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# Susceptibility of *Bactericera cockerelli* Sulc (Hemiptera: Triozidae) nymphs to Sivanto® 200 SL (flupyradifurone)

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**Abstract**

Sivanto® 200 SL (flupyradifurone) was recently introduced into the Mexican market for the control of *Bactericera cockerelli* Sulc (Hemiptera: Triozidae) in different solanaceous crops such as potato, tomato, and pepper, which are important crops in Mexico. The objective of this study was to determine the toxicity of flupyradifurone on 5th instar potato psyllids in 3 field-collected populations and a laboratory susceptible one. There were no differences in susceptibility to flupyradifurone among the evaluated populations. The lethal concentration values (LC) at 50% of mortality ranged from 243.2 to 368.1 mg per L at 24 h, after 48 h of exposure, $LC_{50}$ values were between 51.0 to 62.5 mg per L. The $LC_{95}$ values at 24 h ranged from 2403 to 5265 mg per L, and at 48 h of exposure were between 506.5 to 936.9 mg per L. The relative toxicity (RT) at 50% of mortality was 4.07 to 4.97 times higher after 48 h than after 24 h. Moreover, an antifeedant effect associated with flupyradifurone decreased the amount of excreta after 24 h of feeding by 51, 42, and 75% at the flupyradifurone dosages of 30, 50, and 100 mg per L, respectively, relative to the untreated control.

Key Words: potato psyllid; butenolide; susceptibility; antifeedant effect

**Resumen**

Sivanto® 200 SL (flupyradifurone) fue introducido al mercado en México en el 2015, recomendado para el control de *Bactericera cockerelli* Sulc (Hemiptera: Triozidae) en diferentes solanáceas como papa, jitomate y chile, cultivos de importancia en México. El objetivo de esta investigación fue determinar la toxicidad de flupyradifurone sobre ninfas de 5to instar de paratrioza en tres poblaciones de campo y una susceptible de laboratorio. Los resultados no mostraron diferencias en la respuesta a flupyradifurone entre las poblaciones evaluadas. Los valores de la concentración letal (CL) al 50% de mortalidad variaron de 243.2 a 368.1 mg por L a las 24 h, después de 48 h de exposición, los valores de $CL_{50}$ variaron de 51.0 a 62.5 mg por L. Los valores de $CL_{95}$ a las 24 h variaron de 2403 a 5265 mg por L, y a las 48 h de exposición entre 506.5 y 936.9 mg por L. La toxicidad relativa al 50% de mortalidad fue de 4.07 a 4.97 veces mayor a las 48 h respecto a las 24 h. Así mismo, se observó una reducción en el número de excretas asociada con un efecto antialimentario a las 24 h en un 51, 42 y 75% a las dosis de 30, 50 y 100 mg por L respectivamente de flupyradifurone en relación al control.

Palabras Clave: psílido de la papa; butenolide; susceptibilidad; efecto antialimentario

The potato psyllid, *Bactericera cockerelli* Sulc (Hemiptera: Triozidae), is 1 of the most damaging pests of potato (*Solanum tuberosum* L. [Solanaceae]), tomato (*Solanum lycopersicum* L. [Solanaceae]), and pepper (*Capsicum annuum* L. [Solanaceae]) crops in Mexico, as well as in some regions of the United States and Guatemala (Munyaneza et al. 2007, 2009a, 2009b, 2009c, 2012). Besides the direct attack to the plant, this pest is able to transmit the bacteria *Candidatus* Liberibacter solanacearum (Rhizobiaceae) (Rubio et al. 2006; Liefting et al. 2009), which reduces the fruit quantity and quality in the affected crops (Buchmann et al. 2011). This plant pathogen affects more than 35% of the potato-growing area planted in Texas, USA, causing yearly losses of more than US $25 million (CNAS, 2006).

In Mexico, most growers use insecticides as the only tool against the potato psyllid (Rubio et al. 2006; Vega et al. 2008), generating an intense selection pressure that increases the probability of developing resistance. Sivanto® 200 SL (flupyradifurone) is a new insecticide developed to control different species of sucking insects (Nauen et al. 2015). In Mexico, growers have been using this insecticide since 2015, and it is authorized and recommended for the potato psyllid control in the indicated crops. The objective of this study was to determine, under laboratory conditions, the toxicity of flupyradifurone to 5th instar nymphs of *B. cockerelli* from 3 field-collected populations and to determine if this insecticide exerts an antifeedant effect.

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## Materials and Methods

### INSECTS

One of the populations was field-collected 3 times from the municipality of Tianguistenco, State of Mexico (Toluca Valley), Mexico, during Sep and Oct 2015 in commercial potato fields (19.1202°N, 99.4319°W). The second population was field-collected twice in the municipalities of Yecapixtla, Atlatlahuacán and Totolapan, State of Morelos, Mexico, during Sep 2016 from commercial tomato fields (18.9522°N, 98.8756°W). The third population was field-collected 1 time during Aug 2016 in the Municipality of Galeana, State of Nuevo León, Mexico, from commercial potato fields (25.0505°N, 100.6399°W). At each collection site, 3 sampling points located at least 1 km apart were randomly selected. At each point, ≥ 100 leaflets, 1 per plant, infested with nymphs were collected. At least 500 nymphs were collected per site. The individuals from the different collection sites, of each population, were mixed and placed in cages (70 × 50 × 50 cm) to obtain, under laboratory conditions, enough nymphs from the $F_1$ generation to carry out bioassays. As a susceptible reference population we used a *B. cockerelli* population that had been reared under laboratory conditions free of selection pressure by insecticides since 2008. The insects were reared on > 50-d-old tomato plants, variety Rio Grande. In order to obtain nymphs of similar age, 2 tomato plants were introduced into the cages with adults and infested with at least 500 adults. They then were allowed to lay eggs for 24 h before being removed. Rearing was under greenhouse conditions at a temperature of 27 ± 5 °C and 70 ± 10% relative humidity.

### INSECTICIDE SUSCEPTIBILITY

Laboratory tests were conducted with the commercial formulation of Sivanto® 200 SL (flupyradifurone, 200 g per L, soluble liquid, Bayer de Mexico S.A. de C.V.). To prepare the required insecticide concentrations, distilled water was used.

The leaf-dip test described for the pear psyllid (*Psylla* spp.) and proposed by the Insecticide Resistance Action Committee was used with slight modifications (IRAC 2009). Disks 3.3 cm in diameter were cut from leaflets from mid-height on > 50-d-old tomato plants. On each leaflet, 15–25 healthy 5th instars were deposited and after 30 min the infested leaf disk was dipped in a concentration of Sivanto® 200 SL for 10 s. Afterward, they were left in a laminar flow hood for 30 min to eliminate excess moisture and placed with the underside down in a Petri dish containing 2 mL 2% agar. Initially, we determined the range of concentrations that produced from 0 up to 100% mortality (biological response window). Then, at least 6 intermediate concentrations were included to evenly cover that range. Percent mortality was determined after 24 h and 48 h of exposure; a nymph was considered dead if it did not react to the touch of a paint brush. At least 5 replications were conducted on different days, and each replication included an untreated control. The highest level of acceptable mortality for the untreated control was 10% and corrected by means of the Abbott's formula (Abbott 1925).

In all cases, the treated individuals were maintained under controlled conditions at 27 ± 2 °C, 70 ± 5% RH, and 16:8 h L:D photoperiod. The data on mortality were subjected to a Probit analysis with the Polo-Plus software program (Robertson et al. 2003), to calculate slope, lethal concentration (LC) at 50 and 95% of mortality, confidence limits at 95%, and parallelism (equal slopes) were interpreted using the Chi-square test at a 5% significance level. In addition, we determined the relative toxicity at 50% of mortality ($RT_{50}$), which was obtained by dividing the $LC_{50}$ of the corresponding population after 24 h by the $LC_{50}$ of the same population after 48 h. The responses of the compared populations were considered to be not statistically different when the confidence limits overlap (Robertson & Preisler 1992).

### ANTIFEEDANT EFFECT

The same experimental procedure as described above was used, except that we placed 10 fifth instars of the susceptible population on each leaf disc. Three concentrations of flupyradifurone (30, 50, and 100 mg per L) and 1 control with distilled water were tested. Each treatment was replicated 4 times. After 24 h, the number of excreta in each treatment was counted. The number of excreta were subjected to an analysis of variance by means of the SAS statistical software, version 9.4 (SAS Institute 2016), before analysis, the data were tested for normality, and transformation was not necessary. We used Tukey's means comparison test ($P$ = 0.05) to establish differences among the evaluated treatments. It was assumed that the amount of excreta was positively correlated with the amount of food ingested by the nymph, which therefore reflected feeding intensity.

## Results

There were no significant differences in susceptibility of nymphs to Sivanto® 200 SL among the evaluated populations, including the reference susceptible population, given that the confidence limits overlapped both at the $LC_{50}$ level as well as at the $LC_{95}$ level with both 24 and 48 h of exposure, respectively (Table 1). After 24 h, the $LC_{50}$ values were between 243.2 (Morelos) and 368.1 mg per L (Toluca) (Table 1). After 48 h of exposure, the $LC_{50}$ values decreased and were between 51.0 (Morelos) and 62.5 mg per L (Galeana) (Table 1). The Toluca population was not evaluated at 48 h because the controls had mortality above 10%.

**Table 1.** Toxicity at 24 and 48 h of the insecticide Sivanto® 200 SL (flupyradifurone) applied to 5th instar nymphs in different populations of *Bactericera cockerelli* Sulc.

| Population | n[a] | b ± SE[s] | $LC_{50}$[w] ($CL_{95}$%)[f] | $LC_{95}$[w] ($CL_{95}$%)[f] | $\chi^2$ (df) |
|---|---|---|---|---|---|
| | | | 24 h | | |
| Susceptible | 1054 | 1.43 ± 0.08 | 275.1 (226.1 – 338.8) | 3852 (2549 – 6595) | 6.05 (6) |
| Toluca | 1131 | 1.42 ± 0.08 | 368.1 (316.9 – 429.6) | 5265 (3833 – 7753) | 4.22 (5) |
| Morelos | 804 | 1.65 ± 0.10 | 243.2 (207.5 – 285.8) | 2403 (1783 – 3479) | 4.12 (5) |
| Galeana | 740 | 1.45 ± 0.10 | 254.6 (212.5 – 306.3) | 3455 (2403–5482) | 4.76 (5) |
| | | | 48 h | | |
| Susceptible | 754 | 1.71 ± 0.11 | 55.3 (46.9 – 64.8) | 506.6 (383.5 – 717.1) | 4.93 (5) |
| Morelos | 749 | 1.52 ± 0.13 | 51.0 (33.8 – 69.9) | 613.9 (370.1 – 1423) | 5.35 (4) |
| Galeana | 383 | 1.40 ± 0.15 | 62.5 (43.4 – 81.9) | 936.9 (655.9 – 1561) | 3.45 (4) |

a = number of treated insects; b = slope value; s = Standard Error of slope; w = Lethal Concentration = mg/L; f = Confidence limits at 95%.

After 24 h of exposure, the $LC_{95}$ values were between 2403 (Morelos) and 5265 mg per L (Toluca) (Table 1). After 48 h of exposure, the $LC_{95}$ values decreased relative to the values observed after 24 h and were between 506.5 (susceptible) and 936.9 mg per L (Galeana). When comparing the slopes of the populations, these were statistically equal at both 24 h ($\chi^2_{2,3} = 3.83$, $P = 0.281$) and 48 h ($\chi^2_{2,2} = 2.98$, $P = 0.225$).

Comparison of the values at the $LC_{50}$ level of each population after 24 h with those at $LC_{50}$ after 48 h showed significant differences in the response to flupyradifurone because the confidence limits did not overlap (Table 1). The values of $RT_{50}$ were 4.07 to 4.97 times higher after 48 h than after 24 h.

As the concentration of Sivanto® 200 SL increased, the amount of excreta produced by the 5th instars decreased significantly with respect to the untreated control ($F_{3,12} = 20.8$; df = 3,12; $P < 0.0001$) (Fig. 1). The percentage of excreta inhibition for the dosages of 30, 50, and 100 mg per L were 51, 42, and 75%, respectively, after 24 h.

## Discussion

Insecticides have an important role in agriculture because they reduce yield losses caused by phytophagous insects (Oerke 2006). However, development of resistance due to continuous use of insecticides limits their use (Tabashnik et al. 2014). For this reason, insecticide use should be based on knowledge of the pest susceptibility and other factors such as effects on feeding behavior (Lagunes et al. 2009). Additionally, the needs of today's agriculture requires the development of novel tools and more environment-friendly approaches (Sparks & Lorsbach 2017).

Sivanto® 200 SL is an insecticide that displays both ingestion and contact activity (Nauen et al. 2015) to the potato psyllid. It is classified as a nicotinic receptor agonist and grouped in the new "D" category of group 4 of the insecticide classification proposed by the Insecticide Resistance Action Committee (IRAC 2017). Cross resistance in populations resistant to neonicotinoids, such as imidacloprid, has not been demonstrated.

Exposure time to an insecticide modifies the response values (fFrench-Constant & Roush 1990). As exposure time increases, the LC values decrease and the confidence limits become narrower. The $RT_{50}$ increased from 4.07 to 4.97 when the nymphal exposure was increased from 24 to 48 h. We did not further increase the exposure time due to emergence of adults (MATR, personal observation).

The antifeedant effect of flupyradifurone observed in this study is consistent with the information documented by Nauen et al. (2015), who observed a reduction in the excretion of honeydew by adults of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) after 4 h following exposure on leaves of *Brassica napus* L. (Brassicaceae) treated with a concentration of 250 mg per L of this insecticide. This effect may lead to a decrease in the rate of diseases transmitted by vectors (Castle et al. 2009). In field experiments where Sivanto® has been used, it is associated with reduction in the incidence of zebra chip disease (Lewis et al. 2012; FSG, personal communication), though additional studies are needed to confirm this. The lack of differences in the susceptibility of the psyllids to flupyradifurone in the evaluated populations is likely due to the relatively short period of time that this type of insecticides has been used in Mexico (since the end of 2015).

The results obtained in this study indicate that field populations are susceptible to the insecticide flupyradifurone. This insecticide also reduces the feeding rate of the exposed nymphs of *B. cockerelli*. The results of this research may serve as a baseline reference for later studies.

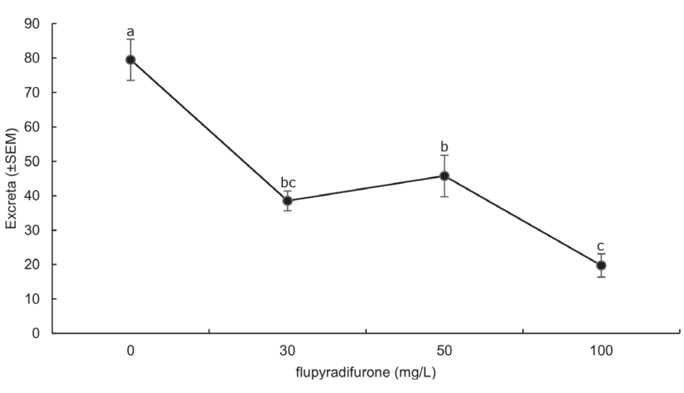


**Fig. 1.** Number of excreta produced after 24 h by 5th instars of *Bactericera cockerelli* Sulc treated with different concentrations of flupyradifurone. Treatments with the same letter are not significantly different ($P \geq 0.05$; Tukey's test). Error bars represent standard error of the mean.

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