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Selection of low toxic insecticides for phytoseiid predatory mites, *Amblyseius cucumeris* and *Amblyseius fallacis*

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Abstract : This study was conducted to select low toxic insecticides against natural enemies, and to evaluate resistance stability and cross-resistance to resistance strain for the fulfillment of integrated pest management development. Toxicity of imidacloprid and spinosad to *Amblyseius cucumeris* was relatively low regardless of the adopting test methods. In addition, those to the *Amblyseius fallacis* was also low by slide dipping method. The slide dipping method was useful to eliminate repellency effect by mites to the tested insecticides. Mortality of *A. fallacis* to deltamethrin recorded in 1994 and 1999 was 21.6% and 7.4%, respectively. Meanwhile, the permethrin-resistanct strain of *A. fallacis* was maintained its resistance to deltamethrin. However, the cross-resistance to the newly introduced insecticides namely imidacloprid, fipronil, chlorfenapyr, abamectin, and spinosad. was relatively low. (Received October 29, 2003; accepted December 23, 2003)

Key words : *Amblyseius cucumeris, Amblyseius fallacis*, pesticide resistance, slide dipping method, potter spray tower method.

Introduction

The value of beneficial organisms, such as entomophagous arthropods and entomopathogenic fungi, has been generally recognized as natural controlling agents regulating pest populations in the field. These natural enemies reduce the population of their host or prey, help in minimizing pest damage, and prevent the outbreak of pests in many instances. Selective use of pesticides without the adverse effect on important natural enemies is basically needed in practicing modern pest management. Besides reducing the population of primary pests, natural enemies help in maintaining secondary pests to be below their economic threshold levels (Hassan, 1989; Croft and Wagner, 1981).

In recent years, there has been a considerable interest in the development of programs that will ensure more compatible use of chemical and biological methods for pest control. It is important to use the selective pesticides that are less toxic to the most important natural enemies to implement successfully such a program. In order to practice selective use of pesticides in integrated pest management, their toxicity levels against the most important natural enemies in different crops should be evaluated.

To establish methodologies to select insecticide-resistant strains of the predatory mites, *Amblyseius cucumeris* and *Amblyseius fallacis,* an integrated pest management was conducted in a greenhouse with the following activities: 1) selection of low toxicity insecticides to *A. cucumeris* and *A. fallacis* using two standard laboratory bioassay techniques. 2) comparison of the resistance levels of *A. fallacis* to insecticides observed in 1994 and 1999 to determine stability of resistance. 3) comparison of the resistance levels of a susceptible

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commercial strain of *A. fallacis* (Applied Bionomics, British Columbia, Canada) with a susceptible strain of *A. fallacis* being cultured in the Southern Crop Protection and Food Research Centre (SCPFRC) at Vineland, Ontario, Canada.

Materials and methods

Rearing predator mites

Three strains (a commercial pyrethroid-resistant strain from Applied Bionomics; and two lab-reared strains maintained at the Vineland site of SCPFRC: Vineland susceptible and Vineland resistant) of *A. fallacis* were used in this experiment. Mites from each population were reared in separate growth chambers at 24±1℃ with 60±5% RH and 16:8 (L:D) photoperiod. Rearing units consisted of a 15 cm petridish containing bean leaf disc infested with both the predators and two-spotted spider mites (TSSM), *Tetranychus urticae*, Koch (Tetranychidae, Prostigmata). The petridishes were lined with tissue paper around the bottom rim and then placed onto a circular sponge (17 cm in diameter by 2 cm in depth) seated in a metal pie plate (19 cm in diameter by 4 cm in depth). The pie plate was filled with water until the sponge and paper were saturated. The pie plate was placed into a plastic sweater box (26 H × 21 W × 10 L cm; Canadian Tire, Mississauga, Ontario) filled with approximately 2.5 cm depth of water. Bean leaves with all life stages of TSSM were harvested and added to the colonies of *A. fallacis* three times a week. Bean plants were transferred 2 to 4 weeks after germination into controlled-environment chambers maintaining at 25±1℃ with 60±5% RH and a photoperiod of 16:8 (L:D), and infested with *T. urticae*. *A. cucumeris* were supplied by Applied Bionomics on a weekly basis and kept in shipping packages at 4℃ before use. *A. cucumeris* packages contained bran flakes that infested with grain mites, *Tyrophagus putrescentiae* as a food source.

Pesticides

For stock solution, technical grade pesticides, imidacloprid, abamectin, fipronil, deltamethrin, and chlorfenpyr were dissolved in 95% acetone-olive oil (19:1, v/v) for the Potter Spray Tower method or acetone-water (1:1, v/v) for slide dipping method. Formulated product of spinosad (NAF 85) was dissolved in water only. Appropriate concentrations for bioassays were made with serial dilution.

Slide dipping assay

The FAO method for testing phytoseiid mites by slide dipping assay (Thistlewood *et al.,* 1992) was used. Fresh solution of each pesticide was prepared by diluting the stock solutions. A piece of double-sided adhesive tape was fixed to a glass slide. A second piece of adhesive tape was located, sticky side up, onto the double-sided tape. Ten females of gravid *A. fallacis* were placed ventral side up on each slide by using a fine camel hair brush. The slide was then dipped into 20 mL beaker containing pesticide solution for 5 seconds to ensure that the tested mites were completely wet. For the control, mites were dipped in a acetone-water (1:1, v/v) solution. Control mites in spinosad bioassays were dipped in water only. When the slide was taken out, excessive water was removed by touching the edge of each slide on a paper towel and kept dry at room temperature for 15 min. The slide was placed into a glass slide holding chamber and held at 24±1℃, 60±5% RH and 16:8 (L:D) photoperiod. After 18 hrs, mites were gently prodded with a fine brush to determine whether the mites were dead or not. When their appendages moved, the mites were considered alive (Herne, 1971; Thistlewood *et al.,* 1992).

Potter spray tower

For potter spray tower test, technical grade of pesticide (>90% purity) was used by spraying directly on the mite. Ten mites were placed on two bean leaf discs (five mites per disc), which were set into a 9 cm petridish lined with filter paper. After placing the petridish into the Potter tower (Burcard, 3000), 3mL of each pesticide solution or acetone-olive oil (19:1, v/v, as check) were pipetted into the reservoir at the top of the tower. Concentrations used in primary bioassays were in logarithmic or log increments. A narrow range of concentrations could be chosen by determining the regression line. The time of spray delivery was set up 15 seconds to ensure optimum coverage. The dishes were removed 15 seconds after the spray cycle was completed and leaf discs were then transferred to 5.5 cm petridishes lined with moistened filter paper. The dishes were covered and placed into a controlled environment chamber (24±1℃, 65±5% RH and 16L:8D photoperiod). Mortality was counted 18 hrs after treatment. Some bioassays were repeated for one or more days depending on the steps of the study and the availability of mites at the proper stage. The mortality 18 hrs after treatment was examined in same manner as the slide dipping method. LC_{50} values were calculated by plotting the data on 3-cycle log-probit graphs using polo-pc analysis program.

Assay

Adults of *A. cucumeris* were collected from rearing colonies using Tullgrin funnel technique. Five adults were placed on the bean leaf discs in a petri-dish lined with filter paper. The outer edge of the bean leaf was coated with sticky material to prevent mites' escape and then 5 mL of a pesticide solution was applied using a Potter spray tower with a fine nozzle. The bean leaf with five mites was placed in a petri-dish containing moistened cotton batten, which was later replaced by moistened filter paper, to prevent leaf discs from drying out. Adult grain mites were added as a food source of *A. cucumeris* during the experiment. The dishes were placed in a growth chamber at 24±1℃, 60±5% RH and 16L:8D photoperiod. Mite mortality was recorded 18 hrs after treatment. Mortality was measured based on the percentage of mortality on the leaf (not the sum of the mortality on leaf plus the percent repellency, as in Nesbitt *et al.*, 1994). Repellency was measured based on the number of insects that had escaped and were stuck on the glue barrier. Averages of mortality and repellency were calculated using Abbott's formula (Abbott, 1925). Values recommended by the International Organization for Biological Control categorize test materials as harmless if adult mortality was less than 30%, slightly harmful if adult mortality was 31 to 80%, moderately harmful if adult mortality was 81% to 99%, and harmful if mortality was greater than 99% and repellency if lower than 40% (Hassan, 1989).

Results

Selection of insecticide with low toxicity to *A. cucumeris* and *A. fallacis*

There was a wide range in toxicity of the insecticides tested to the 2 mite species. The least harmful insecticide to *A. cucumeris* using slide dipping and Potter spray tower was imidacloprid whichwas belonged to neo-nicotinoid among 6 insecticides/acaricides tested, but deltamethrin was showed most toxic against mites (Table 1). The toxicity to *A. fallacis* using slide dipping method was in the order of deltamethrin> fipronil> spinosad> imidacloprid (Table 2). Repellency of *A.*

Table 1. Direct contact toxicity of insecticides against *Amblyseius. cucumeris* using potter spray tower and slide dipping method

Insecticide	LC_{50}, ppm (95% FL[a])	
	Potter spray tower	Slide dipping
Deltamethin	12.0 (4.3～36.5)	2.2 (0.9～3.4)
Fipronil	85.0 (25.2～190.3)	60.0 (20.2～135.3)
Chloyfenapyr	80.0 (30.5～163.6)	90.0 (19.3～230.5)
Abamectin	-	12.0 (3.3～41.5)
Imidacloprid	〉1000.0	2003.5 (1320.0～3525.4)
Spinosad	〉333.3	580.0 (236.3～928.4)

[a]FL : fiducial limit.

Table 2. Direct contact toxicity of insecticides against *Amblyseius fallacis*[a] using slide dipping method

Insecticide	LC_{50}, ppm (95% FL[b])	Slope±SE
Deltamethin	12.5 (3.5-19.7)	1.4±0.2
Fipronil	61.0 (25-143.3)	2.3±0.4
Imidacloprid	〉1000.0	-
Spinosad	960.0 (280-1632.3)	1.3±0.5

[a]*A. fallacis* obtained from Applied Bionomics.
[b]FL : fiducial limit.

cucumeris by abamectin and chlorfenapyr was 7.0% and 10.0%, respectively. Meanwhile, fipronil gave 47% of repellency at the recommended. However, imidacloprid and deltamethrin were showed the most strong repellency (Table 3).

Comparison of the resistance levels of *A. fallacis* to insecticides

To determine resistance stability to pesticides, the resistance level of *A. fallacis*, resistant-Vineland strain tested in 1999 was compared with that observed in 1994(Table 4). The mortality at 4 ppm of deltamethrin by the slide dipping method was 21.6 % in 1994 (Nesbitt *et al.*, 1994). When compared to the mortality (7.4%) obtained in this experiment by the same method at 4 ppm, there was a difference of less than three-fold. But mortality of mite to carbaryl between 1994 and 1999 did not show difference.

Comparison of the resistance level of *A. fallacis* from a commercial company (Applied Bionomics, British Columbia, Canada) with a susceptible strain of *A. fallacis*

The cross-resistance level between a permethrin-resistant strain from Vineland and susceptible strain of *A. fallacis* was compared with various different insecticides (Table 5). Deltamethrin showed high cross-resistance to the permethrin-resistant strain, but recently introduced insecticides, such as imidacloprid, fipronil, chlorfenapyr, abamectin, and spinosad, showed low cross-resistance.

Discussion

The toxicity to *A. cucumeris* using Potter spray tower method was high in the order of deltamethrin>chlorfenapyr>fipronil>spinosad>imidacloprid. The results would differ if the repellency was added to the percentage of mortality. In the slide dipping method, deltamethrin was observed to be the most toxic to *A. cucumeris* and *A.*

Table 3. Repellency and mortality of *Amblyseius cucumeris* at given concentration with potter spray tower method

Insecticide	Concentration (ppm)	Repellency (%)	Mortality (%)
Abamectin	100.0	7.0	93.0
Chloyfenapyr	333.3	10.0	90.0
Fipronil	100.0	47.0	53.0
Imidacloprid	1000.0	85.0	15.0
Deltamethrin	3.3	75.0	25.0

Table 4. Mortality of *Amblyseius fallacis* to selected insecticides tested in 1994 and 1999 by slide dipping method

Insecticide	Concentration (ppm)	Mortality(%)	
		1994[a]	1999
Deltamethrin	4	21.6	7.4
Carbaryl	1130	93.6	97.7

[a]Nesbitt *et al.*, 1994.

Table 5. Resistance ratio between resistant and susceptible strains of *A. fallacis* to several insecticides using slide dipping method.

Insecticide	LC_{50}, ppm (95% FL[a])		RR[b]
	Resistant strain	Susceptible strain	
Chlorfenapyr	37.0 (5.9~69.3)	70.0 (30.5~102.5)	0.5
Imidacloprid	3314.0 (1513.7~4520.3)	3501.0 (1232.5~5903.2)	0.9
Spinosad	5650.0 (953.2~9329.3)	4023.0 (1560.0~7820.3)	1.4
Abamectin	3.1 (1.2~5.8)	2.2 (0.8~4.9)	1.4
Fipronil	65.0 (32.8~98.5)	35.0 (15.9~58.3)	1.9
Deltamethin	27.0 (1.5~63.2)	2.2 (0.4~4.5)	12.2

[a]FL : fiducial limit.

[b]Resistance ratio = LC_{50} of resistant strain/LC_{50} of susceptible strain.

fallacis. Among the pesticides used, imidacloprid and spinosad was showed low toxicity to mites. The results obtained from these two compounds were related to the dose rate recommended in Korea. The recommended dose rate of imidacloprid 10% WP is 10 g/20 L. The pratical concentration of spray solution is 50 ppm. Since the estimated LC_{50} for the three strain mites tested was less than 1000 ppm, Imidacloprid was considered to be the best to recommend for use in integrated pest management program (Tomlin, 1997). The recommended field rate for spinosad was also similar. Therefore, spinosad was also recommended since it showed low toxicity to mites.

In comparison of methods, tests were done with the slide dipping method to eliminate the influence of repellency. The test gave that the higher concentrations caused mortality only and did not cause repellency. The Potter spray tower method combines both direct contact and residual toxicity which is reflected by repellency (Pree *et al*,, 1989). Certain insecticides are known to cause repellent behavior in mites, for instance, pyrethroids (Malezieux *et al.,* 1992) and neo-nicotinoid, (Nauen, 1995). In these studies, mites showed high repellency to deltamethrin and imidacloprid (Table 3)

The resistance level of the present *A. fallacis* resistant-Vineland strain was compared with the one observed in 1994 to find out the stability of its resistance. The data in 1994 showed that the percentage of mortality to deltamethrin using the slide dipping method at 4 ppm was 21.6% (Nesbitt *et al.,* 1994). When compared with the mortality (7.4%) obtained in 1999 by the same method, the difference was only less than three-fold. This result indicated that resistance in this case was stable.

In comparing the LC_{50} values of the susceptible and resistant strains of Vineland to deltamethrin by slide dipping method, deltamethrin was found to be 12.7 times more toxic than the susceptible strain (Table 5). The resistance rate of *A. fallacis* (Applied Bionomics) originated from the permethrin-resistant strain at Vineland, Ontario (Thistlewood *et al.,* 1995) to deltamethrin was about six-fold compared with the *A. fallacis* susceptible strain. The slide dipping method showed that the resistance level to deltamethrin in the R-Vineland strain was greater than two-fold when compared with the Applied Bionomics strain. *A. cucumeris* was similar in susceptibility to the susceptible-Vineland *A. fallacis* strain. Abamectin was highly toxic to both the susceptible and resistant Vineland strains. Fipronil and chlorfenapyr gave average toxicity compared with the other compounds. Since we do not have a recommended rate for these compounds, we cannot tell if they would be harmful to the predatory mites with the rates used in commercial applications.

Finally, it was identified that imidacloprid and spinosad exhibited low toxicity to mite populations (Table 1, 2 and 5). The effects of these two compounds in integrated pest management should be further studied in greenhouse trials where phytoseiid predatory mites are used as biological control agents for mite and insect pests.

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Amblyseius cucumeris 및 _Amblyseius fallacis_ 에 대한 저독성약제 선발

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요약 : 최근 경제작물 재배를 위해 시설재배 면적이 증가되고 있으며, 해충방제를 위해 사용된 농약의 부작용 문제로 인하여 농약과 천적의 조화로운 사용을 강조하는 해충의 종합적 방제 기술이 필요하게 되었다. 따라서 시설재배지에서 많은 피해를 주고 있는 점박이응애 및 총채벌레의 천적인 *Amblyseius cucumeris*와 *Amblyseius fallacis*에 대해 저독성약제를 선발하기 위해 2가지 생물검정기술(slide dipping method, potter spray tower method)을 이용하였다. Potter spray tower method로 살포된 약제는 살충작용과 기피효과를 보였으며, 기피효과를 보이는 살충제는 slide dipping method에 의해 살충율을 효과적으로 평가 할 수 있었다. 1994년에 선발 개발된 permethrin 저항성계통 *A. fallacis* 상품의 저항성 안정성을 평가한 결과 1999년 현재 같은 피레스로이드계통인 deltamethrin에 대한 저항성 감소는 보이지 않았으며, permethrin 저항성계통에 대해 imidacloprid, fipronil, chlorfenapyr, abamectin, spinosad 등의 약제가 비교차저항성을 보였다.

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