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Insecticide-resistance ratios of three populations of *Bactericera cockerelli* (Hemiptera: Psylloidea: Triozidae) in regions of northern Mexico

*Ernesto Cerna Chávez, Omegar Hernandez Bautista, Jerónimo Landeros Flores, Luis Aguirre Uribe, and Yisa M. Ochoa Fuentes**

Abstract

Bactericera cockerelli Šulc (Hemiptera: Psylloidea: Triozidae), also known as the potato psyllid, is a pest of pepper and potato crops in northern Mexico. Growers control it by applying insecticides, without knowing the tolerance or resistance levels to these pesticides. The goal of this study was to determine the resistance ratios of several populations of *B. cockerelli* from regions of northern Mexico. Three populations of *B. cockerelli* were collected from sample sites at Coahuila–Nuevo León, San Luis Potosí, and Aguascalientes and compared with a laboratory susceptible line. Results indicated that populations from Aguascalientes had resistance ratios of 1.69-, 1.26-, and 1.00-fold for the insecticides abamectin, endosulfan, and imidacloprid, respectively. The corresponding ratios were 10.72-, 2.52-, and 3.75-fold for the San Luis Potosí population and 2.57-, 3.75-, and 4.22-fold for the Coahuila–Nuevo León population. We conclude that the *B. cockerelli* population of only San Luis Potosí was resistant to abamectin, but that the other 2 populations were susceptible to it. All 3 populations were fairly susceptible to endosulfan and imidacloprid.

Key Words: resistance; psyllid; solanum

Resumen

El pulgón saltador, *Bactericera cockerelli Š*ulc (Hemiptera: Psylloidea: Triozidae), es una plaga importante afectando las regiones paperas y productoras de chile del Norte de México por los daños directos e indirectos que ocasiona. Su control está basado en la aplicación de insecticidas, sin tener conocimiento de la tolerancia o resistencia para el manejo eficiente de esta plaga. Por lo que, esta investigación tiene como objetivo determinar la proporción de resistencia de diferentes poblaciones *B. cockerelli* provenientes de regiones productoras del Norte de México. Se recolectaron tres poblaciones de campo de *B. cockerelli* (Coahuila–Nuevo León, San Luis Potosí, y Aguascalientes) y se compararon con una línea susceptible de laboratorio. Los resultados indicaron que la población de Aguascalientes presentaron una proporción de resistencia de: 1.69, 1.26 y 1.00 veces para los insecticidas abamectina, endosulfan, e imidacloprid respectivamente, para la población San Luis Potosí los valores fueron de: 10.72, 2.52 y 3.75 veces y para la población Coahuila–Nuevo León: 2.57, 3.75 y 4.22 para los mismos insecticidas respectivamente. Por lo que se concluye que la población de San Luis potosí, presentó problemas de resistencia al insecticida abamectina, al igual que la de Coahuila–Nuevo-León; el resto de los insecticidas mostraron susceptibilidad.

Palabras Clave: resistencia; pulgón saltador; solanáceas

*Bactericera cockerelli Š*ulc (Hemiptera: Psylloidea: Triozidae) is a major pest that affects solanaceous crops in North and Central America, and recently, it was introduced into New Zealand (Munyaneza et al. 2007; Teulon et al. 2009). This insect is widely distributed in the major tomato-producing regions of Mexico: Villa de Arista, San Luis Potosi, Yurecuaro, Michoacán, the region of La Laguna in the states of Durango, Leon, and Coahuila, San Quintín, Baja California, and Morelos, Puebla, Guanajuato, Nayarit, Sinaloa, and Estado de México (Almeyda et al. 2008). The damage caused by this psyllid is associated with chlorosis and shortened and swollen internodes of the upper growth (List 1925). In addition, *B. cockerelli* is an insect vector that transmits '*Candidatus* Liberibacter solanacearum', a bacterial pathogen associated with zebra chip disease (Hansen et al. 2008; Liefting et al. 2008, 2009; Secor et al. 2009). This disease was first documented in Saltillo, Mexico, in 1994 and first identified in Texas, USA, in 2000 (Munyaneza et al. 2007), causing millions of dollars in losses to both potato producers and processors in numerous locations (Hernández-García et al. 2006; Salas et al. 2006). The incidence of this disease has increased substantially in some productive areas of the southern region of Coahuila and Nuevo Leon, reporting a drop in yield by 90% (Flores-Olivas et al. 2004). Presence of this pest can cause damage to crop plants, thus reducing yield, affecting commercialization, and making impossible to use the diseased potatoes as seeds (Cadena et al. 2003). The effective management of this vector insect can significantly reduce the incidence of zebra chip disorder in potatoes (Yang et al. 2010). Reducing the psyllid population is fundamental to the control of this disease (Almeyda et al. 2008). The most common control method used by producers is the application of insecticides (Liu & Trumble 2007). In recent years, in the region of Coahuila and Nuevo Leon, producers have delivered up to 30 applications of insecticides with different modes of action during the crop season (Almeyda et al. 2008). Nevertheless, no resistance problems for this species have been documented. Some studies such as

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those reported by Vega et al. (2008) mentioned that *B. cockerelli* has not developed resistance to products used for its management, and the incidences of non-performance reported by producers are due to poor application and faulty calibration of equipment. Nonetheless, the presence of spontaneous outbreaks, high rates of oviposition, scarcity of natural enemies, and the lack of toxicological information are problematic for the implementation of an integrated pest management program and the development of effective pest management strategies. This study, therefore, aimed to determine the insecticide-resistance ratios of different *B. cockerelli* populations to the major insecticides used for the control of this pest in northern regions of Mexico.

Materials and Methods

Various populations of *B. cockerelli* were collected from Solanaceae-growing areas where damage by this psyllid was reported, i.e., the pepper-production area of Villa de Arista, San Luis Potosi (SLP); those of Rincon de Romos, Aguascalientes (AGS); and the potato production areas of Coahuila and Nuevo Leon (CNL). Three *B. cockerelli* population samples consisting of nymphs and adults were collected from commercial crops in these production areas. These biological materials were gathered in each region using 2.5 × 1 m planting beds covered with organza fabric. The CNL population of *B cockerelli* remained on the potato plants, and the SLP and AGS populations were sampled on 'California Wonder' bell pepper plants. In order to obtain age control of the nymphs, plant materials with *B. cockerelli* eggs were removed from the cage of the adults every 24 h. Rearing of this species was conducted under controlled environmental conditions of 23 ± 3 °C, 70% RH, and a 14:10 h L:D photoperiod. Once the colonies were established, bioassays were performed on the F1 generation using a modified version of the leaf-dip technique for the pear psylla (*Psylla* spp.; Hemiptera: Psyllidae) (IRAC 2005). To do so, leaves with thirty 3rd and 4th instars were selected from the middle strata, and these were immersed for 5 s in various concentrations depending on the insecticide. The treated leaves and nymphs were allowed to dry on paper towels and then placed in plastic trays. The selection of insecticides to be evaluated was performed to directly address questions asked by the producers and by the local board of the State Committee of Plant Health of each locality. The insecticides evaluated were the fermentation product abamectin (Abamectin® 1.8 % CE at 18 g a.i. L^{-1}), the organoclorine endosulfan (Agrosulfan® 35 CE at 350 g a.i. L^{-1}) — reintroduced for protecting the crop after several seasons of discontinued use —, and the neonicotinoid imidacloprid (Picador 70 PH® at 350 g a.i. L^{-1}). To determine the LC_{50} values, serial concentrations were prepared using distilled water and the dispersant Bionex® at 1 mL:1 L of water. The concentration range for the tested insecticides was 0.01 to10 ppm for abamectin, and 100 to 1000 ppm for both endosulfan and imidacloprid. Mortality readings were taken after 24 h except for abamectin, for which they were taken after 48 h because it is a much slower acting product. A nymph was considered to be dead when its appendices were close to the body, it was dehydrated, or it did not react to probing with a brush. Seven concentrations of each insecticide were prepared. Each trial consisted of 4 replications, each of which was carried out on a different day; and each replication included an untreated control. The maximum acceptable level of mortality for the control was 10%, and mortality was corrected using Abbott›s formula (Abbott 1925) whenever mortality occurred in the control. LC_{50} levels were determined for the field lines and the susceptible line (SL). The SL was derived from a population collected on wild plants in Aguascalientes, and it was utilized for calculating resistance ratios. This SL had been reared at the Autonomous University of Aguascalientes under greenhouse conditions free of selection pressure since 2006. The resistance ratios to abamectin and endosulfan were determined by dividing the LC_{50} value in the field line by the LC_{50} in the SL (Georghiou 1962). However, because the LC_{50} of imidacloprid in the AGS field population was less than that in the SL, the LC_{50} of imidacloprid in the AGS field population was used as the basis of comparison to determine the resistance ratios in the 3 populations with regard to imidacloprid. The data obtained were subjected to probit analysis using the maximum likelihood analysis (Finney 1971) and SAS 9.2 for Windows.

Results

The susceptible line, SL, had the lowest LC_{50} values (Table 1) of abamectin and endosulfan, i.e., 0.06 and 55.55 ppm, respectively. However, the LC_{50} value of imidacloprid in the SL, which was derived from *B. cockerelli* collected from wild host plants, was 11.22 ppm, whereas the lowest LC_{50} of imidacloprid was observed in the AGS field population, and this was 8.76 ppm.

The median lethal concentrations of abamectin in the AGS, SLP, and CNL populations were 0.12, 0.74, and 0.17 ppm, respectively, and the corresponding resistance ratios were 1.69 X, 10.72 X, and 2.57 X, respectively (Table 2). These data indicate that resistance to abamectin had developed in the SLP population.

The median lethal concentrations of endosulfan in the AGS, SLP, and CNL populations were 69.86, 140.77, and 208.68 ppm, respectively, and the corresponding resistance ratios were 1.26 X, 2.53 X, and 3.76 X, respectively (Table 3). These data indicate that susceptibility to endosulfan had remained reasonably stable in these 3 populations.

The median lethal concentrations of imidacloprid in the AGS, SLP, and CNL populations were 8.76, 22.86 and 47.41 ppm, respectively, and the corresponding resistance ratios were 1.00 X, 2.61 X, and 5.42 X, respectively (Table 4).

Discussion

Because the LC_{50} of imidacloprid in the AGS field population was 8.76 ppm, which was lower than the 11.72 ppm determined in the SL, the value of 8.76 was used to calculate the resistance ratios in the 3 populations (Table 4). We had not expected that the LC_{50} in the SL would be elevated because this line was collected from wild plants.

Table 1. Median lethal concentrations (ppm) and confidence intervals of the 3 insecticides applied to the susceptible *Bactericera cockerelli* laboratory line, SL.

			ppm					
Insecticide	*n*	df	LC_{50}	C^2	Slope	95% CI	LC_{05}	LC_{95}
Abamectin	480	5	0.07	0.06	y = 1.36 + 1.17(x)	0.053–0.088	0.002	1.73
Endusulfan	480	5	55.54	0.08	y = −2.34 + 1.34(x)	43.69–68.88	3.310	932.07
Imidacloprid	480	5	11.22	0.10	y = −1.33 + 1.27(x)	2.36–26.23	0.570	220.89

n: Number of 3rd to 4th instars of *B. cockerelli*; df: degrees of freedom; C^2: probability that the log dose–probit line fits a straight line; CI: confidence interval.

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Table 2. Median lethal abamectin concentrations (ppm) and confidence intervals in 3 populations of *Bactericera cockerelli*, and their resistance ratios in comparison with the susceptible laboratory line, SL.

Population	*n*	df	ppm LC_{50}	C^2	Slope	95% CI	LC_{05}	LC_{95}	Ratio Pop ÷ SL
AGS	480	5	0.116	0.09	y = 1.15 + 1.23(x)	0.060–0.206	0.005	2.49	1.69
SLP	480	5	0.741	0.10	y = 0.12 + 0.97(x)	0.554–1.000	0.015	36.67	10.72
CNL	480	5	0.167	0.06	y = 0.85 + 1.10(x)	0.091–0.290	0.005	5.13	2.57

n: Number of 4th instars of *B. cockerelli*; df: degrees of freedom; C^2: probability that the log dose–probit line fits a straight line; CI: confidence interval.

Table 3. Median lethal endosulfan concentrations (ppm) and confidence intervals in 3 populations of *Bactericera cockerelli*, and their resistance ratios in comparison with the susceptible laboratory line, SL.

Population	*n*	df	ppm LC_{50}	C^2	Slope	95% CI	LC_{05}	LC_{95}	Ratio Pop ÷ SL
AGS	480	5	69.86	0.3	y = −2.69 + 1.46(x)	43.9–102.8	5.24	931.6	1.26
SLP	480	5	140.77	0.1	y = −3.53 + 1.64(x)	117.7–167.3	14.08	1,406	2.53
CNL	480	5	208.67	0.7	y = −3.42 + 1.47(x)	140.2–310.6	16.08	952,707	3.76

n: Number of 4th instars of *B. cockerelli*; df: degrees of freedom; C^2: probability that the log dose–probit line fits a straight line; CI: confidence interval.

Table 4. Median lethal imidacloprid concentrations (ppm) and confidence intervals in 3 populations of *Bactericera cockerelli*, and their resistance ratios in comparison with the AGS field population.

Population	*n*	df	ppm LC_{50}	C^2	slope	95% CI	LC_{05}	LC_{95}	Ratio Pop ÷ AGS
AGS	480	5	8.76	0.1	y = −1.34 + 1.42(x)	1.87–19.94	0.61	125.7	1.0
SLP	480	5	22.86	0.3	y = −2.09 + 1.53(x)	1.69–79.24	1.95	268.0	2.61
C-NL	480	5	47.41	0.4	y = −2.85 + 1.70(x)	22.2–119.6	5.12	438.8	5.42

n: Number of 4th instars of *B. cockerelli*; df: degrees of freedom; C^2 = probability that the log dose–probit line fits a straight line; CI: confidence intervals.

However, it is possible that some ancestral generations had been exposed to heavy applications of imidacloprid to pepper plants before they dispersed to the wild plants. Cerna et al. (2010) reported that the LC_{50} values of abamectin, endosulfan, and imidacloprid were 0.06, 62.28, and 3.67 ppm, respectively. Thus, the LC_{50} of imidacloprid was about 3-fold greater in the present study than the level reported by Cerna et al. (2010).

With regard to abamectin, Cerna et al. (2010) reported LC_{50} values ranging between 0.02 and 0.29 ppm in Huachichil (Coahuila) and San Rafael (Nuevo Leon) populations. These values are lower than we found in the SLP population (0.74 ppm) in the present study, but higher than we found in the AGS population (0.12 ppm) and in the CNL population (0.17 ppm). Probably, our results differ from those of Cerna et al. (2010) because the insects used in the 2 studies were collected at different locations in the potato production area. Vega et al. (2008) reported an LC_{50} of 0.4 ppm of abamectin in an Arteaga–Galeana population, and a corresponding resistance ratio of 4.0-fold, which demonstrated reduced susceptibility to abamectin.

The perception of unsatisfactory insecticidal control of *B. cockerelli* in Coahuila and Nuevo Leon caused producers to deliver 30 applications of insecticides (Almeyda et al. 2008). Vega et al. (2008) hypothesized that the lack of control might be due to factors such as unsatisfactory spray coverage, faulty calibration, and out-of-order equipment. These concerns were expressed because an earlier study had reported that under controlled conditions abamectin killed 95% of the of *B. cockerelli* nymphs by 48 h after application (Maya et al. 2003). However, the present study showed that the LC_{50} in the SLP population was 10.72-fold greater than that in the SL, and therefore this level of resistance must be considered to be a major factor in the control failures experienced by the growers. Previously, Bujanos & Marin (2007) reported an LC_{50} of 0.03 ppm of abamectin in populations from San Luis Potosi (Juan Carlos Delgado, regional farmer, personal communication, moe_788@hotmail.com) indicating misuse of this insecticide in the pepper area of Villa de Arista, San Luis Potosi, whereas in Aguascalientes the population remained stably susceptible to abamectin.

In the present study, the LC_{50} values of endosulfan in the 3 populations (Table 3), which resulted in resistance ratios ranging from 1.25-fold to 3.76-fold, were found to be lower than those reported by Cerna et al. (2010), who reported a resistance ratio of 13.17-fold. This indicates that some reversion of resistance toward susceptibility had occurred, probably due to little usage of endosulfan, which was reintroduced after having been discontinued for several seasons.

The LC_{50} values of imidacloprid in the present study were 8.76, 22.86, and 47.42 ppm in the 3 populations, AGS, SLP, and CNL, respectively. Particularly in the case of imidacloprid, the AGS population showed a lower LC_{50} value than the SL. These results were used to estimate the resistance ratios, which were 2.61-fold and 5.42-fold in SLP and CNL, respectively (Table 4).

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