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CROP PROTECTION

Susceptibility of *Grapholita molesta* to insecticides in Brazil

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ABSTRACT: *The use of insecticides has been the main tool for Grapholita molesta (Busck) control in Brazil, which is considered one of the most important pests in apple and peach orchards. In order to implement an Insect Resistance Management (IRM) program, studies were conducted to characterize the baseline susceptibility of G. molesta to major insecticides for its control. Then, we conducted an insecticide susceptibility monitoring in thirteen field-collected populations of the pest. Neonates (0-24h old) were exposed to insecticides applied on surface of artificial diet. A high susceptibility was verified when neonates of the Laboratory population of G. molesta were exposed to insecticides with LC_{50} values (*µg *a.i./cm²) of 0.1 (spinetoram), 1.0 (metaflumizone), 1.2 (chlorantraniliprole), 4.8 (novaluron), 5.1 (tebufenozide), 11.3 (phosmet) and 222.5 (pyriproxyfen). Based on the LC_{99} (*µg *a.i./cm²), the diagnostic concentrations of 0.6 (spinetoram), 5.5 (metaflumizone), 5.6 (chlorantraniliprole), 19.6 (tebufenozide), 37.4 (phosmet), 37.8 (novaluron) and 2011 pyriproxyfen) caused high mortality (>95%) of neonates from field populations. These diagnostic concentrations will be used in resistance monitoring programs of G. molesta in Brazil.*
Key words: *chemical control, insect resistance management; oriental fruit moth.*

Suscetibilidade de *Grapholita molesta* a inseticidas no Brasil

RESUMO: *O uso de inseticidas tem sido a principal ferramenta para o controle da Grapholita molesta (Busck) no Brasil, considerada uma das mais importantes pragas em pomares de macieira e pessegueiro. Para implementar um programa de Manejo de Resistência a Insetos (MRI), estudos foram conduzidos para estabelecer uma linha básica de suscetibilidade de G. molesta a inseticidas utilizados para o seu controle. Posteriormente, foi realizado o monitoramento da suscetibilidade a inseticidas em treze populações da praga provenientes do campo. Lagartas (0-24 horas de idade) foram expostas a inseticidas aplicados na superfície da dieta artificial. Verificou-se alta suscetibilidade de lagartas neonatas de G. molesta (população de laboratório) quando foram expostas aos inseticidas, com valores de CL_{50} (***µg** *i.a./cm²) de 0,1 (espinetoram), 1,0 (*metoxifenozida*), 1,2 (clorantraniliprole), 4,8 (novaluron), 5,1 (tebufenozida), 11,3 (fosmete) e 222,5 (piriproxifem). Com base na CL_{99} (***µg** *i.a./cm²), as concentrações diagnósticas de 0,6 (espinetoram), 5,5 (metaflumizona), 5,6 (clorantraniliprole), 19,6 (tebufenozida), 37,4 (fosmete), 37,8 (novaluron) e 2.011 (piriproxifem) ocasionaram alta mortalidade (> 95%) de neonatas provenientes de populações de campo. Essas concentrações diagnósticas poderão ser utilizadas em programas de monitoramento da resistência de G. molesta no Brasil.*
Palavras-chave: *controle químico, manejo de resistência de insetos, mariposa-oriental.*

INTRODUCTION

The oriental fruit moth *Grapholita molesta* (Busck, 1916) (Lepidoptera: Tortricidae) is native from China and is now distributed throughout temperate regions of the world, being considered the most important pest in apple and peach orchards in Brazil (BOTTON et al., 2011). The control of *G. molesta* in Brazil has been carried out mainly using organophosphorate insecticides (chlorpyrifos and phosmet) and insect growth regulators (lufenuron and novaluron) (ARIOLI et al., 2010). However, new insecticides (chlorantraniliprole and spinetoram) have been introduced in the Brazilian market for *G. molesta* management (CHAVES et al., 2014).

Resistance of *G. molesta* to organophosphate insecticides has been identified in Canada, United States (USMANI & SHEARER, 2001) and Brazil (SIEGWART et al., 2011). Resistance evolution is a serious threat for the chemical control sustainability of the oriental fruit moth when we consider the high insecticide selection pressure in Brazil (6-8 treatments per season) (BOTTON et al., 2011), its polyphagy (preference for apple and peach) and its high reproductive ability

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(6 generations per year) (ARIOLI et al., 2010). These aspects are an alert for the risk of resistance evolution of *G. molesta* to insecticides in Brazilian orchards production systems.

In this scenario, the resistance monitoring must be emphasized in Insect Resistance Management (IRM) programs for the preservation of the durability of insecticides and to prevent control failure in the pest management program (ROUSH & MILLER, 1986). Within this context, it is essential to understand the natural response of distinct geographic populations of the target pest, through the characterization of the baseline susceptibility and estimate the diagnostic concentration of each insecticide for the development of an IRM program (ROUSH & MILLER, 1986).

Therefore, objectives of the current study were to characterize the baseline susceptibility to insecticides in *G. molesta* and to validate diagnostic concentration for IRM programs in geographically distinct populations of *G. molesta* collected in the main producing regions of apple and peach in Brazil.

MATERIAL AND METHODS

Insect populations

For insecticides susceptibility characterization, we used a reference susceptible population (population of Laboratory) of *G. molesta* that has been maintained in the laboratory, free from selection pressure by insecticides, for 10 years, reared in the an artificial diet (ARIOLI et al., 2010). To validate the candidate diagnostic insecticide concentrations, we collected larvae from 13 *G. molesta* populations from orchards located in different municipalities of the state of Rio Grande do Sul, Santa Catarina and São Paulo, Brazil (Table 1).

Chemicals

The tested insecticides were chlorantraniliprole (Altacor 350 WG™ - 4.9g a.i. $100L^{-1}$, DuPont do Brasil S.A. - Barueri, SP), metaflumizone (BAS 320I - 14.0g a.i. $100L^{-1}$, Basf S.A., São Paulo, SP), novaluron (Rimon 100 EC™ - 4.0g a.i. $100L^{-1}$, Adama Brasil Londrina, PR), phosmet (Imidan 500 WP™ - Cross Link Consultoria e Comércio, Barueri, SP), pyriproxyfen (Tiger™, 10.0g a.i. $100L^{-1}$, Sumitomo chemical do Brasil Repres. Ltda, São Paulo, SP), spinetoram (Delegate 250 WG™ - 7.5g a.i. $100L^{-1}$, Dow AgroSciences Industrial Ltda, São Paulo, SP), spinosad (Tracer 480 SC™ - 7.5g a.i. $100L^{-1}$, Dow Agro Sciences Industrial Ltda - São Paulo, SP) and tebufenozide (Mimic 240 SC™ - 21.6g a.i. $100L^{-1}$, Dow Agro Sciences Industrial Ltda, São Paulo, SP).

Baseline susceptibility in diet overlay bioassays

For the bioassays, we used the artificial diet proposed by ARIOLI et al. (2010), commonly used for rearing *G. molesta*. After preparation, the artificial diet was kept in bath regulated at 50ºC and, posteriorly, 1.25mL were transferred to 24-well acrylic plates (COSTAR™). Afterwards, the insecticides were diluted in distilled water to prepare the different concentrations to be tested. The surfactant Triton X-100 was added at 0.1% in order to obtain a uniform spread of the solution over the diet surface. The control treatment was composed of distilled water + surfactant. For each insecticide, we tested seven concentrations logarithmically spaced that caused 10-99% mortality in a preliminary test, which were applied on the diet surface with a replication pipette (30 µl per well). After a drying period (90min), one *G. molesta* neonate larvae (0-24h old) was added to each well using a fine brush. The acrylic plates were covered with a lid and were kept in a climatic chamber (temperature 25±2ºC,

Table 1 - Identification of *G. molesta* populations, host, source and collection date to susceptibility monitoring.

Population Code	Host	City, State	Longitude	Latitude	Date
Susceptibility monitoring					
Laboratory	-	Pelotas, RS	31º 48' 8''S	52º 24' 55''W	-
P/SP1	Peach	Paranapanema, SP	48º 39' 17''S	23º 20' 26''W	October 2010
M/SC1	Apple	São Joaquim, SC	49° 55′ 55″ S	28° 17′ 38″W	October 2010
M/RS3	Apple	Vacaria, RS	50º 58.945'S	28º33.003'W	October 2010
P/SP2	Peach	Paranapanema, SP	48º 39' 17''S	23º 20' 26''W	November 2011
M/SC2	Apple	São Joaquim, SC	50º 06.591'S	27º 55011'W	November 2011
M/RS1	Apple	Caxias do Sul, RS	50° 55′ 17″S	27° 01′ 34″W	November 2011
M/RS2	Apple	Antônio Prado, RS	51° 16′ 58″S	28° 51′ 28'W	November 2011
P/RS1	Peach	Pinto Bandeira, RS	51º24.9'65'S	29º07.606'W	December 2012
P/RS2	Peach	Pelotas, RS	52º32.8'84'S	31º25.750'W	December 2012
P/RS3	Peach	Pelotas, RS	52º32.5'45'S	31º25.953'W	December 2012
M/RS4	Apple	Pinto Bandeira, RS	51º24.4'95'S	29º08.203'W	December 2012
M/SP1	Apple	Paranapanema, SP	48° 43′ 22″S	23° 23′ 20″W	December 2012
M/RS5	Apple	Bento Gonçalves, RS	51° 31′ 08″ S	29° 10′ 01″W	December 2012

relative humidity 60±10% and photoperiod16:8 [L:D] h). The assessment of the mortality for the insecticides metaflumizone and phosmet was accomplished 1 day after inoculation (DAI), 3 DAI for the chlorantraniliprole, novaluron, spinetoram and tebufenozide and 5 DAI for pyriproxyfen. Individuals were considered dead if they showed no reaction to a brush touch. The experimental design was completely randomized with five replications (one 24-well acrylic plate for repetition, 48 larvae per acrylic plate) for each concentration, totaling 192 larvae per concentration.

Validation of candidate diagnostic concentrations

The bioassay procedure for the validation of the diagnostic insecticide concentrations for resistance-monitoring programs was identical to that described above. The bioassays used the same susceptible population (laboratory reference population) and 13 populations of *G. molesta* (Table 1). Field populations represent regions with the greatest crop production of apple and peach in Brazil and the orchards were selected based on records of control failure reported by technicians and growers. Larvae were collected from the field during the 2010 to 2012 apple-growing season and reared in the laboratory on an artificial diet according to the procedures described in ARIOLI et al. (2010). For each collected population, neonate larvae (0-24 h old, generation F2) were exposed to a diagnostic concentration (based on the values of LC_{99}) of insecticide defined in the joint analysis of the baseline susceptibility data. For each diagnostic concentration of each insecticide we tested 624 neonates per population (13 replicate of 48 neonates per replicate). Mortality evaluation was performed as described above (baseline susceptibility). The mortality was corrected using the formula ABBOTT's (1925).

Statistical analysis

The LC_{50} and LC_{90} (Lethal Concentration) estimates, the respective confidence intervals (CI 95%) and the angular coefficient data on insect mortality were obtained from Probit analyses (PROC PROBIT, SAS INSTITUTE, 2011). A likelihood ratio test was conducted to test the hypothesis that the LCp values were equal. If the hypothesis was rejected, pairwise comparisons were performed and the significance was declared if confidence intervals did not overlap (ROUSH & MILLER, 1986). The significance of differences among slopes was determined by likelihood ratio test for parallelism and equality (ROUSH & MILLER, 1986).To estimate the LC_{99}, the larval mortality data for each insecticide were analyzed jointly with a binomial model using the log-log complement connection function (gompit; PROC PROBIT) (SAS INSTITUTE, 2011). Through this analysis, LC_{99} values and the respective CIs were estimated to determine the diagnostic concentrations for the resistance monitoring of *G. molesta*. To validate the candidate diagnostic concentrations, the *G. molesta* mortality percentage (x) in each diagnostic concentration and population were transformed in arcsen $\sqrt{x/100}$ and the means submitted to analysis of variance (ANOVA) and, when significant ($P \leq 0.05$), the means were compared using Tukey's test ($P \leq 0.05$) (SAS INSTITUTE, 2011).

RESULTS

Baseline susceptibility in diet overlay bioassays

We observed high susceptibility of *G. molesta* to the tested insecticides (Table 2), especially to spinetoram (LC_{50}=0.1µg a.i./cm^2), in relation to

Table 2 - Lethal concentrations (LC: µg a.i./cm^2) of insecticides to *G. molesta* neonate larvae (Laboratory population) from an artificial diet overlay bioassay.

Insecticide	*n*	Slope (± SE)	LC_{50} (95% FL)[a]	LC_{90} (95% FL)[a]	χ^{2b} (d.f.)[c]
Spinetoram	672	2.5 (±0.3) b	0.1 (0.5-0.10) a	0.3 (0.2-0.4) a	4.8 (3)
Metaflumizone	829	2.5 (±0.2) b	1.0 (0.5-1.3) b	2.9 (2.6-3.4) b	16.2 (4)
Chlorantraniliprole	1.432	2.9 (±0.2) bc	1.2 (1.0-1.3) b	3.2 (2.8-3.5) b	7.2 (7)
Novaluron	760	2.2 (±0.2) ab	4.8 (3.9-5.5) c	17.7 (14.5-23.6) d	7.0 (4)
Tebufenozide	893	3.3 (±0.3) c	5.1 (4.6-5.7) c	12.2 (10.9-14.0) c	4.8 (4)
Phosmet	1250	3.0 (±0.4) c	11.3 (10.2- 12.4) d	24.6 (22.2-27.7) d	1.9 (5)
Pyriproxyfen	978	1.7 (±0.2) a	222.5 (160.3-282.9) e	964.6 (825.0-1199.0) e	13.8 (4)

[a]LC_{50} and LC_{90}: Insecticide concentrations (µg of active ingredient (a.i.)/cm^2) required to kill 50 or 90% of the larvae, respectively. Significance of differences among slopes determined by likelihood ratio test of equality followed by pairwise comparisons using non-overlapping fiducial limits, [b]Chi-square, [c]Degrees of freedom.

metaflumizone (LC_{50}=1.0µg a.i./cm²), chlorantraniliprole (LC_{50}=1.2µg a.i./cm²), novaluron (LC_{50}=4.8µg a.i./cm²), tebufenozide (LC_{50}=5.1µg a.i./cm²), phosmet (CL_{50}=11.3µg a.i./cm²) and pyriproxyfen (LC_{50}=222.5µg a.i./cm²) (Table 2). Similarly, there were significant differences in susceptibility of *G. molesta* based on the values of LC_{90} (Table 2). From the joint analysis of mortality data for each insecticide, we estimated candidate diagnostic concentrations for *G. molesta* resistance monitoring in Brazil were the LC_{99} of 0.6µg a.i./cm² (spinetoram), 5.5µg a.i./cm² (metaflumizone), 5.6µg a.i./cm² (chlorantraniliprole), 19.6µg a.i./cm² (tebufenozide), 37.4µg a.i./cm² (phosmet), 37.8µg a.i./cm² (novaluron), and 2011µg a.i./cm² (pyriproxyfen).

Diagnostic concentrations for resistance monitoring

There was low variation in the susceptibility of the *G. molesta* field populations to the diagnostic insecticide concentrations (Table 3). The M/SP1 of *G. molesta* population from a peach orchard in the municipality of Paranapanema, SP, to phomet, was significantly less susceptible to insecticide (97.5 of mortality) when compared ($F_{7, 96}$=2.80; P<0.0001) to the other populations collected during the season of 2010/11 (Table 3). In the same way, a less susceptibility ($F_{8, 109}$=8.57; P<0.0001) was also observed from M/SC1 (95.5 of mortality) of the *G. molesta* population from an apple orchard in the municipality of São Joaquim/RS, to tebufenozide (Table 3). However, in the 2011/12 season, all populations showed similar susceptibility ($F_{7, 96}$=4.61; P=0.2215) to that observed for Laboratory population (Table 3).

DISCUSSION

The bioassay technique used (surface treatment of artificial diet), the Probit and log-log complementary analysis, showed consistency to describe the biological responses of *G. molesta* larvae to the insecticides evaluated. Furthermore, it is a realistic technique with the pest behavior in the field when *G. molesta* larvae ingest the insecticide that is presented on the surface of the pointers or ripe fruit before penetration in the host (CHAVES et al., 2014). *G. molesta* neonates from the reference population (Laboratory) were susceptible to all tested insecticides on the surface of the artificial diet. Due to these methodological differences, some LC_{50} values reported in this study differ from those obtained by other authors (SIEGWART et al., 2011). However, these authors used other techniques bioassays and a longer exposition time of larvae to insecticides. Therefore, the sum of these factors reflects the difference of values reported in this study compared to those reported in the literature.

Table 3 - Mortality (mean ± SE) of *G. molesta* neonate larvae from apple and peach orchards when compared to the susceptible laboratory population exposed to one candidate diagnostic concentration of insecticides overlaid on an artificial diet.

Population code	% mortality[a]						
	Spi.	Meta.	Chlora.	Nova.	Tebu.	Phos.	Pyri.
Season 2010/11							
Laboratory	99.8±0.5^{ns}	98.5±0.6^{ns}	100.0±0.0^{ns}	99.3±0.0^{ns}	99.0±0.5c	99.8±0.2b	100.0±0.0^{ns}
P/SP1	97.0±0.5	99.7±0.5	100.0±0.0	99.5±0.2	96.6±0.7b	97.5±0.7a	100.0±0.0
M/SC1	96.8±0.9	100.0±0.0	100.0±0.0	99.1±0.4	95.5±0.7a	98.6±0.4b	100.0±0.0
M/SC2	97.7±0.3	98.2±0.8	100.0±0.0	99.8±0.1	99.9±0.4c	98.8±0.2 b	100.0±0.0
M/RS1	99.4±0.3	98.4±0.6	100.0±0.0	98.8±0.3	99.2±0.4c	99.8±0.1b	100.0±0.0
M/RS2	98.1±0.8	98.2±0.7	100.0±0.0	100.0±0.0	99.8±0.2c	99.0±0.4b	100.0±0.0
M/RS3	98.2±0.7	98.0±0.8	100.0±0.0	99.0±0.4	99.4±0.4c	99.6±0.2b	100.0±0.0
Season 2010/11							
Laboratory	99.7±0.3^{ns}	99.4±0.4^{ns}	99.5±0.3^{ns}	99.1±0.4^{ns}	99.0±0.5^{ns}	99.1±0.4^{ns}	100.0±0.0^{ns}
P/RS1	98.9±0.6	99.0±0.4	100.0±0.0	99.1±0.8	99.6±0.8	97.7±0.5	100.0±0.0
P/RS2	99.0±0.4	99.0±0.4	99.2±0.1	99.7±0.6	98.8±0.3	99.8±0.5	100.0±0.0
P/RS3	99.2±0.6	97.7±0.8	99.7±0.2	99.1±0.6	98.8±0.4	99.2±0.3	100.0±0.0
P/SP2	100.0±0.0	99.6±0.2	99.6±0.2	98.8±0.7	98.6±0.6	97.6±0.8	100.0±0.0
M/RS4	99.7±0.2	98.6±0.2	98.8±0.2	99.5±0.5	99.4±0.3	97.4±0.2	100.0±0.0
M/SP1	100.0±0.0	98.5±0.6	99.8±0.2	99.9±0.1	99.8±0.2	98.5±0.5	100.0±0.0
M/RS5	99.6±0.2	99.2±0.3	99.6±0.2	98.8±0.7	99.8±0.6	99.0±0.6	100.0±0.0

[a]Values represent means ± SE. Means followed by the same letter in each season and each candidate diagnostic concentration do not differ statistically (Tukey test, P < 0.05). ^{ns}: not significant in column, Spi: Spinetoram, Meta: Metaflumizone, Chlora: Chlorantraniliprole, Nova: Novaluron. Tebu: Tebufenozide, Phos: Phosmet, Pyri: Pyriproxifen,

Regarding the susceptibility monitoring, a high susceptibility was observed on the *G. molesta* populations collected in different sites to the insecticides (chlorantraniliprole, metaflumizone, pyriproxyfen and spinetoram) (CHAVES et al., 2014). The differences observed in larval survival to the phosmet and tebufenozide insecticides may be due to the presence of natural susceptibility variability in geographically distinct populations. However, we cannot discard the possibility of evolution of resistance to these insecticides, as observed by SIEGWART et al. (2011) in Brazil, for the insecticides organophosphate, pyrethroid and carbamate in Canada (KANGA et al., 2003) and to azinphosmethyl in New Jersey, EUA (USMANI & SHEARER, 2001).

The estimate of baselines and monitoring of susceptibility of *G. molesta* to insecticides provide information to the establishment of proactive IRM program as verified to *Bonagota salubricola* (Meyrick) (Lepidoptera: Tortricidae) in apple orchards in Brazil (BERNARDI et al., 2016). The initiative also allowed monitoring changes in pest susceptibility over time and define IRM strategies,; for example, in the rotation of insecticides with different modes of action (ROUSH & MILLER, 1986; SPARKS & NAUEN, 2015). This management strategy allowed the reduction of the frequency of resistant individuals of *G. molesta* to organophosphate insecticide from 55 to 14% and to pyrethroids from 30 to 10% in peach orchards in Canada (KANGA et al., 2003).

In Brazil, this strategy can also help delay or avoid the evolution of resistance in populations of *G. molesta* due the populations present low genetic variability (SILVA-BRANDÃO et al., 2015). However, the low gene flow between *G. molesta* populations (SILVA-BRANDÃO et al., 2015) may contribute for the occurrence of evolution of resistance more quickly in local populations, since there is no introduction of susceptibility alleles (susceptible individuals) in the population by increasing the frequency of resistant individuals on site (KANGA et al., 2003). Therefore, the use of insecticides in apple and peach orchards should be done with caution, once that the molecules of insecticides available or registered for the *G. molesta* management are practically the same for the two crops.

To subsidize an IRM program, the monitoring of the susceptibility of *G. molesta* to insecticide is of critical importance to maintain the efficiency and control in the field. For susceptibility monitoring, the diagnostic concentrations of each insecticide caused a high mortality of the neonates for these pest insects. These results are very important for IRM programs of *G. molesta*, once with the diagnostic concentrations (obtained from the LC_{99}) reported in the present study for each insecticide are very similar to the concentrations used by the growers to control *G. molesta* in the field.

Therefore, the compilation of this susceptibility database for populations of *G. molesta*, and the validation of the diagnostic concentration to insecticide will be useful in the follow up of possible changes in the susceptibility of this pest. Future efforts should be concentrated on representative samples of populations of these target pests and to give continuity to the studies to evaluate the susceptibility of *G. molesta* insecticide for detecting in advance the possibility of the presence of resistant individuals on apple and peach orchards to prevent or delay the evolution of resistance.

CONCLUSION

The *G. molesta* populations showed high susceptibility to insecticides spinetoram, metaflumizone, chlorantraniliprole, novaluron, tebufenozide, phosmet and pyriproxyfen.

Diagnostic concentrations (µg a.i./ cm^2) 0.6 (spinetoram), 5.5 (metaflumizone), 5.6 (chlorantraniliprole), 19.6 (tebufenozide), 37.4 (phosmet), 37.8 (novaluron) and 2,011 (pyriproxyfen) may be used in IRM programs of *G. molesta* in Brazil.

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