Genetic Control of Dodine Tolerance in Venturia inaequalis

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ABSTRACT

Isolates of *Venturia inaequalis* from apple orchards in which dodine (*n*-dodecylguanidine acetate) had given poor scab control grew on artificial media containing two to four times the dodine concentration that isolates with no previous exposure to dodine would tolerate. Segregation of dodine tolerance in ascospore progenies of tolerant and nontolerant crosses demonstrated that tolerance is genetically controlled. The segregation ratios indicate that at least two genes are responsible for this tolerance. The poor fit of one cross to the ratios suggests that in some isolates additional factors are operating.

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Since its introduction in 1959, the fungicide, dodine (n-dodecylguanidine acetate), has been used extensively in New York State for control of apple scab [*Venturia inaequalis* (Cke.) Wint.], the most economically important disease of apple. In recent years, failure to control apple scab with dodine has been noted by many growers in western New York. In 1969, Szkolnik & Gilpatrick (9) attributed failure of dodine to the development of resistance to dodine by the fungus after approximately ten years' exposure to the compound.

There have been few reports of tolerance of fungi to fungicides and even fewer reports demonstrating genetic control (1). Leben et al. (4) used ultraviolet irradiation to induce antimycin resistance in *V. inaequalis*, and demonstrated that this resistance was controlled by a single gene. Parry & Wood (6, 7) attempted to "adapt" *V. inaequalis* to a variety of fungicides, but in no case were they successful. Ultraviolet or gamma irradiation was used by Kappas & Georgopoulos (2) to induce mutants of *Nectria haematococca* var. *cucurbitae* with resistance to dodine. They reported the presence of four unlinked loci for tolerance to dodine in this fungus (3).

Observations of fungicide tolerance occurring in nature are even rarer. However, this may be because low levels of tolerance are overlooked in the field, rather than because they are absent. The studies reported in this paper deal with naturally occurring dodine tolerance in *V. inaequalis*. The objectives were to verify that control of dodine tolerance is genetic and to determine the genetics.

MATERIALS AND METHODS.—*V. inaequalis* isolates were collected from orchards where apple scab control with dodine had failed. Isolates were also collected from orchards which were abandoned or where dodine had not been used for several years or from roadside trees. Single spore isolations were made from diseased leaves, and were cultured on quarter-strength potato-dextrose agar (Difco).

Response of isolates to dodine was determined on quarter-strength potato-dextrose agar which had been autoclaved and amended with appropriate amounts of dodine in ethanol. Approximately 200 conidia from each single spore isolate were pipetted onto the agar in each of two plates. The plates were examined for growth after 10 days. Each determination was replicated three times.

For the genetic studies, isolates exhibiting tolerance to dodine (growth on dodine at 0.25 or 0.5 μg/ml) were crossed with isolates which exhibited no tolerance (no growth at 0.25 μg/ml). The mating types were determined so that in further studies specific crosses could be made with assurance of compatible matings. The crosses were prepared by a modification of Ross & Hamlin's (8) leaf-disk method in which leaf disks cut from unsprayed mature 'McIntosh' leaves were placed on the surface of 1.7% water agar in deep petri dishes. The petri dishes were then autoclaved for 30 min at 1.05 kg-force/cm^2 (15 psi). Isolates to be mated were macerated together in distilled water in a test tube and pipetted onto the surface of the agar containing the leaf disks. The mixed isolates were incubated at 21 C for 2 weeks under intermittent fluorescent lighting, then at 8 C for 14 to 16 weeks in the dark. Single ascospores were isolated and cultured. Conidia from the single ascospore cultures were used to determine the response to dodine, as described previously.

RESULTS.—Five collections from orchards where dodine had given poor control and eight collections from abandoned orchards, orchards where dodine had not been sprayed for several years, and roadside trees were examined for sensitivity to dodine (Table 1). Most of the isolates from orchards where dodine had

TABLE 1. Growth response of *Venturia inaequalis* isolates from orchards where dodine gave poor apple scab control and from areas where dodine had not been sprayed

Source	Number of collections	Number of isolates examined	Number of isolates which grew on dodine (μg/ml)			
			0	0.25	0.50	1.0
A[a]	5	32	32	30	28	1
B[b]	8	40	40	1	1	0

[a] Orchards where control with dodine was poor.

[b] Orchards where dodine had not been used recently, abandoned orchards, and roadside trees.

TABLE 2. Segregation ratios of growth response of *Venturia inaequalis* progenies to dodine at 0.25 μg/ml and 0.50 μg/ml

	Dodine 0.25 μg/ml		Dodine 0.50 μg/ml	
		X^2 Values		X^2 Values
Cross	Reaction	1:1 segregation	Reaction	1:3 segregation
1	G[a] = 12	0.33[c]	G = 4	1.49[d]
	NG[b] = 15		NG = 23	
2	G = 46	0.76	G = 22	0.06
	NG = 38		NG = 62	
3	G = 21	0.75	G = 10	0.44
	NG = 27		NG = 38	
4	G = 7	0.33	G = 4	0.44
	NG = 5		NG = 8	
Total	G = 86	0.01	G = 40	0.24
	NG = 85		NG = 131	
5	G = 23	9.39	G = 6	10.67
	NG = 29		NG = 66	

[a] Growth in culture after 10 days.
[b] Absence of growth in culture after 10 days.
[c] Expected value at the 5% level of significance is 3.84.
[d] Expected value at the 5% level of significance is 7.84.

failed grew on dodine at 0.25 and 0.5 μg/ml. Only one isolate, of the 40 examined from trees with no recent exposure to dodine, grew at 0.25 or 0.5 μg/ml.

The tolerant parent isolates used in the crosses grew on dodine medium at 0.5 μg/ml, whereas the nontolerant isolates did not grow at 0.25 μg/ml, the lowest level tested. A total of 243 ascospore lines were isolated from the five progenies examined.

Four of the five progenies reacted similarly to dodine, but the fifth was distinct (Table 2). A 1:1 segregation was closely approximated for four progenies on dodine at 0.25 μg/ml. The segregation of these progenies on dodine at 0.50 μg/ml closely approximated 1:3. The reactions of the progeny of the fifth cross yielded X^2 values that are too high for 1:1 segregation at 0.25 μg/ml or 1:3 segregation at 0.50 μg/ml.

DISCUSSION.—The initial screening of the *V. inaequalis* isolates from various sources demonstrated that differences in response to dodine existed. With one exception, all isolates from trees with no recent exposure to dodine failed to grow on the lowest level of dodine tested, 0.25 μg/ml. The isolate which did grow on dodine at 0.25 and 0.50 μg/ml was from an abandoned orchard. It is possible that the isolate was naturally occurring (5), or may have originated in an orchard where dodine had been used previously. The nearest commercial orchard, however, was at least five miles away. Isolates from orchards where control with dodine had failed grew well at 0.25 μg/ml and most of these grew at 0.5 μg/ml. These differences in dodine response remain distinct after prolonged culturing on artificial media.

In four out of five crosses of tolerant and nontolerant isolates, progenies showed good 1:1 segregation (86 tolerant: 85 nontolerant) on dodine at 0.25 μg/ml. This indicates the presence of a single gene for tolerance at this level of dodine. The X^2 values for each individual cross are within acceptable limits as demonstrated in Table 2. Segregation of the progenies at 0.5 μg/ml level of dodine indicates that a second gene is operating, as demonstrated by the good X^2 fit to a 1:3 segregation (Table 2).

The simplest explanation for these data is that two independent genes for dodine tolerance are segregating, as shown in Table 3. The first gene allows growth at the 0.25 μg/ml level of dodine. The second gene is effective only when the first gene is in dominant form, when it allows growth at 0.5 μg/ml dodine.

Although the data from the fifth cross is at variance with the majority of this work, it is presented because of the large size of the sample. The X^2 values for 1:1 segregation at 0.25 μg/ml and 1:3 segregation at 0.5 μg/ml are not within the acceptable limits. This suggests that additional factors are involved in the control of dodine tolerance. A third gene might provide these ratios, but factors such as cytoplasmic control, modifier genes, or several minor genes could be operative as well.

To define further the genes controlling dodine tolerance, selected backcrosses have been made and are being examined. Crosses of tolerant lines to known color mutants have been made to aid in identifying specific loci which control tolerance to dodine.

TABLE 3. Proposed genetic control of dodine tolerance in *Venturia inaequalis*

	Dodine concentration	
Genes	0.25 μg/ml	0.50 μg/ml
AB	Growth	Growth
Ab	Growth	No growth
aB	No growth	No growth
ab	No growth	No growth

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