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ROLE OF THE INTERACTION OF RESISTANCE GENES TO ACARICIDES OF DIFFERENT CHEMICAL CLASSES IN THE POSTERITY OF DIHETEROZYGOUS FEMALES OF THE TWO-SPOTTED SPIDER MITE

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❀ **Background.** Information on the change in the correlation of genotypes in arthropod populations after treatment of crops with pesticides is essential for effective insectoacaricide resistance management. **Materials and methods.** Lines of two-spotted mite the homozygous for the gene of resistance to malathion, bifenthrin, bromopropylate and abamectin were obtained by disruptive selection cycles with treatments diagnostic concentrations of these acaricides. Crossing mites were produced heterozygous females with genes of resistance to malation and one of the other three acaricides, as well as families of their filial generation. Females of the three genotypes were compared the value of the degree resistance when testing mites by diagnostic concentrations of acaricides. **Results.** Combinations of distribution of alleles of resistance to acaricides of different chemical classes in mites of the filial generation of diheterozygous females did not determine the differences from the parent females in the degree of mortality. **Conclusion.** Epistatic interaction of alleles of resistance to toxicants of different chemical classes allows be used only two insectoacaricides in turn for resistance management.

❀ **Keywords:** two-spotted spider mite; *Tetranychus urticae*; acaricide; resistance; inheritance.

ВЗАИМОДЕЙСТВИЕ ГЕНОВ РЕЗИСТЕНТНОСТИ К АКАРИЦИДАМ РАЗЛИЧНЫХ ХИМИЧЕСКИХ КЛАССОВ В ДОЧЕРНЕМ ПОКОЛЕНИИ ДИГЕТЕРОЗИГОТНЫХ САМОК ОБЫКНОВЕННОГО ПАУТИННОГО КЛЕЩА

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❀ Гомозиготные по генам резистентности к отдельным акарицидам линии обыкновенного паутинного клеща были выделены дизруптивным отбором при обработках семей отдельных самок диагностическими концентрациями малатиона, бифентрина, бромпропилата и абамектина. Скрещиванием клещей этих линий получены дигетерозиготные самки с генами резистентности к малатиону и одному из трех других акарицидов, а также семьи их дочернего поколения. Самок трех таких генотипов сопоставляли по количественным показателям проявления признака резистентности при тестировании клещей диагностическими концентрациями акарицидов, гены резистентности к которым присутствовали в геноме. Дочернее поколение дигетерозиготных самок не отличалось от родительских особей по токсикологическим показателям взаимодействующих генов резистентности.

❀ **Ключевые слова:** паутинный клещ; резистентность; акарициды; наследование признака.

INTRODUCTION

Evolutionary changes in arthropod populations after treatment of crops with insectoacaricides tend to increase the number of individuals carrying genes for resistance to the chemicals used. Subsequent treatment for crops against insect pests is recommended to involve an insectoacaricide of different chemical class, to which the number of individuals carrying the resistance gene (R-gene) has not yet increased in the population. Insectoacaricides of 55 chemical classes have been synthesized and are used for the protection of plants against insect pests [1]. Iterations of chemical treatments against pests using compounds of different chemical classes should result in an increase in genotypes that are resistant to multiple pesticides. The opposite results were obtained in experiments conducted on two-spotted spider mites selected according to the presence of genes for resistance to acaricides of different chemical classes. The expression of an R-gene to a toxicant in spider mites with alleles for resistance to acaricides of two different chemical classes was suppressed when another R-gene was present [2]. Quantitative indicators of the epistatic effects of R-genes to acaricides of different chemical classes were used for detection of hereditary changes in spider mites produced by diheterozygous females. Alleles for resistance are split up and independently distributed in the process reproduction, which determines the expression of the characters derived from the parent females.

MATERIALS AND METHODS

Experiments were conducted with females of the two-spotted spider mite *Tetranychus urticae* Koch. Different genotypes of spider mite were obtained by disruptive selection of inbred reproduction for indicators of resistance or sensitivity to acaricides at different concentrations. The following formulations were used: malathion (50 % c. e.[1] of karbofos), bifenthrin (10 % c. e. of talstar), brompropylate (neoron 50 % c. e.) and abamectin (1.8 % c. e. of vertimek). Aqueous solutions of the following concentrations were used as the diagnostic ones ($CK_{95} \times 2$ for spider mites of the sensitive strain, in % of the active fraction); 0.05 malathion, 0.002 bifenthrin, 0.005 brompropylate, and 0.00009 abamectin.

Spider mite offspring from individual females were kept on a bean leaf raft on wet cotton wool. Each generation of spider mites for each treatment were obtained from single females of the tested families. Deutonymphs and one male were put on the bean leaf raft to cross mites of different genotypes.

A piece of bean leaf with the females was tested by immersion in an aqueous solution of the toxicant. The leaf cut-out with spider mites wetted in toxicant was then put on a separate leaf raft. Mortality rate was counted 24 h after immersion.

The error of the mortality rate sampling mean and variation coefficient (ν) of these values was determined [3], and graphed using Microsoft® Excel 2016.

RESULTS

The biology of the two-spotted spider mite produces some peculiarities in the responses of females from some families to the effects of toxicants. Spider mites are a haplo-diploid species of arthropod. Virgin females produce only haploid males, whereas impregnated females produce diploid females and haploid males. Females heterozygous for resistance to specific toxicants produce resistant and sensitive males. A total of 60 % males of the two-spotted spider mite copulate with already-impregnated females [4, 5]. Inbred crossing of the filial females with males of the same generation takes place in isolated mite families obtained from single females. Inbred reproduction of spider mites is not limited by the number of generations and does not produce any signs of inbreeding depression because it is the normal means of reproduction for the species. As a result of these individual traits, the generations of mites selected using disruptive selection for the presence or absence of resistance to any acaricide can produce 15 %–30 % of females without resistance, and 5 %–15 % of females of the sensitive strain survived after treatment with a diagnostic concentration of the acaricide [6].

In the experiments, the occurrence of resistance to each toxicant was compared using the arithmetic mean of the mortality rate of the females of all the families analyzed and by the geographic distribution of the families.

Deutonymphs homozygous for resistance to acaricide were crossed with individuals from the families with the smallest female mortality rate. Males for reciprocal crossing were taken from the same families. Haplodiploid species of arthropods do not produce different genotype combinations in case of reciprocal crossing of males and females; therefore, toxicological indicators in the tables are shown without differentiating between parent females homozygous for the R-gene. Half of the hybrid females of each family that had the R-genes against acaricides of two different chemical groups were separately tested for each of the toxicants.

In all combinations of the R-gene to malathion with R-genes to bifenthrin, brompropylate, and abamectin, when the diagnostic concentration of malathion affected hybrid females, the mean mortality rate was increased by 2–2.5 times in comparison with the parent females homozygous by the R-gene to malathion (Tables 1–3). Resistance to bifenthrin, brompropylate, and abamectin in hybrid females was not manifested at diagnostic concentrations (Tables 1–3). The frequency of occurrence of resistance was uniformly distributed in the families of heterozygous females after treatment with malathion at diagnostic concentrations at all levels of mortality. Somewhat more families in the zone up to 50 % of the section carried resistance alleles against brompropylate (Fig. 1, *b*; 2, *b*; 3, *b*).

All families of heterozygous females having the R-gene to acaricides are distributed on the diagram in the zone of sensitivity to the toxicant, with more than 70 % mortality (Fig. 1, *b*, 2, *b*, 3, *b*), according to the indicator of female mortality due to diagnostic concentrations of bifenthrin, brompropylate, and abamectin.

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[1] c. e. — concentrate of emulsion.

Table 1

The arithmetic mean of the mortality ($\bar{x} \pm S\bar{x}$) and the coefficient of the relative dispersion (ν) in the females of two-spotted spider mite when testing with diagnostic concentration of malathion and bifenthrin

Homozygous parent females for crossing		Crossing variants	Diheterozygous females F_1		Filial generation of diheterozygous females	
R-malathion	r-bifenthrin		malathion	bifenthrin	malathion	bifenthrin
11.5 ± 4.5 $\nu = 39.1 \pm 3.9$ 50♀♀	26.6 ± 6.2 $\nu = 23.3 \pm 2.3$ 50♀♀	♀ R-malathion × × ♂ r-bifenthrin	36.4 ± 6.5 $\nu = 17.8 \pm 0.76$ 274♀♀	88.3 ± 5.2 $\nu = 5.9 \pm 0.29$ 200♀♀	45.3 ± 5.4 $\nu = 11.3 \pm 0.39$ 463♀♀	88.8 ± 3.8 $\nu = 4.3 \pm 0.14$ 476♀♀
		♀ r-bifenthrin × × ♂ R-malathion	38.7 ± 7.5 $\nu = 19.3 \pm 0.89$ 236♀♀	90.2 ± 4.8 $\nu = 5.3 \pm 0.27$ 190♀♀	52.0 ± 6.7 $\nu = 10.8 \pm 0.47$ 265♀♀	82.9 ± 5.4 $\nu = 6.5 \pm 0.29$ 254♀♀

Table 2

The arithmetic mean of the mortality ($\bar{x} \pm S\bar{x}$) and the coefficient of the relative dispersion (ν) n the females of two-spotted spider mite when testing with diagnostic concentration of malathion and brompropylate

Homozygous parent females for crossing		Crossing variants	Diheterozygous females F_1		Filial generation of diheterozygous females	
R-malathion	r-brompropylate		malathion	brompropylate	malathion	brompropylate
19.4 ± 5.6 $\nu = 28.8 \pm 2.9$ 50♀♀	17.1 ± 5.3 $\nu = 31.0 \pm 3.1$ 50♀♀	♀ R-malathion × × ♂ r-brompropylate	40.4 ± 7.6 $\nu = 18.8 \pm 0.85$ 245♀♀	93.4 ± 6.1 $\nu = 6.5 \pm 0.35$ 174♀♀	35.0 ± 5.7 $\nu = 1.6 \pm 0.06$ 290♀♀	94.0 ± 3.9 $\nu = 4.1 \pm 0.18$ 262♀♀
		♀ r-brompropylate × × ♂ R-malathion	45.3 ± 8.7 $\nu = 19.2 \pm 1.0$ 167♀♀	92.1 ± 7.0 $\nu = 7.6 \pm 0.49$ 121♀♀	52.6 ± 6.8 $\nu = 12.9 \pm 0.55$ 269♀♀	95.3 ± 6.1 $\nu = 6.4 \pm 0.29$ 235♀♀

Table 3

The arithmetic mean of the mortality ($\bar{x} \pm S\bar{x}$) and the coefficient of the relative dispersion (ν) in the females of two-spotted spider mite when testing with diagnostic concentration of malathion and abamectin

Homozygous parent females for crossing		Crossing variants	Diheterozygous females F_1		Filial generation of diheterozygous females	
R-malathion	R-abamectin		malathion	abamectin	malathion	abamectin
16.2 ± 5.2 $\nu = 32.1 \pm 3.2$ 50♀♀	18.1 ± 5.4 $\nu = 29.8 \pm 3.0$ 50♀♀	♀ R-malathion × × ♂ R-abamectin	35.9 ± 6.1 $\nu = 17.0 \pm 0.77$ 241♀♀	87.2 ± 5.2 $\nu = 5.9 \pm 0.3$ 189♀♀	40.1 ± 5.9 $\nu = 16.2 \pm 0.55$ 431♀♀	89.5 ± 4.3 $\nu = 4.8 \pm 0.19$ 299♀♀
		♀ R-abamectin × × ♂ R-malathion	43.5 ± 7.5 $\nu = 17.2 \pm 0.85$ 204♀♀	79.9 ± 6.7 $\nu = 8.4 \pm 0.43$ 192♀♀	53.1 ± 5.1 $\nu = 9.6 \pm 0.28$ 567♀♀	90.3 ± 3.9 $\nu = 4.3 \pm 0.17$ 303♀♀

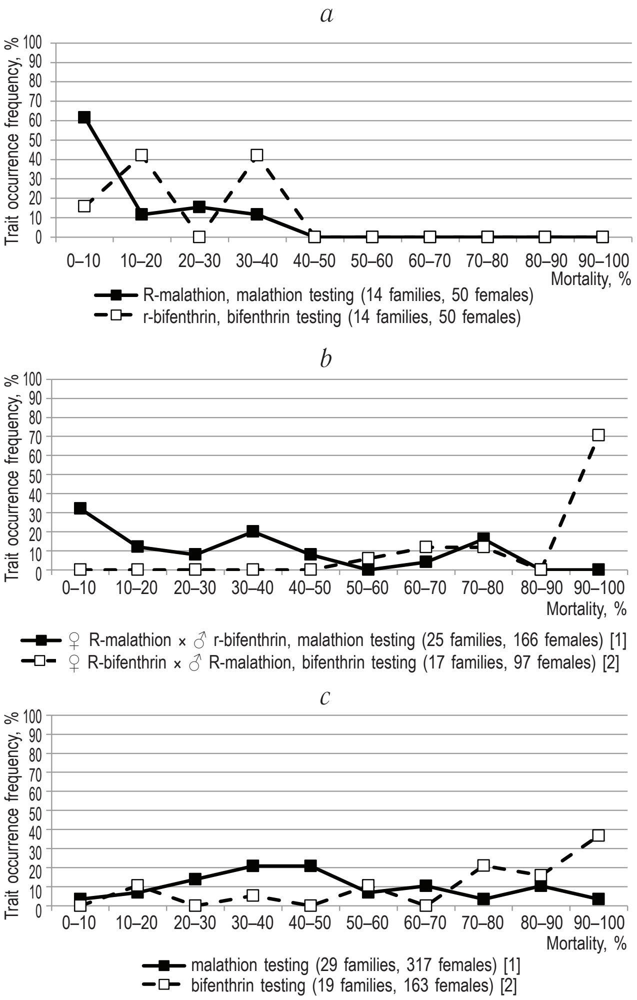

Fig. 1. Distribution of death rates for female parental families (*a*), that are homozygous for genes of acaricide resistance to female acaricides (digits) for these genes of female F_1 generation (*b*) and the genotypes of the offspring from diheterozygote (*c*)

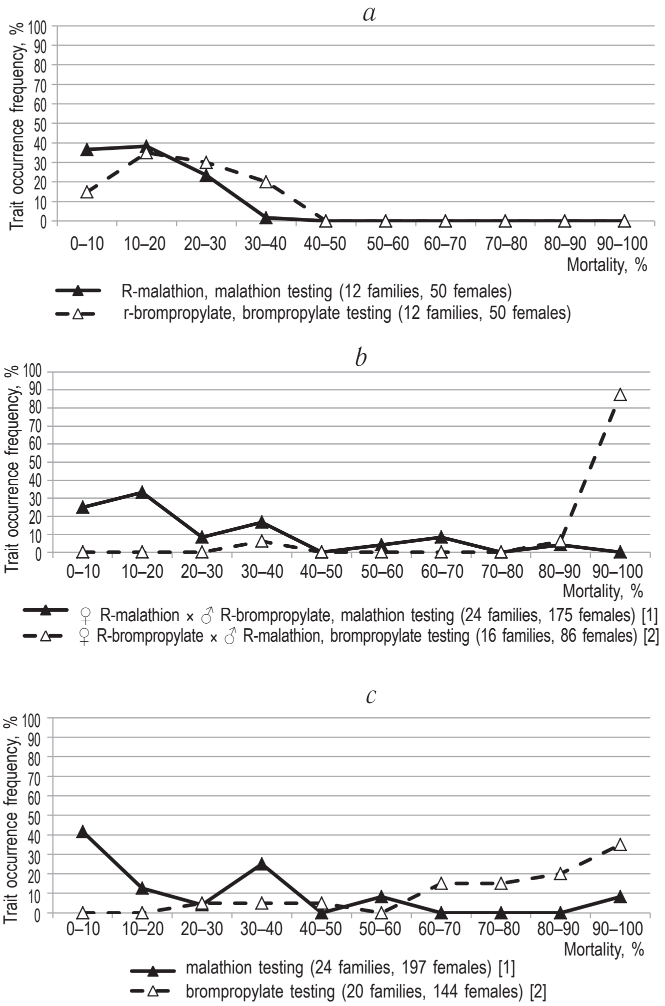

Fig. 2. Distribution of death rates for female parental families (*a*), who are digerozygous for female genes F_1 (*b*) and their offspring's genotypes (*c*)

Tests of females of the first filial generation of the diheterozygous generation did not exhibit statistically significant differences from the females of the parent generation according to the mean arithmetic value of mortality after treatment with diagnostic concentrations of malathion, bifenthrin, brompropylate, and abamectin (Tables 1–3).

The distribution of the mortality of families of diheterozygous parental females and the families of their first filial generation from diagnostic concentrations of all used acaricides was also equal (Fig. 1, *b*, *c*; 2, *b*, *c*; 3, *b*, *c*).

DISCUSSION OF RESULTS

Epistatic interactions between R-genes to acaricides with different mechanisms take place at the third stage of gene expression, phenotypic expression [7].

The combination of alleles for resistance to malathion with alleles for resistance to acaricides of other chemical classes, bifenthrin, brompropylate, or abamectin in the genome of diheterozygous female mites under the effect of toxicants results in full suppression of the deoxidizing metabolism of these genes by the alleles for resistance to malathion.

Processes of the normalization of the transportation function of cells' plasmalemma regulated by the R-gene for malathion, was suppressed by the alleles for resistance to bifenthrin, brompropylate, and abamectin, not completely as a result of the nature of the dominant–recessive relationship between the biochemical reactions controlled by the genes.

Absence of differences in the toxicological indicators of epistatic effects detected by the results of the experiments with diheterozygous females and their filial generations with different combinations of resistance alleles can be explained by the fact that epistatic effect of

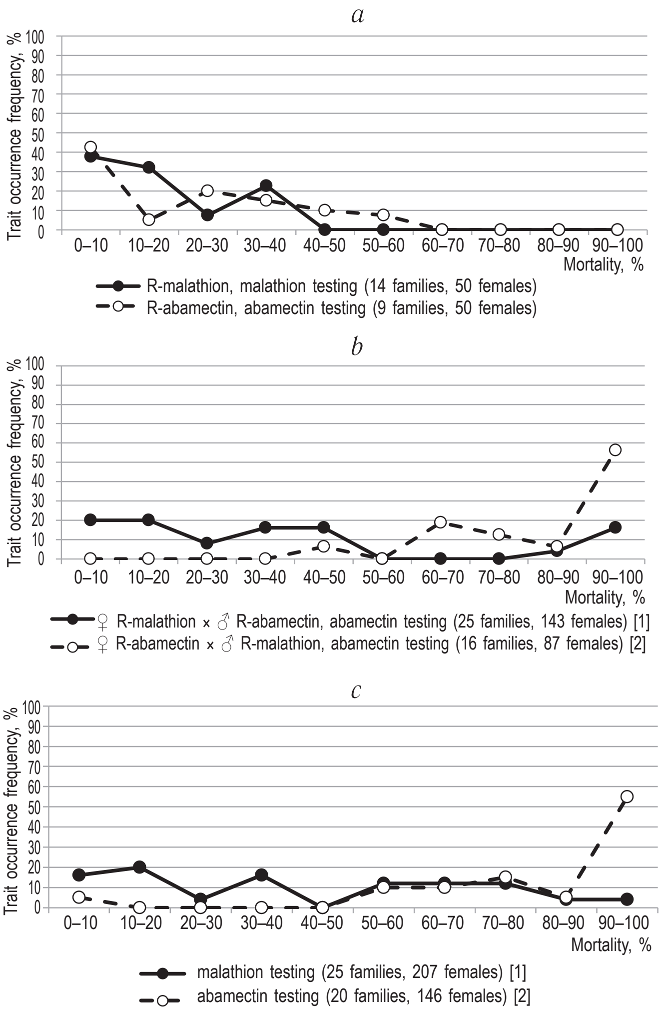

Fig. 3. Distribution of death rates of homozygous genes of resistance to malathion and abamectin in female parents of families (*a*), of the F_1 generation females hybrid to these genes (*b*) and the offspring of genotypes of the offspring from females of the F_1 generation (*c*)

the second gene on the activation of the R-gene to the acaricide is manifested in many possible allelic combinations.

Epistatic interactions of resistance alleles to toxicants of different chemical classes allow the alternative use of two insectoacaricides fast destructing in the environment without reduction in effectiveness. For the insectoacaricides to be ineffective, there have to be conditions for mass occurrence in the population of arthropods of species homozygous for the R-gene. As a result of selectively eliminating the effect of the genetic pool of two insectoacaricides, the dominance of the R-gene heterozygous arthropods will be maintained. Significant reduction in the effectiveness of these toxicants will not occur in the time interval between their sequential uses due to the appearance of new generations of arthropods.

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