Influence of Hexythiazox Resistance on Life History Parameters in the Citrus Red Mite, *Panonychus citri* (McGregor)*

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(Received May 15, 1995; Accepted July 19, 1995)

The life history parameters concerned with population growth were studied in the citrus red mite, *Panonychus citri* (McGregor), among genotypes, or the hexythiazox resistant Haibara-R (RR), the susceptible Haibara-S (SS) and their hybrids (SR and RS) which are the same origin from a citrus orchard at Haibara-cho in Shizuoka Prefecture, Japan. The difference in the intrinsic rate of natural increase (r_m) among genotypes was slight at 25°C where r_m decreased in the order genotype SS (0.2110), SR (0.2098), RS (0.2042), RR (0.1996). However, the fitness disadvantage was severe at 35°C where r_m were 0.1187 and 0.0123 in SS and RR, respectively. At any mite age at 25 and 35°C, reproductive values (V_x/V_o) in RR were smaller than those in SS. The reduction of values of r_m and V_x/V_o at both 25 and 35°C seemed to be ascribed to the significantly shortened longevity and reduced fecundity of adult females in RR, compared with those in SS.

INTRODUCTION

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. 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.

Fitness is one of factors influencing development of insecticide/miticide resistance. And Keiding referred to the fitness as an element of resistance risk assessment. Thus, the life history parameters associated with reproductive potential on mites have been studied in terms of resistance to several miticides. Fitness disadvantage in resistant mites seems to be one of the critical factors affecting the reversion of resistance.

In this study, as a part of the risk assessments of hexythiazox resistance in the citrus red mite, *Panonychus citri* (McGregor), we investigated the effects of hexythiazox resistance and temperature on life history parameters associated with reproductive potential of the species.

MATERIALS AND METHODS

1. Citrus Red Mite

The original strain of citrus red mite, *Panonychus citri* (McGregor), was collected...
from a citrus orchard at Haibara Agricultural Research Station (Nippon Soda Co., Ltd.) in Shizuoka Prefecture, Japan. As reported previously, the strain was selected for resistance and for susceptibility with hexythiazox to obtain resistant (Haibara-R) and susceptible (Haibara-S) ones, respectively. And they have been maintained in the laboratory.

Reciprocal crosses between Haibara-S (SS♀, S♂) and Haibara-R (RR♀, R♂) strains were conducted to obtain heterogeneously resistant individuals (SR♀, RS♂). The detailed method of crosses was the same as that described in the previous report. The susceptibility (LC50 value) of eggs in each genotype to hexythiazox was also reported there as follows: SS♀, 0.462 ppm; SR♀, 8.47 ppm; RS♂, 3.48 ppm; and RR♀, >8000 ppm.

2. Development and Reproduction

Ten to fifteen mated adult females from each cross, SS♀ × S♂, SS♀ × R♂, RR♀ × S♂ and RR♀ × R♂, were inoculated on each detached leaf, where they were allowed to oviposit for 24 hr. After removing the adult females, the eggs were adjusted to 20–30 individuals per leaf to form a cohort that synchronously developed to adults. The number of eggs examined in each genotype, SS+S, SR+S, RS+R and RR+R, are listed in Table 1. Daily monitorings were conducted to investigate the duration of eggs and pre-adult stages which were egg, larva and nymph, and to examine the hatchability of eggs and the survival rate during pre-adult stages.

Adult females, which developed from the eggs in each genotype described above, were individually reared just after emergence to adult on detached leaves with markings. The number of adult females examined in each genotype, SS, SR, RS and RR, are listed in Table 2. Daily observations were made to research the survival rate, mean longevity, the number of eggs oviposited per female per day and the total number of eggs oviposited per female.

During experiments, mites were reared under 16L-8D photoperiod at 25°C with 70% relative humidity or at 35°C without controlling relative humidity. A detached citrus leaf method was adopted for rearing. To prevent the detached leaf from drying, it was put with wet cotton in a glass Petri dish.

3. Life History Parameters

Using the data from methods mentioned above, life history parameters concerned with population growth were estimated in each genotype at 25 and 35°C. In this study the sex ratio in the citrus red mite was assumed to be ♀:♂=2:1.

The age-specific survival rate of females throughout a generation (λ) and the mean number of female eggs produced per day (mx) were estimated. Mean expectation of life (Ex), mean generation time (Tc), doubling time (D), net reproductive rate (R0), intrinsic rate of natural increase (rn), finite rate of natural increase (λ), and reproductive value (Vs/V0) were calculated using the following formulae:

\[ E_x = \frac{\sum (l_x l_{x+1})}{l_x} \]
\[ T_c = \ln R_0 / r_n \]
\[ D = \ln (2) / r_m \]
\[ R_0 = \sum_{x=0}^{\infty} e^{-\tau x} l_x m_x = 1 \]
\[ \lambda = e^{r_n} \]
\[ V_x / V_0 = e^{-\tau x} (\sum_{x=0}^{\infty} e^{-\tau x} l_x m_x) / l_x \]

where x is the age of an individual at a time.

For calculation of relative fitness, \( r_m \) value in each genotype at 25 or 35°C was divided by that in SS at 25°C.

RESULTS

1. Development

Table 1 shows parameters on development of pre-adult stages in the citrus red mite at each genotype, SS+S, SR+S, RS+R and RR+R. At 25°C, there was no significant difference in the hatchability of eggs and the survival rate during pre-adult stages between SS+S and each of other genotypes. However, the durations of eggs and pre-adult stages were significantly longer in RR+R than in SS+S. In SS+S at 35°C compared with at 25°C, the hatchability and the survival rate were low, and the duration of pre-adult stage was short. At 35°C, in RR+R compared with in SS+S, the hatchability and the survival rate were...
Table 1 Parameters on development of pre-adult stages in the citrus red mite at each genotype associated with hexythiazox resistance.

<table>
<thead>
<tr>
<th>Crossing of parents</th>
<th>25°C</th>
<th>35°C</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype of eggs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS × S</td>
<td>293</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>SS × S</td>
<td>152</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td>RR × S</td>
<td>240</td>
<td>292</td>
<td>G&lt;sup&gt;b)&lt;/sup&gt;</td>
</tr>
<tr>
<td>RR × S</td>
<td>273</td>
<td>37.7***</td>
<td></td>
</tr>
<tr>
<td>RR × S</td>
<td></td>
<td></td>
<td>G</td>
</tr>
<tr>
<td>SS × S</td>
<td>240</td>
<td>292</td>
<td></td>
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<tr>
<td>SS × S</td>
<td>152</td>
<td>273</td>
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<tr>
<td>RR × S</td>
<td>240</td>
<td>292</td>
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<tr>
<td>RR × S</td>
<td>273</td>
<td>37.7***</td>
<td></td>
</tr>
</tbody>
</table>

-<sup>a)</sup> Egg + Larva + Nymph.
-<sup>b)</sup> G-test, two-tail test, * (p < 0.05), ** (p < 0.01), *** (p < 0.001). Statistical comparison was made between susceptible-genotype and each genotype at 25 or 35°C.
-<sup>c)</sup> Mann-Whitney’s U-test, see above.<sup>b)</sup>

Table 2 Parameters on survivorship and fecundity of adult female of the citrus red mite at each genotype associated with hexythiazox resistance.

<table>
<thead>
<tr>
<th>Genotype of adult female</th>
<th>25°C</th>
<th>35°C</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. adult females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>63</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>68</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>54</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>RS</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longevity (in days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean ± S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>15.62 ± 0.56</td>
<td>6.91 ± 0.65</td>
<td>3.70 ± 0.50**</td>
</tr>
<tr>
<td>SR</td>
<td>16.18 ± 0.42**</td>
<td>14.96 ± 0.65**</td>
<td>13.79 ± 0.45**</td>
</tr>
<tr>
<td>RR</td>
<td>14.96 ± 0.65**</td>
<td>13.79 ± 0.45**</td>
<td>13.79 ± 0.45**</td>
</tr>
<tr>
<td>Total No. eggs/♀/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mean ± S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>4.26 ± 0.13</td>
<td>2.71 ± 0.41</td>
<td>1.64 ± 0.36**</td>
</tr>
<tr>
<td>SR</td>
<td>3.91 ± 0.15**</td>
<td>3.74 ± 0.12***</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>3.74 ± 0.12***</td>
<td>3.74 ± 0.12***</td>
<td></td>
</tr>
<tr>
<td>Total No. eggs/♀/day</td>
<td>66.92 ± 2.47</td>
<td>20.67 ± 3.58</td>
<td>9.24 ± 2.58*</td>
</tr>
<tr>
<td>(mean ± S.E.)</td>
<td>62.97 ± 2.53**</td>
<td>56.62 ± 3.52**</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>56.62 ± 3.52**</td>
<td>51.40 ± 1.99***</td>
<td></td>
</tr>
<tr>
<td>35°C</td>
<td></td>
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</tbody>
</table>

<sup>a)</sup> Mann-Whitney’s U-test (see Table 1).
significantly low, and the duration of pre-adult stage was significantly long.

2. Reproduction

Table 2 indicates parameters on survivorship and fecundity of adult female in each genotype, SS SR, RS and RR. At 25°C, the longevity in RR was significantly shorter than those in any other genotypes. The number of eggs per female per day and the total number of eggs per females were significantly less in RS and RR than in SS. In SS at 35°C compared with at 25°C, the longevity was short, and the number of eggs per female per day and the total number of eggs per females were few. At 35°C, in RR compared with SS, the longevity was short, and the number of eggs per female per day and the total number of eggs per females were few.

3. Life History Parameters

Age-specific survival rate ($l_x$-curve) was studied among genotypes, SS, SR, RS and RR, at 25 and 35°C (Fig. 1). At 25°C, the $l_x$-curves in SR and RS were similar to that in SS, while the curve in RR existed below that in SS. In SS, the $l_x$-curve at 35°C was distinctly below that at 25°C because of higher mortality of pre-adult stage at 35°C. At 35°C, also, the curve in RR was below that in SS.

Age-specific fecundity ($m_x$-curve) was studied at 25 and 35°C (Fig. 2). The fecundity ($m_x$) is the number of female eggs per adult female at age $x$ in days. At 25°C, the $m_x$-curves in SR and RS were similar to that in SS, while the curve in RR existed below that in SS. In SS, the $m_x$-curve at 35°C was drastically below that at 25°C. At 35°C, the curve in RR was below that in SS.

Age-specific reproductive value ($V_x/V_o$) was studied at 25 and 35°C (Fig. 3). The value ($V_x/V_o$) is the relative number of female eggs which will have been oviposited by adult female since age $x$. In other words, it means contribution of adult female at age $x$ to the next generation. There was no or little differences in the shape of curve of reproductive value ($V_x/V_o$-curve) between genotypes, at 25
and 35°C, respectively. At 25°C, the values \( \frac{V_x}{V_0} \) at each mite age were slightly smaller in SR and RS than in SS, while they were clearly smaller in RR than in SS. In SS at 35°C compared with at 25°C, the values were drastically small. At 35°C also, the values at each mite age in RR were not as high as that in SS.

Table 3 indicates the parameters on survivorship and reproduction in these genotypes at 25 and 35°C. At 25°C, the values of mean longevity \( E_0 \), mean generation time \( T_e \), net reproductive rate \( R_0 \), intrinsic rate of natural increase \( r_m \) and finite rate of natural increase \( \lambda \) were smaller in RR than those in SS, and the doubling time \( D \) was longer in RR than SS. But there were only a few differences in the values of these 6 parameters among genotypes SS, SR and RS. In SS and RR, respectively, the values of these parameters except for \( D \) were drastically smaller at 35°C than 25°C, and the doubling time was longer at 35°C than 25°C. At 35°C, the values of \( E_0, R_0, r_m \) and \( \lambda \) were smaller in genotype RR than those in SS, and \( D \) was longer in RR than SS. There was slight difference in \( T_e \) between SS and RR.

**DISCUSSION**

Reproduction in spider mites is extremely sensitive to a variety of intrinsic and extrinsic conditions. Individual life history parameters, or fitness traits, are associated with reproductive potential including fecundity, hatchability, oviposition and longevity periods, rate of development, survivorship and certain aspect of sex ratio. Extrinsic factors influencing these parameters are temperature, humidity, light, level of predation, intra- and inter-specific competition, quantity, quality and application timing of pesticides, and various features of host plants such as strain, plant and soil nutrition and plant age. Intrinsic factors affecting reproductive potential are mite strain, level of inbreeding, colony density, age of female and of population, female’s fertilization status, quality of male, duration of insemination, and various aspects of behavior.

Therefore, to study effect of resistance on life history parameters, or fitness, the investigated strains should be maintained under similar intrinsic and extrinsic conditions as possible. And especially, the genetic background should be the same between the strains.

There were no or little differences of fitness between resistant and susceptible genotypes of mites in several cases of miticide resistance. On the contrary, the reproductive disadvantage in miticide resistant mites have been reported in cases of resistance to the following miticides: dimeton, parathion, dicofol, and fenpropathrin.

Also in the hexythiazox resistant Haibara-R strain of citrus red mite, the reproductive disadvantage was observed. Because reproductive potential in the species was known to be affected by temperature, those in genotype SS, SR, RS and RR were studied at 25 and 35°C. In this species, the higher the temperature increased up to about 30°C, the higher the intrinsic rate of natural increase \( r_m \) be-
came. But the $r_m$ was suppressed over about 30°C.\textsuperscript{21,23}

The intrinsic rate of natural increase ($r_m$) and the reproductive value ($V_x/V_o$) were adopted for comparisons of reproductive potentials between these genotypes. The value of $r_m$ at 25°C decreased in the order SS (0.2110), SR (0.2098), RR (0.2042), RS (0.1996) (Table 3). It was considered that the differences in longevity and fecundity of adults affected the differences in $r_m$ between genotype SS and the others (Tables 2 and 3). The fitness disadvantage, or the reduction of $r_m$, was evident but seemed to be slight in SR, RS and RR at 25°C where the citrus red mite seemed to reproduce suitably.\textsuperscript{24,25} However, at 35°C where it seemed to be too hot for the mite to reproduce easily,\textsuperscript{24,25} $r_m$ of 0.0123 in RR was smaller than that of 0.1187 in SS. The fitness disadvantage under this condition in RR was considered to be severe. The same tendency was observed in the reproductive values ($V_x/V_o$) at 25 and 35°C. And at any mite ages at 25 and 35°C, reproductive potential in RR was suggested to be inferior to that in SS.

Recently, the relative fitness advantage was reported in the two-spotted spider mite, \textit{Tetranychus urticae} (Koch), which was hexythiazox-clofentezine resistant.\textsuperscript{16} The resistant strain QRPH had the $r_m$ value of 0.292 which was higher than the $r_m$ values of the susceptible strain S (0.285) and the hybrid strain S-QRPH (0.268). This result in the two-spotted spider mite did not agree with our result in the citrus red mite, \textit{Panonychus citri} (McGregor). However, the QRPH and S strains were collected from different region, and their genetic backgrounds were also different. Thus, the effect of resistance to hexythiazox and clofentezine on fitness were not always clear in the species.

A relative fitness disadvantage associated with the resistant gene might retard resistance development primarily by reducing the reproduction of heterogeneous and homogeneous resistant individuals. And immigration might provide a source of susceptible individuals, and thus would greatly increase the effectiveness of a reproductive disadvantage.\textsuperscript{26}

In the Haibara-R strain of citrus red mite, as the relative fitness disadvantage was slight the rate of resistance reversion might not be so rapid under suitable conditions for reproduction. In the field, however, a reproductive success such as the magnitudes of intrinsic rate of natural increase or reproductive value would vary depending on various intrinsic and extrinsic factors described before. Especially under hard conditions for population increase such as high temperature, the hexythiazox resistance might be expected to retard because of severe fitness disadvantage.

Rotation programs using hexythiazox and other miticides, which have no cross-resistance with hexythiazox or distinct mode of action, would be practically recommended to manage the hexythiazox resistance especially in the citrus orchard where the Haibara-R strain was collected, because the reversion of resistance would be expected due to some factors including the fitness disadvantage in the strain. In some resistant strains to miticide,\textsuperscript{12,14,27} the reversion of resistance was observed under field conditions, and seemed to be ascribed to the reproductive disadvantage in resistant individuals as well as immigration of susceptible individuals. However, reversion of hexythiazox resistance in the citrus red mite remains to be investigated.

**ACKNOWLEDGMENTS**

We wish to express our thanks to Dr. Tadashi Miyata of Nagoya University, and the staffs of Biological Laboratory and Haibara Agricultural Research Station of Odawara Research Center (Nippon Soda Co., Ltd.) for their critical reading, helpful comments and continued encouragement.

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要約
ミカンハダニにおけるヘキシチアソックス抵抗性の生活史パラメータに及ぼす影響 *

* 植食性ハダニ類のヘキシチアソックス抵抗性に関する研究 (第 4 報)