

Laboratory Tests to Determine the Intrinsic Toxicity of Four Fungicides and Two Insecticides to the Predacious Mite *Agistemus fleschneri*

N.J. Bostanian and N. Larocque¹

Laboratory tests showed that trifloxystrobin (Flint[®] 50WG), myclobutanil (Nova[®] 40WP), flusilazole (Nustar[®] 50DF) and kresoxim-methyl (Sovran[®] 50WG) were harmless to adult mites and did not affect adversely the reproductive capacity of *Agistemus fleschneri* (Summers) (Acari: Stigmaeidae). Among the insecticides evaluated, by the same technique, λ -cyhalothrin (Warrior T[®] 12%) and imidacloprid (Admire[®] 24%) were also non-toxic.

KEY WORDS: Trifloxystrobin; myclobutanil; flusilazole; kresoxim-methyl; λ -cyhalothrin; imidacloprid; toxicity; *Agistemus fleschneri*.

INTRODUCTION

Several reports have indicated that stigmatid mites contribute to the biological control of phytophagous mites in orchards (2,6,13-15,20,21).

In integrated pest management programs, it is essential that pesticides that are effective against target species are at the same time relatively harmless to non-target parasitic and predatory arthropods. In apple orchards, the greatest impact of pesticides on non-targets is in summer, because most of the non-target arthropods will reach maximum abundance at that time (4,10). In Wooster, Ohio (USA), sulphur applied three times to control powdery mildew in an orchard was shown to be incompatible with the biological control of the European red mite *Panonychus ulmi* (Koch) by *Zetzellia mali* (Ewing) and *Agistemus fleschneri* (Summers) (13). Nelson *et al.* (19) reported the toxicity of 20 insecticides and five fungicides, used in Michigan apple orchards, to *A. fleschneri*. In apple orchards under a 'supervised chemical control' program, in Verona, Italy, the fungicides mancozeb and metiram were toxic to *Amblyseius andersoni* (Chant) and this toxicity led to *P. ulmi* (Koch) outbreaks (12). Croft (9) and Bostanian *et al.* (5) reported that the response of a non-target arthropod to the toxic action of a pesticide varied from one locality to another and postulated that this may be a consequence of the way the pesticide may have been used in the past. Consequently, the intrinsic toxicity of pesticides to biocontrol agents should be established before, or at the latest as soon as, these pesticides become commercially available. Our study was planned to establish the intrinsic toxicity of four fungicides and two insecticides to field-collected *A. fleschneri* prior to the widespread use of these pesticides in apple orchards.

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¹Horticultural Research and Development Centre, Agriculture and Agri-Food Canada, St. Jean-sur-Richelieu, Quebec, Canada J3B 3E6 [Fax: +1-450-346-7740; e-mail: bostaniannj@em.agr.ca].

MATERIALS AND METHODS

Field collection Females of *Agistemus fleschneri* (Summers) (Acari: Stigmaeidae) were collected from a commercial orchard at St. Alexandre, Quebec, Canada, from early June to the end of September 1999. Throughout the study, leaves with mites were picked from apple trees and within 30 min brought to the laboratory in paper bags placed in a cooler. None of the pesticides evaluated in this study had ever been used in this orchard. The low rate of reproduction among *A. fleschneri* reared in the laboratory and the large numbers required for this study forced us to abandon the use of laboratory-reared mites.

Toxicity to females The standard slide-dip method (1) was used to assess the toxicity of the various pesticides. Thirty females for each concentration were transferred with a fine camel-hair brush to double-sided tape (48 mm × 4 m, Cantech,[®] Canadian Technical Tape Ltd., Quebec, Canada) on microscope slides. The slides were dipped in appropriate concentrations of the pesticide for 5 sec and air-dried for 30 min. They were then placed on top of metal gratings shaped like tables (28 × 20 × 10 cm) and lowered into plastic containers (34 × 21 × 21 cm) of water; the depth of the water was 5 cm. The cover of the container was placed diagonally so as to allow a certain amount of air exchange between the inside and the outside of the container. The setup was then placed in a growth chamber at 21°C., 80% r.h. and 16:8 L:D. With this arrangement the r.h. within the container was $\sim 93 \pm 2\%$. Twenty-four hours following the application of pesticides, the treated slides were placed under a stereomicroscope and dead mites were counted. Death was determined by the absence of appendage movements when the mites were prodded with a camel-hair brush. Percent mortality was calculated by probit analysis (18). In this series, the experimental unit for each concentration consisted of a slide treated with a particular pesticide; it was replicated three times.

Reproductive capacity (ability to produce viable eggs) Fresh fully expanded apple leaves grown in a growth chamber were used throughout this series of tests. The blade of each leaf was cut to a rectangle (4 × 3 cm) and its axil was inserted in a small vial containing water. The top of the vial was sealed with Parafilm[®] (American National Can, Chicago, IL, USA) to reduce water evaporation. A thin coat of insect trapping adhesive (The Tanglefoot Co., Grand Rapids, MI, USA) was applied at the point of insertion of the axil into the vial. Twenty two-spotted spider mite (*Tetranychus urticae* Koch) females were transferred onto each leaf for oviposition. After 24 h, three *A. fleschneri* females were introduced onto each leaf. The infested leaves (prey + predator) were treated with a pesticide at different concentrations with a thin layer chromatography sprayer set at 10.34 kPa (1.5 PSI) and allowed to dry at room temperature. Once the pesticide residue had dried, the vials with the leaves were inserted into polystyrene racks that held the vials at an angle of 45° with the floor. The experimental setting was then placed in a growth chamber at 21°C, 80% r.h. and 16:8 L:D. After 48 h of exposure, any *T. urticae* females that had not been consumed by *A. fleschneri* were removed, along with *A. fleschneri* females. The predator eggs were counted under a stereomicroscope and kept in the growth chamber until hatching. After 7 days, egg hatch was recorded for 3 days and percent mortality was calculated by probit analysis (18). In this series, the experimental unit for each concentration consisted of five leaves treated with a particular pesticide; it was replicated five times.

Pesticides under evaluation The fungicides kresoxim-methyl (Sovran® 50WG, BASF Canada, Toronto, Ont., Canada), trifloxystrobin (Flint® 50WG, Bayer Inc., Etobicoke, Ont., Canada), flusilazole (Nustar® 50DF, DuPont Canada, Mississauga, Ont., Canada) and myclobutanil (Nova® 40WP, Rohm & Haas Canada, Westhill, Ont., Canada) and the two insecticides λ -cyhalothrin (Warrior T® 12%, Syngenta Crop Protection Inc., Guelph, Ont., Canada) and imidacloprid (Admire® 24%, Bayer Inc., Etobicoke, Ont., Canada), were evaluated from 4x to 1/8x in decreasing geometric progression. The letter x represented the manufacturer's recommended concentration for the pesticide (g a.i./l) (Table 1). The controls throughout this study were treated with tap water.

RESULTS AND DISCUSSION

The data (Fig. 1 and Table 1) reveal that none of the four fungicides evaluated had any adverse effects on adult *A. fleschneri* even when these compounds were applied at four times the recommended concentration. The slopes were near zero and even negative in some cases (Fig. 1 and Table 1). Walker *et al.* (23) had reported no adverse effects of myclobutanil on *Typhlodromus pyri* Scheuten in New Zealand. Likewise, no effects of myclobutanil on *Amblyseius fallacis* (Garman) adults and eggs were reported (8). In greenhouse studies, the chemically related flusilazole was reported to be slightly toxic to the aphid endoparasite *Aphidius rhopalosiphi* DeStefani-Perez on wheat (16).

The present data (Fig. 1 and Table 1) also reveal that imidacloprid and λ -cyhalothrin have no toxic effects on *A. fleschneri* adults. Bostanian and Racette (7) had found that the residual toxicity of λ -cyhalothrin to *A. fallacis* was less than 50% 10 days after treatment, and the rapid dissipation of this pyrethroid was an asset for IPM programs in Quebec apple orchards. On the other hand, earlier pyrethroids such as cypermethrin, fenvalerate and deltamethrin had caused over 86% mortality to *A. fallacis* (3). As for imidacloprid, its non-toxic effect on *Typhlodromus pyri* Scheuten had been reported from apple orchards in Germany, Italy and Spain (11), and on *Neoseiulus longispinosus* (Evans) from apple orchards in South Korea (22). Figure 2 and Table 2 summarize the effects of these six pesticides on the reproductive capacity of treated females and we note no adverse effects by any of the products.

When *A. fleschneri* is the only predator of phytophagous mites in an orchard, then the intrinsic harmlessness of kresoxim-methyl, trifloxystrobin, flusilazole, myclobutanil, λ -cyhalothrin and imidacloprid to adults and their ability to lay viable eggs, suggests that these pesticides can be used to manage other pests and diseases without interfering with the biological control program for phytophagous mites. Nevertheless, restrictions imposed by the manufacturer on the label should be observed. For example, the Canadian label for kresoxim-methyl states that only four treatments may be made per season, with no more than two treatments in sequence. Furthermore, the last treatment should not be within 30 days before harvest. In the presence of other predacious mites additional restrictions would be applicable to these pesticides. Thus, λ -cyhalothrin may be used pre-bloom only if in addition to *A. fleschneri* substantial numbers of *A. fallacis* appear in July (7). Furthermore, the manufacturer's labels for λ -cyhalothrin and imidacloprid in Canada restrict the use of these insecticides to a single treatment per season. Moreover, neither of these insecticides should be applied when the trees are in bloom, or near ponds, lakes, streams or rivers. Finally, although myclobutanil and kresoxim-methyl have been shown to be harmless to *A. fallacis* (17), possible toxic effects to *A. fallacis* of the remaining three pesticides involved in this study have not yet been established.

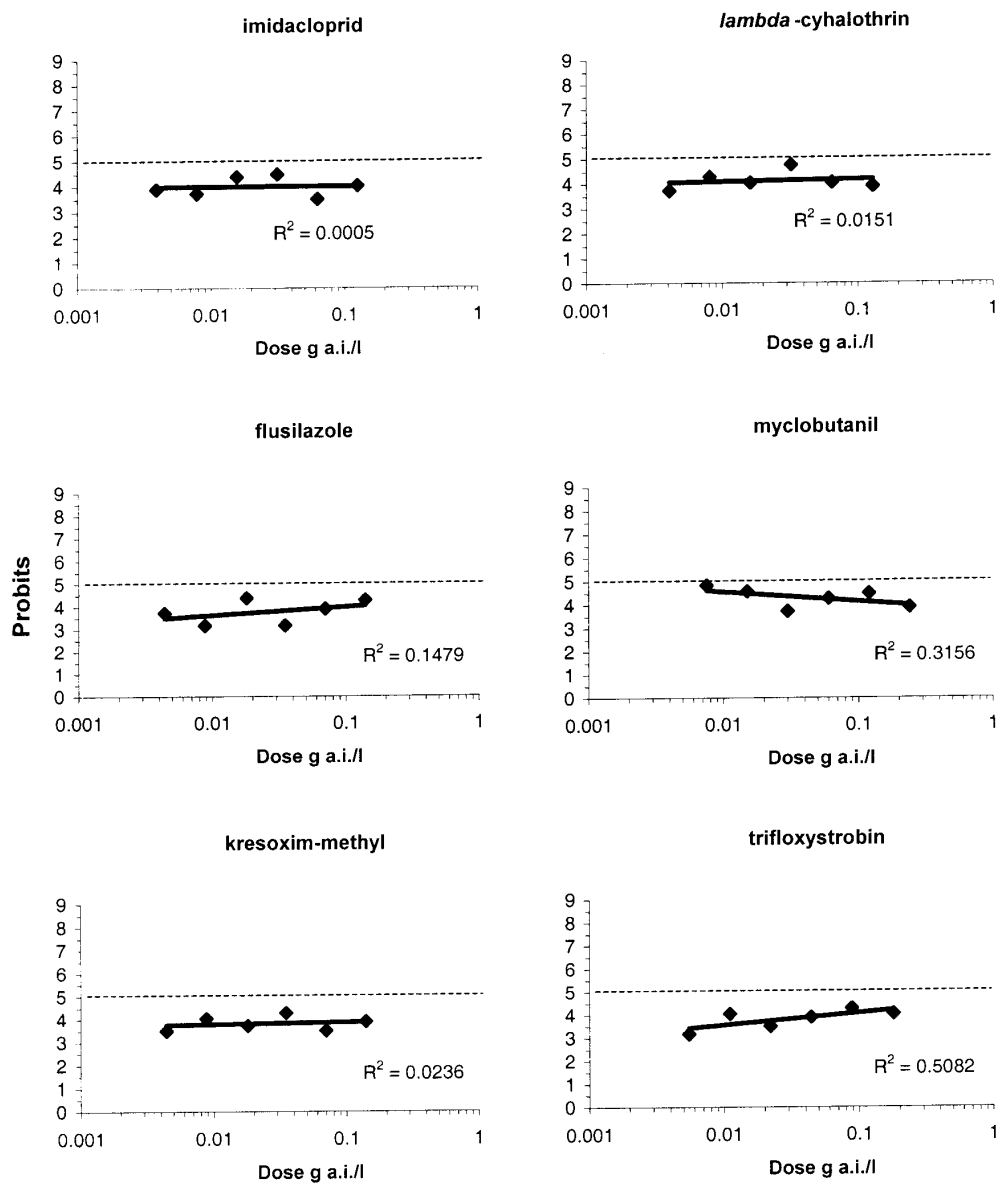


Fig. 1. Acute toxic effects of fungicides (flusilazole, myclobutanil, kresoxim-methyl and trifloxystrobin) and insecticides (imidacloprid and λ -cyhalothrin) on *Agistemus fleschneri* adults. The dotted lines represent 50% mortality on the probit scale.

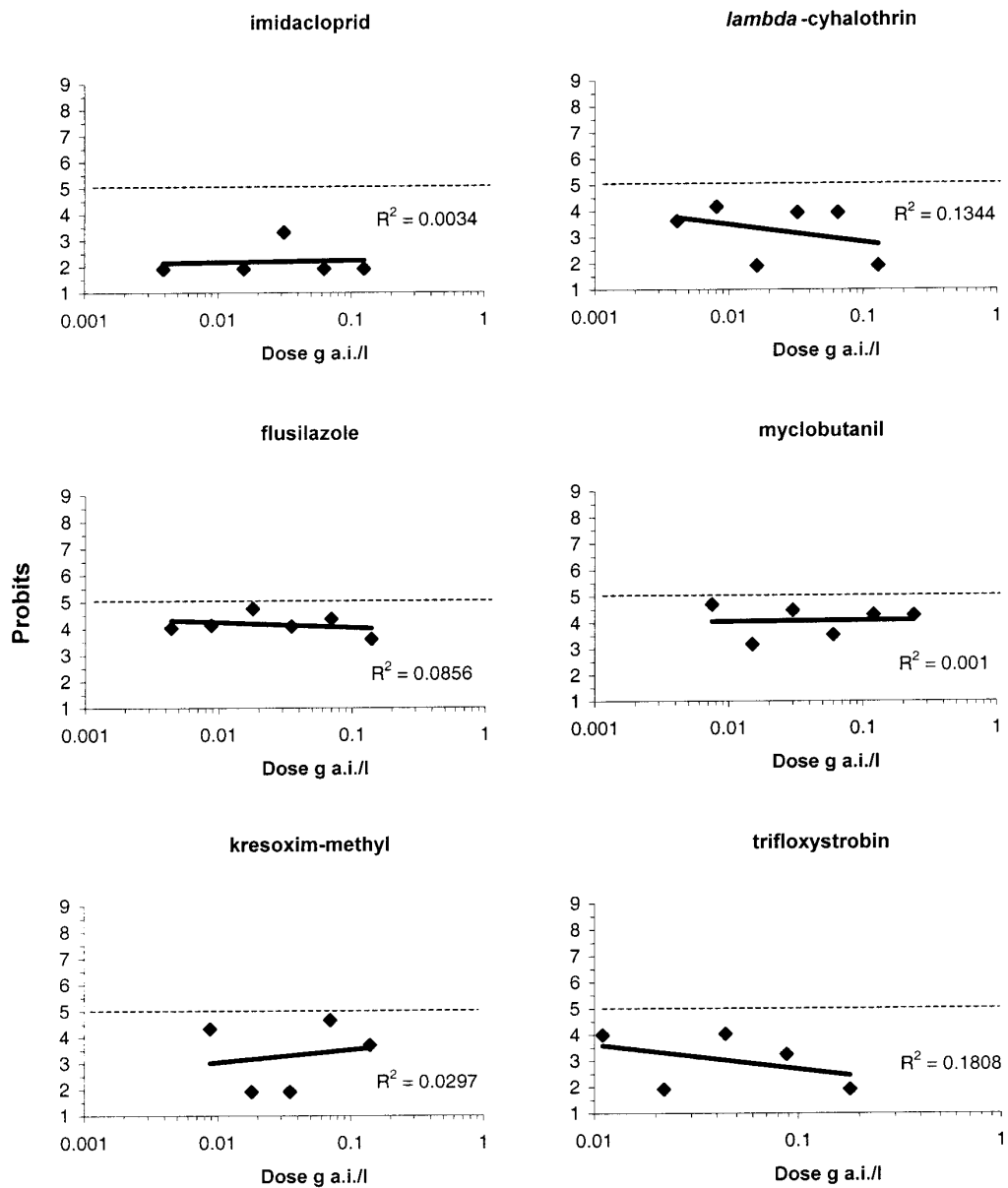


Fig. 2. Effects of fungicides (flusilazole, myclobutanil, kresoxim-methyl and trifloxystrobin) and insecticides (imidacloprid and λ -cyhalothrin) on the ability of *Agistemus fleschneri* adults to produce viable eggs. The dotted lines represent 50% mortality on the probit scale.

TABLE 1. Effects of orchard pesticides on *Agistemus fleschneri* adults

Treatment	% Mortality of adults, by pesticide concentration ^z						R ²	Slope	
	4x	2x	x ^y	$\frac{1}{2}x$	$\frac{1}{4}x$	$\frac{1}{8}x$			Control
<i>Fungicides</i>									
Kresoxim-methyl 50WG	13.3	6.7	23.3	10.0	16.7	6.7	16.7	0.02	0.68±0.13
Trifloxystrobin 50WG	16.7	23.3	13.3	6.7	16.7	3.3	0.0	0.51	0.43±0.24
Flusilazole 50DF	23.3	13.3	3.3	26.7	3.3	10.0	0.0	0.15	0.33±0.23
Myclobutanil 40WP	13.3	30.0	23.3	10.0	33.3	43.3	16.7	0.32	-2.69±1.46
<i>Insecticides</i>									
λ-cyhalothrin 12%	13.3	16.7	40.0	16.7	23.3	10.0	0.0	0.02	0.07±0.21
Imidacloprid 240 g/l	16.7	6.7	30.0	26.7	10.0	13.3	0.0	0.01	0.04±0.22

^zFor each concentration n=90.

^yThe value of x (g a.i./l) is as follows: kresoxim-methyl, 0.035; trifloxystrobin, 0.044; flusilazole, 0.035; myclobutanil, 0.060; λ-cyhalothrin, 0.034; imidacloprid, 0.031.

TABLE 2. Effects of orchard pesticides on *Agistemus fleschneri* eggs

Treatment	% Non-hatched eggs, by pesticide concentration ^z						R ²	Slope	
	4x	2x	x ^y	$\frac{1}{2}x$	$\frac{1}{4}x$	$\frac{1}{8}x$			Control
<i>Fungicides</i>									
Kresoxim-methyl 50WG	9.5 (21)	36.4 (11)	0.0 (6)	0.0 (6)	25.0 (4)	∞	4.3 (23)	0.03	0.07±0.73
Trifloxystrobin 50WG	0.0 (14)	4.0 (25)	16.7 (6)	0.0 (10)	15.8 (19)	–	4.3 (23)	0.18	-23.03±7.4×10 ⁶
Flusilazole 50DF	8.0 (25)	26.3 (19)	17.9 (28)	40.0 (10)	19.0 (21)	16.7 (24)	0.0 (25)	0.09	-0.13 ±0.24
Myclobutanil 40WP	23.5 (17)	24.1 (29)	7.1 (28)	30.0 (10)	3.4 (29)	37.9 (29)	0.0 (25)	0.01	-0.13±0.23
<i>Insecticides</i>									
λ-cyhalothrin 12%	0.0 (5)	14.3 (14)	14.3 (7)	0.0 (3)	20.0 (5)	8.3 (12)	0.0 (5)	0.13	-0.03±0.46
Imidacloprid 240 g/l	0.0 (17)	0.0 (2)	4.5 (22)	0.0 (4)	–	0.0 (8)	0.0 (5)	0.01	-0.08±0.86

^zWithin each parentheses is the value of n for that concentration.

^yThe value of x (g a.i./l) is as follows: kresoxim-methyl, 0.035; trifloxystrobin, 0.044; flusilazole, 0.035; myclobutanil, 0.060; λ-cyhalothrin, 0.034; imidacloprid, 0.031.

^xNot calculated.

In Quebec, biological control of phytophagous mites in apple orchards is achieved by the presence of *A. fallacis*, *A. fleschneri* and *Amblydromella caudiglans* (Schuster). In some orchards all three predators occur concurrently whereas in others they occur at different times of the season. There are also orchards where only one or two species of these predators are present. Furthermore, the predacious mite fauna changes qualitatively from season to season (Bostanian, N.J. and Racette, G., unpublished data). Therefore, the intrinsic toxicity of the pesticides reported in this study to the three predacious mites has to be documented before these pesticides can be used to manage other pests and diseases across the province without interfering with the biological control program for phytophagous mites.

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REFERENCES

1. Anon. (1968) First conference on test methods for resistance in insects of agricultural importance. Method for the boll weevil and tentative method for spider mites. *Bull. Entomol. Soc. Am.* 14:31-35.
2. Berkett, L.P. and Forsythe, H.Y. (1980) Predaceous mites (Acari) associated with apple foliage in Maine. *Can. Entomol.* 112:497-502.
3. Bostanian, N.J., Belanger, A. and Rivard, I. (1985) Residues of four synthetic pyrethroids and azinphos-methyl to *Amblyseius fallacis* (Acari: Phytoseiidae). *Can. Entomol.* 117:143-152.
4. Bostanian, N.J., Dondale, C., Binns, M.R. and Pitre, D. (1984) Effects of pesticide used on spiders (Aranea) in Quebec apple orchards. *Can. Entomol.* 116:663-675.
5. Bostanian, N.J., Larocque, N., Vincent, C., Chouinard, G. and Morin, Y. (2000) Effects of five insecticides used in apple orchards on *Hyaliodes vitripennis* (Say) (Hemiptera: Miridae). *J. Environ. Sci. Health B35*:143-155.
6. Bostanian, N.J., Lasnier, J. and Racette, G. (1999) Biological control of mites in Quebec apple orchards. *Can. Fruit Grower* Sept./Oct. 8:8-10.
7. Bostanian, N.J. and Racette, G. (1997) Residual toxicity of λ -cyhalothrin on apple foliage to *Amblyseius fallacis* and the tarnished plant bug, *Lygus lineolaris*. *Phytoparasitica* 25:193-198.
8. Bostanian, N.J., Thistlewood, H. and Racette, G. (1998) Effects of five fungicides used in Quebec apple orchards on *Amblyseius fallacis* (Garman) (Phytoseiidae: Acari). *J. Hortic. Sci. Biotechnol.* 73:527-530.
9. Croft, B.A. (1990) Arthropod Biological Control Agents and Pesticides. John Wiley & Sons, New York, NY.
10. Croft, B.A. and McGroarty, D.L. (1977) The Role of *Amblyseius fallacis* in Michigan Apple Orchards. *Res. Rep. Mich. Agric. Exp. Stn.* no. 33.
11. Elbert, A., Nauen, R. and Leight, W. (1998) Imidacloprid, a novel chlorotynyl insecticide: Biological activity and agricultural importance. *in*: Ishaaya, I. and Degheele, D. [Eds.] *Insecticides with Novel Modes of Action*. Springer-Verlag, Berlin, Germany. pp. 50-73.
12. Gambaro, P.I. (1991) Comparison between the population dynamics of *Amblyseius andersoni* in different apple orchards protected by "Supervised Control" Programmes. *Inf. Agrar.* 47:73-75.
13. Holdsworth, R.P. (1972) Major Predators of the European Red Mite on Apple in Ohio. *Ohio Agric. Exp. Stn. Res. Circ.* 192.
14. Holdsworth, R.P. and Ellingsen, I.J. (1973) The role of stigmatid mites in the biological control of European red mite in Ohio apple orchards. *Proc. North Central Branch, ESA* 28:104-105.
15. Hoyt, S.C. (1969) Population studies of five mite species on apple in Washington. *Proc. 2nd Int. Congr. Acarology* (Sutton-Bonnington, UK, 1967), pp. 117-133.
16. Jansen, P. (1999) Effects of wheat foliar fungicides on the aphid endoparasitoid *Aphidius rhopalosiphii* DeStefani-Perez (Hymenoptera: Aphidiidae) on glass plates and on plants. *J. Appl. Entomol.* 123:217-223.
17. Larocque, N., Bostanian, N.J., Racette, G. and Lasnier, J. (2000) Toxicité des pesticides sur les prédateurs d'acariens des vergers de pommiers au Québec. *Sommaires des conférences. Journ. Pomicole St. Hyacinthe* Fév.:45-47.
18. LeOra Software. (1987) POLO- PC. Probit and Logit Analysis. Berkeley, CA, USA.

19. Nelson, E.E., Croft, B.A., Howitt, A.J. and Jones, A.L. (1973) Toxicity of apple orchard pesticides to *Agistemus fleschneri*. *Environ. Entomol.* 2:219-222.
20. Parent, B. (1967) Population studies of phytophagous mites and predators on apple orchards in southwestern Quebec. *Can. Entomol.* 99:771-778.
21. Parent, B. and Leroux, E.J. (1956) Note on *Mediolata mali* (Ewing) (Acarina: Raphignathidae) as a predator of the European red mite. *Can. Entomol.* 88:487.
22. Park, C.G., Yoo, J.K. and Lee, L.O. (1996) Toxicity of some pesticides to two-spotted spider mite (Acari: Tetranychidae) and its predator *Amblyseius womersleyi* (Acari: Phytoseidae). *Korean J. Appl. Entomol.* 35:232-237.
23. Walker, J.T.S., Baynon, G.T., Shaw, P.W. and Cassidy, D. (1988) Evaluation of fungicide programmes compatible with integrated control of European red mite *Proc. N.Z. Weed and Pest Control Conf.* 41:193-197.