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Insecticide use and organophosphate resistance in the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae)

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Abstract

Increasing rates of insecticide use against the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville) and field reports on insecticide resistance led to an investigation of the possible occurrence of resistance of this species to some of the oldest insecticides used against it in Brazil: chlorpyrifos, disulfoton, ethion and methyl parathion. Insect populations were collected from ten sites in the state of Minas Gerais, Brazil and these populations were subjected to discriminating concentrations established from insecticide LC_{99}s estimated for a susceptible standard population. Eight of the field-collected populations showed resistance to disulfoton, five showed resistance to ethion, four showed resistance to methyl parathion, and one showed resistance to chlorpyrifos. The frequency of resistant individuals in each population ranged from 10 to 93% for disulfoton, 53 to 75% for ethion, 23 to 76% for methyl parathion, and the frequency of resistant individuals in the chlorpyrifos resistant population was 35%. A higher frequency of individuals resistant to chlorpyrifos, disulfoton and ethion was associated with greater use of insecticides, especially other organophosphates. This finding suggests that cross-selection, mainly between organophosphates, played a major role in the evolution of insecticide resistance in Brazilian populations of *L. coffeella*. Results from insecticide bioassays with synergists (diethyl maleate, piperonyl butoxide and triphenyl phosphate) suggested that cytochrome P450-dependent monooxygenases may play a major role in resistance with minor involvement of esterases and glutathione *S*-transferases.

Introduction

The coffee leaf miner *Leucoptera (=Perileucoptera) coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) is a monophagous insect that is the main pest of one of the major agriculture commodities of Brazil – coffee (Reis & Souza, 1984; Souza *et al.*, 1998). This insect species is also an important coffee pest in several other coffee-producing countries in South and Central America, as well as in the Caribbean (Green, 1984; Thomaziello, 1987). Its presence in Brazil was reported soon after 1851, probably following its introduction with a batch of coffee seedlings from Bourbon Island in the West Indies (Souza *et al.*, 1998).

At first, *L. coffeella* was assumed to be the same coffee leaf miner species that occurs in West Africa (Box, 1923; Notley, 1948) and is recognized today as two different species – *L. meyricki* Ghesquière and *L. caffeina* Washburn (Lepidoptera: Lyonetiidae) (Bradley, 1958). The true *L. coffeella*, however, is

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restricted to the Neotropical region (Reis & Souza, 1984) and commonly referred to in Brazil by its synonym – *Perileucoptera coffeella* (Guérin-Méneville), following a suggestion based on insects from Trinidad (Silvestri, 1943). However, the latest review of the genus *Leucoptera*, carried out by Mey (1994), recognized *Perileucoptera* as a junior synonym of *Leucoptera*. Therefore the use of *P. coffeella* should be avoided in favour of *L. coffeella*, as earlier acknowledged by Green (1984) and more recently by Mey (1994).

Despite earlier reports of its introduction into Brazil, the outbreaks of *L. coffeella* in the country were sporadic up to 1970 (Reis & Souza, 1984). Only after the expansion of the coffee plantations in Brazil and the modifications introduced to the traditional cultivation practices with the Renewal and Strengthen Plan of Coffee Plantations put forward by the Federal Government from 1969–1970 to 1979–1980, did *L. coffeella* become a problem. The new coffee plantations with reduced stands and modified cultivation practices, such as frequent fungicide sprayings for control of coffee rust *Hemileia vastratrix* (Berk. et Br.) (Pucciniaceae), seem to have favoured more frequent and longer outbreaks of *L. coffeella* (Souza *et al.*, 1998). This species seems to benefit from warmer and drier conditions (Reis & Souza, 1986) and, once favoured by the new cultivation practices, has become the main pest of coffee in Brazil (Reis & Souza, 1984; Souza *et al.*, 1998).

The leaf miner larva attacks coffee plants in the mid and upper parts of the canopy, where it mines the leaves reducing plant photosynthetic area (Reis & Souza, 1986; Souza *et al.*, 1998). As a result of such injury, the mined leaves drop prematurely from the plant, especially during the dry season, leading to a reduction in coffee yield and, if sufficiently frequent, reducing plant longevity (Reis & Souza, 1986; Souza *et al.*, 1998).

Adults of *L. coffeella* are reported to be active at twilight and during the night (Reis & Souza, 1984; Souza *et al.*, 1998), but recent investigations have shown that the peak of female capture in pheromone traps occurs at midday (Michereff, 2000), casting doubts on these earlier reports. Predatory wasps are commonly reported as the main natural enemies of *L. coffeella* in Brazil (Reis & Souza, 1986; Souza *et al.*, 1998). However, it seems that parasitism in this pest species is underestimated due to frequent predation of parasitized larvae (Avilés, 1991; Reis *et al.*, 2000). The interaction between predatory wasps (*Brachygastra lecheguana* Latreille, *Polybia scuttelaris* (White) and *Protonectarina silveirae* (de Saussure) (all Vespidae)) and parasitic wasps (*Colastes* sp., *Eubadizon* sp. and *Mirax* sp. (Braconidae); and *Cirrospilus* sp., *Closterocerus* sp., *Horismenus* sp., *Proacrias* sp. and *Tetrastichus* sp. (Eulophidae)) and their apparent overall inefficiency in containing outbreaks of *L. coffeella* (Avilès, 1991; Reis *et al.*, 2000), aided by the non-existence of commercial coffee varieties resistant to this species, has led to a heavy reliance on insecticide use for its control from the 1970s.

Organophosphates are the main insecticide group used against *L. coffeella* in Brazil after it reached its current status as the main coffee pest in the country (Souza *et al.*, 1998; Andrei, 1999). Pyrethroid use in Brazilian coffee plantations began later in the 1980s and the use of representatives of other insecticide groups is even more recent (Reis & Souza, 1984; Andrei, 1999). As a result of the heavy insecticide use on Brazilian coffee, the third largest crop for insecticide use in the country with over $50 million annual expenditure (Conceição, 2000), control failures and insecticide resistance have already been reported (Alves *et al.*, 1992; Guedes & Fragoso, 1999). Insecticide resistance has also been reported in *L. meyricki* from Tanzania (Bardner & Mcharo, 1988). Owing to increasing complaints of control failures with organophosphate use against *L. coffeella*, the present study was carried out to: (i) detect resistance to major representatives of this insecticide group; (ii) determine how this phenomenon relates to insecticide use, and; (iii) provide preliminary information on the involvement of detoxification enzymes as resistance mechanisms in Brazilian populations of *L. coffeella*.

Materials and methods

Insects

Ten populations of *L. coffeella* from the state of Minas Gerais (table 1), the largest coffee producing state in Brazil, were used in this study. This state can be divided into four major coffee producing regions: west, south, southeast and mid-north. The two first regions produce top quality coffee and are represented in our study by the populations from Araguari, Bambuí, Patrocínio and São Gotardo in the west, and Cambuquira in the south. The southeast region is

Table 1. Origin, year of collection, and characteristics of the collection sites of populations of *Leucoptera coffeella*.

Code no.	County	Month and year collected	Average annual temperature (°C)	Annual rainfall (mm)	Number of insecticide applications per year (average of 1998–1999 and 1999–2000)					
					Chlorpyrifos	Disulfoton	Ethion	Methyl parathion	Organo-phosphates (total)	Grand total
1	Araguari	Aug. 1998	21	1484	0	0	0	5	10	20
2	Bambuí	Apr. 1999	21	1448	2	1	1	0	7	18
3	Cambuquira	Feb. 1999	20	1346	0	1	0	0	1	1
4	Caparaó	Aug. 1999	20	1600	0	0	0	0	0	0
5	Capelinha	Aug. 1998	23	1300	1	1	0	0	2	2
6	Guiricema	Sep. 1998	20	1200	0	0	1	3.5	4.5	4.5
7	Patrocínio	Mar. 1999	22	1700	2	0	2.5	0	10	22
8	Ponte Nova	Oct. 1999	20	1060	0	0	0.5	0	0.5	0.5
9	São Gotardo	May 1999	22	1300	1	1	0	0	2	10
10	Simonésia	Sep. 1999	20	1140	0	0	0	0	0	0

represented by Caparó, Guiricema, Ponte Nova, Simonésia and Viçosa, where coffee is produced in hilly areas. The mid-north region is represented by Capelinha, where the coffee production is recent and the drink quality does not reach the standards from the west and south of the state. A further population was collected in Viçosa county, Minas Gerais, Brazil, at the coffee nursery of the Federal University of Viçosa. The coffee plantation at this site has never received insecticide use and is located in a relatively isolated area of a region where insecticide use against *L. coffeella* is rare. Therefore, it was used as a standard susceptible population in our study, as in a previous survey (Alves *et al.*, 1992; Guedes & Fragoso, 1999). Colonies of *L. coffeella* were established from at least 200 larvae obtained from infested leaves collected from each site. The individual populations were reared on coffee seedlings cv. Catuaí, without insecticide exposure, enclosed in cages and maintained in a greenhouse. The populations were maintained for two generations under these conditions before starting the bioassays. Individuals of these populations were sent to Dr W. Mey (Institute of Zoological Systematics, Humboldt University, Berlin, Germany) for identification.

Insecticide use and climate data

Information on insecticide use was obtained from appropriate growers and state extension personnel for each of the collection sites. The climatic data were obtained from local Agriculture State Offices.

Chemicals

The four technical grade insecticides used were chlorpyrifos (96% a.i., Dow AgroSciences, São Paulo, Brazil), and disulfoton (97.1% a.i.), ethion (97.8% a.i.) and methyl parathion (80.7% a.i.) (Bayer, São Paulo, Brazil). The three insecticide synergists used were diethyl maleate (DEM, 97% a.i.), piperonyl butoxide (PBO, 90% a.i.) and triphenyl phosphate (TPP, 99% a.i.), which were all obtained from Sigma-Aldrich Química Brasil Ltda. (São Paulo, Brazil). Analytical quality acetone was purchased from ISOFAR Ind. Com. Ltda. (Jacaré, Brazil).

Insecticide bioassays

Concentration–mortality insecticide bioassays were carried out following methods adapted from Siqueira *et al*. (2000a,b) using insecticide-impregnated filter papers (9 cm diameter). Five to seven different insecticide concentrations ranging from 10^{-4} to 10^{2} mg a.i. ml^{-1} depending on the insecticide, were applied for each bioassay against the standard susceptible (Viçosa) population; treatments with acetone only were included as controls. Three replicates, each with 20 third instar larvae of *L. coffeella* were used at each concentration. For each replicate, a filter paper was impregnated with 1 ml of insecticide dissolved in acetone. All filter papers were allowed to dry before use. The same procedure was used for the bioassays using insecticides in mixture with synergists. The use of technical grade insecticides on dry filter paper has been shown to reduce insecticidal pick-up by insects at low relative humidities (< 50%) (Samson & Keating, 1987), but the bioassays reported here were carried out at 70 ± 5% r.h. and 25 ± 2°C to minimize this effect. The proportion of insecticide:synergist was 1:10 and low mortality (< 5%) was observed in preliminary assays with the synergists used alone at concentrations of up to 3 mg cm^{-2}, higher than those used in this investigation when in mixture with organophosphates. The use of a constant insecticide synergist ratio in mixtures was chosen instead of pre-exposing larvae to a constant synergist concentration in order to: (i) speed up the bioassay, as the larvae do not survive long out of their mines; (ii) avoid excessive handling of the larvae, thereby minimizing any control mortality; and (iii) allow uniform availability of both insecticide and synergist to the insects. Insecticide concentrations were calculated as μg a.i. cm^{-2} of treated surface. Mortality assessment was made after 6 h and insects were counted as dead if they were unable to walk after prodding. Such a short exposure time may lead to overestimated levels of resistance because many compounds may not achieve complete kill so quickly. However the insecticides used in this study are known to be fast acting compounds which, aided by the less restrictive mortality criteria adopted (i.e. inability to walk), reduced this possibility. The LC_{99}s estimated from the concentration–mortality curves for the standard susceptible population were used as discriminating concentrations to screen the field-collected populations for resistance. Five replicates with 20 insects each were used for the monitoring bioassays.

Statistical analyses

Concentration–mortality data were subjected to probit analysis (PROC PROBIT; SAS Institute, 1989). The resistant populations in the monitoring bioassays were identified using LC_{99}s as discriminating concentrations, by the one-sided Z test at 95% confidence level with correction for continuity (Roush & Miller, 1986). The effect of the synergists was investigated using Dunnett's test at $P < 0.05$ by comparing the insecticidal activity with and without synergists against each of the selected populations (SAS Institute, 1989). Path analyses were used to test the hypothesized relationships between the rate of insecticide use and frequency of individuals resistant to each one of the organophosphate insecticides under study. These analyses were carried out using the procedures PROC REG and PROC CALIS from SAS (SAS Institute, 1989), following guidelines provided by Mitchell (1993). The variables, the total number of insecticide applications per year, the number of organophosphate applications per year, the number of applications per year of each insecticide under study, and the mortality at the discriminating concentrations of these insecticides did not satisfy the normality assumptions (PROC UNIVARIATE; SAS Institute, 1989), and transformation was necessary. The first three variables were transformed to $\log(x + 1)$, while the last was transformed to $\arcsin(x(\%)/100)$.

Results

Resistance detection

Concentration–mortality regression lines for the standard susceptible population of *L. coffeella* exposed to chlorpyrifos, disulfoton, ethion and methyl parathion are presented in table 2. The probit model adopted was suitable for the concentration–mortality analyses as mortality values predicted by the probit model did not differ significantly

Table 2. Toxicity of organophosphate insecticides, alone and in mixture with synergists (1:10 ratio), against a standard susceptible population of *Leucoptera coffeella*.

Insecticide + synergist	Number of insects	Slope ± SEM	LC$_{50}$ (CL 95%) μg a.i. cm^{-2}	LC$_{99}$ (CL 95%) μg a.i. cm^{-2}	SR	χ^2	Prob. (P)
Chlorpyrifos	300	0.77 ± 0.08	0.013 (0.012–0.015)	0.071 (0.050–0.110)	–	4.79	0.18
Chlorpyrifos + DEM	360	0.09 ± 0.07	0.004 (0.003–0.005)	0.020 (0.016–0.029)	3.25	0.79	0.93
Chlorpyrifos + PBO	280	1.03 ± 0.08	0.005 (0.004–0.006)	0.016 (0.013–0.022)	2.60	5.02	0.16
Chlorpyrifos + TPP	300	1.00 ± 0.09	0.003 (0.003–0.004)	0.011 (0.009–0.014)	4.33	1.55	0.67
Disulfoton	360	0.65 ± 0.07	0.011 (0.0097–0.013)	0.082 (0.057–0.139)	–	2.63	0.62
Disulfoton + DEM	360	0.89 ± 0.04	0.023 (0.017–0.031)	0.140 (0.08–0.46)	0.47	8.95	0.06
Disulfoton + PBO	300	0.85 ± 0.11	0.036 (0.031–0.043)	0.21 (0.15–0.34)	0.30	5.70	0.12
Disulfoton + TPP	360	0.83 ± 0.05	0.028 (0.020–0.038)	0.14 (0.08–0.42)	0.39	8.91	0.06
Ethion	360	0.52 ± 0.02	3.08 (2.37–3.86)	83.9 (47.4–202)	–	0.81	0.93
Ethion + DEM	300	0.86 ± 0.07	0.82 (0.72–0.92)	3.76 (2.83–5.74)	3.75	2.36	0.50
Ethion + PBO	300	0.81 ± 0.11	77.6 (70.1–84.	239 (184–386)	0.03	5.53	0.13
Ethion + TPP	420	0.38 ± 0.02	4.34 (3.21–5.68)	293 (164–652)	0.71	3.20	0.66
Methyl parathion	300	0.57 ± 0.05	0.023 (0.0024–0.029)	0.43 (0.27–0.87)	–	4.87	0.18
Methyl parathion + DEM	300	0.88 ± 0.07	0.013 (0.011–0.015)	0.076 (0.053–0.131)	1.76	4.42	0.21
Methyl parathion + PBO	360	0.70 ± 0.06	0.014 (0.012–0.016)	0.071 (0.053–0.107)	1.64	5.60	0.23
Methyl parathion + TPP	360	0.81 ± 0.05	0.011 (0.009–0.012)	0.081 (0.058–0.132)	2.09	2.74	0.60

The synergists used were diethyl maleate (DEM), piperonyl butoxide (PBO) and triphenyl phosphate (TPP). Synergism ratio (SR) is the ratio between LC$_{50\ \text{(unsynergized insecticide)}}$ and LC$_{50\ \text{(synergized insecticide)}}$.

from values observed in the bioassays (low χ^2-values and $P > 0.05$). The screening of the field-collected populations with the discriminating concentrations allowed the recognition of resistant populations (table 3). All but two field populations (Ponte Nova and Simonésia), showed apparent resistance to disulfoton. Five populations showed resistance to ethion (Araguari, Bambuí, Capelinha, Patrocínio and São Gotardo), four showed resistance to methyl parathion (Araguari, Bambuí, Patrocínio and São Gotardo), and one population showed resistance to chlorpyrifos (Araguari). The frequency of resistant individuals in these populations ranged from 10 to 93% for disulfoton, 53 to 75% for ethion, 23 to 76% for methyl parathion, and 35% for chlorpyrifos. A comparison across compounds for individual populations in table 3 shows great variation in the frequency of resistant individuals among the different field populations and suggests differences in cross-resistance patterns among them.

Association between insecticide use and resistance

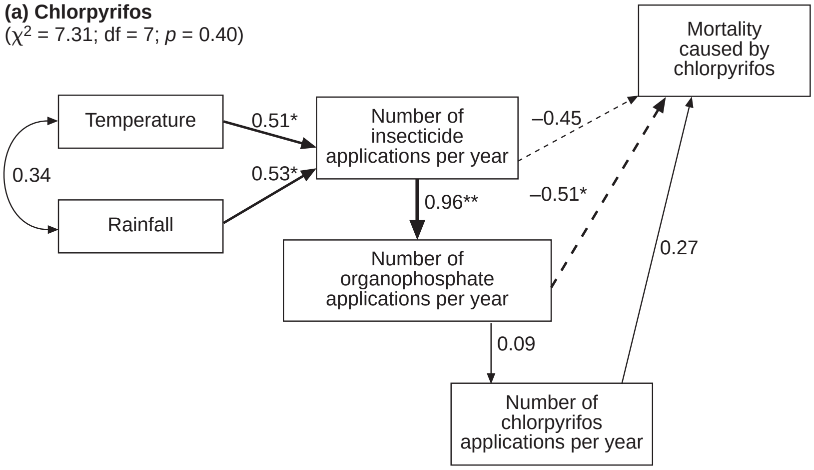
Insecticide use varied widely between sites, ranging from 0 to 22 applications per year (table 1). As *L. coffeella* prefers warm, dry conditions (Reis & Souza, 1986), it was hypothesized that temperature and rainfall would affect the frequency of insecticide use eventually leading to an increase in the frequency of resistant individuals in each population, measured as a decrease in mortality to insecticides. This would occur because higher temperatures and lower rainfall favour infestations of the leaf miner, necessitating a higher frequency of insecticide use in these regions thereby favouring the selection of insecticide resistance in this species. The model was tested for all four insecticides studied and significant models ($P > 0.05$) by the χ^2 goodness of fit test were obtained for chlorpyrifos, disulfoton and ethion (fig. 1), but not for methyl parathion ($\chi^2 = 23.15$; df = 11; $P = 0.02$), probably due to its use at only two of the sites surveyed (table 1). Each model variable of

Table 3. Control corrected mortality of field-collected populations of *Leucoptera coffeella* at discriminating concentrations.

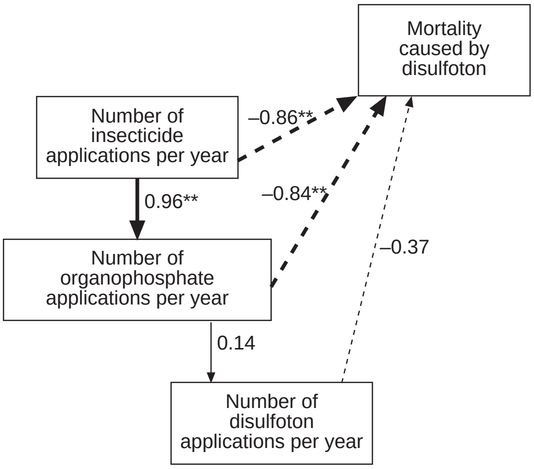
Population	Number of insects	Mortality (%)			
		Chlorpyirifos	Disulfoton	Ethion	Methyl parathion
Araguari	100	65*	7*	21*	10*
Bambuí	100	95	17*	15*	14*
Cambuquira	100	95	59*	87	100
Caparaó	100	95	90*	98	95
Capelinha	100	95	81*	29*	90
Guiricema	100	100	20*	98	99
Patrocínio	100	92	55*	29*	76*
Ponte Nova	100	100	98	98	99
São Gotardo	100	100	21*	47*	70*
Simonésia	100	100	98	98	99
Viçosa	100	96	99	85	95

Mortalities followed by an asterisk are significantly different from mortalities observed in the standard susceptible population by the 1-sided Z test at 95% CL with correction for continuity.

(a) Chlorpyrifos
($\chi^2 = 7.31$; df = 7; $p = 0.40$)



(b) Disulfoton
($\chi^2 = 10.18$; df = 7; $p = 0.18$)



(c) Ethion
($\chi^2 = 10.88$; df = 7; $p = 0.14$)

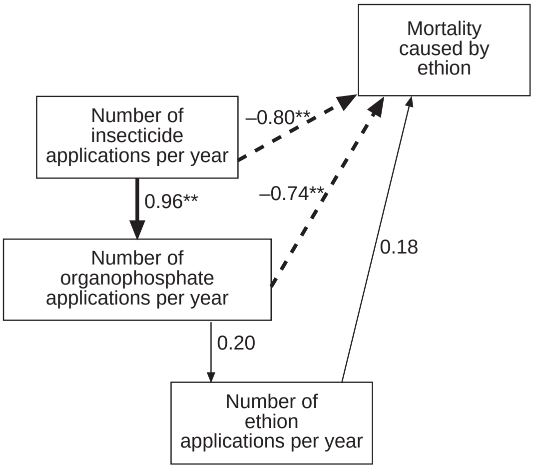


Fig. 1. Path analysis diagrams for (a) chlorpyrifos, (b) disulfoton, and (c) ethion. The results of χ^2 goodness of fit for each model diagram are indicated. Path (direct effects) and correlation coefficients are indicated for each interaction. A one-headed arrow indicates a causal interaction of one variable on another; a doubled-headed arrow indicates a correlation. Solid lines denote positive effects and dashed lines denote negative effects. Width of each line is proportional to the strength of the relationship, and paths significant at $P < 0.10$ and $P < 0.01$ are indicated with one or two asterisks respectively. The path from temperature and rainfall to the number of insecticide applications per year is the same in all diagrams and was represented only on the path diagram for (a) chlorpyrifos. Direct, indirect and total values for path coefficients are fully presented in table 4.

the path analysis may have direct and/or indirect effects on the derived path variable, which may even be antagonistic, and their added effects result in the total effect on the path. Temperature and rainfall for instance, create conditions that lead to a higher number of insecticide applications per year and have indirect effects on all other variables. Direct, indirect and total effects for the path diagrams (fig. 1) are shown in table 4. The magnitude of the total explained variance of each dependent variable is represented by the R^2-values in table 4.

There was no significant correlation between average annual temperature (°C) and annual rainfall (mm), but an increase in both showed a significantly positive trend with the total number of insecticide applications per year ($P < 0.10$) (fig. 1). However, the additive effect of these components was not significant ($R^2 = 0.39$; $P = 0.14$) (table 4). Greater insecticide use in general led to a greater use of organophosphates ($R^2 = 0.91$; $P < 0.0001$), but greater use of organophosphates was not correlated specifically with the usage of chlorpyrifos ($R^2 = 0.01$; $P = 0.80$), disulfoton ($R^2 = 0.02$; $P = 0.69$) or ethion ($R^2 = 0.04$; $P = 0.55$) (fig. 1; table 4). This suggests that additional organophosphate products were used by farmers at times of high insecticide use. Increase in insecticide usage had a significant negative effect on the levels of mortality achieved by disulfoton ($R^2 = 0.78$; $P = 0.009$) and ethion ($R^2 = 0.70$; $P = 0.02$), but not on mortality caused by chlorpyrifos ($R^2 = 0.36$; $P = 0.34$) (table 4) probably due to the little variation in mortality caused by this insecticide (table 3). Total number of insecticide applications and, in particular, the number of organophosphate applications appeared to be the major factors reducing mortality caused by disulfoton and ethion, probably by favouring selection for resistance to these compounds throughout the year. Nonetheless, the number of disulfoton and ethion applications per year had no significant effect on the levels of mortality achieved by these compounds, suggesting that cross-selection by other insecticide(s) may be leading to disulfoton and ethion resistance (fig. 1; table 4).

Synergism and resistance suppression

All synergists were able to significantly potentiate the insecticidal activity of chlorpyrifos, methyl parathion and chlorpyrifos (table 2). In contrast, the synergists did not enhance the activity of disulfoton, reducing its potency, and the synergists showed contrasting effects on ethion with diethyl maleate existing synergism, piperonyl butoxide exhibiting antagonism and triphenyl phosphate marginally reducing activity (table 2).

The % mortality of strains of *L. coffeella* exposed to 1:10 mixtures of insecticide:synergist at the estimated LC_{99} concentrations for the standard susceptible population are presented in table 5. No synergist was able to suppress the only instance of chlorpyrifos resistance suggesting target site insensitivity as the likely resistance mechanism in this case. In contrast, piperonyl butoxide provided nearly complete suppression of disulfoton resistance, followed by diethyl maleate and triphenyl phosphate, but all synergists reduced disulfoton activity on the susceptible population. This was also the case for piperonyl butoxide suppression of ethion resistance. Triphenyl phosphate and diethyl maleate provided partial suppression of ethion resistance in two populations and piperonyl butoxide was the only synergist to provide partial suppression of resistance to methyl parathion in one population (table 5).

Discussion

Resistance to the organophosphates chlorpyrifos, ethion and fenthion in Brazilian populations of *L. coffeella* was reported previously by Alves *et al*. (1992). However, the frequency of resistant individuals was relatively lower (< 50% for ethion, < 35% for ethion, and < 12% for chlorpyrifos) than reported here. Resistance to disulfoton, the main systemic insecticide used against *L. coffeella* in Brazil, and to methyl parathion, one of the oldest insecticides still in use against this coffee pest are reported here for the first time.

Resistance to ethion and chlorpyrifos does not appear to have spread much in the last ten years based on the results reported here compared with those of Alves *et al*. (1992). Resistance to methyl parathion has spread to the western part of the State of Minas Gerais and disulfoton resistance was observed throughout Minas Gerais, except in the southeast. Curiously, the resistance problems appear more serious in the west reflecting the distinct climate and agricultural practices of this region. In Araguari (west of Minas Gerais) the situation appears particularly serious with the highest frequencies of resistant individuals to all four insecticides studied. This trend was first reported by Guedes & Fragoso (1999) and is reinforced in this study. The variation in resistance patterns and the lack of a synergism pattern among different populations of *L. coffeella* suggest different regional patterns of cross-resistance with possible involvement of target site insensitivity as an additional resistance mechanism.

Leucoptera coffeella is deeply affected by weather conditions (Reis & Souza, 1984, 1986; Nestel *et al*., 1994). Greater infestations are observed in warmer areas during the drier months (Reis & Souza, 1984, 1986). The western coffee producing region of Minas Gerais is warm with two distinct dry periods – a shorter one (≈ 30 days), around February/March, during the rainy season, and a longer one corresponding to the dry season which lasts from June to October. As a consequence, there are two *L. coffeella* outbreak peaks in this region, in April/May and September/October (Souza *et al*., 1998), requiring intensive insecticide use (up to 22 applications per year concentrated during the outbreak periods). Outbreaks of *L. coffeella* take place only during the dry season in the other coffee producing regions requiring less insecticide use to contain them, especially in southeast Minas Gerais (Reis & Souza, 1984; Souza *et al*., 1998). This pattern of insecticide use was observed in our survey and an association between weather conditions, insecticide use, and resistance was hypothesized in a path diagram model.

There appeared to be a positive but weak influence of temperature and rainfall on the farmers' frequency of insecticide use. The temperature effect was expected because higher temperatures favour *L. coffeella* outbreaks (Reis & Souza, 1986) requiring more insecticide applications to achieve control. Rainfall, in contrast, may wash away the applied insecticides so reducing their efficiency and requiring additional applications. However, the added effect of both weather variables was not significant suggesting the involvement of other factors, possibly relative humidity as this is also reported to have a strong negative effect on *L. coffeella* abundance (Reis & Souza, 1986).

Table 4. Direct (DE), indirect (IE) and total (TE) effects for the path diagrams of fig. 1 which model resistance to chlorpyrifos, disulfoton and ethion as a result of weather conditions (temperature and rainfall) and local patterns of insecticide use.

Variable	General						Chlorpyrifos						Disulfoton						Ethion					
	Number of insecticide applications per year			Number of organophosphate applications per year			Number of chlorpyrifos applications per year			Mortality caused by chlorpyrifos			Number of disulfoton applications per year			Mortality caused by disulfoton			Number of ethion applications per year			Mortality caused by ethion		
	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE
Temperature (°C)	0.36	–	0.36	–	0.35	0.35	–	0.03	0.03	–	−0.17	−0.17	–	0.05	0.05	–	−0.32	−0.32	–	0.07	0.07	–	−0.29	−0.29
Rainfall (mm)	0.38	–	0.38	–	0.37	0.37	–	0.03	0.03	–	−0.18	−0.18	–	0.05	0.05	–	−0.31	−0.31	–	0.07	0.07	–	−0.30	−0.30
Number of insecticide applications per year	–	–	–	0.96	–	0.96	–	0.08	0.08	1.09	−0.62	−0.47	–	0.13	0.13	−0.49	−0.35	−0.84	–	0.19	0.19	-0.92	0.14	−0.78
Number of organophosphate applications per year	–	–	–	–	–	–	0.09	–	0.09	−0.68	0.03	−0.65	0.14	–	0.14	−0.34	−0.03	−0.37	0.20	–	0.20	0.09	0.06	0.15
Number of applications of a specific insecticide per year	–	–	–	–	–	–	–	–	–	0.30	–	0.30	–	–	–	−0.21	–	−0.21	–	–	–	0.28	–	0.28
R^2		0.39			0.91**			0.01			0.36			0.02			0.78**			0.04			0.70*	
P		0.14			< 0.0001			0.80			0.34			0.69			0.009			0.55			0.02	

The effects of number of insecticide applications per year and number of organophosphate applications per year are the same for all three insecticides. Direct effects are path coefficients. An asterisk indicates that the path is significant at 5%.

Table 5. Control corrected mortality of field-collected populations of *Leucoptera coffeella* exposed to discriminating concentrations of insecticide in mixture with synergists (1:10 ratio).

Population	Mortality (%)		
	Chlorpyrifos		
	Piperonyl butoxide	Triphenyl phosphate	Diethyl maleate
Araguari	52	48	50
	Disulfoton		
	Piperonyl butoxide	Triphenyl phosphate	Diethyl maleate
Araguari	93*	23	36*
Bambuí	88*	52*	63*
Cambuquira	79*	51	95*
Caparaó	100	92	73*
Capelinha	86	69	76
Guiricema	96*	50*	61*
Patrocínio	89*	20*	29*
	Ethion		
	Piperonyl butoxide	Triphenyl phosphate	Diethyl maleate
Araguari	41*	0*	25
Bambuí	76*	57*	8
Capelinha	90*	51*	89*
Patrocínio	35	12	42
	Methyl parathion		
	Piperonyl butoxide	Triphenyl phosphate	Diethyl maleate
Araguari	22	24	26
Patrocínio	44*	54*	45*
Bambuí	40*	11	24

Mortalities that differ significantly between insecticides with or without a synergist are followed by an asterisk (Dunnett's test, $P < 0.01$). Underlined mortality values indicate significant synergism and non-underlined values indicate significant antagonism.

Farmers who relied heavily on insecticides tended to prefer to use organophosphates. This greater use of organophosphates favoured resistance development, as shown by our path analyses for disulfoton and ethion. The frequency of use of chlorpyrifos, disulfoton, ethion and methyl parathion did not appear to be sufficient to select for resistance to these compounds at present, suggesting cross-selection with other insecticides, possibly other organophosphates based on our path analyses. Therefore, cross-resistance (i.e. resistance to two or more insecticides due to the same mechanism) appears to be present in Brazilian populations of *L. coffeella* resistant to organophosphates. Bardner & Mcharo (1988) also suggested the occurrence of cross-resistance in organophosphate resistant populations of *L. meyricki*.

Insects use enzymes not only to maintain homeostasis, but also to protect themselves against xenobiotics (Taylor & Feyereisen, 1996). Among the known insecticide resistance mechanisms, enhanced activity of detoxification enzymes is common, as is target site insensitivity (Scott, 1990, 1999; Taylor & Feyereisen, 1996; ffrench-Constant, 1999). Preliminary evidence of their involvement in insecticide resistance can be obtained by using synergists (Brindley & Selim, 1984; Scott, 1990; Bernard & Philogène, 1993). The synergists diethyl maleate, piperonyl butoxide, and triphenyl phosphate are reported as inhibitors of the main detoxification enzymes commonly involved in insecticide resistance: glutathione *S*-transferases, cytochrome P450-dependent monooxygenases, and esterases, respectively (Raffa & Priester, 1985; Bernard & Philogène, 1993; Scott, 1999). However, there are confounding effects of synergists acting to increase insecticidal uptake or interfering with non-target detoxification enzymes (Scott, 1990).

Resistance suppression by a particular synergist suggests the involvement of the enzyme blocked by it as the resistance mechanism. This was achieved in our study mainly by the synergist piperonyl butoxide, suggesting a major involvement of cytochrome P450-dependent monooxygenases as the resistance mechanism to organophosphates in *L. coffeella*, with minor involvement of glutathione *S*-transferases and esterases. Since partial suppression of resistance was achieved with different synergists in most, but not all populations, suggesting the involvement of two or even three separate detoxification enzymes, multiple resistance (i.e. resistance to a compound due to the co-involvement of two or more different mechanisms) may also occur in *L. coffeella* populations resistant to organophosphates. However, the antagonistic activity of synergists that decrease insecticide potency against the susceptible population casts doubt on some of these conclusions. Chlorpyrifos resistance in the leaf miner population from Araguari was not affected by synergist use suggesting the likely involvement of altered acetylcholinesterase leading to target site resistance as one of the resistance mechanisms in this population. Target site resistance may also occur in other populations, a possibility not properly explored in this investigation. Further studies on organophosphate resistance mechanisms in *L. coffeella* using *in vitro* colorimetric assays may shed light on this important aspect and help in the design of further resistance management strategies. Such information could, for example, help in the selection of suitable insecticides for use in rotation, or in mixtures, and even allow the use of insecticides with negative cross-resistance, as suggested for other insect pests (Zhu & Clark, 1995; Guedes *et al.*, 1997).

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References

Alves, P.M.P., Lima, J.O.G. & Oliveira, L.M. (1992) Monitoramento da resistência do bicho-mineiro-do-café, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), a inseticidas em Minas Gerais. *Anais da Sociedade Entomológica do Brasil* **21**, 77–91.

Andrei, E. (1999) *Compêndio de defensivos agrícolas*. 506 pp. São Paulo, Organizações Andrei.

Avilés, D.P. (1991) *Avaliação das populações do bicho-mineiro do cafeiro* Perileucoptera coffeella *(Lepidoptera: Lyonetiidae) e de seus parasitóides e predadores: metodologias de estudo e flutuação estacional*. 127 pp. MS thesis, Universidade Federal de Viçosa, Brazil.

Bardner, R. & Mcharo, E.Y. (1988) Confirmation of resistance of the coffee leafminer *Leucoptera meyricki* Ghesquière (Lepidoptera: Lyonetiidae) to organophosphate insecticide sprays in Tanzania. *Tropical Pest Management* **34**, 52–54.

Bernard, C.B. & Philogène, B.J.R. (1993) Insecticide synergists: role, importance, and perspectives. *Journal of Toxicology and Environmental Health* **38**, 199–223.

Box, H.E. (1923) The bionomics of the white coffee-leaf miner, *Leucoptera coffeella*, Guér. (Lepidoptera: Lyonetidae), in Kenya colony. *Bulletin of Entomological Research* **14**, 133–145.

Bradley, J.D. (1958) Taxonomic notes on *Leucoptera meyricki* Ghesquière and *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera, Lyonetiidae). *Bulletin of Entomological Research* **49**, 417–419.

Brindley, W.A. & Selim, A.A. (1984) Synergism and antagonism in the analysis of insecticide resistance. *Environmental Entomology* **13**, 348–353.

Conceição, M.Z. (2000) Manejo integrado em defesa vegetal. pp. 1–80 *in* Zambolim, L. (*Ed.*) *Manejo integrado de doenças, pragas e plantas daninhas*. Viçosa, Brazil, UFV.

ffrench-Constant, R.H. (1999) Target site mediated insecticide resistance: what questions remain? *Insect Biochemistry and Molecular Biology* **29**, 397–403.

Green, D.S. (1984) A proposed origin of the coffee leaf-miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae). *Bulletin of the Entomological Society of America* **86**, 664–667.

Guedes, R.N.C. & Fragoso, D.B. (1999) Resistência a inseticidas: bases gerais, situação e reflexões sobre o fenômeno em insetos-praga do cafeeiro. pp. 99–120 *in* Zambolim, L. (*Ed.*) *Livro de palestras do primeiro encontro sobre produção de café com qualidade*. Viçosa, Brazil, UFV.

Guedes, R.N.C., Zhu, K.Y., Kambhampati, S. & Dover, B.A. (1997) An altered acetylcholinesterase conferring negative cross-insensitivity to different insecticidal inhibitors in organophosphate-resistant lesser grain borer, *Rhyzopertha dominica*. *Pesticide Biochemistry and Physiology* **58**, 55–62.

Mey, W. (1994) Taxonomische bearbeintung der westpaläarktischen arten der gattung *Leucoptera* Hübner, [1825], s. l. (Lepidoptera, Lyonetiidae). *Deustche Entomologische Zeitschrift* **41**, 173–234.

Michereff, M.F.F. (2000) *Comportamento reprodutivo do bicho-mineiro-do-cafeeiro,* Leucoptera coffeella *(Guérin-Méneville, 1842) (Lepidoptera: Lyonetiidae)*. 46 pp. MS thesis, Universidade Federal de Viçosa, Brazil.

Mitchell, R.J. (1993) Path analysis: pollination. pp. 211–231 *in* Scheiner, S.M. & Gurevitch, J. (*Eds*) *Design and analysis of ecological experiments*. New York, Chapman & Hall.

Nestel, D., Dickschen, F. & Altieri, M.A. (1994) Seasonal and spatial population loads of a tropical insect: the case of the coffee leaf-miner in Mexico. *Ecological Entomology* **19**, 159–167.

Notley, F.B. (1948) The *Leucoptera* leaf miners of coffee on Kilimanjaro. I – *Leucoptera coffeella*, Guér. *Bulletin of Entomological Research* **46**, 399–416.

Raffa, K.F. & Priester, T.M. (1985) Synergists as research tools and control agents in agriculture. *Journal of Agricultural Entomology* **2**, 27–45.

Reis, P.R. & Souza, J.C. (1984) Pragas do café. *Informe Agropecuário* 10, 3–57.

Reis, P.R. & Souza, J.C. (1986) Influência das condições do tempo sobre a população de insetos e ácaros. *Informe Agropecuário* **12**, 25–30.

Reis, R., Jr., DeSouza, O. & Vilela, E.F. (2000) Predators impairing the natural biological control of parasitoids. *Anais da Sociedade Entomológica do Brasil* **29**, 507–514.

Roush, R.T. & Miller, G.L. (1986) Considerations for design of insecticide resistance monitoring programs. *Journal of Economic Entomology* **79**, 293–298.

Samson, P.R. & Keating, J.A. (1987) Effect of relative humidity on the biological activity of insecticides in impregnated paper assays. *Journal of Stored Products Research* **23**, 177–181.

SAS Institute (1989) *SAS/STAT user's guide, version 6*. Cary, North Carolina, USA, SAS Institute.

Scott, J.G. (1990) Investigating mechanisms of insecticide resistance: methods, strategies, and pitfalls. pp. 39–57 *in* Roush, R.T. & Tabashnik, B.E. (*Eds*) *Pesticide resistance in arthropods*. New York, Chapman & Hall.

Scott, J.G. (1999) Cytochromes P450 and insecticide resistance. *Insect Biochemistry and Molecular Biology* **29**, 757–777.

Silvestri, F. (1943) *Conpendio di entomologia applicata. Parte speciali, vol. II (Fogli 1–32)*. 512 pp. Portici, Italy, F. Silvestri.

Siqueira, H.A.A., Guedes, R.N.C. & Picanço, M.C. (2000a) Insecticide resistance in populations of *Tuta absoluta* (Lepidóptera: Gelechiidae). *Agricultural and Forest Entomology* **2**, 147–153.

Siqueira, H.A.A., Guedes, R.N.C. & Picanço, M.C. (2000b) Cartap resistance and synergism in populations of *Tuta absoluta* (Lep., Gelechiidae). *Journal of Applied Entomology* **124**, 233–238.

Souza, J.C., Reis, P.R. & Rigitano, R.L.O. (1998) *Bicho-mineiro do cafeeiro: biologia, danos e manejo integrado*. 48 pp. Belo Horizonte, Brazil, EPAMIG.

Taylor, M. & Feyereisen, R. (1996) Molecular biology and evolution of resistance to toxicants. *Molecular Biology and Evolution* **13**, 719–734.

Thomaziello, R.A. (1987) Manejo integrado de pragas, doenças e plantas daninhas em café. pp. 155–170 in *Anais do simpósio internacional de manejo integrado de pragas, doenças e plantas daninhas,* Campinas, Brazil, ANDEF.

Zhu, K.Y. & Clark, J.M. (1995) Comparisons of kinetic properties of acetylcholinesease purified from azinphosmethyl-susceptible and resistant strains of Colorado potato beetle. *Pesticide Biochemistry and Physiology* **51**, 57–67.

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