was constituted of a coffee field with a circular area measurung 22,500m² (Fig. 1).
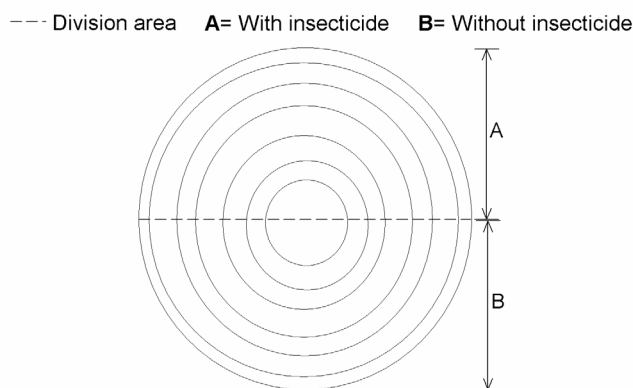


Fig. 1. Experimental area in a coffee system irrigated by central pivot. The letter A is the side with insecticide spraying and the letter B is the side without insecticide spraying. The dashed line shows the division between the two areas. The solid line shows the rows of plants.

### Treatments

After the experimental area was delimited, the treatments were assigned. The experimental area was divided in 2 parts, which received two treatments: Area A received Treatment 1 (chlorpyrifos 1.5L/ha) and Area B received treatment 2 (without insecticide) (Fig. 1). Both areas were separated by a distance of 60 meters, in order to avoid contamination of the treatment 2 area by insecticide drift. Each treatment had five replicates, each one comprised of 65 contiguous coffee plants. Within each coffee plant, two leaves were marked in the mid-third part in the coffee plants, summing up 130 leaves/replicate or 650 leaves/treatment. Each replicate (rows with 65 plants), in both treatments, were separated by a distance of 28 meters to assure independence.

In order to simulate the real effect in the field, the insecticide chlorpyrifos 480 BR was used in the recommended dose of 1.5 L/ha. Insecticide spraying was carried out once, in August. We used a prior compression sprayer with 5L capacity, conic beak and manometer to apply the insecticides. The presence of active leaf mines (with larvae present) was an *a priori* criterion to include a leaf in the experimental replicate.

### Predatory wasps species identification

The monitoring of predatory wasps was carried out on a weekly basis, during the whole period of the experiment. The collection of specimens was made in points with 20m spacing. The observations were made for 3h at the hottest periods (periods specified in Table 1) when the wasps were more present. The wasp adults found in the coffee crop were collected, stored in alcohol at 70% concentration and then, they were taken to the Insect Taxonomy Laboratory at the Federal University of Viçosa in order to be identified.

Table 1. Occurrence and frequency of Vespidae predators in *Coffea arabica* during the trial period.

| Months of the year | Time of evaluation | Species of wasps | F* |
|---|---|---|---|
| May | 09:30-12:30 | *Polybia* sp.<br>*Protonectarina sylveirae* | 05<br>14 |
| June | 13:00-16:00 | *Protonectarina sylveirae* | 10 |
| July | 09:34-12:34 | *Protonectarina sylveirae*<br>*Polistes versicolor* | 21<br>01 |
| August | 10:25-13:25 | *Protonectarina sylveirae*<br>*Polybia* sp.<br>*Brachigastra lecheguana* | 25<br>02<br>12 |
| September | 12:00-15:00 | *Protonectarina sylveirae* | 01 |
| October | 11:23-14:23 | *Protonectarina sylveirae* | 0.5 |

* Frequency of visits (%)

*Chlorpyrifos toxicity on L. coffeella and vespidae predators*

During the whole experimental period (before and after the insecticide application), the coffee leafminer attack intensity and its predation by wasps were weekly monitored. In order to assess the chlorpyrifos toxicity on this pest and on predators action, the mines were opened with metalic blade. So, in order to evaluate the coffee leafminer attack, the numbers of active mines (alive larvae), inactive mines (dead larvae by insecticide) and mines with predation evidence (injuries made by the wasps) in the leaves were recorded.

*Behavior of predatory wasps*

Behavior activities collect of four colonies *P. sylveirae*, *B. lecheguana*, *Polybia* sp. and *Polistes versicolor* works were quantified. All colonies were in the adult phase. The colonies were observed by 12h, from 6:00 a.m. to 6:00 p.m. The number of wasps that leaved and returned of the nest, as well as the type of food sources collected (glucidic foods, prey larvae, matherial for construction of the nest) were recorded.

The identification of the material collected by wasps was evaluated based on their behavior and the collected material. The wasps that collected prey and/our material for the construction of the nest were identified by flight and source

collected. Trophallaxis was used to identify the wasps that collected glucidic resource. The behavior was evaluated before and after insecticide application on coffee plants.

*Statistical Analysis*

The data on the observation of adult predator wasps were used in order to calculate the frequency of the species presence at the experimental area. In order to determine the densities of *L. coffeella* and of Vespidae predators, the number of alive larvae and dead larvae/100 leaves and the number of ripped mines per wasps/100 leaves were calculated and graphs were made with the monthly average of the populational densities. The results were corrected in relation to the mortality obtained in the outgroup using the formula (Abbott, 1925). The results were transformed to arcscene (x/100)*0.5 in order to run the analysis of variance (ANOVA) and to compare the average by the clustering average test by Scott-knott 5% significance (Scott & Knott, 1974).

Based on the number of species that returned to the nest, we calculated the eficiency index in the search for resources used in the construction of the nest, glucidic foods and prey: Eficiency Index (EI) = Number of wasps that returned with resources/number of wasps that left the nest x 100.

**Results and Discussion**

*Physiological selectivity*

The organophosphate insecticides showed no selectivity in favour of predatory wasps. Chlorantraniliprole showed selectivity to all species of wasps (*P. sylveirae*, *B. Lecheguana* and *Polybia* sp.) with mortalities of 10.32, 7.01 and 9.15, respectively. In contrast, the insecticide chlorpyrifos was about 8.44, 10.55 and 10.56 times more toxic to *P. sylveirae*, *B. lecheguana* and *Polybia* sp. than chlorantraniliprole. On the other hand, insecticides triazophos and pyridaphenthion were more toxic to *Polibia* sp. than to other species of wasps, being 7.57 and 6.32 times more toxic than chlorantraniliprole (Table 2). In general, no significant differences in the toxicity of insecticides between wasp species were found, except for the insecticide pyridaphenthion, which was more toxic to *P. sylveirae* (50.66%) and

Table 2. Mortality (%) of adults of *Protonectarina sylveirae*, *Brachygastra lecheguana* and *Polybia* sp. treated with old and new organophosphorate insecticides.

| Insecticides | Mortality (%) | | |
|---|---|---|---|
| | *Protonectarina sylveirae* | *Brachygastra lecheguana* | *Polybia* sp. |
| Chlorpyrifos | 87.12±5.10Aa | 74.01±13.98Aa | 97.36±10.05Aa |
| Triazophos | 55.22±6.40Ba | 67.00±0.14Ba | 78.13±15.12Aa |
| Pyridaphenthion | 50.66±1.65Ba | 35.11±1.20Cb | 65.19±7.79Aa |
| Chlorantraniliprole | 10.32±5.10Ca | 7.01±0.72Da | 9.15±5.03Ba |

[1]Averages followed by the same capital letter in a column and lower case in a line belong to the same group according to the Scott-Knott test at $P < 0.05$.

*Polibia* sp. (65.19%) than to *B. lecheguana* (35.11%).

The bioassays were carried out under extreme conditions of insect exposure to the insecticide. However, low mortality rates are expected to be found in field conditions due to problems such as insecticide drifting, degradation and loss during application. Thus, an insecticide that showed some high selectivity could be used selectively (physiological selectivity), in order to reduce the exposure predatory wasps.

The selectivity of clorantraniliprole to wasps may be related to an increased rate of metabolism of the compound by natural enemies than the pest, or to changes in the target of action of insecticides against the natural enemy (Yu, 1987). The cloronantraniliprole has shown high selectivity for parasitoids and predators such as predatory mites (Dinter et al., 2008), parasitoid wasps (Preetha et al., 2009) and predatory stinkbugs (Lahm et al., 2009). Possible mechanisms of physiological selectivity of these insecticides are not properly clarified due to few biochemical and physiological studies that may elucidate these mechanisms. Moreover, the insecticide chlorpyrifos has showed greater toxicity than other insecticides (Gusmão et al., 2000; Fragoso et al., 2001; Bacci et al., 2006). Also, the insecticide triazophos is one of the organophosphate pesticides extensively used in agricultural practices throughout the world. Triazophos is a broad spectrum systemic insecticide. Lipophilic compounds have higher affinity to the insect cuticle and are more easily absorbed and translocated to the site of action. This hypothesis is based on the low water solubility of the insecticide chlorpyrifos (2 ppm) and triazophos (40 ppm) (Kidd & James 1991; Berg et al., 2003).

The low toxicity of the insecticide pyridaphenthion to the species *B. lecheguana* has not yet been elucidated. However, it showed low toxicity to other species, which may be due to its low solubility (100ppm) (Berg et al., 2003; Kidd & James 1991).

### Predatory wasps frequencies in coffee crops insecticide

During the experimental period the species and frequencies of wasp predators found were: *P. sylveirae* (71.50%), *B. lecheguana* (12.00%), *Polybia* sp. (7.00%) and *P. versicolor* (1.00%) (Table 2).

These found species were pointed out by several authors as the most important predator species of *L. coffeella* (Parra et al., 1977; Pereira et al., 2007). The biggest frequency of *P. sylveirae* was reported in works of Pereira et al. (2007) who pointed out this species as a key predator of coffee leafminer in this crop. This species cut the tissue in the lesion upper part taking the *L. coffeella* larvae off through the superior part. On the other hand, *B. lecheguana* e *P. paulista* cut the tissue through the lesion lower part. The predation behavior of *B. lecheguana* e *P. paulista* was reported by Fragoso et al. (2001). Therefore, the nest preservation of this species would be really important in order to keep its predation potencial in high levels.

The biggest species diversity in August can be related to the density dependent relation between coffee leafminer and predator Vespidae species. Fernandes et al. (2008b) verified that the spatial distribution of predatory wasps, as well as the dependent density relation between these species present positive correlation. But the low frequency can be a result of possible mortalities due to the use of organophosphate insecticide.

### Chlorpyrifos selectivity to predatory wasps

We detected a significant effect of the insecticides on the predatory activity of wasp species (F = 40.65, df = 2,58, P<0.001). Furthermore, there was a significant difference of predatory activity of Vespidae predators (F = 33.11, df = 5,53, P<0.001) over time.

The larval mortality in the treatment without insecticide was low during all the experimental period. Thus, the infestation of leaf miners reached levels of 35.23% in July, 30.34% in August, 25.09% in September and 39.12% in October, an infestation level that overpasses the economic damage level -EDL (Fig. 2A). On the othe hand, in the treatment with inseticide, populations of *L. coffeella* remained bellow the EDL (Fig. 2A). The predation by wasps increased up to 10% in September (Fig. 2B), while in the area treated with insecticide thare was a reduction of predation by wasps that reached 0.5% in October (Fig. 2C).

These results showed that the insecticide chlorpyrifos did not present selectivity to the predatory wasps. The findings of Fragoso et al. (2001) confirmed chlorpyrifos high toxicity on *L. coffeella* and low selectivity to the predatory wasps *B. lecheguana*, *P. paulista* and *P. exigua*. Gusmão et al. (2000) observed that the insecticide chlorpyrifos caused mortality of 100% in the species *Apoica pallens*, *B. lecheguana* and *P. versicolor*. Fernandes et al. (2008a) when studying the seletivity of the organophosphate Dimethoate 400 EC found mortality of 88% to *P. sylveirae* with the use of the recommended dose.

The nonselectivity of this product to the predatory wasps can be related to the consume of poisoning larvae, bigger mobility, so being more exposed to the pesticides and higher percentage of product penetration in its cuticle.

The high toxicity of organophosphate to predatory wasps can be related to the good perfomance of this insecticide group activity. In penetrating in the organism, these compounds suffer reactions and become more toxic. Other factor possibly related to organophosphate toxicity is the lipofilic character of some insecticides associated to the thickness and the insect cuticule lipidic composition. This relation is responsible for the product penetration in the cuticule and the translocation up to the action target (Leite et al., 1998). The lipofilic compounds present more afinity with the insect cuticule and are easily absorbed and translocated up to the action site. This hypothesis is based on the low chlorpyrifos insecticide solubility
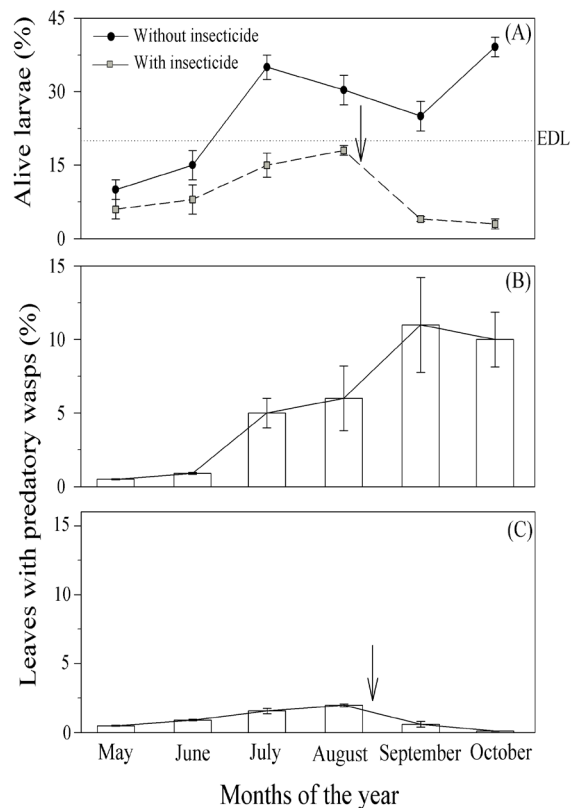
Fig. 2. Average percentage ± standard error of alive Vespidae predators in a central pivot irrigated coffe system without and with (A) insecticide spraying and presence of preadatory wasps (B and C). Arrows pointing to the bottom indicate the moment of chlorpyrifos spraying; EDL= economic damage level. Rio Paranaíba-MG, 2010.

in water (2 ppm) (Kidd & James, 1991; Berg et al., 2003).

*Behavior of predatory wasps*

Before insecticide application: collection of glucidic food was the more important activity of *P. sylveirae, B. lecheguana, Polybia* sp. and *P. versicolor* colonies. The colonies of these four species had eficiency indexes (EI) of 67.10, 55.78, 45.09 and 89.77 for the collection of glucidic food, 31.08, 44.22, 25.01 and 71.31 for prey search and 1.82, 0.00, 18.97 and 35.08 for the search for material to build the nest, respectively (Fig. 2).

After insecticide application: *P. sylveirae, B. lecheguana, Polybia.* sp. and *P. versicolor* colonies, had eficiency indexes (EI) of 35.74, 40.18, 22.09 and 86.17, respectively, for the collection of glucidic food, 1.08, 4.33, 4.87 and 70.09 for the search of prey and 0.00, 0.00, 0.00 and 28.98 for the search of material to build the nest, respectively (Fig. 2).

We observed a reduction of activities in all behaviors after insecticide application in two colonies of predatory wasps (Table 3). However, *P. versicolor* was the less affected species. In general, the effect of insecticides on behavior is a syndrome that affects motility, orientation, feeding, oviposition and learning. In many cases, insecticides act as repellents that are associated to the behavior of food searching. In some cases, repellence is the result of the contact with the host plant

or prey treated with insecticides (Desneux et al., 2007).

In the present work, we have shown that chlorpyrifos clearly is not selective and interferes with the behavioral response of predatory wasps. Because chlorpyrifos largely contributes to environmental pollution, and considering its broad spectrum of action, this insecticide can have important effects on nontarget insects and thus seriously modifies the equilibrium in natural populations. In addtion, the chlorantraniliprole belongs to the chemical group of anthranilic diamides and it has selectivity to the predatory wasp *P. sylveirae*.

Table 3. Predation (%) of *Leucoptera coffeella* according to the chlorpyrifos insecticide application in *Coffea arabica*.

| Year Month 2010 | Insects[2] |
|---|---|
| | Predation (%) |
| May | 3.43±0.04 a |
| June | 5.90±0.06 a |
| July | 9.86±0.20 b |
| August | 11.97±0.10 b |
| September[1] | 2.60±0.20 a |
| October | 0.75±0.01 c |

[1] Month of chlorpyrifos insecticide spraying;
[2] Means followed by the same letter in the column are not different according to the Skott-Knott test, $P < 0.05$.

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