Susceptibility of *Phthorimaea operculella* (Zeller) to Insecticides[1]

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ABSTRACT Seven technical grade insecticides were evaluated against a susceptible population and against two field populations of the potato tuberworm *Phthorimaea operculella* (Zeller) by topical application to third-instar larvae. Using a standard susceptible laboratory colony, we calculated median lethal doses (LD_{50}) of 0.39, 0.059, 0.12, 0.00022, 0.18, 0.010 and 0.0007 µg/larvae for the insecticides DDT, methyl parathion, ethyl parathion, azinphosmethyl, malathion, carbaryl and permethrin, respectively. By comparison, we found that a wild population from Oyameles, Puebla was susceptible to the insecticides while a population from Leon, Guanajuato was susceptible to DDT, methyl parathion, ethyl parathion, malathion and permethrin, but was resistant to azinphosmethyl and carbaryl.

KEY WORDS *Phtorimaea operculella*, insecticide susceptibility, insecticide resistance

The potato tuberworm *Phthorimaea operculella* (Zeller), is an insect species of worldwide distribution which causes considerable losses to the potato crop (Arx et al. 1988). Its eggs are deposited on the underside of the leaf, and the emerging larvae eat their way through the leaf. As the leaves are consumed the larvae abandon the tunnels and mine the leaves adjacent to the stem, which causes the death of the terminal leaves (Burton 1989). The larvae may also burrow into the stems and tubers when these are exposed by cracks that form in clay and dry soils (Raman 1980, Valencia 1986, Povolny and Valencia 1986). When infestations are intense, the cost of eliminating infested tubers makes harvesting uneconomical. If infested tubers are stored without treatment, the larvae continue extensive boring, filling the tubers with excrement which promote the development of microorganisms and decomposition of the tubers (Awate and Pokharkar 1976, Arx et al. 1988).

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The use of insecticides to control *P. operculella* and other insect pests of the potato has dramatically increased often resulting in overapplication and the use of insecticide mixtures. On the other hand, because field applications do not prevent infestation in stored tubers, the insects survive and reproduce, making direct treatment during storage necessary, which also provides only a partial control (Arx et al. 1988). Finally, the over use of insecticides promotes resistance development in pest populations and leaves residues that pose a health hazard for consumers (Raman 1986). The resistance of *P. operculella* to DDT was documented in 1965 in Australia, South Africa, and Zimbabwe, and in 1968 in Florida and Zambia, and to parathion in Florida (Georghiou and Lagunes 1986). Guerrero and Aranda (1988) reported resistance to methyl parathion and to azinphosmethyl in Coahuila and Nuevo Leon, Mexico. The purpose of the present study was to establish the baselines for seven insecticides in a susceptible colony of the potato turberworm kept at Chapingo, Mexico, as well as determining the susceptibility to insecticides in populations of *P. operculella* from two potato growing regions using different insecticides for insect control.

Materials and Methods

The investigation was conducted in the Laboratory of Insect Physiology of the Program of Entomology and Acarology of the Instituto de Fitosanidad, Colegio de Postgraduados at Montecillo, State of Mexico.

Collecting and Breeding of Insects. An insect colony kept in the laboratory for more than seven years without application of insecticides was used as a susceptible population. The field populations were collected on potato foliage as well as on tubers in two potato growing regions: Oyameles, State of Puebla, a rainfed cropping system that includes three to eight applications of insecticides every season (Bahena 1992), and in Leon, State of Guanajuato, where as many as 19 applications per season have been reported (Salazar 1990). The larvae from the leaves were placed on healthy, Alpha-variety tubers to develop. When the tubers were infested, they were confined to cages until adult emergence. The adults were placed in acrylic boxes covered with nylon for mating. They were fed a solution of 5% saccharose.

Eggs deposited on the nylon were collected daily with a brush and transferred to Petri dishes. After eclosion, the larvae were placed on tubers previously washed an disinfected with 2% sodium hypochlorite. Prior to this, the tubers had been perforated by rotating them on a board with nails, thus insuring uniform infestation. The infested tubers were introduced into plastic containers covered with nylon to prevent larval escape. After 2 wks, the larvae nearing pupation were removed from the tubers and dispersed onto rolled up sheets of flannel to pupate. Pupae were collected and placed in acrylic boxes. After emerging, the adults were collected and confined in boxes for oviposition. The third-instar larvae used in the bioassays were obtained by dissecting the tubers approximately 10 days after infestation.

Insecticides and Bioassays. The technical grade products used were DDT (100%), methyl parathion (82%), ethyl parathion (99%), azinphosmethyl (90%), malathion (84%), carbaryl (100%) and permethrin (94%). Acetone was used as a solvent. Third-instar larvae (22 ± 3 mg) were treated by topical application of 0.5 µl with a 250 µl Hamilton microsyringe. Seven to 11 doses of each insecticide were used with 10 insects per dose and five replications. The treated larvae were kept in Petri dishes (5 cm diam) with a piece of potato of approximately 1.0 cm^3. Mortality was recorded 24 h after treatment.

Statistical Analysis. The corrected mortality data (Abbott 1925) were processed by means of the program PCPROBIT (Camacho 1990). The differences among populations and among insecticides were considered significant when there was no overlapping of fiducial limits of the calculated median lethal doses (LD_{50}). The range of adjustment of the model was defined by means of the Chi-square (χ^2) test. The resistance factor was calculated by dividing the LD_{50} of each wild population by the LD_{50} of the susceptible laboratory colony.

Results

Table 1 shows LD_{50}s, the slopes of the regression lines, and their fiducial limits as well as the resistance ratio for the different populations tested. For the susceptible laboratory colony, the least toxic product was DDT, followed by malathion, ethyl parathion, methyl parathion, carbaryl, permethrin and azinphosmethyl. No overlapping of the fiducial limits for the LD_{50} values occurred. The most homogeneous response among insect populations was to ethyl paration; the most heterogeneous response was to carbaryl.

For the population from Oyameles, Puebla, the most toxic insecticides also were azinphosmethyl and permethrin, followed by carbaryl, ethyl parathion and methyl parathion, DDT and malathion. Malathion was the least toxic and exhibited the most homogeneous response, whereas carbaryl showed the highest heterogeneity. Based on the overlapping of fiducial limits of the LD_{50}, DDT and malathion were equally toxic, as well as ethyl parathion and methyl parathion. In the population derived from Leon, Guanajuato, toxicity at the LD_{50} level was higher for permethrin, followed in decreasing order by azinphosmethyl, methyl parathion, ethyl parathion, malathion and DDT, whereas the value obtained for cabaryl was extremely high and cannot be considered reliable. It was not possible to calculate the standard error for carbaryl, therefore only the slope is given.

In the case of DDT, the LD_{50} for the Puebla population was lower than for the susceptible Chapingo population; therefore, the resistance factor was <1, while it was 1.15X for the Leon population. Due to the overlapping fiducial limits for the Chapingo and Leon populations, both are considered susceptible to DDT.

The variation in susceptibility of field populations to methyl parathion is evident, but the heterogeneous response of the susceptible population caused minimum differences in the LD_{95} across locations. The LD_{50} values for ethyl parathion in the Chapingo and Puebla populations were very similar (0.12 and 0.13 µg/larva, respectively), and the difference was not statistically significant. The Leon colony presented a resistance factor of 2.25X with respect to the susceptible one. The slopes obtained for the Chapingo and Puebla populations with azinphosmethyl were equal, and the resistance ratio was 2.2. But, in the case of Leon, there was a more heterogeneous response and a 136.3-fold increase for LD_{50}, indicating the population resistance.

Table 1. Comparative susceptibility of potato tuber moth populations at various locations.

Insecticide	Population	Slope ± SE	LD_{50}* (95% CL)	LD_{95}* (95% CL)	RR**, LD_{50}*	RR**, LD_{95}*
DDT	Chapingo-s	1.57 ± 0.12	0.39(0.32-0.47)	4.35(3.01-7.03)	1.0	1.0
	Puebla	1.40 ± 0.10	0.20(0.16-0.26)	3.10(2.06-5.27)	0.51	0.71
	León	1.56 ± 0.13	0.45(0.36-0.55)	5.04(3.42-8.42)	1.15	1.16
Methyl parathion	Chapingo-s	1.50 ± 0.11	0.059(0.048-0.073)	0.73(0.50-1.19)	1.0	1.0
	Puebla	2.77 ± 0.24	0.16(0.14-0.18)	0.65(0.53-0.86)	2.7	0.89
	León	3.15 ± 0.30	0.23(0.21-0.26)	0.78(0.63-1.04)	3.8	1.07
Ethyl parathion	Chapingo-s	4.92 ± 0.41	0.12(0.11-0.13)	0.27(0.24-0.32)	1.0	1.0
	Puebla	2.02 ± 0.18	0.13(0.11-0.16)	0.90(0.65-1.4)	1.08	3.33
	León	1.58 ± 0.14	0.27(0.21-0.33)	3.0(2.12-4.77)	2.25	11.1
Azinphosmethyl	Chapingo-s	2.36 ± 0.20	0.00022(0.00019-0.00026)	0.0011(0.0009-0.0015)	1.0	1.0
	Puebla	2.36 ± 0.20	0.00049(0.00043-0.00056)	0.0024(0.0019-0.0034)	2.2	2.18
	León	1.61 ± 0.17	0.030(0.024-0.037)	0.31(0.20-0.57)	136.3	281.8
Malathion	Chapingo-s	3.07 ± 0.27	0.18(0.16-0.20)	0.62(0.51-0.81)	1.0	1.0
	Puebla	4.97 ± 0.54	0.24(0.22-0.26)	0.51(0.45-0.62)	1.33	0.82
	León	1.44 ± 0.14	0.38(0.31-0.47)	5.24(3.34-10.03)	2.1	8.45
Carbaryl	Chapingo-s	1.22 ± 0.12	0.010(0.007-0.013)	0.22(0.13-0.43)	1.0	1.0
	Puebla	1.06 ± 0.09	0.020(0.014-0.028)	0.71(0.40-1.53)	2.0	3.22
	León	0.95	27.26(20.27-39.61)	1427.0(514-8882)	2726.0	6486.0
Permethrin	Chapingo-s	1.89 ± 0.16	0.0007(0.00058-0.00084)	0.0052(0.0038-0.0078)	1.0	1.0
	Puebla	1.49 ± 0.12	0.0008(0.00065-0.001)	0.010(0.0069-0.017)	1.15	1.92
	León	1.42 ± 0.13	0.0048(0.0038-0.006)	0.069(0.043-0.130)	6.8	13.3

* In µg/larva.
** Resistance ratio relative to Chapingo-s insecticide-susceptible strain.

For malathion, LD_{50} values indicate that the Chapingo and Puebla populations are statistically equal since their fiducial limits overlap. The Leon population had a LD_{50} very close to values for other two populations, but because of the heterogeneity represented in the low value of the slope, the LD_{95} was very high. The tolerance of the Puebla population to carbaryl was twice that of the susceptible population. On the other hand, in the Leon population the mortality did not show a logical proportion of change according to the dose, and dilution problems at high doses reduced product penetration. The maximum mortality achieved was 65% with a dose of 41.6 µg/larva. The resistance factor was 2726X at the LD_{50}. In the bioassays with permethrin, there were no significant differences between the Chapingo and Puebla populations. In contrast, the Leon population showed a resistance factor of 6.8X with respect to the susceptible one.

Discussion

Insecticides use in potato-growing regions of Mexico are quite variable. In the State of Puebla, where rainfed farming is predominant, the number of insecticide applications to control *P. operculella* varies from 3 to 8 per season (Bahena 1992). The most frequently used products are methyl parathion, methamidophos, and carbofuran, as well as mixtures of methyl parathion + methamidophos and carbofuran + methamidophos. In Leon, Guanajuato, under irrigated conditions farmers use as many as 19 applications per season of products such as azinphosmethyl, methamidophos, permethrin, cypermethrin and mixtures of chlorpyrifos + permethrin, carbaryl + mevinphos, metamidophos + azinphosmethyl and mevinphos + methomyl (Salazar 1990), or mixtures of pyrethroids with methamidophos or with methomyl (Rocha, personal communication).

Based on our results, a baseline of LD_{50} values of 0.39, 0.059, 0.12, 0.00022, 0.18, 0.010 and 0.007 µg/larva was established for DDT, methyl parathion, ethyl parathion, azinphosmethyl, malathion, carbaryl and permethrin, respectively. The LD_{50} for azinphosmethyl reflects its high toxicity, as confirmed in field populations of potato tuberworm (Foot 1976). This product, as well as permethrin and methyl parathion, proved to be effective against eggs, larvae and adults of a colony of *P. operculella* at doses 50% lower than the commercially recommended ones (Guerrero and Gálvez 1990). By comparing the values obtained for the susceptible colony, it was observed that the Puebla population is susceptible to all tested insecticides.

The Leon population was found to be susceptible to DDT, malathion, ethyl parathion and methyl parathion. The 6.8X resistance factor for permethrin indicates the importance of periodically verifying changes in susceptibility of *P. operculella* to this product to plan long-term utilization, especially in mixtures with organophosphates and carbamates. Azinphosmethyl has largely been used to control potato tuberworm, but the resistance detected argues against its continued use in the Leon area. It is well known that the main mechanism of resistance to azinphosmethyl involves esterases (Bush et al. 1993), but resistance can be diminished by not applying the product for several generations of *P. operculella* (Guerrero and Villa 1992). The high increase of the doses of carbaryl required to obtain mortality in the Leon population indicates a remarkable degree of resistance; thus its use is not recommended in this region. Moldenke et al. (1992) mention that this product is detoxified by various enzymatic routes which include hydrolysis, oxidation and conjugation, in addition to mechanisms which increase tolerance to the insecticide, such as reduced penetration caused by changes in the cuticular lipids. More work with *P. operculella* is needed to corroborate the data for mortality at high doses, due to poor penetration of the insecticide.

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