

Resistance Status of the Colorado Potato Beetle, *Leptinotarsa decemlineata*, to Endosulfan in East Azarbaijan and Ardabil Provinces of Iran

Authors: Mohammadi Sharif, M., Hejazi, M. J., Mohammadi, A., and Rashidi, M. R.

Source: Journal of Insect Science, 7(31) : 1-7

Published By: Entomological Society of America

URL: <https://doi.org/10.1673/031.007.3101>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.



Resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, to endosulfan in East Azarbaijan and Ardabil provinces of Iran

M. Mohammadi Sharif¹, M. J. Hejazi^{1,a}, A. Mohammadi² and M. R. Rashidi³

¹ Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

² Department of Plant Breeding, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

³ Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Three Colorado potato beetle, *Leptinotarsa decemlineata*, populations, from Ardabil, Bostanabaad and Ajabshir, were collected from potato fields in East Azarbaijan and Ardabil provinces in Iran and assayed for resistance to endosulfan. Possible resistance mechanisms were investigated using synergism studies and biochemical assays. Laboratory tests showed that the Bostanabaad strain was 220 and 109 times resistant compared with the susceptible strain in 2003 and 2004, respectively. The resistance ratios for the Ajabshir and Ardabil strains were 19 and 18, respectively. Since considerably more resistance was observed in the Bostanabaad strain compared with the other two, further investigation of the origin of resistance was done on this strain. Two insecticide synergists, piperonyl butoxide and S,S,S-tributylphosphorotrithioate, reduced resistance 2.3 and 3.5 times, respectively. These small degrees of synergism suggest that metabolism is not the source of the considerable difference in susceptibility between the two strains. This was supported by the results obtained from the biochemical assays that showed that glutathione S-transferase activity in the Bostanabaad strain did not significantly differ from the susceptible strain. These results suggest that target site insensitivity may be involved.

Keywords: synergism, S,S,S-tributylphosphorotrithioate, piperonyl butoxide, resistance mechanism

Abbreviations: CDNB 1-chloro-2, 4-dinitrobenzene, DEF S,S,S-tributylphosphorotrithioate

Correspondence: ^a mjhejazi@tabrizu.ac.ir

Received: 3 March 2006 | **Accepted:** 10 August 2006 | **Published:** 14 May 2007

Copyright: This is an open access paper. We use the Creative Commons Attribution 2.5 license that permits unrestricted use, provided that the paper is properly attributed.

ISSN: 1536-2442 | Volume 7, Number 31

Cite this paper as:

Mohammadi Sharif M, Hejazi MJ, Mohammadi A, Rashidi MR. 2007. Resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, to endosulfan in East Azarbaijan and Ardabil provinces of Iran. 7pp. *Journal of Insect Science* 7:31, available online: insectscience.org/7.31

Introduction

The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a major pest of potatoes in most potato growing regions of the world, including Iran. In the absence of control practices, *L. decemlineata* populations can completely defoliate potato plants. In general, potato plants are sensitive to beetle defoliation up to 60 days after planting (Zehnder and Evanylo 1989). However, most of the damage is caused at the beginning of blooming, which coincides with tuber formation and filling (Hare 1990). This insect was a quarantine pest in Iran, but in 1984 was reported from Ardabil, the major potato production region of Iran (Nouri Ganbalani 1986).

L. decemlineata control mainly involves the use of insecticides. Development of resistance to organochlorine insecticides by *L. decemlineata* occurred quickly (McDonald 1976; Harris and Svec 1976; Harris and Svec 1981) and is widespread. Resistance to organophosphorous, carbamate and pyrethroid insecticides has been documented in many locations (Harris and Svec 1981; Heim et al. 1990; French et al. 1992; Pap et al. 1997). This insect has a high potential for the development of resistance against different insecticidal chemicals including recently registered products (Zhao et al. 2000; Cutler et al. 2005). Three main reasons for this high potential are its high capability for increasing its population in a short period of time (Senanayake et al. 2000), the selection of resistant individuals due to a higher chance of exposure of all active stages to insecticides (Follett et al. 1993), and cross resistance to several insecticides as a result of a single resistance mechanism or the co-existence of several resistance mechanisms leading to multiple resistance to several insecticides (French et al. 1992). Besides these, other factors such as the absence of crop rotation in some regions, and the occurrence of local populations of *L. decemlineata*, may lead to homozygosity for insecticide resistance.

Since the development of resistance can affect the efficiency of available insecticides for *L. decemlineata* control, understanding the resistance status of *L. decemlineata* populations would be useful (French et al. 1992). A successful resistance management strategy involves a thorough knowledge of resistance mechanisms (Ioannidis et al. 1992).

Application of insecticide synergists is one of the easiest and fastest procedures to obtain preliminary information about potential mechanisms of resistance (Scott 1990). Piperonyl butoxide inhibits cytochrome P₄₅₀ microsomal monooxygenases, and S,S,S-tributylphosphorothioate (DEF) inhibits esterases and glutathione S-transferase. Since endosulfan does not have an ester bond, in such synergism studies, DEF acts as glutathione S-transferase inhibitor (Rufingier et al. 1999).

Endosulfan is both an effective insecticide against *L. decemlineata*, and is economical, so it has been well accepted and used for several years by potato growers in many potato growing regions of northwestern Iran. Hence, determining the existence and possible causal mechanisms of resistance to this insecticide is of prime importance. To achieve this goal, endosulfan was tested on 4th instar larvae of the *L. decemlineata* populations and possible mechanisms of resistance were investigated using synergism studies and biochemical assays.

Materials and Methods

Insect strains and rearing conditions

During 2003 and 2004, populations of Colorado potato beetle were collected from potato fields of Ardabil county in the province of Ardabil, and Bostanabad and Ajabshir counties in East Azarbaijan province. In Bostanabad the field sampled in 2004 was different from the sampled field in 2003. A susceptible strain was supplied by M. S. Goettel, Agriculture and Agri-Food Canada Research Center, Lethbridge. Colonies of these insects were reared in greenhouse at 26 ± 2° C, 50 ± 5% RH and 16:8 (L:D) photoperiod on unsprayed potato foliage, and were monitored for insecticide resistance.

Chemicals

Technical grade endosulfan (96% purity) was supplied by Bayer CropScience (bayercropscience.com). S,S,S-tributylphosphorothioate (DEF) and piperonyl butoxide were purchased from Chem Service (chemservice.com) and Fluka Chemie (sigmaaldrich.com), respectively. 1-chloro-2, 4-dinitrobenzene (CDNB) and reduced glutathione were purchased from Merck (merck.de), copper sulfate and bicinehoninic acid from Sigma-Aldrich Chemie (sigmaaldrich.com) and bovin serum albumin and ethylene diamine tetraacetic acid were purchased from Carl Roth (carl-roth.de).

Table 1. Resistance status of *Leptinotarsa decemlineata* populations

Population	n	Slope ± SE	LD 50 (µg/larva) (95% CL)	Resistance ratio *
Ajabshir (2003)	350	0.8 ± 0.13	1.9 (0.81–3.18)	19
Ardabil (2003)	365	0.8 ± 0.15	1.8 (0.81–2.81)	18
Bostanabaad (2003)	358	0.7 ± 0.14	22.0 (13.6–45.7)	220
Bostanabaad (2004)	360	0.9 ± 0.14	10.9 (7.10–16.3)	109
Susceptible (2004)	360	1.9 ± 0.23	0.1 (0.07–0.12)	-

* LD₅₀ of resistant strain/LD₅₀ of susceptible strain

Bioassays

Bioassays were conducted using 24–48 hour old 4th instar larvae. To obtain uniform larvae for use in bioassays, up to 24 hour old larvae were fed for 24 hours under rearing conditions. The insecticide was dissolved in acetone and 0.5 (in 2003) or 1.0 µl (in 2004) aliquots were deposited on the mesonotum. The control larvae were treated with 0.5 or 1.0 µl acetone. The ranges of endosulfan doses for susceptible and resistant strains were 0.05–0.5 and 1–80 µg/larva, respectively. Technical difficulties made it necessary to change the droplet size to 1 µl in 2004, which is likely to have increased the area of the cuticle surface treated.

The treated larvae were placed in 13 x 6.5 x 4 cm transparent plastic containers and supplied with untreated potato foliage. The treated insects were kept at 26 ± 1° C and 16:8 (L:D) photoperiod and mortality was recorded 24 h after treatment. The affected larvae were considered alive if they could move their legs and body after touching one leg with a needle. Five levels of the insecticide were used in all treatments and each treatment consisted of 4–5 replicates. The probit option of SPSS was used for analyzing dose-mortality data (SPSS 1999).

Synergism studies

The synergists used in this study were DEF and piperonyl butoxide. These chemicals were dissolved in acetone and applied topically one hour prior to treatment with endosulfan using the same procedure described above. Each treated larva received 5 µg DEF or 10 µg piperonyl butoxide. These were the highest doses of the

synergists that showed no mortality on the susceptible strain. The control larvae were treated with piperonyl butoxide or DEF. The insecticide doses and the procedure used for data analysis were similar to those described above. Each treatment was replicated four times.

Biochemical assays

Glutathione S-transferase activity was measured using CDNB (Habig et al. 1974). Abdomens of fourth instar larvae were homogenized in phosphate buffer (100 mM, pH 6.5) containing 5 mM reduced glutathione. The homogenate was centrifuged at 11000 g and 4°C, and the supernatant was used as the enzyme source. In a final volume of 3 ml, the reaction mixture consisted of 50 µl supernatant, 50 µl 1 mM CDNB in methanol and 2.9 ml of the buffer. After adding CDNB, the reaction was assayed spectrophotometrically (Shimadzu, UV-2550) at 340 nm for 3 min and the activity was calculated using the extinction coefficient of 9.6 mM⁻¹cm⁻¹. A blank consisted of 50 µl CDNB and 2.95 ml of the phosphate buffer; each treatment was replicated four times. To determine Michaelis-Menten parameters, the initial velocity of reactions resulting from six different concentrations of CDNB (0.05–1 mM) with the enzyme fraction were measured separately in both susceptible and resistant strains. The K_m and V_{max} values were obtained from Lineweaver-Burk plot. These treatments were replicated three times.

Protein content was measured with bicinchoninic acid procedure (Smith et al. 1985) using bovine serum albumin as the standard.

Table 2. Synergism of endosulfan with DEF and PBO in resistant and susceptible populations of *Leptinotarsa decemlineata*

Treatment	n	Slope ± SE	LD 50 (µg/larva) (95% CL)	SR *
Susceptible (2004)	360	1.9 ± 0.23	0.1 (0.07–0.12)	–
DEF	366	1.7 ± 0.27	0.2 (0.17–0.26)	0.5
PBO	341	2.0 ± 0.24	0.1 (0.09–0.14)	1
Bostanabaad (2004)	360	0.9 ± 0.14	10.9 (7.10–16.3)	–
DEF	360	1.0 ± 0.12	3.1 (2.10–4.41)	3.5
PBO	358	0.9 ± 0.10	4.7 (3.10–7.0)	2.3

* Synergism ratio: LD₅₀ /s LD₅₀

Table 3. Activity and kinetic parameters of GSH-transferase of susceptible and resistant strains of *Leptinotarsa decemlineata*

Parameter	Susceptible	Resistant (Bostanabaad strain)
Activity ^a (4) ^b	73.2 ± 7.6	61.9 ± 12.4
K _m ^c	154 ± 31.3	279 ± 54
V _{max} ^a	66.5 ± 16.3	72.5 ± 6.6

^aMean value (μM/min/mg protein) ± SD.^bNumber of replicates (n).^cMean value (μM) ± SD.

Results

Compared with the susceptible strain, the Bostanabaad strain was 220 and 109 times resistant to endosulfan in 2003 and 2004, respectively. The resistance ratios for Ajabshir and Ardabil strains were 19 and 18, respectively (Table 1). As shown in Table 2, piperonyl butoxide and DEF did not synergize endosulfan in the susceptible strain, but enhanced the toxicity of endosulfan in the resistant Bostanabaad strain. piperonyl butoxide and DEF reduced resistance 2.3 and 3.5 times, respectively. Glutathione S-transferase activity of susceptible and resistant *L. decemlineata* strains was measured using *in vitro* CDNB (Table 3). No statistical difference ($P > 0.05$) was observed between the two strains. There was also no difference ($P > 0.05$) in V_{max} for glutathione S-transferase activity toward CDNB between the susceptible and resistant strains. Affinity of glutathione S-transferase for CDNB, as addressed by higher K_m in resistant vs. susceptible strains, was significantly ($P < 0.05$) lower in the resistant strain.

Discussion

Monooxygenase and glutathione S-transferase based metabolism have been shown to be associated with insecticide resistance in *L. decemlineata*. Argentine et al. 1989 demonstrated that these enzyme systems were involved in resistance to azinphosmethyl and permethrin. Other investigations have also shown the importance of detoxifying mechanisms in *L. decemlineata* resistant strains (Argentine et al. 1992; Ahammad-Sahib et al. 1994; Zhao et al. 2000).

Our results indicate that oxidases and glutathione S-transferases may be involved in endosulfan metabolism in the resistant Bostanabaad population, but may not be solely responsible for endosulfan resistance in this strain because the treatment with the synergists could not cope with

the resistance problem completely. This suggests that other mechanisms may be involved as well. Lin et al. 1993 also did not find metabolic detoxification as an important mechanism for dieldrin resistance in *Tribolium castaneum*.

Although glutathione S-transferase is a major detoxifying enzyme system in insecticide detoxification (Hemingway 2000; Kostaropoulos et al. 2001), our results from the biochemical assays are in agreement with the synergism data and confirm only a minor effect of glutathione S-transferase in detoxifying endosulfan in the resistant strain of the *L. decemlineata*. These results are somewhat similar to those reported by Lin et al. 1993.

Although crop rotation is not a common practice used in potato growing fields of East Azarbaijan and Ardabil provinces, some crop rotation is done using wheat and alfalfa in rotation with potatoes. Since the size of agricultural fields in the region is usually small (0.5–1 ha) it does not seem this fairly uncommon practice would considerably hinder development of *L. decemlineata* resistance to insecticides. Among the agricultural crops grown in the region, none is considered as an alternate host for *L. decemlineata*, which means the host difference as well as the difference in the type of insecticide used for different hosts would not affect development of resistance in this insect. The history of endosulfan usage and potato production practices in the regions monitored may explain the variation in the observed resistance levels.

Bostanabaad

The pest found its way to the region in 1992. Agria which is a medium to late maturing cultivar is dominantly planted in Bostanabaad and harbors *L. decemlineata* for over 80 days. The potential for long term damage requires sprays up to four times a year of which two to three of the sprays include endosulfan. Endosulfan has been the major chemical for *L. decemlineata* control since

1997 in this region. The use of this chemical reached its peak in 2003, but has declined in the past three years due to lack of acceptable control. High selection pressure could have been the main cause of the high level of resistance for endosulfan found in Bostanabaad. The high frequency of insecticide application as an important cause of resistance development has also been reported by others (Zhao et al. 2000). Development of resistance in such a fairly short time is not unexpected. Rapid development of *L. decemlineata* resistance to organochlorine (Harris and Svec 1981) and some other insecticides such as carbofuran (Ioannidis et al. 1992) and abamectin (Argentine and Clark 1990) have been reported. Although in 2004 the use of endosulfan was somewhat diminished, the considerable decrease in resistance to endosulfan in 2004 could not be justified solely by the decrease in usage. Instead, it is possible that variation between fields in insect response to the insecticide could have been the major factor involved in this regard. This phenomenon has also been demonstrated in other investigations (Heim et al. 1990; Tissler and Zehnder 1990).

Ardabil

In Ardabil, chemical control of *L. decemlineata* had relied on phosalone and to a less extent, endosulfan, after its initial outbreak. The use of endosulfan became the major management approach after a considerable decrease in phosalone efficiency around 1992. Heavy spraying with endosulfan for several years in a row was followed by control failures that resulted in abandonment of this chemical in Ardabil after 1997. The main chemicals used for controlling this pest after the decline in efficacy of endosulfan have been organophosphorus, carbamates and nicotinoid insecticides which would eliminate the anticipation of cross resistance to endosulfan. Lower level of resistance in Ardabil compared to Bostanabaad population, is likely due to reversion of a high level of resistance that had led to control failure in 1995–1996. Such a decrease in resistance levels has been reported for DDT-resistant populations of *L. decemlineata* (McDonald 1976). Reduction of resistance level in absence of the pesticide previously used is the basis for resistance management (French-Constant et al. 1993a). Scott et al. 2000 stated that resistant populations of the Australian sheep blowfly, *Lucilia cuprina* lost their resistance to dieldrin in absence of exposure, possibly due to the fitness cost involved. Also, resistance to permethrin (Follett et al. 1993), azinphosmethyl (Zhu et al. 1996) and *Bacillus*

thuringiensis Cry3A toxin (Whalon et al. 1993; Alyokhin and Ferro 1999) was associated with fitness cost in resistant strains of *L. decemlineata*. Considering these findings, reduced resistance in the Ardabil population could have been related to a fitness cost involved. The other possibilities for partial justification of this reduction in resistance could have been the cessation of endosulfan application and selection pressure in the area.

Ajabshir

L. decemlineata was observed in potato fields of Ajabshir for the first time in 1989, but its damage was negligible and no regular chemical control was required until 1997. In this region short season potato cultivars are planted and the *L. decemlineata* adults emerge around early May. Because potato plants are harvested in late July, only the first generation requires chemical control and the second generation does not cause major damage because the approaching harvest time usually prevents completion of larval development. Hence, exposure of only a small proportion of the population to insecticides may result in a low frequency of selection toward resistance. The use of insecticides belonging to various groups of chemicals in different fields and years also makes selection pressure for resistance less of a problem in this region. This is in agreement with Heim et al. 1990 who stated that the presence of 3rd and 4th generations of *L. decemlineata* with no spraying was one of the main reasons for the delay in development, or low levels of resistance to insecticides. The difference in the number of imidacloprid applications (2–4 times per year) in Long Island populations of *L. decemlineata* has also been suggested as the cause of higher level of resistance to this insecticide compared with a Michigan population with imidacloprid application of once a year (Zhao et al. 2000). Although the availability of the host plant for a limited time and consequently reduction in selection pressure can delay development of resistance due to the high capability of *L. decemlineata* to adapt to local climatic and potato growing conditions (Senanayake et al. 2000), regular monitoring of the resistance status in this population must also be taken into consideration. Fipronil is a newly registered insecticide in Iran. Since in some cyclodiene-resistant insects cross resistance to fipronil has been found (Bloomquist 2001; Kristensen et al. 2005), considering fipronil for use in *L. decemlineata* management programs should be done with a thorough knowledge of the resistance status of this pest to cyclodiene insecticides.

Conclusion

As the high level of endosulfan resistance in the Bostanabaad strain could not be justified by synergism and biochemical studies, further investigation should be done. Target site insensitivity has been reported as the major cause of resistance to cyclodienes (ffrench-Constant et al. 1993b; Anthony et al. 1995; Bass et al. 2004). Additional experiments are required to further investigate mechanisms involved in the endosulfan resistant strain of *L. decemlineata*.

Acknowledgements

Support for this work was provided by the University of Tabriz Board of Graduate Studies. We thank M. S. Goettel and R. Talaei for kindly providing the *L. decemlineata* susceptible strain and Iranian Agency of Dow-Elanco for supplying technical grade endosulfan. The authors wish to thank S. Abbasi for her help in caring for the insect colonies and conducting experiments and A. Gharamaleki for arranging trips for sampling.

References

- Ahammad-Sahib KI, Hollingworth RM, Whalon ME, Ioannidis PM, Grafius EJ. 1994. Polysubstrate monooxygenases and other xenobiotic-metabolizing enzymes in susceptible and resistant Colorado potato beetle. *Pesticide Biochemistry and Physiology* 49: 1-12.
- Alyokhin AV, Ferro DN. 1999. Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. *Journal of Economic Entomology* 92: 510-515.
- Anthony NM, Brown JK, Markham PG, ffrench-Constant RH. 1995. Molecular analysis of cyclodiene resistance-associated mutations among populations of the sweet potato whitefly *Bemisia tabaci*. *Pesticide Biochemistry and Physiology* 51: 220-228.
- Argentine JA, Clark JM, Ferro DN. 1989. Genetics and synergism of resistance to azinphos-methyl and permethrin in the Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 82: 698-705.
- Argentine JA, Clark JM. 1990. Selection for abamectin resistance in Colorado potato beetle (Coleoptera: Chrysomelidae). *Pesticide Science* 28: 17-24.
- Argentine JA, Clark JM, Lin H. 1992. Genetics and biochemical mechanisms of abamectin resistance in two isogenic strains of Colorado potato beetle. *Pesticide Biochemistry and Physiology* 44: 191-207.
- Bass C, Schroeder I, Turberg A, Field L, Williamson MS. 2004. Identification of the *Rdl* mutation in laboratory and field strains of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). *Pest Management Science* 60: 1157-1162.
- Bloomquist JR. 2001. GABA and glutamate receptors as biochemical sites for insecticide action. In: Ishaaya I, editor. *Biochemical Sites of Insecticide Action and Resistance*, pp. 17-41. Springer-Verlag.
- Cutler GC, Tolman JH, Scott-dupree DS, Harris CR. 2005. Resistance potential of Colorado potato beetle (Coleoptera: Chrysomelidae) to novaluron. *Journal of Economic Entomology* 98: 1685-1693.
- ffrench-Constant RH, Steichen JC, Ode PJ. 1993a. Cyclodiene insecticide resistance in *Drosophila melanogaster* (Meigen) is associated with a temperature-sensitive phenotype. *Pesticide Biochemistry and Physiology* 46: 73-77.
- ffrench-Constant RH, Steichen JC, Rocheleau TA, Arnostein K, Roush R. 1993b. A single-amino acid substitution in γ -aminobutyric acid subtype A receptor locus is associated with cyclodiene insecticide resistance in *Drosophila* populations. *Proceedings of the National Academy of Sciences USA* 90: 1957-1961.
- Follett PA, Gould F, Kennedy GG. 1993. Comparative fitness of three strains of Colorado potato beetle (Coleoptera: Chrysomelidae) in the field: spatial and temporal variation in insecticide selection. *Journal of Economic Entomology* 86: 1324-1333.
- French NM, Heim DC, Kennedy GG. 1992. Insecticide resistance patterns among Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) populations in North Carolina. *Pesticide Science* 36: 95-100.
- Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249: 7130-7139.
- Hare JD. 1990. Ecology and management of the Colorado potato beetle. *Annual Review of Entomology* 35: 81-100.
- Harris CR, Svec HJ. 1976. Susceptibility of the Colorado potato beetle in Ontario to insecticides. *Journal of Economic Entomology* 69: 625-629.
- Harris CR, Svec HJ. 1981. Colorado potato beetle resistance to carbofuran and several other insecticides in Quebec. *Journal of Economic Entomology* 74: 421-424.
- Heim DC, Kennedy GG, Van Duyn JW. 1990. Survey of insecticide resistance among North Carolina Colorado potato beetle (Coleoptera: Chrysomelidae) populations. *Journal of Economic Entomology* 83: 1229-1235.
- Hemingway J. 2000. The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. *Insect Biochemistry and Molecular Biology* 30: 1009-1015.

- Ioannidis PM, Grafius EJ, Wierenga JM, Whalon ME, Hollingworth RM. 1992. Selection, inheritance and characterization of carbofuran resistance in the Colorado potato beetle (Coleoptera: Chrysomelidae). *Pesticide Science* 35: 215-222.
- Johnston RL, Sandvol LE. 1986. Susceptibility of Idaho populations of Colorado potato beetle to four classes of insecticides. *American Potato Journal* 63: 81-85.
- Kostaropoulos I, Papadopoulos AI, Metaxakis A, Boukouvala E, Papadopoulou-Mourkidou E. 2001. Glutathione S-transferase in the defense against pyrethroids in insects. *Insect Biochemistry and Molecular Biology* 31: 313-319.
- Kristensen M, Hansen KK, Jensen KV. 2005. Cross-resistance between dieldrin and fipronil in the German cockroach (Dictyoptera: Blattellidae). *Journal of Economic Entomology* 98: 1305-1310.
- Lin H, Bloomquist JR, Beeman RW, Clark JM. 1993. Mechanisms underlying cyclodiene resistance in the red flour beetle, *Tribolium castaneum* (Herbst). *Pesticide Biochemistry and Physiology* 45: 154-165.
- McDonald S. 1976. Evaluation of several new insecticides for the control of the Colorado potato beetle and the status of DDT resistance in Southern Alberta. *Journal of Economic Entomology* 69: 659-664.
- Nouri Ganbalani G. 1986. *Colorado potato beetle* (in Persian). University of Tabriz Publications.
- Pap L, Toth A, Karikas S. 1997. A survey of the insecticide resistance status of the Colorado potato beetle, *Leptinotarsa decemlineata*, in Hungary between 1987 and 1991. *Pesticide Science* 49: 389-399.
- Rufingier C, Pasteur N, Lagnel J, Martin C, Navajas M. 1999. Mechanisms of insecticide resistance in the aphid *Nasonovia ribisnigri* (Mosley) (Homoptera: Aphididae) from France. *Insect Biochemistry and Molecular Biology* 29: 385-391.
- Scott JG. 1990. Investigating mechanisms of insecticide resistance: Methods, strategies, and pitfalls. In: Roush RT, Tabashnik BE, editors. *Pesticide Resistance in Arthropods*, pp. 39-57. Chapman and Hall.
- Scott M, Diwell K, McKenzie JA. 2000. Dieldrin resistance in *Lucilia cuprina* (the Australian sheep blowfly): chance, selection and response. *Heredity* 84: 599-604.
- Senanayake DG, Radcliffe EB, Holliday NJ. 2000. Oviposition and diapause behavior in Colorado potato beetle (Coleoptera: Chrysomelidae) populations from East Central Minnesota and the valley of the Red River of the North. *Environmental Entomology* 29: 1123-1132.
- Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry* 150: 76-85.
- SPSS. 1999. *SPSS 10.0.1 for Windows*. SPSS Inc., Chicago, Illinois.
- Tissler AM, Zehnder GW. 1990. Insecticide resistance in Colorado potato beetle (Coleoptera: Chrysomelidae) on the Eastern shore of Virginia. *Journal of Economic Entomology* 83: 666-671.
- Whalon ME, Miller DL, Hollingworth RM, Grafius EJ, Miller JR. 1993. Selection of a Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. *Journal of Economic Entomology* 86: 226-233.
- Zehnder GW, Evanylo GK. 1989. Influence of extent and timing of Colorado potato beetle (Coleoptera: Chrysomelidae) defoliation on potato tuber production in Eastern Virginia. *Journal of Economic Entomology* 82: 948-953.
- Zhao J, Bishop BA, Grafius EJ. 2000. Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 93: 1508-1514.
- Zhu KY, Lee SH, Clark JM. 1996. A point mutation of acetylcholinesterase associated with azinphosmethyl resistance and reduced fitness in Colorado potato beetle. *Pesticide Biochemistry and Physiology* 55: 100-108.