GENETIC ANALYSIS OF DODINE RESISTANCE IN *NECTRIA HAEMATOCOCCA* (SYN. *HYPOMYCES SOLANI*)*

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THERE has been a small amount of published information on the genetics of the heterothallic ascomycete causing root and fruit rot of cucurbits. In these publications the fungus has been referred to as *Hypomyces solani* f. *cucurbitae*. However, according to DINGLEY (1961), this organism belong to the species *Nectria haematococca* and should be called *N. haematococca* var. *cucurbitae*. Genetic knowledge available includes inheritance of mating type, of male and female sterility, of perithecial and ascospore color, and of resistance to some aromatic hydrocarbon fungitoxicants (HANSEN and SNYDER 1946; EL-ANI 1954a; GEORGOPOULOS 1963b).

In an earlier communication (KAPPAS and GEORGOPOULOS 1968) we reported on the genetic basis of resistance to n-dodecylguanidine acetate in the cucurbit pathogen. This compound, commonly known as dodine, cyprex, or melprex, has been in use for many years as a successful agricultural fungicide. Its mechanism of action has been studied mainly by BROWN and SISLER (1960), SOMERS and PRING (1966), and BARTZ and MITCHELL (1970a, 1970b). Recently, resistance to dodine has become a matter of much practical concern in New York State (SZKOLNIK and GILPATRICK 1969) where the fungicide is no longer effective against apple scab (*Venturia inaequalis*). Details on the genetic control of dodine resistance in *N. haematococca* var. *cucurbitae* will be presented in this paper.

MATERIALS AND METHODS

Strains No. 10 and 14 of race 1 (TOUSSON and SNYDER 1961) were the wild-type strains from which dodine-resistant mutants were obtained following UV and γ radiation treatments (KAPPAS and GEORGOPOULOS 1968). Stock cultures were maintained on potato dextrose agar (PDA) slants at 10°C and transferred every month.

Testing dodine resistance was most safely done on plates of PDA to which the proper amount of an ethanol solution of dodine had been added to give dodine concentrations differing by 0.5 μg/ml increments. Toxicity depends largely on the amount of fungus per unit dodine (KAPPAS and GEORGOPOULOS 1968) and it is therefore necessary to use a standard inoculum. In this work point inoculations on the surface of the poisoned agar were made with a single germinated conidium (Fusarium imperfect stage) or ascospore. Under our conditions, the highest concentration on which wild-type spores were able to produce colonies was 0.5 μg/ml of medium.

Techniques for crossing compatible strains, progeny analysis, and linkage detection have been described (GEORGOPOULOS 1963a, 1963b).

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TABLE 1

Random ascospore analyses of crosses of dodine†-resistant strains of Nectria haematococca *to compatible wild types*

Cross	Ascospores tested	Resistant	Sensitive	Chi-square (1:1)
$RD_4 \times 5$	28	13	15	0.14
$RD_6 \times 10$	30	17	13	0.53
$RD_9 \times 10$	32	15	17	0.12
$5 \times RD_{10}$	31	15	16	0.03
$10 \times RD_{12}$	30	16	14	0.13
$10 \times RD_{14}$	29	15	14	0.03
$RD_{15} \times 10$	29	15	14	0.03
$9 \times RD_{19}$	27	12	15	0.33
$RD_{22} \times 10$	26	14	12	0.15
$10 \times RD_{23}$	31	14	17	0.29
$9 \times RD_{26}$	28	13	15	0.14
$RD_{27} \times 10$	28	12	16	0.57

† Dodine concentration 1 μg/ml.

RESULTS

Strains obtained on the dodine-containing medium were transferred to PDA slants and were given isolation numbers in a series that was characterized by RD. Twelve of these dodine-resistant strains were selected and crossed to compatible wild-type strains (characterized as 5, 9, and 10 in Table 1). Random ascospore analysis for dodine resistance *vs.* sensitivity gave a segregation of 1:1 independent of whether the resistant strain was used as the protoperithecial or the spermatizing parent. Therefore, the dodine-resistant phenotype was the result of mutation of a single Mendelian factor in each case. In subsequent tests mutants were crossed among themselves for allelism. Two mutants were considered to be

TABLE 2

Loci, strains of origin, and level of resistance of dodine-resistant mutants

Isolation number	Locus	Strain of origin	Maximal dodine concentration tolerated (μg/ml)
RD_4	*dod-1*	10	2.5
RD_6	*dod-2*	14	1.5
RD_9	*dod-2*	14	1.5
RD_{10}	*dod-1*	10	2.5
RD_{12}	*dod-1*	14	2.0
RD_{14}	*dod-4*	14	6.5
RD_{15}	*dod-3*	14	5.5
RD_{19}	*dod-1*	14	2.5
RD_{22}	*dod-2*	14	1.5
RD_{23}	*dod-2*	14	2.0
RD_{26}	*dod-1*	14	2.5
RD_{27}	*dod-1*	14	2.5

allelic if no wild type (sensitive) was observed in a small (usually around 40) population of ascospores from the mutant × mutant cross. In this way the twelve dodine-resistant mutants were assigned to four chromosome loci: *dod-1*, *dod-2*, *dod-3*, and *dod-4*. Each resistance gene corresponded to a different level of resistance to the fungicide (Table 2), the differences being distinct and reproducible. Strain RD_{12} has been found to carry a modifier which reduces the resistance of *dod-1*. Another modifier which increases the resistance of *dod-2* is present in strain RD_{23}. Testing the resistant segregants from crosses of either of these strains to wild type on varying dodine concentrations showed two resistance classes in a 1:1 ratio. Half of the resistant ascospores possessed the resistance specified by the respective resistance gene and half a lower or a higher resistance level depending on the modifier present.

When the progeny of crosses involving two loci for dodine resistance were tested on 1 μg dodine per ml, the ratio of resistant to sensitive ascospores did not significantly differ from 3:1, indicating that the four genes segregate independently (Table 3). However, of the resistant progeny from such crosses, one-third showed the resistance level of the one parent, one-third that of the other parent, and the remaining third appeared to possess approximately the sum of the two resistances. Table 4 shows analyses of two unordered tetratype tetrads from the cross *dod-2* × *dod-1*. In each ascus two ascospores could not grow on medium containing 1.0 μg dodine per ml, thus exhibiting the sensitivity of the wild-type strains originally used. The corresponding concentration for the remaining three pairs of ascospores was 1.5, 2.5, and 4.0 μg/ml, respectively. Table 5 gives random spore analyses for *dod-1* × *dod-3* and a ratio of approximately 3 resistant to 1 sensitive at the concentration tolerated by both parents, 1 resistant to 1 sensitive at the concentration tolerated only by the one parent, and 1 resistant to 3 sensitive at a concentration corresponding to the sum of the two. This is exactly what would be expected if the phenotypic effects of the dodine resistance genes were additive. Appropriate crosses have shown that segregants obtained from a cross carry the resistance gene indicated by their resistance level; the highest degree of resistance in each case is exhibited by the double-resistant recombinant ascospores. For example, when recombinant no. 1 of ascus I of Table 4 was crossed to

TABLE 3

Analysis of random progeny of dihybrid crosses between dodine†-resistant strains

Cross	Ascospores tested	Resistant	Sensitive	Chi-square (3:1)
dod-1 × *dod-2*	84	64	20	0.06
dod-1 × *dod-3*	68	52	16	0.07
dod-1 × *dod-4*	33	25	8	0.01
dod-2 × *dod-3*	28	21	7	0.00
dod-2 × *dod-4*	34	26	8	0.03
dod-3 × *dod-4*	25	19	6	0.01

† Dodine concentration 1 μg/ml.

TABLE 4

Response of two unordered tetratype tetrads from the cross* dod-1 × dod-2 *to different concentrations of dodine*

Ascus no.	Ascospore no.	Dodine concentration (μg/ml) 0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
I	1	+	+	+	+	+	+	+	+	+	—
	2	+	+	+	+	+	+	—	—	—	—
	3	+	+	—	—	—	—	—	—	—	—
	4	+	+	+	+	—	—	—	—	—	—
	5	+	+	—	—	—	—	—	—	—	—
	6	+	+	+	+	—	—	—	—	—	—
	7	+	+	+	+	+	+	+	+	+	—
	8	+	+	+	+	+	+	—	—	—	—
II	1	+	+	—	—	—	—	—	—	—	—
	2	+	+	—	—	—	—	—	—	—	—
	3	+	+	+	+	+	+	—	—	—	—
	4	+	+	+	+	+	+	+	+	+	—
	5	+	+	+	+	—	—	—	—	—	—
	6	+	+	+	+	—	—	—	—	—	—
	7	+	+	+	+	+	+	+	+	+	—
	8	+	+	+	+	+	+	—	—	—	—

* + = ability; — = inability of single spores to form colonies on medium containing the dodine concentration indicated.

wild type and 24 random ascospores were transferred on 1 μg dodine per ml, a segregation of 17 resistant to 7 sensitive was obtained, indicating the presence of two resistance genes in the resistant parent.

Linkage relationships between each of the four dodine-resistance loci and the loci for mating type (*A*), male sterility (*m*), and perithecial color (*w*), were sought; the results are summarized in Table 6. In this way *dod-1* and *dod-2* have been assigned to the first linkage group and *dod-4* to the third linkage group (GEORGOPOULOS 1963a). No linkage involving *dod-3* and either of the two modifiers has yet been detected.

DISCUSSION

A multigenic system of resistance to n-dodecylguanidine acetate in *N. haematococca* var. *cucurbitae* has been recognized which involves at least four, but

TABLE 5

Response of 38 random ascospore isolates from the cross dod-1 × dod-3 *to different concentrations of dodine*

Dodine concentration (μg/ml)	Number of ascospore isolates Resistant	Sensitive
2.5	31	7
5.5	20	18
8.0	9	29

TABLE 6

Summary of random spore analyses from crosses involving two loci

Loci	Number of ascospores tested			Percent recombination
	Total	Parental	Recombinant	
A and *dod-1*	109	83	26	23.85
A and *dod-2*	88	58	30	34.09
A and *dod-3*	25	12	13	52.00
A and *dod-4*	31	15	16	51.61
m and *dod-1*	27	14	13	48.14
m and *dod-2*	28	14	14	50.00
m and *dod-3*	31	16	15	48.38
m and *dod-4*	29	14	15	51.72
w and *dod-1*	27	13	14	51.85
w and *dod-2*	21	11	10	47.61
w and *dod-3*	20	10	10	50.00
w and *dod-4*	36	29	7	19.44

probably numerous, resistance genes and at least two modifiers. In the same fungus, resistance to aromatic hydrocarbon fungitoxicants (GEORGOPOULOS and PANOPOULOS 1966) is also controlled by many loci (*cnb* loci). However, the *cnb* genes do not show the positive interaction in recombinants which was observed in the case of dodine (Tables 4 and 5) resistance. This interaction is reminiscent of the multigenic systems for resistance to cycloheximide in Saccharomyces (WILKIE and LEE 1965) and Neurospora (HSU 1963). It is logical that whether or not introducing more than one resistance gene will increase the resistance level does not depend on the organism studied but rather on the kind of toxicant and on the mechanism(s) by which resistance can develop. A possible way in which the positive interaction between resistance genes could be explained has been proposed by SISLER and SIEGEL (1967).

The recombination studies included in this paper (Table 6) show that the loci *dod-1* and *dod-2* are linked to the mating-type locus (*A*). Because there is no linkage between *dod-1* and *dod-2* (Table 3), *A* must be located between the two dodine resistance loci at a distance of approximately 24 and 34 units, respectively. Considering that *A* is about 21 units from its centromere (GEORGOPOULOS 1963), one has to accept that either *dod-1* or *dod-2* must also show centromere linkage. The only other definite linkage recognized in this study is that of *dod-4* and the *w* locus. The *w* locus determines the color of perithecia and is closely linked to a locus for perithecial sterility, approximately 27 units from its centromere (GEORGOPOULOS 1963). Whether *dod-4* is on the centromere side of *w* has not been determined. On the basis of cytological observations EL-ANI (1954b) accepts n = 4, but HOWSON, MCGINNIS and GORDON (1963) use 5 as the haploid chromosome number in this fungus. The knowledge of linkage groups is still too incomplete to help resolve this controversy.

No extensive studies on the physiological basis of dodine resistance in *N. haematococca* var. *cucurbitae* have been conducted. BARTZ and MITCHEL (1970a, 1970b) found that when conidia of *F. solani* f. *phaseoli*, a closely related fungus, are incubated in a solution of ^{14}C-dodine, the amount of radioactivity bound by

the fungus increases to a maximum and then drops due to the release of a less toxic labeled compound. We have used ^{14}C-dodine in similar experiments and have observed a similar drop of the radioactivity of the conidia in wild type and a *dod-3* mutant. The drop was somewhat sharper in the case of the mutant and it is possible that resistance may be due to an increase in the detoxifying ability.

SUMMARY

Four unlinked loci for resistance to n-dodecylguanidine acetate (dodine) have been recognized in *N. haematococca* var. *cucurbitae*. The resistance varies according to the mutant gene present and, in recombinants, the genes interact to give higher resistance levels. Three of the dodine resistance genes have been assigned to linkage groups. Analyses have also shown the presence of modifiers.

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