

## Article

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## Differential susceptibility to insecticides by *Leptinotarsa decemlineata* [Coleoptera: Chrysomelidae] populations from western Canada

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The susceptibility of Colorado potato beetles (*Leptinotarsa decemlineata*) (CPB) from three provinces in western Canada was measured using a filter paper bioassay to substantiate the reported insecticide resistance by the beetle in Manitoba, and to compare the situation there to beetle populations from Saskatchewan and Alberta. Susceptibility of beetles was measured against five insecticides: the organophosphates, azinphos-methyl (Guthion), and methamidophos (Monitor); the pyrethroid, permethrin (Ambush); the organochlorine, endosulfan (Endosulfan); and the carbamate, carbaryl (Sevin). All 12 populations tested from Manitoba were found to have resistance to one or more of the insecticides. All populations were classified as either having resistance or intermediate resistance to permethrin; two of the populations were classified as having resistance to azinphos-methyl and three to methamidophos. Two of four populations from Saskatchewan were classified as having intermediate resistance to azinphos-methyl and methamidophos. Intermediate resistance to permethrin was recorded in 12 of the 13 populations from Alberta, with only one being highly susceptible. Two populations showed evidence of intermediate resistance to azinphos-methyl and three to methamidophos. In all three provinces, survival rate from different egg masses within the susceptible populations ranged from 0-100%, indicating the presence of individuals with either resistance, intermediate or high susceptibility within these populations. With the expanding potato acreage in western Canada and the detection of the CPB populations with resistance to insecticides, a resistance management program must be implemented to prevent the rapid selection of resistant populations.

### [Sensibilités variées aux insecticides de populations de *Leptinotarsa decemlineata* [Coleoptera : Chrysomelidae] de l'Ouest canadien]

La sensibilité du doryphore de la pomme de terre (*Leptinotarsa decemlineata*) de trois provinces de l'Ouest canadien a été mesurée par bioessai sur

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papier filtre pour corroborer les comptes rendus faisant état de doryphores résistants aux insecticides au Manitoba, et pour comparer la situation au Manitoba à celles de la Saskatchewan et de l'Alberta. La sensibilité des doryphores a été mesurée pour cinq insecticides : les organophosphates azinphos-méthyl (Guthion) et méthamidophos (Monitor), le pyréthroïde perméthrine (Ambush), l'organochloré endosulfan (Endosulfan) et le carbamate carbaryl (Sevin). Les 12 populations du Manitoba examinées démontraient de la résistance à au moins un des insecticides. Toutes les populations ont été classées comme étant résistantes ou moyennement résistantes à la perméthrine; deux des populations ont été classées comme résistantes à l'azinphos-méthyl et trois au méthamidophos. Deux des quatre populations de la Saskatchewan ont été classées comme étant moyennement résistantes à l'azinphos-méthyl et au méthamidophos. Une résistance intermédiaire à la perméthrine a été trouvée dans 12 des 13 populations de l'Alberta, alors qu'une seule était très sensible. Deux populations se sont montrées moyennement résistantes à l'azinphos-méthyl et trois au méthamidophos. Dans les trois provinces, le taux de survie de diverses masses d'oeufs provenant de populations sensibles variait de 0 à 100 %, ce qui montre que des individus résistants, moyennement résistants et très sensibles coexistent dans ces populations. Avec l'expansion de la culture de la pomme de terre dans l'Ouest canadien et la détection d'une résistance aux insecticides dans les populations du doryphore de la pomme de terre, un programme de gestion de la résistance doit être mis en place pour éviter la sélection rapide de populations résistantes.

## INTRODUCTION

Potato is the most important vegetable crop in Canada, making up 60% of all vegetable farm cash receipts (Statistics Canada 1999). The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) [Coleoptera: Chrysomelidae] is considered one of the most destructive foliage feeding pests of potatoes. The application of chemical insecticides has been the primary method used to control this insect in North America (Hare 1990; Martel 1987; Roush *et al.* 1990). However, widespread and repeated use of chemicals as a control method have resulted in the selection of insecticide resistant populations. In some potato producing areas in North America, nearly all the previously effective insecticides are no longer able to reduce beetle populations and new insecticides lose their effectiveness within a few yr because of cross resistance (French *et al.* 1992; Harris and Turnbull 1986). Thus, the selection of insecticide resistant populations is a major threat to the potato industry and a continuing problem in Colorado potato beetle manage-

ment (Forgash 1981; Grafius 1997; Martel 1987).

In Canada, insecticide resistant populations have been reported from most of the eastern provinces where potatoes are grown (Boiteau 1988; Boiteau *et al.* 1987; Harris and Svec 1976; Stewart *et al.* 1997). The first reports of resistance were to organochlorine insecticides (Harris and Svec 1976; McClanahan 1975; McDonald 1976). By 1981, populations showing resistance to organophosphates and carbamates were found in Quebec. In 1979, most populations tested in Ontario were susceptible to pyrethroids (permethrin, fenvalerate and cypermethrin), but by 1982, a 22-37 fold resistance was reported after just 2 yr of use of these insecticides (Harris and Turnbull 1986). In New Brunswick, there was a 70% increase in beetle populations between 1974 and 1980 which coincided with an increase in insecticide resistant populations (Boiteau *et al.* 1987). Some populations in these areas have developed resistance to insecticides in all classes, resulting in the emergency registration in 1995 of imidacloprid (Admire™),

which belongs to the new chloronicotinyl class of insecticide.

In western Canada there have been recent reports of insecticide resistance to three of the four classes of insecticides tested from Manitoba (Gavloski 1997). In Alberta, the last report of the presence of resistance was to DDT (McDonald 1976) but since then there have been no surveys conducted.

Over the last 10 yr, Canadian potato production has increased by 55% and the area planted by 40% (Statistics Canada 1999). The future growth of the potato industry is expected to be in western Canada, including Alberta, as more potato processors establish there. This increasing demand for potatoes requires an increase in acreage, and may result in a decrease in rotation, factors that will favour Colorado potato beetle populations and the need for an increase in the use of insecticide treatments. This will consequently provide ideal conditions for the selection of insecticide resistant beetle populations.

The objective of this study was to measure the susceptibility to insecticides, of beetles from three western Canadian provinces, to substantiate the reported occurrence of insecticide resistance to the beetle in Manitoba, to compare the situation there to beetle populations from Saskatchewan and Alberta, and to provide base-line data for future survey.

## MATERIALS AND METHODS

Insecticide resistance in beetle populations from Manitoba, Saskatchewan, and Alberta was measured using a filter paper bioassay (French *et al.* 1992; Heim *et al.* 1990). Due to the low numbers of beetles found throughout parts of western Canada, we were unable to collect sufficient numbers of egg masses directly from the field. Consequently, during the summer of 1998, laboratory cultures of 35 egg-laying females and 15 males were established on potted "Russet Burbank" potato plants from field collected adults or fourth instar larvae from commercial fields in each province (Table 1). Beetles from each commercial field were considered separate populations. The egg masses from each population were collected over a 1 to 3-month period and used in the assays. For the most part, eggs were collected from adults that arose directly from the field collected larvae. However, for four populations (Portage la Prairie, Selkirk 2, Winkler and Lake Diefenbaker), we had to add a few beetles from the first generation to the cage with the original population towards the end of experimentation because egg laying had slowed down considerably in these populations.

Each bioassay unit consisted of a filter paper (5.5 cm diam., Fisher P-5) that was pre-treated with a commercial insecticide dissolved in acetone using the diagnostic concentrations calculated by

**Table 1. Collection localities for *Leptinotarsa decemlineata* from western Canada**

No.	Alberta	Saskatchewan	Manitoba
1	Edmonton-1	Lake Diefenbaker	Lauder
2	Edmonton-2	Outlook	Hartney
3	Nobleford	Saskatoon	Bagot
4	Lethbridge	Nipawin	Portage la Prairie-1
5	Cranford		Portage la Prairie-2
6	Enchant-1		Winkler
7	Enchant-2		Gretna
8	Taber-1		Selkirk-1
9	Taber-2		Selkirk-2
10	Vauxhall		Steinbach-1
11	Hays-1		Steinbach-2
12	Hays-2		Unknown
13	Grassy Lake		

**Table 2. Insecticides tested for resistance in Colorado potato beetle populations from western Canada using a filter paper bioassay**

Insecticide class	Insecticide	Diagnostic concentration <sup>a</sup> ( $\mu\text{g ai cm}^{-2}$ )	Mean mortality of susceptible strain <sup>b</sup> (%)	Mean mortality of resistant strain <sup>b</sup> (%)
Organophosphate	Azinphos-methyl (Guthion <sup>TM</sup> ) Bayer	1.05	95 $\pm$ 5	61 $\pm$ 13
	Methamidophos (Monitor <sup>TM</sup> ) Bayer	1.05	97 $\pm$ 9	72 $\pm$ 10
Pyrethroid	Permethrin (Ambush <sup>TM</sup> ) Zeneca	0.16	93 $\pm$ 3	0
Organochlorine	Endosulfan (Endosulfan <sup>TM</sup> ) Aventis	0.11	100	0
Carbamate	Carbaryl (Sevin <sup>TM</sup> ) Aventis	30.00	100	92 $\pm$ 2

<sup>a</sup> As used by French *et al.* 1992.

<sup>b</sup> Susceptible and resistant laboratory strains were obtained from S. Hilton, Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada, London, Ontario (Hilton *et al.* 1998). Ten egg masses of each strain were tested against each insecticide.

French *et al.* (1992) (Table 2). A single egg mass, with the excess foliage removed, was placed on the insecticide treated filter paper in a Petri dish and incubated at 23°C and 16L:8D photoperiod. When at least 50% of the eggs hatched, the filter paper was moistened with about 0.3 mL water. The dish was sealed with parafilm, and the number of dead and live larvae were counted 24 h later. A larva that did not return to its upright position after having been placed on its dorsal side was considered dead. Egg masses for the controls were placed on acetone treated filter paper. Ten egg masses (> 15 eggs per egg mass) per population per insecticide were tested. We followed the classification scheme of Kennedy and French (1994); egg masses showing < 50% mortality were classified as resistant. The proportion of egg masses within each population showing resistance (*i.e.* < 50% mortality) to the diagnostic concentrations of French *et al.* (1992) was used to classify the populations according to their susceptibility as follows: resistant (> 80% of the egg masses had < 50% mortality); intermediate (between 20 to 79% of the egg masses had < 50% mortality); and sus-

ceptible (between 0 to 19% of the egg masses had < 50% mortality). Controls for all populations from the three provinces were run simultaneously.

To verify the diagnostic concentrations against known resistant and susceptible populations of the beetle, we tested 10 egg masses per insecticide each from susceptible and resistant populations originating from populations from southern Ontario (Hilton *et al.* 1998). The resistant population was resistant to organochlorine, pyrethroid and organophosphate insecticides, with the exception that it had lost its resistance to carbofuran and resistance to azinphos-methyl had decreased (S. Hilton, personal communication).

## RESULTS

Populations from all three western provinces demonstrated presence of individuals with resistance to one or more of the insecticides (Table 3). Of the 12 populations tested from Manitoba, 6 were classified as having resistance and 6 showed intermediate susceptibility to permethrin (Fig. 1; Table 3). Popula-

**Table 3. Mean percent mortality and range per egg mass of *Leptinotarsa decemlineata* for each insecticide and population tested. Numbers in bold representant resistant populations**

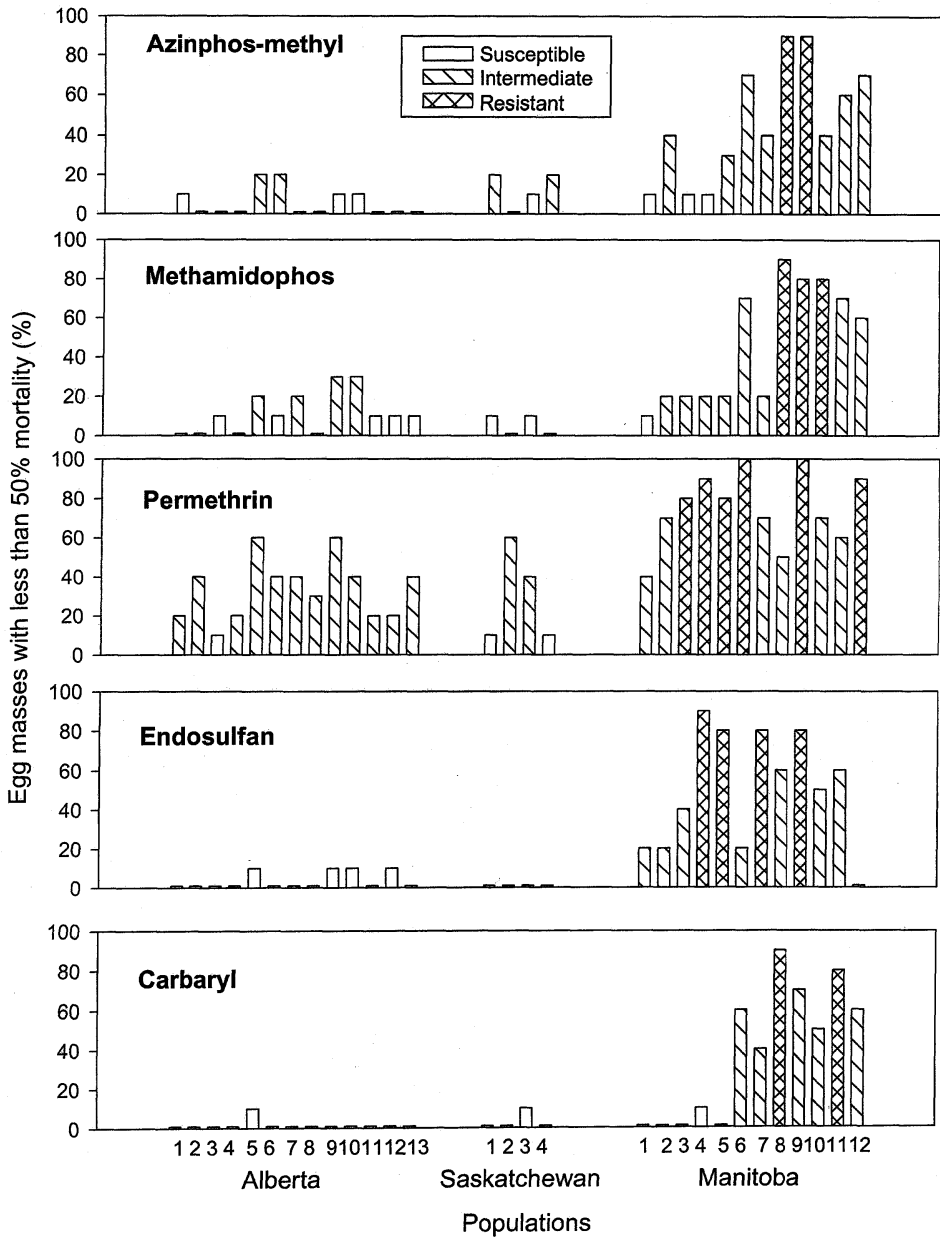
Population number	Mean percent mortality (range) per insecticide				
	Azinphos-methyl	Methamidophos	Permethrin	Endosulfan	Carbaryl
<b>Alberta</b>					
1	81 (5-100)	99 (93-100)	77 (11-100)	100	100
2	84 (60-100)	86 (54-100)	55 (14-100)	100	100
3	92 (77-100)	71 (3-100)	80 (40-95)	99 (96-100)	99 (92-100)
4	97 (74-100)	99 (95-100)	82 (27-100)	100	100
5	78 (24-100)	74 (3-100)	27 (0-84)	80 (0-100)	92 (24-100)
6	80 (38-100)	90 (18-100)	69 (14-100)	99 (96-100)	100
7	88 (66-100)	88 (45-100)	62 (15-100)	97 (85-100)	99 (96-100)
8	86 (67-100)	93 (65-100)	68 (16-100)	99 (96-100)	100
9	83 (17-100)	70 (26-100)	49 (0-86)	76 (22-100)	98 (75-100)
10	87 (33-100)	67 (28-100)	42 (0-95)	85 (11-100)	99 (95-100)
11	92 (75-100)	82 (3-100)	73 (23-100)	98 (90-100)	97 (83-100)
12	90 (72-100)	71 (19-100)	63 (9-100)	95 (50-100)	99 (87-100)
13	83 (32-100)	74 (23-100)	54 (5-88)	93 (79-100)	97 (79-100)
<b>Saskatchewan</b>					
1	74 (41-100)	84 (30-100)	87 (38-100)	100	99 (97-100)
2	87 (60-100)	88 (66-100)	46 (0-100)	97 (83-100)	99 (96-100)
3	77 (47-97)	88 (45-100)	48 (0-95)	95 (80-100)	94 (48-100)
4	82 (40-100)	98 (92-100)	76 (46-100)	100	99 (94-100)
<b>Manitoba</b>					
1	87 (29-100)	92 (29-100)	64 (0-100)	82 (17-100)	99 (95-100)
2	57 (0-96)	69 (23-100)	39 (0-100)	85 (25-100)	95 (73-100)
3	78 (4-100)	78 (9-100)	<b>30 (0-69)</b>	56 (9-100)	99 (91-100)
4	78 (39-100)	69 (6-100)	<b>26 (0-52)</b>	<b>17 (0-93)</b>	83 (23-100)
5	74 (7-100)	82 (13-100)	<b>14 (0-67)</b>	<b>28 (0-94)</b>	97 (79-100)
6	39 (0-100)	43 (15-90)	<b>&lt;1 (0-3)</b>	88 (39-100)	43 (0-89)
7	57 (21-92)	73 (0-100)	32 (5-67)	<b>35 (0-100)</b>	57 (0-95)
8	<b>19 (0-100)</b>	<b>17 (0-100)</b>	48 (0-98)	50 (19-86)	<b>29 (0-100)</b>
9	<b>28 (0-71)</b>	<b>30 (0-67)</b>	<b>1 (0-7)</b>	<b>39 (6-82)</b>	40 (0-89)
10	52 (0-100)	<b>36 (0-93)</b>	38 (0-100)	57 (13-100)	57 (8-100)
11	39 (0-80)	34 (0-100)	45 (4-100)	37 (0-100)	<b>13 (0-71)</b>
12	40 (0-81)	47 (8-92)	<b>12 (0-67)</b>	77 (53-100)	46 (6-100)

tions showed highest susceptibility to carbaryl, with only two of the 12 populations classified as having resistance to this insecticide. Four of the 12 populations were classified as having resistance and 7 showed an intermediate level of susceptibility to endosulfan. Two populations showed resistance to azinphos-methyl and 3 populations to methamidophos, with 7 and 8 showing intermediate levels of susceptibility. One population (population 1) of the 12 tested was classified as highly susceptible to all three chemicals, azinphos-methyl, methamidophos, and carbaryl; no populations were found to be highly susceptible to permethrin. Several populations demonstrated multiple resistance with population 8 demonstrat-

ing resistance to azinphos-methyl, methamidophos, and carbaryl and population 9 to azinphos-methyl, methamidophos, permethrin and endosulfan.

From Saskatchewan, only two populations of the four tested showed intermediate susceptibility to permethrin and azinphos-methyl (Fig. 1). All four populations were highly susceptible to the methamidophos, endosulfan and carbaryl.

In Alberta, an intermediate level of susceptibility to permethrin was recorded in 12 of the 13 populations tested (Fig. 1). Of the two organophosphates tested, two populations showed intermediate susceptibility to azinphos-methyl and four populations to metha-



**Figure 1. Proportion of egg masses (%) of *Leptinotarsa decemlineata* from the Canadian prairie provinces with less than 50% mortality after exposure to insecticides.** Susceptibility categories are defined as follows: Resistant when > 80% of the egg masses had < 50% mortality; Intermediate when between 20 and 79% of the egg masses had < 50% mortality; and Susceptible when between 0 and 19% of the egg masses had < 50% mortality (see Kennedy and French 1994). Insecticide concentrations used were those previously determined as diagnostic for resistant and susceptible populations from North Carolina (French *et al.* 1992).

midophos. All 13 populations were classified as highly susceptible to endosulfan and carbaryl; 10 of the populations were highly susceptible to at least three insecticide classes.

Controls for all populations from the three provinces showed no mortality over the 24-hr assay period. The application of the French *et al.* (1992) diagnostic concentrations to laboratory-reared Canadian susceptible and resistant strains of potato beetles confirmed the suitability of these diagnostic concentrations used for this survey (Table 2). The intermediate resistance response of the resistant population to azinphos-methyl and methamidophos was as expected for this colony (S. Hilton, personal communication).

## DISCUSSION

Populations from all three western provinces demonstrated some level of resistance to one or more of the insecticides tested. Resistance was most prevalent in populations from Manitoba, which is consistent with the results of a previous survey, where populations from 21 of 55 potato fields in Manitoba were found to be resistant to at least one of the nine insecticides tested (Gavloski 1997). Although the diagnostic concentrations used in this study were calculated for resistant and susceptible populations from North Carolina, they were verified by Stewart *et al.* (1997) for populations from Prince Edward Island and Ontario, and for a resistant and susceptible population from Ontario in this study. However, we have chosen to report our results according to level of susceptibility to French *et al.*'s (1992) diagnostic concentrations rather than to probability of field control (Kennedy and French 1994), because base-line data on the susceptibility of beetle populations from western Canada had not yet been determined and consequently, local resistant and susceptible populations were not available for us to verify these diagnostic concentrations. The results of the present study can now be used as a base-line to establish local dose: response regressions in order to fine

tune, if necessary, the diagnostic concentrations we used for further monitoring of the evolution of resistance in the prairie provinces. These results can also be used as a base to measure the future development of resistance to insecticides by Colorado potato beetles in the Canadian prairie provinces.

In our study, populations most commonly showed some level of resistance to the pyrethroid, permethrin. Resistance to pyrethroids can develop quite rapidly. Harris and Svec (1981) reported low levels of resistance to pyrethroids in field populations of CPB from Sherbrooke, Quebec in 1979, however by 1982, greater than 23-fold resistance was reported in this population (Harris and Turnbull 1986). In a laboratory bioassay, CPB selected sequentially for fenvalerate exhibited a 1700 fold increase in resistance to this insecticide within eight generations (Huang *et al.* 1994, 1995). The prevalence of populations with low susceptibility to permethrin throughout all three western provinces and especially in Manitoba, should be a warning signal to producers that pyrethroids should be used cautiously.

Our data also shows that the development of resistance to organophosphate insecticides may soon occur in western Canada. In Manitoba, 17% of the populations were classified as having resistance to azinphos-methyl and 25% to methamidophos. However, 58 and 67% of the populations were classified as having intermediate levels of susceptibility to these two insecticides in Manitoba, and 15 and 23% had intermediate susceptibility in Alberta. This is an indication that the selection of resistant beetles has already started and these populations typify a "mixed" population.

In all three provinces, there were populations classified as highly susceptible to at least one or more insecticides. The numbers of highly susceptible populations were much greater in Alberta and Saskatchewan than in Manitoba. However, even within these susceptible populations, the range of survival within the bioassay units varied considerably, ranging from 0-100% for some insecticides. Although such



natural variations are common, it underscores that there is always the potential for the rapid development of resistance, even in a highly susceptible population and that the development of resistance can be very localized. A similar variation was observed in the populations classified as having intermediate levels of susceptibility. Continuous pressure by the use of insecticides within the same class, especially on these intermediate populations, would result in the eventual selection of a resistant population.

The presence of larger numbers of populations with low susceptibility in Manitoba as compared to Alberta and Saskatchewan may be attributed to the recent rapid increase in potato acreage in Manitoba (62% since 1992) (Statistics Canada 1999) resulting in an increase in Colorado potato beetle populations, and the need to control these populations using insecticides (J. Gavloski, personal communication). In Saskatchewan and Alberta, Colorado potato beetle populations have been traditionally low, but with the expanding potato industry, an increase in the Colorado potato beetle population is expected, which in turn could result in increased insecticide applications and the eventual rapid development of resistance. Thus caution must be exercised and a resistance management program should be implemented immediately in order to prevent, or at least delay, further selection of insecticide resistant populations.

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