



Szent István University

COMPLEX LABORATORY METHODOLOGY FOR  
TESTING ACARICIDE SUBSTANCES

PhD Theses

By

Edit Bleicher

Gödöllő, Hungary  
2003

Doctoral school:  
Crop Production and Horticultural Sciences

School leader:

Prof. Dr. Ferenc Virányi, DSc  
Faculty of Agricultural and Environmental Sciences  
Department of Plant Protection

Secretary     Dr. Gábor Gyulai, PhD  
Associate Professor. PhD Biology  
SZIU, Department of Genetics and Plant Breeding  
Gödöllő

Supervisor:

Prof. Dr. Gábor Jenser, DSc  
Department of Entomology  
Plant Protection Institute, HAS

.....  
School leader

.....  
Supervisor

## 1. The intention of the research

Because of the growing economic importance of phytophagous mites new potent and selective acaricides are continuously needed for mite management.

A new tetrazine type acaricide molecule flufenzin (kode: SZI-121), formulation Flumite200, has been developed by AGRO-CHEMIE, formerly Chinoin.

A large number of molecules have been synthesised, mostly tetrazine analogues. A comprehensive screening system was applied to recognise some effects important in terms of field performance. The chemical structure was designed to get improvements in mode of action and efficacy simultaneously. We used seven laboratory methods for investigation efficacy of acaricides in different mode of action and on different developmental stages of the spider mite *Tetranychus urticae* Koch.

## 2. Materials and methods

### 2.1. Test animal

A sensitive strain of two-spotted spider mite (*Tetranychus urticae* Koch) was used for testing the new molecules beside standard acaricides obtained from the market. The strain originated from the Plant Protection Institute, Hungarian Academy of Science, Budapest and has been reared in our laboratory for 14 years.

### 2.2. Test methods

#### 2.2.1. Ovicidal methods

We investigated ovicidal activity in three different mode of actions using four methods. These methods were described by Pap et al. (1994).

##### 2.2.1.1. The contact mode of action

We treated leaf discs (*Phaseolus* sp.) of 15 mm diameter infested with 0-24 h-old eggs by immersion for a period of 5 secundum. Egg hatching was assessed after 7 days.

##### 2.2.1.2. The translaminar mode of action

We applied two methods.

Using the first method we investigated the translaminar effect on females ability to lay viable eggs:

Upper surface of leaves was treated with test solution for 5 secundum. After 24 h incubation the lower surface of leaves were infested with females to lay eggs for a period of 24 h. Egg hatching was assessed after 7 days.

By the second method we investigated translaminar effect on egghatch:

Upper surface of leaves was treated with appropriate test solution for 5 secundum. After 24 h incubation the lower surface of leaves were infested with 0-24 h-old eggs. Egg hatching was assessed after 7 days.

##### 2.2.1.3. The transmaternal mode of action:

Females fed on treated leaves for 48 h were transferred to untreated leaf discs to lay eggs for a period of 7 h. Egg hatching was assessed after 7 days.

### 2.2.2. Effect on chrysalis stages

Leaf discs treated by immersion were infested with just hatched larvae. The motile and chrysalis forms were recorded every day until the 95% of control group had become adults. Total mortality was expressed as a proportion of mites which died in chrysalis form. This method was described by Pap et al. (1994).

### 2.2.3. Effects on females

We investigated effects on females in two different mode of actions using two methods. These methods are described first in the present dissertation.

#### 2.2.3.1. The contact mode of action

Leaf discs treated by immersion were settled with females. Mortality and eggproduction inhibitor activity were assessed after 48 h incubation.

#### 2.2.3.2. The translaminar mode of action:

Upper surface of leaves was treated with test solution for 5 secundum. After 24 h incubation the lower surface of leaves were settled with females. Mortality and eggproduction inhibitor activity were assessed after 48 h incubation.

### 3. Results

The results of laboratory studies demonstrate that SzI-121 is one of the most effective acaricide on eggs and on chrysalis stages. It is not able to kill adult mites but females sucking the treated leaves lay unviable eggs.

We compared the activities of ovicides, the three most active Table 1. and their activities on juvenile stages Table 2.

Table 1. Ovicidal activities in different mode of action

Ovicidal activities			
<i>Applied method</i>	$\approx$ LC50 ppm (AI)		
	hexythiazox 10 WP (10% AI)	clofentezin 500 SC (50% AI)	flufenzin 200 (20% AI)
The contact mode of action	0.03	0.15	0.03
Translaminar effect on females ability to lay viable eggs	18	> 300	4
Translaminar effect on egghatch	> 200	> 300	7
The transmaternal mode of action	3	> 300	6

Table 2. Activities on chrysalis stages

Activities on chrysalis stages			
<i>Applied method</i>	$\approx$ LC50 ppm(AI)		
	hexythiazox 10 WP (10% AI)	clofentezin 500 SC (50% AI)	flufenzin 200 (20% AI)
Effect on chrysalis stages	0.8	5	0.6

We hypothesised that all of these premium effects are important in practical conditions and confer higher potency, wider efficacy spectra and more flexible timing of applications to flufenzin over clofentezine and hexythiazox.

### 3.1. The new results for the laboratory work

#### 3.1.1. Innovation in methodology

I elaborated a new treating procedure for investigation the translaminar mode of actions. This procedure is easy to carry out and it allowed to test a large number of molecules in laboratory screen methods.

#### 3.1.2. The fotoperiod as a possible selecting agent in laboratory *T. urticae* populations

The authenticity of results based on the standard susceptibility of the referent strains. The environment of referent strains must be free from any selection agents. Acting as selection agent can be not only chemicalien, but fotoperiod is a possible selection agent too. I propose applying fotoperiod insensitive strains as referent strain.

#### 3.1.3. The transmaternal mode of action of ovicides

I propose the expression “transmaternal mode of action” instead of “transovarian activity” [Brooker, P. J., J. H. Parsons, J. Reid, and P. J. West (1997): Acaricidal 1, 2, 4, 5,- Tetrazines . *Pestic. Sci.* 18, 179-190. p.] or “sterilising effect” [Chapman, R. B. and J. W. Marris (1986): The sterilising effect of clofentezine and hexythiazox on female twospotted mite. *Reprinted from Proceedings of the 39<sup>th</sup> N.Z. Weed and Pest Control Conference.* 237-240 p.]. We have not any information about the way of contamination of eggs with ovicides. The biologie of *T. urticae* allowed to suppose that the injury of genital organs is not indispensable in the case of transmaternal activity.

#### 4. Conclusion

The new acaricide Flumite 200 has been on the market since 1996.

The field rate of Flumite 200 is 80-100 gramm a.i./ha and the field rate of the contact acaricide Apollo 500 SC is 200-300 gramm a.i./ha. In the laboratory estimated premium effects realized in the practice.

The excellent translaminar activity is the main improvement of flufenzin (SzI-121). Following the treatment of the upper surface of leaves flufenzin enters not only into the mesophyllum, it occurs on lower surface of leaves too. It is an unique property among the investigated ovicides.

During the biological evaluation of flufenzin and Flumite 200 the formulated acaricid we gain experiences which can be useful at researches of resistance.

## 5. Publications

Pap, L., J. Hajimichael, E. Bleicher, S. Botár, and I. Székely (1994): SzI121 – Chemical and biological evaluation of a new acaricide. *Brighton Crop protection Conference – Pest and Diseases*. 2-8 75-82. p.

Pap, L., J. Hajimichael, E. Bleicher. Biological evaluation of SzI121, a new miticide. (1995): 5th European Conference on „Chemistry and the Enviroment, Pesticide chemistry for sustainable agriculture” Outlook for the 21st Century. Budapest. 15-18 p.

Pap, L., J. Hajimichael, E. Bleicher, S. Botár, and I. Székely. (1995): SzI121 a new versatile miticide. XIII International Plant Protection Congress, Hague, July 2-7. Congress Issue: 983

Pap, L., J. Hajimichael, E. Bleicher, S. Botár, and I. Székely (1995): Notes about testing of the sterilitant activity of ovicides. XIII International Plant Protection Congress. Hague July 2-7. Congress Issue: 982

Pap, L., E. Bleicher, M. Molnár, and M. Kelemen (1995): Flufenzin, egy új, fejlesztés alatt álló akaricid az integrált termesztés számára. „Integrált termesztés a kertészeti kultúrákban” tanácskozás Budapest november 28.

Pap, L., J. Hajimichael and E. Bleicher (1996): Biological evaluation of SzI121, a new miticide. *J. Environ. Sci. Healt.* B31 (3) 521-526 p.

Pap L. , Molnár M., Koleva R., Kelemen M., és Bleicher E. (1997): Flumite 200, új környezetkímélő atkaölő szer. Növényvédelmi fórum Keszthely, 70. o.

Pap L., Molnár M., és Bleicher E. (2000): A Flumite 200 transzlamináris aktivitásának vizsgálata a különböző kultúrákban. 46. Növényvédelmi Tudományos napok, Budapest, 68. o.

Szabadalmi okirat. (1993 július 21.): Tetrázinszármazékok, eljárás a vegyületek előállítására, a vegyületeket hatóanyagként tartalmazó akaricid, lárvicid és ovidicid készítmények, valamint eljárás az előállításukra és alkalmazásukra. Szabadalmi okirat. Szolgálati találmány. Magyar Szabadalmi Hivatal. Lajstrom szám: 212 613 B. ügyszám: P 9302098 10 p.