Inheritance of Resistance to Cyhexatin in the Kanzawa Spider Mite, *Tetranychus kanzawai* KISHIDA (Acarina: Tetranychidae)

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The genetics of resistance to cyhexatin in *Tetranychus kanzawai*, one of the major pests of tea plants in Japan, was investigated using homogeneously resistant and susceptible strains of the mite. Similar dosage-mortality lines obtained in the F_1 progenies from reciprocal crosses indicated the absence of sex-linked inheritance. The dosage-mortality line in the F_2 progeny from backcrossing the F_1 hybrid to susceptible males was significantly different from that in the expected response estimated on the basis of monogenic inheritance. These results for F_1 and the backcross indicate that cyhexatin resistance in *T. kanzawai* is due to autosomal, polyfactorial genes.

INTRODUCTION

Resistance to acaricides is a serious problem in the control of the Kanzawa spider mite, *Tetranychus kanzawai*, which is one of the major pests of tea plants in Japan. Resistance of mites to acaricides develops rapidly for several reasons such as high biotic potential, high mutation rate and an arrhenotokous manner of reproduction. HELLE and VAN ZON (1967) investigated the mutation rate of six visible characters in haploid offsprings from unfertilized females of the Pacific spider mite, *T. pacificus*, and found a relatively high mutation rate ranging from 0.8×10^{-4} to 2.8×10^{-4} . This mutation rate is remarkably high in comparison with that ranging from 10^{-5} to 10^{-7} in other arthropod species having generation turnover similar to spider mites (ASADA, 1978).

Although recessive genes may remain undetected in bisexual zygotenic species for long periods, both profitable and detrimental mutation will appear in the haploid sex with the possibility of selection in haplo-diploid species. Consequently, detrimental recessive genes tend to be eliminated and profitable mutation for biotic potential will rapidly spread over a population of haplo-diploid species (ASADA, 1978).

The rate of resistance development is dependent on the initial frequency of alleles conferring resistance, the degree of dominance, the fitness of resistance alleles to natural selection, and the intensity of selection pressure (METCALF, 1955; CROW, 1957).

The genetics of acaricide resistance has been investigated in detail, and the mode of inheritance, e.g., the degree of dominance, the number of factors associated with the resistance, was clarified for some specific acaricide resistance in spider mites (TAYLOR and SMITH, 1956; DITTRICH, 1972; OVERMEER and VAN ZON, 1973; KUWAHARA, 1977). Also, there are instances where the level of resistance can be increased by maternal

factors or modifiers (VAN ZON et al., 1964; OVERMEER, 1967; OVERMEER and HARRISON, 1969).

GEORGHIOU and TAYLOR (1977) investigated the genetics and biological influences in the evolution of insecticide resistance and found that gene frequency increased most rapidly when the resistance gene was completely recessive. Also, the rate at which the resistant population increased was highest when the resistance allele was completely dominant.

Cyhexatin is a selective acaricide which has been used extensively in integrated pest management because it enables effective control of pest mites and is moderately to slightly toxic to predatory phytoseiid mites, which allows appreciable biological control of pest mites (CROFT et al., 1984). Cyhexatin was introduced into Japan in the 1970's and has been used to control spider mites on a wide variety of crops. Recently, resistance to cyhexatin has been observed with the two-spotted spider mite, *T. urticae*, in Australia and in the United States (EDGE and JAMES, 1982, 1983; CROFT et al., 1984). Such resistance has also been observed in local populations of *T. kanzawai* in Japan (HAMAMURA, 1985). The present paper deals with the genetics of resistance to cyhexatin in *T. kanzawai*.

MATERIALS AND METHODS

Breeding procedures. Mites were collected in 1985 from the Haibara district, Shizuoka Pref., where high levels of resistance to cyhexatin have been observed in *T. kanzawai* (KUMAYAMA et al., unpublished data). The following procedures, described by DITTRICH (1972), were employed to establish a resistant strain from the mites. Mites were selected six times with a discriminating concentration of 1,000 ppm cyhexatin, and twenty fertilized females from the colony were transferred singly to a bean leaf and subjected to inbreeding over seven generations. These inbred lines were selected further with a discriminating concentration of 2,000 ppm cyhexatin. The females that survived were transferred singly to a bean leaf placed on 0.3% agar gel and allowed to lay eggs. The resultant teliochrysalia were transferred to newly prepared leaves on agar gel for molting to take place, and each individual was allowed to oviposit until a batch of ca. 30 haploid eggs was obtained. Next, the females were transferred singly to newly prepared leaves and stored at 10°C with constant illumination and 30–50% R.H. In the meantime, eggs were kept at 30°C. As soon as the males appeared, they were mated with their unfertilized females. The resultant brood of females was used partly for the toxicological tests with a discriminating concentration of 2,000 ppm cyhexatin. When all specimens survived, the remaining mites were used to establish the strain homozygous for the resistance gene. This procedure was repeated twice, and a new strain was propagated after the 2nd cycle. This strain was used as a resistant one (R).

Susceptible mites were collected from our laboratory field in Shiga Pref. in 1985, where no acaricide had been used for a long period. They were then reared in our laboratory without exposure to acaricides. Two hundred fertilized females from the stock culture were transferred singly to a bean leaf placed on agar gel as described above, and allowed to lay eggs until a batch of ca. 30 eggs was observed. When the brood developed to the adult stage, they were selected with a discriminating concentration of 10 ppm cyhexatin. When they all died at the concentration, the remaining mites were

used to establish the strain homozygous for the susceptible gene. This procedure was repeated three times. This strain was used as the susceptible one (S).

Toxicological test. A commercial product of cyhexatin (25% W.P.) was used for the toxicological tests. The toxicant was sprayed on the surface of detached bean leaves on which adult mites had been inoculated. The detached leaf was placed singly on agar gel (0.3%) in a plastic cup (MATSUNAGA and FURUHASHI, 1972). Ten concentrations of toxicant with six replications of 15 mites each were tested, and the tests were repeated four times. Mortality was assessed 48 hr after application, with correction using ABBOTT's formula (ABBOTT, 1925). Experiments showing more than 10% control mortality were disregarded. The expected dosage-mortality response curve for the backcross progeny estimated on the basis of monofactorial inheritance was calculated as described by GEORGHIOU (1969). The degree of conformity between the observed and expected mortality was tested by chi-square analysis and the *t*-distribution with 95% confidence limits. Mating procedures for genetic analysis were similar to those reported by HERNE and BROWN (1969), CROFT et al. (1976) and CROFT and WHALON (1983).

RESULTS AND DISCUSSION

The results of susceptibility tests with cyhexatin against S and R strains and their offsprings are summarized in Table 1 and Fig. 1, respectively. The resistant ratio to cyhexatin in the R strain was 282.4 at LC₅₀ and 2,589.8 at LC₉₅. The slope of the regression line was steep in the S strain (4.39) in comparison with that in the R strain (1.23). As pointed out by CROFT et al. (1984), the flat slope in the resistant population is highly variable in response to increasing concentrations of toxicant. The flat slope in the R strain may thus reflect a polygenic response or a very complicated response due to a monogenic factor with modifiers.

Table 1. Responses of S and R strains of *T. kanzawai* and their F₁ and backcross progenies to cyhexatin

Strain or progeny	<i>n</i>	LC ₅₀ (ppm)	(95% CL) (ppm)	LC ₉₅ (ppm)	(95% CL) (ppm)	Slope	RR LC ₅₀
S	2343	10.2	8.6–12.2	24.2	18.6–38.1	4.4	—
R	1873	2883.1 ^a	—	62674.0 ^a	—	1.2	282.4
F ₁ progeny							
S female × R male	2306	66.4	57.9–75.8	373.1	295.5–502.1	2.2	
S male × R female	2441	79.4	71.1–88.9	429.8	353.6–544.7	2.2	
Backcross progeny							
F ₁ (S female × R male) female × S male	2045	24.9	20.7–29.1	164.8	125.4–217.5	2.2	
F ₁ (S male × R female) female × S male	2309	27.5	21.2–33.6	165.6	111.4–223.9	2.2	

n: Number of mites examined, RR: Resistance ratio (LC₅₀ S/LC₅₀ R).

^a Tests could not be done at cyhexatin concentrations of more than 2,000 ppm in this toxicological experiment, because at the higher dosages mite death was caused by vehicles contained in the cyhexatin formulation (25% W.P.). Consequently, the LC₅₀ and LC₉₅ values were extrapolated based upon the d-m line of the R strain with a limited range of cyhexatin concentrations.

Fig. 1. Dosage-mortality relationships for cyhexatin against S and R strains, their F_1 and backcross (B_1) progenies of *T. kanzawai*. Part of the regression line (dotted) for the R strain was extrapolated based upon the line obtained (solid) in this toxicological experiment. $\circ$: S strain, $\bullet$: R strain, $\triangle$: F_1 , $\blacktriangle$: B_1 .

When the R strain was reciprocally crossed with the S strain, the F_1 female progenies from the reciprocal crosses showed almost an identical dosage-mortality (d-m) line and an intermediate response between the S and R strains. This suggests that cyhexatin resistance is not sex-linked but autosomal. When the F_1 females from the reciprocal crosses were backcrossed with males of the S strain, the observed d-m line of the F_2 progenies differed significantly from that of the expected one estimated on the basis of monofactorial inheritance except for small regions near LC_{50} . Consequently, it was concluded that cyhexatin resistance in *T. kanzawai* was not due to monogenic but to polygenic factors.

CROFT et al. (1984) investigated cyhexatin resistance from the genetic viewpoint in *T. urticae* and observed different responses to cyhexatin between reciprocal crosses. However, such a response was not found in our experiments. As suggested by CROFT et al. (1984), the different responses described above may be attributable to considerable heterozygosity in the diploid female. The present breeding procedures may be available for homogeneity of the resistance gene in the case where resistance is monofactorial. Our observations suggest that cyhexatin resistance is due to polygenic factors. Consequently, we cannot deny the possibility that our R strain is composed of genetically heterogeneous mites.

CROFT et al. (1984) observed the rapid loss of cyhexatin resistance through inbreeding of heterozygous mites when selection was discontinued. However, the resistance to cyhexatin of the strain supplied for this study remained at the same level through ca. 20 generations without exposure to acaricides. These observations indicate that our R strain is almost homogeneous for the resistance genes.

The polygenic basis of cyhexatin resistance will retard the rate at which the resistant population grows. The characteristics of cyhexatin resistance makes it possible to detect the resistance at an early stage.

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