Resistance of the Bulb Mite, *Rhizoglyphus robini* Claparede, to Organophosphorus Insecticides

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The bulb mite, *Rhizoglyphus robini* Claparéde, is commonly found world wide in association with corms of flowering bulbs, and is regarded as a key pest for these crops in Japan. Some of the organophosphorus (OP) insecticides such as disulfoton and dimethoate were officially registered and have been used for over 20 years in Japan against the bulb mite with no evidence of resistance for the first decade of their introduction. But in the recent decade, mite control with these insecticides has been becoming difficult year by year, and some researchers conjecture that the bulb mite might have developed resistance to these insecticides. Resistance of various degrees, however, may go undetected for considerable periods. Thus there have been no confirmed records of resistance to these insecticides in the bulb mite.

Recently, it was demonstrated that bulb mite populations collected from various fields in Kochi Prefecture showed the resistance to disulfoton which has been mainly used for bulb mite control in that area[10]. This might be the first record which showed resistance to insecticides in the bulb mite. However, the problem of the rates at which the bulb mite develops resistance to various OP insecticides or the extent to which the use of these registered insecticides produces resistance to other OP insecticides is quite obscure.

This paper summarizes the result of experiments on the resistance to various OP insecticides and the genetics of resistance to disulfoton in relation to the development and stability of the resistance in the bulb mite.

Materials and methods

1) *Toxicity test*

Several populations of the bulb mite collected from various parts of Japan and one reference (S) strain were used in the present experiment. The origin of these populations is shown in Table 1. Mites were isolated from host plants and their rhizosphere by using the Tullgren's equipment and were reared in moist plastic containers (9 cm diam.) with onion powder as food[6]. The origin and response to several insecticides of the S strain were already described elsewhere[10]. Various kinds of OP insecticides listed in Table 2 were used in this experiment. Most of them were emulsified and commercial products except monocrotophos and acephate. A series of concentrations of the insecticides were prepared by diluting with distilled water. Acephate and monocrotophos were diluted directly with distilled water.

Sensitivity of the bulb mite to OP insecticides was tested by the filter paper method developed by Kuwahara et al.[5] Three sheets of paper (3 cm diam.) to which thin layers of onion powder adhered were placed into each

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Table 1. Data on the bulb mites used in this study

Strain	Locality	Host	Time of collection
Oki	Ie Is., Okinawa Pref.	Easter lily (*Lilium longiflorum*)	Dec. 1984
Ka	Okinoerabu Is., Kagoshima Pref.	Freesia (*Freesia* sp.)	Apr. 1985
Shi	Shimonoe, Tosashimizu, Kochi Pref.	Rakkyo (*Allium chinense*)	June 1985
Nuno	Nuno, Tosashimizu, Kochi Pref.	〃	Aug. 1985
Hata	Hata-gun, Kochi Pref.	〃	Feb. 1984
Fukui	Sakai-gun, Fukui Pref.	〃	Sept. 1984
Chiba	Katori-gun, Chiba Pref.	Chinese chive (*Allium tuberosum*)	Sept. 1984

petri-dish (3 cm diam.) and 0.5 m*l* of insecticide solution was poured on it. Thereafter, 40 females were transferred into each petri-dish and the lid was put on it. These dishes were kept at 25°C in the moist chamber. Mortality was checked at 48 hr after treatment, and LC_{50} values were determined graphically.

2) Cross experiment

The females of mixed populations of Ka and Shi strains, whose sensitivity to OP insecticides is shown in Table 2, were selected further at every two generations with two repetitions of 200 and 400 ppm disulfoton suspension prepared from emulsified concentrates. The resulting strain was the parent of the R strain, which was used without further selection for crossing with the reference S strain. The tritonymphs in dormant stage were removed from culture containers to separate virgin females, and were isolated individually in short and fine glass tubes (3 mm diam.). These glass tubes were sealed with cotton to prevent contamination and kept at 25°C and 90–95% relative humidity for molting to take place. The sex ratio of molted adults was approximately 1 : 1. The freshly molted females were picked out from glass tubes, and approximately 150 females and the similar number of males were placed for mass mating on moist onion powder placed in a container. Mating usually occurred as soon as adults had began to feed, and often recurred. Females began to oviposit one day after mating. Females and males were removed from the container 12 days after crossing, and the resulting females were tested for sensitivity to disulfoton.

Present status of resistance to OP insecticides

LC_{50} for insecticides in the S strain and resistance factors in the field-collected populations of the bulb mite on several crops are shown in Table 2. It is obvious from the data that the field-collected populations except Chiba population showed a similar resistance pattern and a high level of resistance to a wide range of OP insecticides, especially to aromatic phosphorothionate (cyanophos, fenitrothion, fenthion, methyl parathion, dichlofenthion), aliphatic phosphorothiolothionate (malathion, phenthoate, dimethoate, formothion, mecarbam, disulfoton, thiometon), phosphoramidothiolate (acephate), aliphatic derivatives of phosphate (dichlorvos, naled, monocrotophos, chlorfenvinphos, propaphos), phosphonate (trichlorfon) and phosphorothiolate (ESP, vamidothion). However, the resistance ratio to heterocyclic phosphorothionate (pyrimiphos-methyl, chlorpyrifos, pyridaphenthion, salithion), aromatic phosphonothionate (EPN, S-seven, cyanophenphos), phosphorothiolothionate and phosphate forming an ester bond with heterocyclic compound and enol (methidathion, phosmet, azinphos-methyl, dimethylvinphos, tetrachlorvinphos), *S*-propyl phosphorothiolothionate and phosphorothiolate (prothiophos, TIA-230) was less than five times as that of the susceptible strain. These results indicated that the bulb mites have developed lower level of resistance to heterocyclic compounds.

This resistance spectrum is quite different from that of insect pests such as the smaller brown planthopper, *Laodelphax*

Table 2. Response of the susceptible strain (S) to various organophosphorus insecticides and resistance factors in the field-collected strains of the bulb mite

Insecticide	LC_{50} (ppm)	Resistance factor						
	S	Oki	Ka	Shi	Nuno	Hata	Fukui	Chiba
Phosphorothionate								
1 Cyanophos	16		106	122	201	87	60	
2 Fenitrothion	11	156	291	307	417	145	131	2.7
3 Fenthion	13	120	230	298	369	251	184	
4 Methyl-parathion	28	207	250	313	372	266		
5 Dichlofenthion	240	19	32	46	43	27	17	
6 Pyrimiphos-methyl	46		2.2	3.5	4.0	2.6	2.2	1.1
7 Chlorpyrifos-methyl	21	1.8	4.8	5.6	6.9	2.5	2.1	1.0
8 Chlorpyrifos	15		2.8	3.4	3.9	2.3		
9 Diazinon	37	4.3	6.2	15	24	6.1	4.3	1.0
10 Pyridaphenthion	76	1.9	1.4	3.2	4.4	2.6	2.0	
11 Isoxathion	13	7.4	4.6	13	21	6.9	7.4	
12 Salithion	4.6	1.9	2.7	2.9	2.7	2.3	1.7	1.0
Phosphorothiolothionate								
13 Malathion	64	8.0	13	24	20	16	9.5	
14 Phenthoate	28	12	16	24	27	13	11	
15 Dimethoate	7.8	56	314	380	427	64	79	2.2
16 Formothion	8.1	67	198	178	207	91	63	
17 Mecarbam	49		17	23	15	18	11	
18 Disulfoton	1.2	30	65	105	126	42	42	2.1
19 Thiometon	1.7	128	135	370	335	165	211	3.5
20 Methidathion	9.6	1.8	1.3	3.4	2.9	2.7	2.6	
21 Phosmet	18	1.6	1.7	3.4	2.6	1.7	1.4	
22 Azinphos-methyl	26	1.9	2.2	4.9	4.3	2.6		
23 Phosalone	73	5.7	6.6	8.7	8.9	6.6	3.9	1.7
24 Dialifor	28	3.2	3.9	8.5	12	4.9	4.7	
25 Azinphos-ethyl	17	11	7.4	14	19	11	7.7	
26 Prothiophos	9.0	1.8	2.3	3.0	3.6	2.7	1.9	0.9
Phosphonothionate								
27 EPN	7,250		1.8	1.5	1.7	1.2		
28 S-seven	5,710		1.2	2.1	2.3	2.0		
29 Cyanophenphos	1,530	4.7	3.8	5.6	5.2	3.6		
Phosphoramidothiolate								
30 Acephate	57	46	84	91	107	41		
Phosphate								
31 Dichlorvos	28	8.6	16	20	18	8.9	7.1	1.3
32 Naled	36		128	204	177	83	40	
33 Monocrotophos	9.6	89	113	131	142	108		
34 Dimethylvinphos	128	2.0	2.1	3.0	1.6	2.0	1.8	
35 Chlorfenvinphos	47	51	83	116	137	66	34	
36 Tetrachlorvinphos	3,760	1.4	1.9	1.8	2.1	1.6		
37 Propaphos	820	>26	>26	>26	>26	>26	>26	
Phosphonate								
38 Trichlorfon	35	>286	>286	>286	>286	>286	>286	
Phosphorothiolate								
39 ESP	10	27	34	47	55	22	27	1.9
40 Vamidothion	14	43	79	111	163	77	24	2.7
41 TIA-230[a]	2.1	2.5	3.0	2.8	3.0	2.3	2.6	0.9

a) : *O*-[1-(4-chlorophenyl)-4-pyrazolyl] *O*-ethyl *S*-*n*-propyl phosphorothiolate.

striatellus[8], the green rice leafhopper, *Nephotettix cincticeps*[2], the rice stem borer, *Chilo suppressalis*[3] and the housefly, *Musca domestica*[4]. The response of the bulb mite to OP insecticides is thus characterized by limited resistance levels to OP insecticides having heterocyclic configuration in their structure.

Genetics of resistance

The response of the susceptible (S) and resistant (R) strains and their offsprings to disulfoton is shown in Fig. 1. The F_1 female progeny from the reciprocal crosses did not differ each other significantly in resistance to disulfoton, indicating that sex-linked or cytoplasmic inheritance was not involved. Their regression line is considerably reduced but there remains resistance. The resistance, therefore, is determined by semidominant factors, and the resistance gene is incompletely dominant over its normal allele.

Backcrossing the F_1 resistant hybrids to susceptible males produced the F_2 female progeny which segregated clearly into a 1 : 1 ratio of susceptible and resistant phenotypes, i.e., an average of 50% mortality was produced over a large range of concentrations in the intermediate range. The observed response and the expected backcross response were statistically examined by χ-square tests, and these figures yielded a goodness of fit. These results provided the presence of the major gene for resistance, and the degree of dominance estimated on the basis of Stone's formula[9] was 0.557.

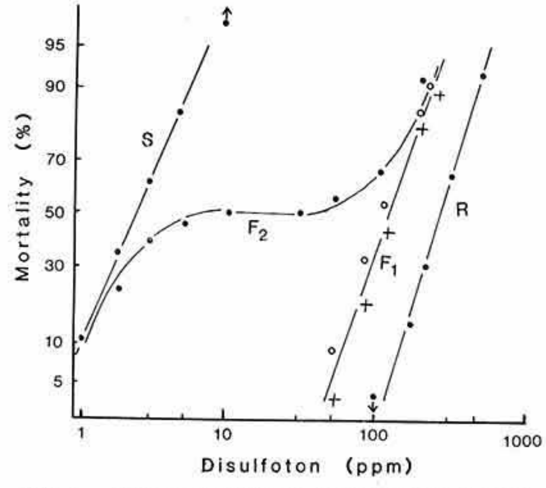

Fig. 1. Dosage-mortality curves to disulfoton in F_1 of S ♀×R ♂ (○), R ♀×S ♂ (+) and F_2 of F_1 ♀ (S ♀×R ♂)×S ♂

These results for F_1 and backcross thus support that disulfoton resistance in this strain is due to a single, incompletely dominant, autosomal gene.

Discussion

In this experiment it is revealed that resistance of the bulb mite to OP insecticides is commonly found in various mite populations on several crops in Japan. It is reasonable to guess that most of the populations have developed resistance to OP insecticides including disulfoton and dimethoate which have been mainly used for over 20 years against the bulb mite in Japan, and that application of disulfoton or dimethoate is no longer a useful tool to control the bulb mite. The further use of these insecticides would result in full resistance.

These populations also showed a similar resistance pattern to various OP insecticides. These results indicate that there is a cross resistance to OP insecticides, and suggest that major resistance factors are involved in OP resistance in the bulb mite. The populations, however, have developed a lower level of resistance to OP insecticides having heterocyclic configuration in their structure, and that heterocyclic nature of OP insecticides should be concerned with a lower level of resistance in the bulb mite. These insecticides should be considered as candidates for a practical alternative against bulb mites which have developed resistance to disulfoton or dimethoate.

Dosage-mortality test to determine the mode of inheritance of disulfoton resistance provided the evidence of monofactorial inheritance with high degree of dominance value (D=0.557). It is well known that a rare dominant gene is selected much more rapidly than a rare recessive gene. According to the computor analysis, development of resistance will be faster to evolve when the resistance is determined by the dominant or incompletely dominant factor than when determined by the recessive factor[1,11]. Dominance of the resistance gene in the bulb mite would thus favor more rapid selection, and the degree of resistance conferred by a resistance gene would determine the gene frequency at which control failures are experienced in field.

Resistance to disulfoton and to dimethoate is fairly stable; resistance strains maintained their resistance to these insecticides without any selection during 3 and a half years in laboratory culture, corresponding to more than 70 generations[7]. It indicates that the resistance is clearly the stable type. Although biological comparisons between the resistant and susceptible strains have been meagre, the persistence of OP-resistant phenotypes in laboratory culture under relaxation of the selection pressure indicates that any biotic disadvantage related to the resistance to these insecticides must be minor. Thus, dominant factors in the resistance gene and biological stability of resistant phenotypes should be concerned with faster rates of build-up and slower rates of break-down of the resistance in the bulb mite.

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