AGRICULTURAL BIOLOGY,
2018, V. 53, № 5, pp. 1045-1053
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 632.7:632.951:57.084.1

doi: 10.15389/agrobiology.2018.5.1045eng
doi: 10.15389/agrobiology.2018.5.1045rus

SPECIFIC FEATURES OF DEVELOPMENT OF SPIDER MITE *Tetranychus urticae* Koch RESISTANCE TO ACARICIDE FLORAMITE® (BIFENAZATE)

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The authors declare no conflict of interests
Received May 15, 2016

Abstract

Currently, chemical method is deemed the most effective for plant protection against two-spotted spider mite *Tetranychus urticae* Koch. However, pest resistance when using chemicals for a long time remains among the main challenges. Rather short list of insectoacaricides approved for greenhouse farming in Russia aggravates the problem, since effectiveness of pest resistance control via rotation of pesticides decreases. One more thing is imported propagating material of ornamental crops infested by spider mites resistant to commonly used pesticides. Bifenazate is being successfully applied in different countries for phytophagous mite control. This work is the first rationale for the use of a novel acaricide Floramite® (bifenazate, 240 g/l) against spider mites in Russia. Our objective was to estimate the rate of Floramite®-resistance formation and cross-resistance to most used insectoacaricides in laboratory spider mite lines which are highly resistant to commonly used insectoacaricides. These lines were S-vniif (no contact to pesticides), S-floramite (derived from S-vniif to study resistance to Floramite® in the course of selection), R-vertimec (resistant to abamectin, R-fitoverm (resistant to aversectin C), R-talstar (resistabt to bifentrin), R-actellik (resistant to pirimiphos methyl), and R-BTB (resistant to bitoxibacilline biologicals based on *Bacillus thuringiensis* var. *thuringiensis*). The resistance ratios (RR_{50} and RR_{95}) were calculated from the ratio of average lethal concentrations of LC_{50}/LC_{50} and $LC9_5/LC_{95}$ for the selected line and susceptible parental line. The ratio of 10-fold (RR_{50} = 10×) and higher values stand for true resistance, those less than 10-fold value stand for tolerant. We studied formation of Floramite® resistance for 3.5 years. As per our findings, the mites show more than 5000-fold resistance to Floramite® after 53 treatments with this acaricide during 120 generations (for the majority of the most known insectoacaricides maximum resistance appears during 17-25 generations). No cross-resistance to floramite is detected in the lab lines of mites resistant to bitoxybacillin (*B. thuringiensis*) and Vertimec® (abamectin), with 1.2-1.8-fold RR_{50}, respectively. The mite lines resistant to fitoverm, Vertimec® and Actellic® show tolerance to Floramite® at RR_{50} of 2.8×, 2.9× and 3.6×, respectively. Thus, due to slow growth of *T. urticae* resistance to Floramite® and its potential in eradication of mite populations resistant to different pesticides, Floramite® should be approved in domestic protocols for greenhouse farming.

Keywords: two-spotted spider mite, *Tetranychus urticae*, Floramite®, bifenazat, resistance, cross-resistance, insectoacaricides, greenhouses

The two-spotted spider mite *Tetranychus urticae* Koch (*Acariformes: Tetranychidae*) is one of the most dangerous pests of crops in fields and greenhousees. For many decades, it has been controlled by pesticides. However, the ability of phytophages to develop resistance to them quite rapidly often creates unconquerable difficulties in the plant protection practice. This challenge concerns not only the agricultural industry but also other human activities. The issues of the resistance of the spider mites have been widely reported. It has been established that they may develop resistance to almost all groups of pesticides, irrespective of their origin [1-6]. The Insecticide Resistance Action Committee (IRAC) has included the two-spotted spider mite into the list of 20 arthropod species that have reached critical resistance to pesticides in the pest areas [7]. In Russia, the resistance of *Tetranychus* mites is of particular danger in the protected ground, where beneficiary conditions for massive pest irruptions are provided. The situation is additionally worsened by an insufficiently wide arsenal of insectoacaricides approved for use in the Russian territory.

Over the recent years, a significant problem has been the almost country-wide resistance of the two-spotted spider mite to avermectin agents, which have been continuously used for over 20 years in floral and vegetable facilities. Monitoring of the abamectin resistance carried out by the authors in 2011-2013 has shown that in the vegetable crops the resistance ratio (RR_{50}) of the two-spotted spider mite varied from 48- to 1300-fold; in greenhouse roses, it was from 3.7- to extremely high 3300-fold. Together with this, in the greenhouses the transportation of the avermectin-resistant spider mite populations (on the rose planting material) from abroad was frequently registered [8, 9]. During extensive chemical treatments, the resistant pest populations rapidly restore and significantly increase their numbers, which adversely affects the phytosanitary state of the greenhouses and leads to additional economic costs.

Practice demonstrates that the measures for successful prevention and overcoming of the pest resistance should be based on continuous improvement of the pesticide arsenal, taking into the account the modern physiological, biochemical and genetic impressions about its formation [10]. An important role belongs to the investigation of the population biology of developing resistance to novel pesticides. This provides a possibility to predict the emergence of resistance and take operative measures on selection and rational application of pesticides.

The bifenazate-based acaricides were registered at the end of the last century in the USA and United Kingdom. Currently, these are positioned in many countries as effective agents for controlling the herbivore mites of different crops [11-14]. It is important to note that bifenazate, having a selective activity, is more toxic to herbivore mites than to carnivorous *Phytoseiidae*. Therefore, it may be used in combination with biological means for controlling the pests [15]. In China, monitoring of field populations of the two-spotted spider mite resistant to abamectin has shown that the pests have retained sensitivity to bifenazate [16]. The similar results were obtained at studying various crops in greenhouses and in open ground in Cyprus, where the tested mite populations exhibited sensitivity to bifenazate [17].

The literature data about the emergence of bifenazate-resistant populations of spider mites are rare. Development of resistance to bifenazate at the early stage has been found in two-spotted spider mite in cucumber plants in Jordan [18]. In Korea under laboratory conditions, the acaricides sensitivity was determined in eight populations of two-spotted spider mite collected on roses in greenhouses; two of these were resistant to bifenazate [19]. Further laboratory selection of one of such populations for 4 years has led to the formation of 248.8-fold bifenazate resistance. Cross-resistance of female mites to acenoquinocyl and fenpyroximate, and that of eggs to amitraz, emamectin benzoate, fenpyroximate, milbemectin, pyridaben and spirodiclofen was found. At the same time, the absence of cross-resistance to emamectin benzoate and milbemectin in females and that to abamectin in pest eggs was found [20].

In the studies by T. van Leeuwen et al. [21], the artificial selection of 36 generations of two-spotted spider mite has led to more than 164-fold resistance to bifenazate. The bifenazate-resistant line had no cross-resistance to other acaricides [22]. In other studies, there was no cross-resistance to bifenazate in the laboratory lines of two-spotted spider mite with 580-fold resistance to chlorfenapyr [23]. A possibility of inhibiting bifenazate activity by insectoacaricides from the organophosphate and carbamate classes has been found at using them against spider mites [24].

In the present papre, the authors have substantiated a possibility of using for the first time the Floramite® 240 SC acaricide, novel in Russia, against populations of two-spotted spider mite, resistant to insectoacaricides from various groups used in the protected ground. It was shown that the resistance to this acaricide develops in mites very slowly, and the resistance to other recommended acaricides does not cause cross-resistance to Floramite.

The aim of the study was to identify the features of forming resistance to Floramite® agent in two-spotted spider mite.

Techniques. The following insectoacaricides allowed for application in Russia were used in this study: Vertimec® EC, 18 g/l (abamectin) (Vertimec® 018 EC, abamectin, Syngenta AG, Switzerland); Phytoverm® EC, 2 g/l, active ingredient aversectin C (Farmbiomed LLC, Russia); Talstar® EC, 100 g/l, active ingredient bifenthrin (Talstar 10 WP, bifenthrin, FMC Chemicals, Belgium); Actellic® CE, 500 g/l, active ingredient pirimiphos-methyl (Actellic® 50 EC, pirimiphos-methyl, Syngenta AG, Switzerland); Bitoxibacillin® P, BA 1500 EA/mg, *Bacillus. thuringiensis* var. *thuringiensis* (Sibbiofarm LLC, Russia).

The experiments were carried out using the standard (sensitive) line of the two-spotted spider mite (*Tetranychus urticae* Koch) cultured in laboratory and never exposed to pesticides (S-vniif); a subline isolated therefrom for studying the rate of forming the Floramite resistance (S-floramite); mite lines selected in the laboratory by resistance to Vertimec® (R-vertimec), Fitoverm® (R-fitoverm), Talstar (R-talstar), Actellic® (R-actellic) and Bitoxibacillin® (R-BTB). The lines were maintained by stabilizing treatments with the said pesticides and were further used for studying cross-resistance. The mites were maintained in isolated glass boxes on the young plants of Sax bush bean at the temperature of 22±3 °C, relative air humidity of 55-70% and 18 h light period.

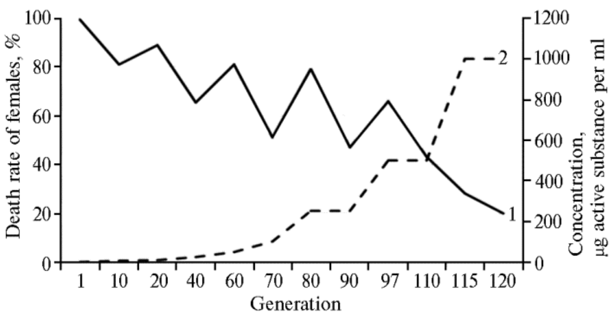
In the experiments on the study of the development of resistance to Floramite® 240 SC (Floramite, 240 g/l, active substance bifenazate, Floramite® 240 SC, bifenazate, Chemtura AgroSolutions, USA), the starting mite subline (S-floramite) was subjected to sequential selective treatment with the pesticide. The bean plants populated by spider mites (about 2000 mobile mites on 100 leaves) were cut and immersed into an aqueous solution of the pesticide for 3 s; the survived mites were allowed to move to the untreated plants. For the starting treatment, the acaricide concentration of 0.0001% AI (or 1.0 μg AI/ml) was selected in such a way so as to cause the death of at least 85% of mites. At the restoration of numbers of the mite to the conditionally starting one, the treatment was repeated. As the mortality of mites decreased, the norm of the pesticide was increased at the next treatment to a value sufficient to maintain its high efficiency. Mites at all stages of development were simultaneously subjected to acaricide, and the results were taken into account with respect to females only. The mortality was assessed after each treatment; the resistance of females to Floramite® was assessed after every 8-10 generations. The criterion of resistance development was the resistance ratios (RR_{50} and RR_{95}), established from the ratio of lethal concentrations LC_{50}/LC_{50} and LC_{95}/LC_{95} in the selected and parental (sensitive) lines. The true resistance was the ten-fold (RR = 10×) and higher, the tolerance was the parameter of less than 10-fold.

In order to determine the lethal concentrations of the pesticides CL_{50} and CL_{95} at determining the rate of resistance development and investigation of cross-resistance, the bean plants populated with female mites (at least 100 mites each) at the phase of 2 true leaves were immersed into aqueous acaricide solutions of 5-7 increased concentrations (from minimal, causing death of 5-10%

sensitive specimens, to maximal, leading to death of 90% specimens and more). Death of females was taken into the account after 24 h (Actellic®), 72 h (Vertimec®, Fitoverm®, Talstar, Floramite®) and 5 days (Bitoxibacillin®) after treatment.

All experiments were carried out in accordance with methodical recommendations for entomological and toxicological studies [25, 26] in 4-fold frequency. The experimental data were processed by the statistical method of probit analysis.

Results. The Floramite® 240 SC (bifenazate) is not yet registered in Russia, but is of interest as a prospective acaricide. It belongs to a new group of chemical compounds with a different mechanism of action and may be used in the pesticide alternation system in order to overcome resistance to avermectins, and it is of low hazard to predatory mites and beneficial insects.



Mortality of female two-spotted spider mites (*Tetranychus urticae* Koch) treated with Floramite® 240 SC (240 g/l, bifenazate) at various concentrations (laboratory experiment).

Formation of resistance to Floramite® in two-spotted spider mite was quite prolonged. In 20 generations, the female mortality was almost constant (81-89%), although the pesticide concentration was twice increased 5- and 10-fold compared to the starting one for this period (Fig.). By the time of development of the 40th generation, the 2.5-fold concentration increase has led to a drastic decrease in female mortality (65%). Further in the selection, the same trend was observed in the mite reaction to the pesticide. In the 60th generation, in response to another 2-fold concentration, an increase (81%) and then a rapid decrease in mortality followed (up to 51%). Alteration of mortality decrease rates indicates a beginning of a new stage of resistant genotype accumulation. However, from the 97th generation, the female mortality began to decrease at a continuous increase in the Floramite® concentration (the pesticide norm was adjusted to 0.1% AI, or 1000 μg AI/ml). This period may be regarded as the beginning of pest resistance stabilization.

The first notable, 5.1-fold, alteration of the average lethal concentration of Floramite® was detected at the 13th generation (Table 1). By the 31st generation, the resistance of the treated line exceeded the control 76.4-fold, by the 45th generation 180.6-fold. Such resistance held for quite a long time, up to the 70th generation; the mites were characterized by an almost identical reaction to the wide range of the Floramite® concentrations. A continuation of selection has led to a rapid increase in resistance (5125-fold to the 113th generation), an extremely high value for the studied pesticide. The experiment took 3.5 years: before the line achieved stable resistance, 53 Floramite® treatments were carried out. The selection was carried out up to the 120th generation. At the final phase of selection, the stabilization of the resistance development process occurred and no reliable alterations in the resistance ratio were observed (RR_{50} 5160). As a rule, at the artificial selection, resistance to most of the known insectoacaricides (organophosphorus, sulfurous and others) emerges in mites quite rapidly, in 17-25 generations on the average [2]. The resistance to Floramite® formed more slowly; however, it should be taken into account that, as is seen from the obtained data (see Table 1), at laboratory selection the use of Floramite® was effective only up to the 45th generation of the pest (at about one and half years). The subsequent treatments (up to the 70th generation) have led to the development and maintaining of almost 200-fold resistance. It is obvious that at the formation of such resistance in the population, further use of the pesticide is not effective. At approximation under commercial greenhouse conditions, the development of analogous resistance at continuous use of Floramite® may occur approximately in at least 2 to 2.5 years. The similar processes were noted by the authors for the avermectin pesticides in 2-3 years and in 10-15 years in industrial greenhouses [8, 28, 29].

1. Ratios of resistance to the Floramite® 240 SC (240 g/l, bifenazate) pesticide in female two-spotted spider mites (*Tetranychus urticae* Koch) during the selection process (laboratory experiment)

Generation	Number of treatments	LC_{50}, μg AI/ml	LC_{95}, μg AI/ml	Resistance ratio	
				RR_{50}	RR_{95}
P	0	0.07 (0.07÷0.13)	0.57 (0.25÷1.34)	1	1
13	5	0.37 (0.31÷0.76)	2.70 (1.99÷3.33)	5.1	4.7
31	15	5.5 (3.41÷6.00)	32.00 (29.00÷36.00)	76.4	56.1
45	20	13.00 (12.00÷15.30)	440.00 (320.00÷600.00)	180.6	771.9
65	28	12.50 (11.00÷13.10)	260.00 (110.00÷420.00)	173.6	456.1
70	31	13.90 (12.20÷14.40)	490.00 (476.00÷503.00)	193.1	859.6
100	48	130.00 (110.00÷150.90)	2700.00 (1300.00÷5600.00)	1806	4737
113	51	369.00 (300.00÷452.00)	2460.00 (1100.00÷5940.00)	5125	4316
120	53	372.00 (331.00÷491.00)	2808.00 (1900.00÷6900.00)	5167	4926

N o t e. P — parental (sensitive) line, LC_{50} and LC_{95} — lethal concentrations of pesticide causing death of 50% and 95% females, RR_{50} and RR_{95} — the resistance ratios determined from the ratios of LC_{50}/LC_{50} and LC_{95}/LC_{95} in the selected and parental lines. The confidence limits at p = 0.05 are recited in parentheses

2. Resistance to Floramite® 240 SC (240 g/l, bifenazate) pesticide in female two-spotted spider mites (*Tetranychus urticae* Koch) from the lines resistant to other insectoacaricides (laboratory experiment)

Line	LC_{50}, μg AI/ml	LC_{95}, μg AI/ml	Resistance ratio	
			RR_{50}	RR_{95}
S-vniif	0.07 (0.07÷0.13)	0.57 (0.25÷1.34)	1	1
R-vertimec	0.13 (0.12÷0.15)	3.30 (1.80÷60.0)	1.8	5.8
R-fitoverm	0.21 (0.17÷0.26)	3.16 (1.30÷7.70)	2.9	5.5
R-talstar	0.26 (0.22÷0.31)	6.10 (3.27÷10.11)	3.6	10.7
R-actellik	0.20 (0.17÷0.24)	3.62 (1.71÷7.69)	2.8	6.4
R-BTB	0.08 (0.07÷0.09)	0.84 (0.42÷1.68)	1.2	1.5

N o t e. See the description of lines in the Techniques section. LC_{50} and LC_{95} — lethal concentrations of pesticide causing the death of 50% and 95% females, RR_{50} and RR_{95} — resistance ratios determined from the ratios of LC_{50}/LC_{50} and LC_{95}/LC_{95} in the selected and parental lines. The confidence limits at p = 0.05 are recited in parentheses.

In commercial farming, an extension of the useful life of the pesticide is probable. In the protected ground, at low diversity of pesticides allowed for use against spider mites, it is possible to overcome the resistance at a combination of non-related pesticides. However, the manifestation of cross-resistance significantly decreases the effect of protective measures and sometimes it makes impossible the use of even those pesticides the phytophages never interacted with

[2, 3, 30, 31].

At the present time, the problem of effective control of spider mites resistant to insectoacaricides, firstly, to avermectin pesticides, is of utmost importance. For example, Vertimec® (active ingredient — abamectin) was widely used and exhibited high efficiency for a long time [32, 33], but further, it did not provide the required protective activity [9]. This determines the interest in the assessment of resistance to Floramite® in laboratory lines of two-spotted spider mites resistant to other pesticides. In particular, in the current experiment, the authors used the lines which were highly resistant to Vertimec® (1660-fold), Phytoverm® (1020-fold), Actellic® (2200-fold), Talstar (283-fold) and Bitoxibacillin® (the mortality of resistant females did not exceed 5-7% at treatment with sub-lethal concentration) (Table 2).

The authors hereof have established the absence of cross-resistance to Floramite® in the two-spotted spider mite lines resistant to Bitoxibacillin and Vertimec® (the resistance ratios are correspondingly 1.2× and 1.8×). In the lines resistant to Fitoverm®, Talstar and Actellic®, the 2.8 to 3.6-fold tolerance to Floramite was found. The results concerning Vertimec® and Actellic® obtained in the studies correspond to the literature data. For example, in the studies by Lee *et al.* [19] the populations of two-spotted spider mite resistant to abamectin (3822-fold) and to pirimiphos-methyl (increase to 77-fold) exhibited resistance to bifenazate. There are reports about the absence of cross-resistance to bifenazate in the abamectin-resistant (1294-fold increase) *T. urticae* line [27].

Therefore, at laboratory selection of the sensitive sub-line of two-spotted spider mite (S-floramite) for resistance to the Floramite® 240 SC pesticide, resistance develops quite slowly (RR_{50} over 5,000 for the time of development of 120 generations). In the mite lines that are resistant to Bitoxibacillin® and Vertimec®, no cross-resistance to Floramite® is found. The mites from the lines resistant to Fitoverm®, Talstar 10 WP and Actellic® exhibite tolerance to Floramite®. These results indicate that Floramite® 240 SC (Floramite EC, 240 g/l, bifenazate), if registered in the Russian territory, may be effectively employed against two-spotted spider mite, including its populations resistant to insectoacaricides.

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