

## Original Article

# Realized Heritability Estimates of Hexythiazox Resistance in the Citrus Red Mite, *Panonychus citri* (MCGREGOR)\*

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Estimations of realized heritabilities were conducted for the character of hexythiazox resistance by performing artificial laboratory selections in the citrus red mite, *Panonychus citri* (MCGREGOR), which were collected from a citrus orchard at Haibara Agricultural Research Station (Nippon Soda Co., Ltd.) in Shizuoka Prefecture, Japan. Realized heritabilities were estimated to be 0.835 for the selection for resistance using a strain collected from the orchard after 17 field selections during 6 years, and 0.051 for the selection for susceptibility using a greenhouse-maintained susceptible strain collected from the orchard before the field selections. These results suggested that hexythiazox resistance would be highly heritable in the strain whose initial resistance level was moderate.

## INTRODUCTION

Estimation of heritability<sup>1)</sup> provides a standardized way to analyze and summarize results from artificial selection experiments to pesticides.<sup>2)</sup> For resistance risk assessment,<sup>3)</sup> the estimation can be a good candidate for quantitative evaluation for the ability of pest to succeed pesticide resistance.<sup>2, 4-6)</sup> The calculation method for estimating heritability of pesticide resistance was reported by Tanaka & Noppun,<sup>4)</sup> Firko & Hayes,<sup>5)</sup> and Tabashnik.<sup>2)</sup> And a few studies have estimated the heritability of insecticide resistance in experimental populations of insects.<sup>2, 4, 7-10)</sup> However, there have not been any reports on the heritability of miticide resistance in phytophagous mites.

Hexythiazox, *trans*-5-(4-chlorophenyl)-*N*-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide, is a selective miticide which is active against various phytophagous mites of agricultural importance, and has no or little effects on their natural enemies.<sup>11)</sup> The miticide interferes with mite growth and reproduction, and does not show cross-resistance to conventional miticides. Thus, hexythiazox has been used for controlling mites on various crops under the integrated pest management system. The development of hexythiazox resistance,

however, was recently reported in several species of mites.<sup>12-15)</sup>

In this study, as a part of risk assessment of hexythiazox resistance in the citrus red mite, *Panonychus citri* (MCGREGOR), we estimated realized heritability of hexythiazox resistance from an aspect of quantitative genetics.

## MATERIALS AND METHODS

### 1. Citrus Red Mite

As previously reported,<sup>16)</sup> the strain of citrus red mite selected for hexythiazox resistance in the laboratory was collected from a citrus orchard at Haibara Agricultural Research Station (Nippon Soda Co., Ltd.) in Shizuoka Prefecture, Japan, on August 27th, 1986. The orchard had been successively sprayed with hexythiazox 17 times since 1981 as the field selection experiment.<sup>15)</sup> At the time of collection, the resistance level to hexythiazox seemed to be moderate. The selection for susceptibility was conducted using the strain collected from the same orchard before the field selection, and the strain have been reared on potted young citrus trees in a greenhouse under hexythiazox-free conditions.

During the laboratory selection experiments, the mites were reared at 25°C and 70% relative humidity under 16L-8D photoperiod. A detached citrus leaf method<sup>16)</sup> was adopted for rearing.

\* Studies on Hexythiazox Resistance in Phytophagous Mites (Part 6). For Part 5, see Ref. 21).

## 2. Laboratory Selection for Resistance and for Susceptibility

The detail methods of selections for resistance and for susceptibility with hexythiazox were reported previously.<sup>16)</sup>

The selection for resistance was initiated with about 15,000 eggs (0–2 day-old) oviposited by 2000 females. Eggs on the detached leaves were dipped for 10 sec in hexythiazox solution. The mites which successfully hatched from treated eggs and developed to adults on the treated leaves were used for the next selection. The intensity of selection was estimated as percentage of mites unable to develop to adult after the selection application on 10 leaves drawn randomly.

The selection for susceptibility was initiated with 381 females. The mites were placed individually on the detached leaves to oviposit eggs for 2 days, and then transferred to new leaves with individual markings for other 2 days. Eggs laid for the first 2 days on the leaves were dipped in the hexythiazox solution for 10 sec as a selection treatment. The females whose eggs completely died by the selection treatment were selected, and their eggs, which had been laid for other 2 days were used for the next generation. The intensity of selection was calculated as percentage of the selected adults.

To monitor the change in susceptibility to hexythiazox during selection experiments, ovicidal tests were conducted. Fifty to eighty eggs laid by 6 to 10 adult females for 2 days on each detached leaf were dipped for 10 sec in different concentrations of hexythiazox solutions containing 0.033% spreader, Rabiden SS®. Four to eight different concentrations with 2 to 3 replicates were tested. The number of unhatched eggs were counted 7 days after treatments, and the percent mortality was calculated. Data were analysed by probit regressions<sup>17)</sup> after Abbott's correction for natural mortality.<sup>18)</sup>

## 3. Statistical Genetic Methods

Realized heritability was estimated according to the procedure described by Tanaka & Noppun.<sup>4)</sup>

The character of resistance is assumed to be log-normally distributed and referred to as susceptibility ( $x$ ). The susceptibility of individual insect is defined as a resistible limit for certain dosage density. It is normally distributed in the logarithmic scale ( $X = \log(x)$ ) as follows:

$$P(X) = (1/\sigma\sqrt{2\pi}) \exp[-\{(X - \bar{X})/\sigma\}^2/2],$$

where  $P(X)$  is the probability distribution of phenotypic value,  $\bar{X}$  and  $\sigma$  is the population mean and the standard deviation of susceptibility after logarithmic transformation.

In quantitative genetics, values of a phenotypic trait are assumed to be subject to the following linear model if interactions between loci is disregarded:  $P = A + D + E$ ,

where  $P$ , phenotypic value;  $A$ , additive genetic value;  $D$ , dominance deviation and  $E$ , environmental deviation. Among the casual effects the additive genetic effect contributes uniquely to inheritance of trait values between generations. The amount of variation is expressed as variances, and total phenotypic variance is decomposed into variance components caused by  $A$ ,  $D$  and  $E$  as follows:  $V_P = V_A + V_D + V_E$ , where  $V_P$ , phenotypic variance;  $V_A$ , additive genetic variance;  $V_D$ , dominance variance and  $V_E$ , environmental variance.<sup>1)</sup>

Narrow sense heritability ( $h^2$ ) is the regression of breeding values on phenotypic values, and equivalent to the ratio of additive genetic variance to total phenotypic variance:  $h^2 = V_A/V_P$ . The response ( $R$ ) to selection is  $R = h^2S$ , where  $S$  denotes the selection differential. Thus realized heritabilities can be estimated by  $h^2 = R/S$  with the artificial selection technique.<sup>1)</sup> The selection differential is usually expressed as the product of selection intensity ( $i$ ) and phenotypic standard deviation, i.e.  $S = i\sigma$ . The response in every generation was determined as difference in population means between adjacent generations with the probit analysis:

$$R_t = \log \bar{x}_{t+1} - \log \bar{x}_t,$$

where  $R_t$  is the response at  $t$ -th generation,  $\bar{x}_t$  the value of  $LC_{50}$  at the  $t$ -th generation. Directional artificial selections are regarded as truncation selections, assuming that the character of susceptibility is expressed on a threshold manner.<sup>1)</sup> The selection intensity was calculated from the proportion of surviving individuals in the examined population using Appendix table of Falconer.<sup>1)</sup> In the case of downward selection (i.e. selection for susceptibility), the selection intensity was determined from proportion of dead individuals. The concentration mortality curve, which was estimated at every generation, could be transformed by the traditional probit method as follows:  $Y_t = a_t + b_t (\log(x_t) - \log(x_t))$ . The regression slope is the inverse of the standard deviation of log susceptibility.<sup>17, 19, 20)</sup> Thus phenotypic standard deviation at  $t$ -th generation ( $\sigma_t$ ) was obtained as the inverse of the regression slope:  $\sigma_t = 1/b_t$ . The parameters ( $R$ ,  $i$  and  $\sigma$ ) were determined at every generation and realized heritability was estimated as the regression coefficient of cumulative responses ( $CR_{t+1} = \sum_n^{t+1} R_n$ ) on cumulative selection differentials ( $CS_t = \sum_n^t S_n$ ).<sup>1)</sup>

## RESULTS

Table 1 shows the results of laboratory selection experiments with hexythiazox for resistance (the resistant selection line) and for susceptibility (the susceptible selection line) in the Haibara strain of citrus red mite. For estimation of realized heritability, the table was reconstructed using necessary data from original tables reported previously.<sup>16)</sup> Because of a few lacks of data on the

Table 1 Results of laboratory selection experiments with hexythiazox for resistance and for susceptibility in the Haibara strain of citrus red mite.

SG <sup>c)</sup>	Resistant selection line <sup>a)</sup>				Susceptible selection line <sup>b)</sup>			
	Selection		Susceptibility		Selection		Susceptibility	
	Conc. <sup>d)</sup> (ppm)	Intensity <sup>e)</sup> (%)	Slope <sup>f)</sup>	LC <sub>50</sub> <sup>g)</sup> (ppm)	Conc. (ppm)	Intensity <sup>h)</sup> (%)	Slope	LC <sub>50</sub> (ppm)
P	5.0	95.5	2.56	4.81	5.0	39.1	3.03	0.93
G1	2.5	58.4	2.30	4.24	3.9	75.9	3.34	0.61
G2	4.0	60.4	1.54	11.9	3.0	69.2	3.02 <sup>i)</sup>	0.59 <sup>i)</sup>
G3	10	78.4	0.94	19.0	1.95	28.9	3.02 <sup>i)</sup>	0.58 <sup>i)</sup>
G4	20	33.5	0.35	1280 <sup>i)</sup>	1.95	49.8	3.02 <sup>i)</sup>	0.56 <sup>i)</sup>
G5	1000	23.4	0.67	20,500 <sup>i)</sup>	—	—	2.70	0.54
G6	10,000	28.2	1.03	21,400	—	—	—	—

a) A strain collected from a hexythiazox treated orchard was used as a parental generation (P) for the selection.

b) The unselected strain, which was collected from the same orchard before treatments with hexythiazox, was used as a parental generation (P) for selection.

c) Selection generation.

d) Selection concentration.

e) Percent of eggs unable to develop to adult after selection.

f) Slope of regression equation.

g) Ovicidal test using 0–2 day-old eggs.

h) Percent of adult females whose eggs completely died by selection.

i) Assumed value (see text).

The table was reconstructed from original tables<sup>16)</sup> for estimation of realized heritability.

Table 2 Basic parameters of the resistant selection line in the Haibara strain of citrus red mite.

SG <sup>a)</sup>	log $\bar{x}$ <sup>b)</sup>	$\sigma$ <sup>c)</sup>	$i$ <sup>d)</sup>	$S$ <sup>e)</sup>	$\sum S$ <sup>f)</sup>	$R$ <sup>g)</sup>	$\sum R$ <sup>h)</sup>
P	0.682	0.391	2.107	0.824	0.824	—	—
G1	0.627	0.435	0.931	0.405	1.229	-0.055	-0.055
G2	1.076	0.649	0.973	0.631	1.860	0.449	0.394
G3	1.279	1.064	1.356	1.443	3.303	0.203	0.597
G4	3.107	2.857	0.548	1.566	4.869	1.828	2.425
G5	4.312	1.493	0.400	0.597	5.466	1.205	3.450
G6	4.330	0.971	0.470	0.456	5.922	3.051	3.648

a) Selection generation.

b) Mean susceptibility in the logarithmic scale.

c) Standard deviation of susceptibility.

d) Selection intensity.

e) Selection differential.

f) Cumulated selection differential.

g) Response.

h) Cumulated response.

Table 3 Basic parameters of the susceptible selection line in the Haibara strain of citrus red mite.

SG	log $\bar{x}$	$\sigma$	$i$	$S$	$\sum S$	$R$	$\sum R$
P	-0.032	0.330	0.982	0.324	0.324	—	—
G1	-0.215	0.299	0.410	0.123	0.447	-0.183	-0.183
G2	-0.227	0.331	0.508	0.168	0.615	-0.013	-0.196
G3	-0.240	0.331	1.182	0.391	1.006	-0.013	-0.209
G4	-0.254	0.331	0.801	0.265	1.272	-0.014	-0.222
G5	-0.268	0.370	—	—	—	-0.014	-0.235

original results, additional data were assumed as follows. In the resistant selection line, the LC<sub>50</sub> values of G4 and G5 were assumed to be 1280 ppm and 20,500 ppm, respectively, though each mortality at those highest concentrations tested was below 50%. In the susceptible selection line, all of LC<sub>50</sub> and slope in G2–G4 were

assumed from the data of the former G1 and the latter G5 by eye measurement.

Tables 2 and 3 indicate basic parameters of the resistant and susceptible selection lines, respectively. They were calculated from the results of laboratory selection experiments of Table 1. Figure 1 shows the relations

Table 4 Realized heritability estimates of hexythiazox resistance in the Haibara strain of citrus red mite.

Line	Regression equation of $\sum R$ on $\sum S$	Realized heritability
Resistant selection	$Y = -0.700 + 0.835X$ ( $r^2 = 0.98, p < 0.001$ ) <sup>a)</sup>	0.835
Susceptible selection	$Y = -0.172 - 0.051X$ ( $r^2 = 0.96, p < 0.01$ ) <sup>a)</sup>	0.051

a) ANOVA in the regression analysis.

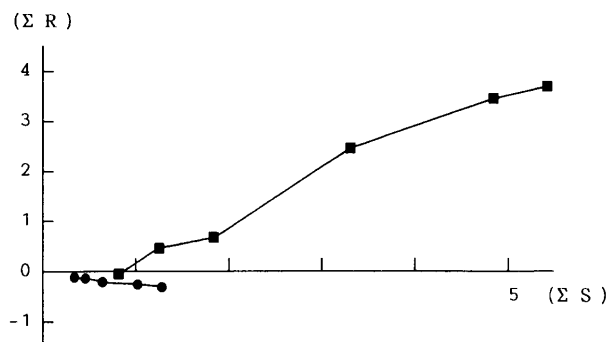


Fig. 1 Relation between cumulated selection differential ( $\sum S$ ) and cumulated selection response ( $\sum R$ ) in Haibara strain of citrus red mite.

Symbols indicate as follows: ■, resistant selection line; ●, susceptible selection line. The solid lines connect points of successive generations.

between cumulative responses ( $\sum R$ ) and cumulative selection differentials ( $\sum S$ ) in the two lines. The cumulated responses ( $\sum R$ ) of the two lines systematically changed along cumulative selection differentials ( $\sum S$ ), and asymmetrical responses were found in the two-way selections. Table 4 represents the results of linear regression of cumulative responses ( $\sum R$ ) on cumulative selection differentials ( $\sum S$ ). And the realized heritabilities, or the regression coefficients, were estimated to be 0.835 and 0.051 in the resistant and susceptible selection lines, respectively.

## DISCUSSION

The realized heritability of the trait of hexythiazox resistance was lower in susceptible selection line (0.051) than that in resistance selection line (0.835) in the citrus red mite (Table 4, Fig. 1). The asymmetry might arise from several causes *e.g.* gene frequency biases (*i.e.* low initial frequency of resistant alleles at the majority of the loci involved).<sup>4)</sup> For resistance risk assessment, the estimation of realized heritability would be practically more significant in the selection for resistance than that for susceptibility, because it is one of attempts to predict the rate of resistance development.

The laboratory selection for resistance did not initiate with a susceptible strain but with a field-collected strain which was about 5-fold resistant to hexythiazox (Table 1). Then, the realized heritability of selection for resist-

ance from completely susceptible level was not estimated. Thus, it was uncertain whether the realized heritability would change during selections with hexythiazox.

Changes of realized heritability during selection were reported in laboratory selection experiments with *Bacillus thuringiensis* in insects such as *Plutella xylostella* (L.), *Helicoverpa virescens* (F.) and *Leptinotarsa decemlineata* (SAY).<sup>2)</sup> Parameters, which were response to selection ( $R$ ), selection differential ( $S$ ) and realized heritability ( $h^2$ ), could be calculated separately for the first and second half of selection experiment. The  $R$  and the  $h^2$  were higher for the first half than the second half, while the  $S$  did not differ significantly between halves. It was suggested that substantial additive genetic variation was present initially (*i.e.* alleles for resistance were not rare) and then declined as selection proceeded in these cases.<sup>2)</sup>

A heritability estimates obtained by laboratory experiments has some difficulties when we extrapolate it to field conditions.<sup>2, 4, 5)</sup>

For example, Tanaka & Noppun<sup>4)</sup> described the following three difficulties. (1) Differences in environmental effects between laboratory and field conditions might exist. Experiments under uniform laboratory conditions might result in overestimations for a real heritability under natural field conditions where the environmental parameters would not be constant. As previously reported on field selection experiment with hexythiazox resistance in the citrus red mite,<sup>15, 21)</sup> the levels of resistance and the degree of resistance reversion varied depending on the sub-populations on the different trees within the orchard in spite of the same times of selection application. This result indicated that the heritabilities of hexythiazox resistance might differ among sub-populations irrespective of the same genetical background, because environmental variance ( $V_E$ ) probably differ among them. Heritability ( $h^2$ ) is the ratio of additive genetic variance ( $V_A$ ) to total phenotypic variance ( $V_P$ ) which is the sum of genotypic variance ( $V_G$ ) and environmental variance ( $V_E$ ), *i.e.*  $h^2 = V_A / (V_G + V_E)$ . Therefore, heritability estimates partly depend on the value of environmental variance ( $V_E$ ). (2) Genotype-environment interactions along some environmental parameters, *e.g.* temperature, photoperiod and density, which fluctuate under field conditions might exist. (3)

There might be pleiotropic effects of resistant genes to several fitness components. If there are antagonistic pleiotropic effects between insecticide resistance and fitness components, positive selection on fitness components should result in negative indirect responses of resistance. In the hexythiazox resistant citrus red mite, the fitness disadvantage was observed.<sup>22)</sup>

When realized heritability ( $h^2$ ) is estimated as  $h^2 = R/S$  from selection for pesticide resistance, the selection differential ( $S$ ) is not calculated directly but is estimated from the equation,  $S = \sigma_i$ . And there are some potential factors biasing the estimation of  $S$ .<sup>6)</sup>

One of these factors is the unequal selection on males and females if their resistance to a pesticide is different.<sup>6)</sup> The citrus red mite is arrhenotokous.<sup>23)</sup> Thus haploid males are susceptible ( $S\sigma$ ) or resistant ( $R\sigma$ ), whereas diploid females are susceptible ( $SS\phi$ ), resistant ( $RR\phi$ ) or heterozygous ( $RS\phi$ ,  $SR\phi$ ). There was no difference in susceptibility to hexythiazox between  $S\sigma$  and  $SS\phi$ , or between  $R\sigma$  and  $RR\phi$  in the citrus red mite. However, the heterozygous females were almost susceptible to hexythiazox, because the mode of hexythiazox resistance was incompletely recessive.<sup>24)</sup> Therefore, overall mortality of females might be higher than that of males.

Difference in reproductive potential between genotype associated with resistance also might bias the  $S$ . The reproductive disadvantage in the hexythiazox resistant genotype of citrus red mite was observed.<sup>22)</sup>

There were limitations, as described in MATERIALS AND METHODS, in estimating realized heritability.<sup>2, 4, 6)</sup> But, the realized heritability could be useful for understanding and managing resistance development particularly if one recognize the limitations.<sup>2)</sup> The trait of hexythiazox resistance in the citrus red mite was suggested to be highly heritable from a moderate resistance level. For resistance management from a stand point of quantitative genetics, the additive genetic variance ( $V_A$ ) and the dominance variance ( $V_D$ ) is considered to be beyond man's control. Thus, tactics increasing the environmental variance ( $V_E$ ) seemed to be one of the recommendations for managing the hexythiazox resistance in this species.

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#### 要 約

#### ミカンハダニにおけるヘキシチアゾクス抵抗性の実現遺伝率の推定\*

山本敦司, 米田 渥, 波多野連平, 浅田三津男  
静岡県榛原郡の日本曹達(株)榛原農業研究所内の柑橘園において, 6年間で17回のヘキシチアゾクスによる圃場淘汰を行なった後に採集したミカンハダニ (*Panonychus citri* MCGREGOR) を用いた抵抗性への室内淘汰における実現遺伝率は0.835と推定された。一方, 圃場における淘汰を開始する以前にこの柑橘園から採集した系統を用いた感受性への室内淘汰における実現遺伝率は0.051と推定された。この結果から, 抵抗性レベルが中程度の榛原系ミカンハダニの集団において, ヘキシチアゾクス抵抗性は遺伝性が高いことが示唆された。

\* 植食性ハダニ類のヘキシチアゾクス抵抗性に関する研究 (第6報)