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Resistance of oriental fruit moth (Lepidoptera: Tortricidae) to insecticides in apple orchards in southern Brazil

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Abstract - Oriental fruit moth (*Grapholita molesta*) is the most important pest of apple orchards in Brazil. For many years, insecticides have been the only tool used for its control. The aim of this work was to characterize the management of apple orchards by the system for Assessment of Environment Impact of Agricultural Technology Innovations (AMBITEC-AGRO) and to relate it with the resistance status of *G. molesta* populations. Insecticide resistance was estimated by toxicology and enzymatic activities of glutathione S-transferase (GST), esterases (α-β EST), and acetylcholinesterase (AChE). The phytosanitary strategies adopted by the four apple production orchards in southern Brazil were analyzed in: Vacaria (RS population), Fraiburgo (SC1 population), São Joaquim (SC2 population), and Porto Amazonas (PR population). Oriental fruit moth field populations were reared in laboratory for bioassays with chlorpyriphos, carbaryl, deltamethrin, and tebufenozide insecticides and were compared with a reference susceptible population. Larval mortality rates were recorded for seven days. GST, α-β EST, and AChE assays were performed with third- and fourth-instar larvae. Results indicated that oriental fruit moth populations collected in Vacaria, Fraiburgo, and São Joaquim showed tolerance to carbaryl and chlorpyriphos, whereas the population collected in Porto Amazonas was more susceptible. SC2 and PR populations were more tolerant to deltamethrin, while RS and SC1 populations were more susceptible. No population was tolerant to tebufenozide. RS population showed the highest activity for AChE, GST, and α-EST. The findings of this study suggest that the AMBITEC-AGRO system was a suitable method to evaluate the phytosanitary activity of apple orchards and to relate it to the resistance status of *G. molesta* populations.

Index Terms: Resistance management; *Grapholita molesta*; susceptibility, apple orchard

Resistência de mariposa oriental (Lepidoptera: Tortricidae) a inseticidas em pomares de maçã no sul do Brasil

Resumo- Mariposa-oriental (*Grapholita molesta*) é a praga mais importante de pomares de maçã no Brasil. Por muitos anos, o inseticida foi a única ferramenta usada como controle. O objetivo deste trabalho foi caracterizar o manejo dos pomares por meio da Avaliação de Impacto Ambiental da Inovação Tecnológica Agropecuária (AMBITEC-AGRO) e relacionar com o status da resistência de populações de *G. molesta* nos pomares de maçã, por bioensaios toxicológicos e atividades enzimáticas de glutatione S-transferase (GST), esterases (α-β EST) e acetilcolinesterase (AChE). A estratégia fitossanitária adotada por quatro pomares de maçã no Sul do Brasil foi analisada em: Vacaria (população RS), Fraiburgo (população SC1), São Joaquim (população SC2) e Porto Amazonas (população PR). As populações foram criadas em laboratório para bioensaios com clorpirifós, carbaril, deltametrina e tebufenozide. A mortalidade larval foi avaliada durante sete dias. A atividade das enzimas GST, α-βEST e AChE foi realizada com larvas de terceiro e quarto instares. O resultado indicou que as populações coletadas em Vacaria, Fraiburgo e São Joaquim mostraram tolerância ao carbaril e ao clorpirifós, enquanto em Porto Amazonas se mostraram mais suscetíveis. As populações de SC2 e PR foram mais tolerantes à deltametrina, enquanto que nenhuma foi tolerante a tebufenozide. A população RS foi a que apresentou maior atividade para AChE, GST e α-EST. Os resultados deste estudo podem sugerir que o sistema AMBITEC-AGRO foi um método adequado para avaliar as atividades fitossanitárias de um pomar de maçã e relacioná-lo ao estado de resistência das populações de *G. molesta*.

Termos para indexação: manejo da resistência, *Grapholita molesta*, manejo integrado, macieira.

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Introduction

Oriental fruit moth, *Grapholita molesta* (Busk, 1916) (Lepidoptera: Tortricidae), is the most important peach pest worldwide (SHEARER, USMANI, 2001; LLANOS; MARIN, 2004; TIMM et al., 2008). In Brazil, the occurrence of *G. molesta* was first recorded in 1929 in Rosaceae orchards (GONZALES, 1986); being also found in an apple orchard in early 1980s (LORENZATO, 1988). The same occurred in apple it was found in the eastern U.S. in the 1990s (FELLAND; HULL, 1998; BERGH; ENGELMAN, 2001). The larvae attack the peach and apple fruits and, the damages are similar in both plant species (NATALE et al., 2003). Due to the high value of host crops and strict quality standards for domestic and export markets, there is a near zero tolerance regarding fruit damage. Therefore, chemical control plays a key role in the management of *G. molesta*, as well as of two other economic pests: Brazilian apple leafroller, *Bonagota salubricola* (Lepidoptera: Tortricidae) and South American fruit fly, *Anastrepha fraterculus* (Diptera: Tephritidae).

In Southern Brazil, *G. molesta* and *B. salubricola* control requires up to 10 treatments with broad-spectrum insecticides per year (BOTTON et al., 2009; MONTEIRO et al., 2009, CHAVES et al., 2014). Of these, 76% of insecticide applications are performed with organophosphates (WITT, 2016), although new insecticides have been registered, including neonicotinoids (acetamiprid), spinosyns (spinetoram), anthranilic diamides (chlorantraniliprole), benzoylphenylureas (novaluron), juvenile hormone mimics (pyriproxyfen), pyrethroid ethers (etofenprox), and diacylhydrazines (tebufenozid) (AGROFIT, 2017). Furthermore, two to three additional applications are performed with organophosphates for the control of fruit fly. Despite the high pesticide use, there are reports of control failures (MONTEIRO et al., 2008). Identification of potential resistance mechanisms is necessary for the success of IPM.

In the last 10 years, a new pheromone formulation for the management of *G. molesta* and *B. salubricola* was available in Brazil (MONTEIRO et al., 2008, 2013, PASTORI et al., 2012). Although mating disruption technology reduces the use of insecticides for these pests, insecticide applications are necessary for other pests (PASTORI, 2012). Despite the evolution of pest management in apple trees in southern Brazil, the importance of new technologies on control efficiency is not measured due to the absence of indicators. The proposal of indicators checklist on the AMBITEC-AGRO system (RODRIGUES et al., 2003) could be used as a tool for evaluating the efficiency of technological contributions.

Common mechanisms for measuring insect resistance to insecticides are performed using toxicological and biochemical biomarkers. Activities of glutathione S-transferase, esterase, and acetylcholinesterase enzymes have been used as biochemical biomarkers of resistant Tortricidae populations in North (KANGA et al., 1997; 2003) and South America (SIEGWART et al., 2011).

The aim of this work was to characterize the management of apple orchards and to relate it with the resistance status of *G. molesta* populations collected in apple orchards in Southern Brazil that were exposed to four insecticides (chlorpyriphos, deltamethrin, tebufenozide, and carbaryl). Biochemical analysis was also used to evaluate the enzymatic activity of glutathione s-transferase, esterases (α and β), and acetylcholinesterase.

Materials and Methods

Insects. *Grapholita molesta* specimens were sampled in apple orchards and were selected due to the economic importance and history of insect fluctuation. Orchards were located in Porto Amazonas (state of Paraná - PR) (25º32'8"S, 49º53'33"W, 854 m a.s.l.) - *G. molesta* population was named PR; in Vacaria (state of Rio Grande do Sul - RS) (28º 30' 44" S, 50º 56' 02" W , 971 m a.s.l.) - population named RS; in Fraiburgo (state of Santa Catarina – SC) (27° 01' 34" S, 50° 55' 17" W, 1048 m a.s.l.) - population named SC1; and in São Joaquim (SC) (28º15'S e 49º54' W, 1.360 m a.s.l.) - population identified as SC2. Larvae were collected in fruits in 2010 and maintained on artificial diet for breeding (GUENNELON et al., 1981). Larvae were re-infested in 2011. Larvae used in toxicological bioassays were from the fourth and fifth laboratory generation, depending on the replicate. Larvae were pooled for biochemical analysis immediately after field collection, and were preserved in freezer at -80ºC. A susceptible laboratory strain (SS) originated from Italian peach orchards (Provided by Fabio Molinari, University of Piacenza, Italy) was used as reference. This strain was maintained on artificial diet for 18 years without the introduction of new genetic material.

Phytosanitary profile of orchards. Management practices in orchards were analyzed during five years (2006 to 2011). They served to characterize the profiles of orchards by the system for Assessment of Environment Impact of Agricultural Technology Innovations (AMBITEC-AGRO), according to Rodrigues et al. (2003). The AMBITEC-AGRO consists of an integrated indicator scaling checklist that was adapted in this study to represent the cultural and management strategies of apple orchards that may influence the pest's status (phytosanitary profile). Change coefficients were applied (CC = +3, +1, -1 and -3) to these indicators to analyze the performance of management practices. Positive values (+1 and +3) express factors or measures of good agricultural practices, while negative values indicate factors that promote selection of resistant individuals. The phytosanitary profile (Table 1) was established based on six indicators, as follows:

Table 1. Indicators that characterize the phytosanytary profile of apple orchards in Southern Brazil using a change coefficient system[1]

Indicator	Change Coefficients			
	3	1	-1	-3
Monitoring[2]	*G. molesta* and *A. fraterculus*	*G. molesta* or *A. fraterculus*	irregular	absent
Mating disruption[3]	> four years	3 years	2 years	absent
Phytophagus mite control[4]	phytoseiid mite	phytoseiid mite + acaricide	one acaricide	> 2 acaricides
Frequency of insecticide[5]	4 to 6	7 to 9	10 to 12	> 13
Rotation of insecticide[6]	4	3	2	1
Flutuation post-treatment[7]	0 to 5	6 to 10	11 to 19	> 20

[1]According Rodrigues et al. (2003); [2]Use of monitoring of *G. molesta* and *A. fraterculus* for spraying of insecticides per year; [3]Presence of mating disruption for minimum four months; [4]Use phytoseiids mites for biological control; [5]Frequency of insecticides per year; [6]Number of chemical groups of insecticides; [7]Number of *G. molesta* on the 10th day after spraying with insecticides.

I. i) Pest monitoring. Pest monitoring is used to define the spraying (KOVANCI, WALGENBACH, 2005) unlike pre-defined timetable. When monitoring was performed for the three main pests, both tortricids and fruit fly, a very positive factor was considered (CC = +3). When monitoring was performed for a single species, moderately positive value was considered (+1), while in partial sampling in a few weeks during the production cycle, CC value of -1 was observed. Completely negative value (-3) without monitoring.

II. ii) Mating disruption to *G. molesta* control. This strategy provides significant reduction of Oriental fruit moth populations (MONTEIRO et al., 2008). Therefore, orchards that had never used or stopped using mating disruption were strongly negative (-3), but when the technique was used during two productive cycles before evaluation, orchards were moderate (-1). The factor was considered positive when mating disruption was used three or more than four times, +1 and +3, respectively;

III. iii) Biological control of *Panonychus ulmi* (Acari: Tetranychidae). Apple producers in Southern Brazil multiply *Neoseiulus californicus* (Acari: Phytoseiidae) for *P. ulmi* biocontrol. Phytoseiid mite was used as a biomarker for the use of less toxic insecticides (MONTEIRO, 1994). If only phytoseiid mites were used for *P. ulmi* control, it was positive (+3), and when phytoseiid and acaricides were used in 50% orchard area, the CC value is +1. Control with a single acaricide treatment in total area was -1. CC was negative (-3) when two or more acaricides were applied per production cycle.

IV. iv) Frequency of insecticide application. The number of insecticide applications has negative impact on natural enemies (MONTEIRO, 2001) and exerts selection pressure on tortricids (KANGA et al., 2003, SIEGWART et al, 2011, BERNARDI et al., 2016). The largest number of insecticides applied (more than 13) was considered a negative ecological impact (-3), and CC was -1 between 10 and 13 applications. Positive values (+3) represent less environmental impact (< 7 treatments) and moderate (+1) for seven to nine applications.

V. v) Insecticide rotation with different chemical groups. Application of insecticides within the same chemical group is directly related to resistance selection (KANGA et al., 1997). Thus, control of tortricids and fruit flies with insecticides of the same chemical group per productive cycle was considered highly negative (C = -3) and moderately negative when insecticides of two groups were used. When chemical control was performed with three and four chemical groups, it was considered positive, +1 and +3, respectively;

VI. vi) Post-treatment fluctuation of insects. The number of *G. molesta* captured on the 10th day after insecticide application was considered completely negative (-3); when 11 to 19 adults were captured, value was moderately negative (-1). Captures ranging from 0 to 10 were positive (+3 to +1).

The sum of CCs, considering the positive and negative values of the six indicators, calculated cumulatively for five years totalize 90 positive and negative points (i.e., CC = ± 3 $_*$ 6 indicators $_*$ 5 years). The level of pest management adoption was divided into five profiles with equal amplitude, as follows: (i) Integrated Pest Management (IPM), whose CC sum of indicators results in values from 55 to 90; (ii) Good Agricultural Practices (GAP), in the range from 19 to 54; (iii) Conventional management (CM), ranging from -18 to 18; (iv) Unbalance Management (UM), ranging from -19 to -54; (v) Unsuitable management (UM), ranging from -55 to -90. The possible influences of these environmental factors were not considered in the plant profile.

Bioassays. Bioassays were carried out in microplates (96 wells, Kasvi, China), with wells filled with 150µl of artificial diet (Soybean-Wheat Germ Insect Diet, Stonefly Industries, TX, EUA), and 6µL of each insecticide solution concentration was applied on the diet's surface. Chlorpyriphos (Lorsban® 480 BR, Dow AgroSciences), deltamethrin (Decis®, Bayer CropScience), carbaryl (Sevin® SL, Bayer CropScience), and tebufenozide (Mimic® 240 SC, Dow AgroSciences) were tested in seven concentrations defined from pilot tests. After 20 minutes of drying at 22 ± 2 °C, one *G. molesta* larva was placed in each well per concentration (n = 24) per replicate (n= 168).Three replicates (n= 504) were used, each one with its respective control. Newly hatched larvae (0-3 h old) were placed in wells with the aid of a brush. The microplate wells were sealed with parafilm to prevent larvae escape and diet dehydration. Larval mortality was observed after seven days, and larvae were considered dead when they did not respond to the touch of the brush. The corrected mortality was calculated based on the highest insecticide dose relative to mortality observed in control using the formula of Abbott (ABBOTT, 1925). The lethal concentration ratio (LCR) for each insecticide was calculated with LC_{50} and LC_{95} values of field and susceptible populations.

Enzymatic activity. Acetylcholinesterase (AChE), glutathione S-transferase (GST), and esterases (α and β-Est) analyses were carried out with pools (n = 10) of five larvae from the 3rd to 5th instars (n = 50). Pools were homogenized at ratio of 1:10 (weight: volume) in potassium phosphate buffer 0.1 M (pH 7.0) using microhomogenizer, and then centrifuged for 20 min (10.000 *g* at 4°C). The supernatant was used to determine the enzymatic activity.

The glutathione S-transferase activity was measured according to method of Keen et al. (1976). A solution containing 0.6 mM GSH and 0.5 mM CDNB was prepared and 180 µl was added to the extract (20 µL) in the microplate. Activity was measured at 340 nm. The acetylcholinesterase activity was measured using method of Ellman et al. (1961) and modified to microplate by Silva de Assis (1998). The extract (50 µL) and 200 µL DTNB (0.75 mM 5. 5–ditio-bis-2-nitrobenzoate) were added in the microplate, followed by 50 µL of 10 Mm acetylcholine. The activity was measured at 405 nm. The esterase activity was measured in end point at 570 nM, according to method proposed by Brasil (2006). For α-esterase, 10 uL of sample and 200 µL of l alpha-naphthyl acetate/Na phosphate were added, and kept at room temperature. After 15 min, 50 uL of fast blue were added. Negative control was distilled water and positive control was a solution of 0.5 mg uL^{-1} alpha-naphthol in (~3.5 nmol uL^{-1}). For β-esterase, the concentrations of reagents were the same as those used for α-esterase. However, the β-naphthyl acetate/Na phosphate reaction was used as base, and positive control was 0.5 mg uL^{-1} β-naphthol.

Protein concentration was determined by the Bradford method (BRADFORD, 1976) using bovine serum albumin as standard. A microplate spectrophotometer TECAN A 5082 was used for enzymatic measurements.

Statistical analysis. LC_{50} and LC_{95} estimates and the angular coefficients of insect mortality data were analyzed by probit (POLO PLUS Data). Mortality percentage in each concentration and population, and enzymatic activities were expressed as mean ± standard error of the mean. Data were tested for normality of residuals using the Shapiro-Wilk test and for homoscedasticity by Hartley and Bartlett tests. Analysis of variance (ANOVA) was applied to determine the differences of results in groups. P values < 0.05 were considered significant by the Tukey test.

Results

Phytossanitary profile of orchards. The phytossanitary profile of orchards is presented in Table 2. Monitoring was carried out for both pests for five years, exception for Vacaria orchard. Only Porto Amazonas (Porto) orchard used mating disruption throughout the evaluation period; Vacaria and São Joaquim orchards never applied this technique. Porto and Fraiburgo orchards produced phytoseiid mites for control of *P. ulmi*. Vacaria and São Joaquim orchards used only acaricides for mite control. Porto Amazonas orchard used the fewest insecticides (between 7 and 9), whereas Vacaria and São Joaquim orchards applied more than 13 insecticides per year. São Joaquim orchard used more than two chemical groups for pest control, and in Porto Amazonas orchard, insecticides used were of one group during the first three years, but three groups were used on the next two years. The number of *G. molesta* populations was higher in Vacaria orchard 10 days after application; in the other orchards, pest populations in general were less than 10 catch. There was no orchard without *G. molesta* (+3) 10 days after application. There was a positive trend toward mating disruption and reduced insecticide use.

The management in Porto Amazonas orchard was considered as Good Agricultural Practice (GAP), whereas São Joaquim and Fraiburgo orchards were characterized as Conventional Management (MC); Vacaria orchard was classified as Unsuitable Management (UM). Insecticide rotation was the main negative factor to reduce performance in the first three years in Porto Amazonas orchard.

Toxicology bioassays. The corrected mortality values among three replicates were not different, thus data were grouped for statistical analysis (n = 504) (Table 3). The equality hypothesis between field populations and SS was rejected for all insecticides. There was a strong negative correlation between LC_{50} and the correct mortality among populations for chlorpyriphos (r = -0.93) and deltamethrin (r = -0.97). RS, SC1, and SC2 populations were significantly more resistant to chlorpyriphos than the SS population, whereas PR was more susceptible than other field populations (CI for $LC_{50:}$ 5.89 to 7.83).

RS, SC1, and SC2 populations were also resistant to carbaryl (CI: 16.35 – 25.33) in relation to SS, whereas PR was more susceptible than the other populations (CI: 5.89 – 7.83). Only SC2 population was resistant to deltamethrin (CI: 23.57 – 36.62).

Surprisingly, RS and SC1 were less susceptible to deltamethrin (LCR 0.46 and 0.92, respectively) and to tebufenozide (LCR 0.67 and 0.76, respectively) than the SS strain; however, only RS was significant. Likewise, PR population was significantly different for tebufenozide (LCR 0.70).

Differences in the selection pressure among four field populations were related to each insecticide. The PR population was 1.5 times more susceptible to chlorpyriphos and 1.9 times more susceptible to carbaryl than the average of RS, SC1 and SC2 LCR_{50} populations (x=21.01). Likewise, RS population was 2, 2.9 and 5.7 times more susceptible to deltamethrin than SC1, PR, and SC2, respectively. The SC2 population was 1.5 times less susceptible to tebufenozide than the other populations.

Enzymatic activities. The GST activity of RS population was significantly different than that of the other populations (F = 8.27, df = 6, P <0.0001); GST of PR, SC1, and SC2 activities (ranging from 40.4 to 50.8) were similar to the activity of the SS population (34.7) (Fig.1A). RS population exhibited 4.1-fold higher GST activity than SS. GST activity followed pattern similar to AChE for SC1, SC2 and SS, but not to PR. The AChE activity was statistically higher in RS and PR populations than in SS, while it was not different in SC1 and SC2 (F = 15.3, df = 6, P < 0.0001) (Fig. 1B). The AChE activity of RS and PR was 1.6-fold higher than that of SS. The highest α Est activity was measured in RS (F = 10.57, df = 6, P < 0.0001) and PR populations, and SC2 showed activity similar to SS; on the other hand, SC1 presented lower activity compared to the SS population (Fig 1C). β Est activity in field populations did not differ from that of SS population (Fig 1C).

Tables 2. Sum of change coefficients of six indicators of apple orchard phytosanitary profile in Southern Brazil during five productions years (2006-2011)

Years	Indicator	Apple orchard			
		Vacaria	Fraiburgo	São Joaquim	P. Amazonas
2006/07	Monitoring	-1	3	3	3
	Mating disruption	-3	-1	-3	3
	Phytophagus mite control	-3	3	-3	3
	Frequency of insecticide	-3	-3	-1	1
	Rotation of insecticide	-1	-1	3	-3
	Flutuation post-treatment	-3	1	-1	1
	Subtotal	-14	2	-2	8
2007/08	Monitoring	-1	3	3	3
	Mating disruption	-3	-1	-3	3
	Phytophagus mite control	-3	3	-3	3
	Frequency of insecticide	-1	-3	-1	1
	Rotation of insecticide	-1	-1	3	-3
	Flutuation post-treatment	-1	1	-1	1
	Subtotal	-10	2	-2	8
2008/09	Monitoring	-3	-3	3	3
	Mating disruption	-3	3	-3	3
	Phytophagus mite control	-3	-3	-3	3
	Frequency of insecticide	-3	3	-1	1
	Rotation of insecticide	-1	1	1	-3
	Flutuation post-treatment	-1	1	1	1
	Subtotal	-14	2	-2	8
2009/10	Monitoring	-3	3	3	3
	Mating disruption	-3	-3	-3	3
	Phytophagus mite control	-3	3	-3	3
	Frequency of insecticide	-3	-3	-1	1
	Rotation of insecticide	-1	-1	1	1
	Flutuation post-treatment	-1	1	1	1
	Subtotal	-14	0	-2	12
2010/11	Monitoring	-3	3	3	3
	Mating disruption	-3	-3	-3	3
	Phytophagus mite control	-3	3	-3	1
	Frequency of insecticide	-3	-3	-1	1
	Rotation of insecticide	-1	-1	1	1
	Flutuation post-treatment	-1	1	1	1
	Subtotal	-14	0	-2	10
	Total	-66	6	-10	46
	Phytosanytary profile*	UM	CM	CM	GAP

*IPM - Integrated Pest Management, CC sum of indicators results in values from 55 to 90; GAP - Good Agricultural Practices, in the range from 19 to 54; CM - Conventional management, ranging from -18 to 18; UM - Unbalance Management.

Table 3. Concentration-mortality relathionship for Oriental fruit moth larvae in apple orchards in Southern Brazil

Insecticide	Pop[1]	N	Corrected mortality[2]	X^2	LC50 (95% CL)	LCR[3]	LC95 (95%CL)	LCR[4]	Slope ± SE
Chlorpyriphos	SS	504	91.49	0.83	0.49 (0.27-0.92)		1,262.00 (256.00-1,548.10)		0.48±0.05
	RS	504	81.44	3.90	9.78(8.29-11.43)	19.95*	50.59 (36.48-83.72)	0.04	2.30±0.27
	PR	504	87.50	0.08	6.84 (5.89-7.83)	13.95*	39.13 (28,96-61.09)	0.03	2.17±0.23
	SC1	504	83.33	0.56	9.77 (8.43-11.24)	19.93*	59.94 (43.03-98.63)	0.04	2.08±0.22
	SC2	504	80.12	0.27	11.43 (9.69-13.25)	23.32*	57.66 (43.23-88.42)	0.04	2.34±0.25
Carbaryl	SS	504	85.41	0.27	1.13 (0.68-1.99)		769.99 (216.00-4,941.40)		0.58±0.05
	RS	504	85.12	1.96	21.61 (18.25-25.31)	19.12*	129.10 (91.53-216.40)	0.16	2.11±0.24
	PR	504	81.94	0.91	10.91 (8.40-14.23)	9.65*	231.77 (125.00-589.00)	0.30	1.23±0.14
	SC1	504	85.00	1.84	19.03 (16.35-22.02)	16.84*	96.85 (71.50-152.14)	0.12	2.32±0.25
	SC2	504	85.50	1.07	21.41 (17.76-25.33)	18.67*	124,72 (88.55-212.42)	0.16	2.14±0.25
Deltamethrin	SS	504	91.29	0.05	10.97 (8.03-14.26)		168.25 (105.90-333.81)		1.38±0.15
	RS	504	92.26	1.49	5.06 (2.67-8.00)	0.46*	817.43 (294.02-4,287.17)	4.85	0.74±0.09
	PR	504	91.66	1.39	14.83 (12.25-17.85)	1.35	142,13 (98.97-233.00)	0.84	1.67±0.14
	SC1	504	94.80	2.25	10.17 (7.79-12.79)	0.92	127.17 (84.00-227.45)	0.75	1.50±0.15
	SC2	504	88.89	4.30	29.80 (23.57-36.62)	2.64*	180.20 (116.42-401,20)	1.07	2.10±0.22
Tebufenozide	SS	504	78.00	1.45	11.73 (9.81-13.89)		69.02 (48.43-119.66)		2.13±0.25
	RS	504	86.00	0.46	7.89 (6.12-9.76)	0.67*	83.29 (50.17-201.59)	1.20	1.60±0.23
	PR	504	75.50	0.41	8.27 (7.71-10.25)	0.70	112.25 (62.65-615.47)	1.62	1.45±0.21
	SC1	504	83.33	1.19	8.93 (7.71-10.25)	0.76	52.73 (38.34-84,81)	0.76	2.13±0.23
	SC2	504	81.83	0.34	13.00 (11.26-14.97)	1.10	60.86 (45.55-93.25)	0.88	2.45±0.26

[1]SP – susceptible population; RS – Vacaria population; PR – Porto Amazonas population; SC1 – Fraiburgo population; SC2 – São Joaquim population; [2]Correted mortality was calculated by the highest concentration by Abbott (ABBOTT, 1925); [3]Lethal concentration ratio: field population LC_{50}/susceptible population LC_{50} – asterisk indicates significant at P=0.05; [4]Lethal concentration ratio: field population LC95/ susceptible population LC_{95}.

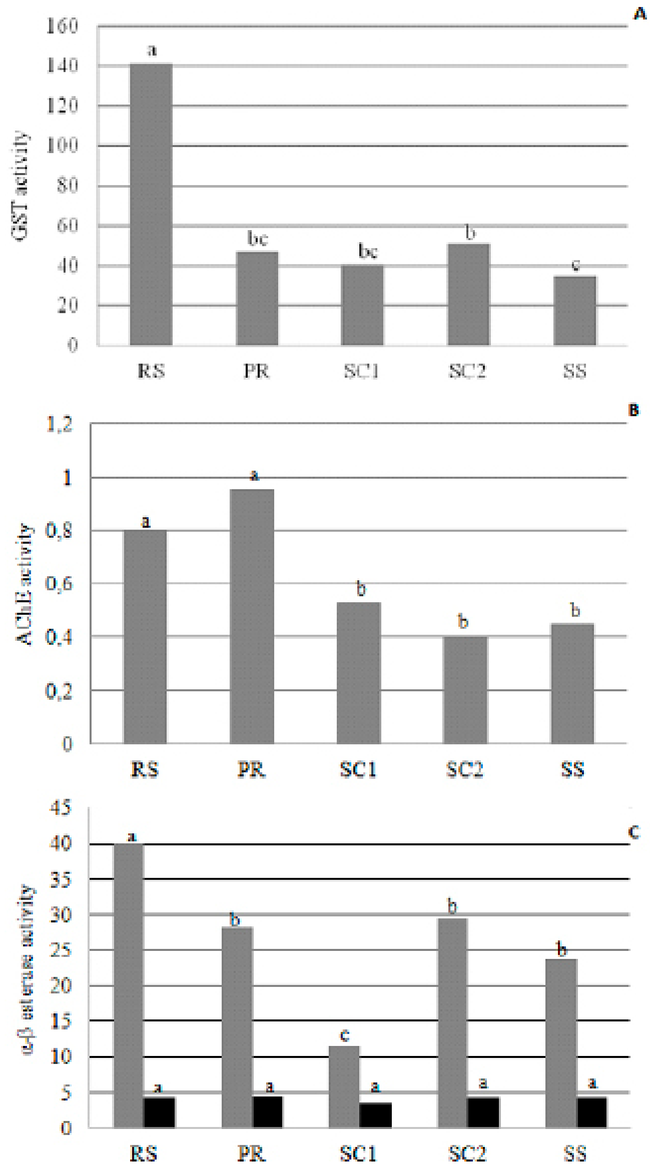

Figure 1. GST activity (1A), AChE activity (1B) and α-β EST (1C) expressed in nmol per minute per microgram of protein, in Vacaria (RS), Porto Amazonas (PR), Fraiburgo (SC1), São Joaquim (SC2), and Susceptible Strain (SS) of oriental fruit moth populations. Bars topped with different letters indicate significant difference by the Tukey's test ($P<0.05$).

Discussion

Resistance to insecticides is the development of the ability of a pest population to tolerate concentrations that would be lethal to most individuals of that species. Resistance to conventional insecticides is widespread in insects, and performance of resistance management is frequently neglected (SIEGWART et al., 2015). In Southern Brazil, crop protection strategies in apple orchards include applications of organophosphate insecticides for the control of tortricids and fruit flies. This can change the resistance status of insects in orchards. Because of the history of occurrence of infestations in apple orchards in Southern Brazil, *G. molesta* can be used as a bioindicator for the presence of resistant populations.

Vacaria orchard had the most important insecticide frequency indicator (more than 13 insecticide applications), and the insecticide rotation indicator showed that insecticides were of two groups, mostly organophosphates (80%). The post-treatment fluctuation indicator showed that the number of *G. molesta* in Vacaria was higher than in other orchards, as the large number of insecticides did not reduce pest populations on the 10th day after treatment. This suggests that pest management was unsuitable in the Vacaria orchard, which was motivated by the lack of monitoring and decision-making on pest control based on timetable. The insecticide frequency and insect fluctuation indicators were the best in Porto Amazonas orchard; however, the insecticide rotation indicator remained negative in the first three years. In this case, the rotation indicator was influenced by *A. fraterculus* control that was done only with organophosphates. Control was sufficient to maintain fewer pest collections on the 10th day after application, possibly because there was less selection pressure when phytosanitary strategies were interrupted by the use of non-chemical methods, like mating disruption (GEORGHIOU, 1994; THOMSON et al., 2001). This hypothesis on the combination of chemical control and mating disruption was reported by Rodriguez et al. (2012) in Spain and Kovanci & Walgenbach (2005) in the USA. Fraiburgo and São Joaquim phytosanitary profiles were intermediate in relation to Porto Amazonas and Vacaria profiles.

The AMBITEC-AGRO system was a suitable method to evaluate the phytosanitary-related activities of an apple orchard. Through the indicator checklist, it was possible to verify how the producer guides his strategies and, thus will allow establishing which has influence on the pest status.

All *G. molesta* field populations were more tolerant to chlorpyriphos and carbaryl than the SS population. Such reduced susceptibility of organophosphate may partially explain because 75% of insecticides spraying are of this group (Witt, 2016). The phytosanitary profile of Porto Amazonas orchard was confirmed by the results of bioassays, in which the Porto Amazonas orchard used the fewest insecticides and the PR population was the most susceptible to organophosphates and carbamates than the other three field populations. Rodriguez et al. (2010) reported the influence of crop protection history on toxicology status. Results with other populations were coherent with the frequency of organophosphate application in Fraiburgo, São Joaquim, and Vacaria. These results agree with those of studies on tortricids resistance in Spain (RODRIGUEZ et al., 2010, 2012) and Canada (KANGA et al., 2003).

The RS population was four times less tolerant to deltamethrin than the PR population, probably because in the PR orchard, pyrethroid application was limited to an annual application in the two last years and was not applied in the first three years. Jones et al. (2011) reported that insect survival in bioassays is not necessarily related to pest resistance, but to unexpected levels of natural tolerance. It may be the case for the SC2 population, which was 5.7 times more resistant than the RS population, although no history of deltamethrin application was recorded over the last five years in SC2 orchard. Despite the absence of deltamethrin use, tolerance of tortricids to pyrethroids was detected in other researches (REYES et al. 2007).

All *G. molesta* populations were susceptible to tebufenozide, maybe because applications with this insecticide were reduced in apple trees in Southern Brazil. Nonetheless, resistance of *B. salubricola* to tebufenozide was recently detected in Vacaria (BERNARDI et al., 2016). Nevertheless, it was surprising that the SS population had higher LC_{50} than that of most field populations; the SS population was bred in laboratory for 18 years, and when they were collected in peach orchards in Italy, tebufenozide was no available for sale.

The four population exhibited tolerance to chlorpyriphos and carbaryl compared with SS population, but only PR and RS populations showed significant different for AChE activity, 2.2 and 1.8 times more than SS, respectively. AChE activity has been consistently associated with resistance to organophosphate and carbamates (SHEARER; USMANI, 2001; KANGA et al. 1997, 2003, SIEGWART et al, 2011). The high AChE activity in PR populations is probably because organophosphate was the only insecticide group used in the first three years, mainly to control fruit fly. However, it received fewer insecticide applications and the bioassay showed that it was more susceptible than SC1 and SC2 populations. Such activities of PR populations were not related to increased tolerance to any of the tested insecticides because the post-treatment *G. molesta* fluctuation was smaller in Porto Amazonas orchard than in Vacaria orchard and corroborated the difference of mortality between PR and RS populations (average 30%) in toxicological biossays. Both SC1 and SC2 AChE activities no differ from the SS population possibly because reduction of selection pressure by rotation of insectides during five years (average 2 to 3 and 3 to 4 chemical groups for SC1 and SC2, respectively). The GST and α-Est activities in SC2 population does not confirm possible tolerance to deltamethrin obtained by bioassay, when SC2 was significantly more tolerant among populations. β-Est activity did not express any relationship with population tolerance to insecticides.

The high AChE, GST and α-EST activities in the RS population corroborated results of toxicological bioassays. So, this suggest that more two mechanisms analyzed (GST and α-EST) may be involved with RS population and this maybe related with deltamethrin resistance. GSTs are multifunctional enzymes with detoxification function, promoting resistance to organophosphates and pyrethroids by conjugation of reduced glutathione to insecticide molecules (HEMINGWAY, 2000).

Finally, the AMBITEC-AGRO system indicators defined very well the phytosanitary profile of apple orchards and the relationship with toxicological and biochemical bioassays, demonstrating that RS population was more resistant to organophosphates, and PR population was the most susceptible to insecticides tested.

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