Ascospore Tolerance to Dodine in Relation to Orchard Control of Apple Scab

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ABSTRACT

Ascospores of *Venturia inaequalis* from orchards with varying histories of fungicide use and performance were tested for sensitivity to dodine in spore germination tests on glass slides. LD_{50}'s for inhibition of germination were 0.3 - 0.65 μg/ml of dodine for spores obtained from an experimental orchard in which dodine controlled apple scab satisfactorily. LD_{50}'s were 0.75 - 1.25 μg/ml for spores from an orchard where dodine gave inadequate scab control in 1967-69. In each test, ascospores from the dodine-tolerant source were 2-3 times less sensitive to dodine than those from the nontolerant source. Ascospores from other orchards where dodine failed to control scab in 1969 were also found to be dodine-tolerant, but spores from wild trees or abandoned orchards with no previous dodine exposure were not tolerant to dodine. Spores from orchards with less exposure to dodine than tolerant sources were semitolerant. These studies support the hypothesis that failure of dodine to control apple scab in many orchards of western New York in 1969 was due to a developed tolerance of *V. inaequalis* to this fungicide, and that this is related to the intensive and almost exclusive use of dodine in problem orchards in the previous decade.

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Szkolnik and Gilpatrick (7) reported that despite excellent control of apple scab [*Venturia inaequalis* (Cke.) Wint.] during the previous decade, the fungicide dodine (*n*-dodecylguanidine acetate) failed to control this disease in many orchards in the Lake Ontario fruit belt of New York State in 1969. These authors tentatively concluded that this event was caused by the development of lines of *V. inaequalis* with tolerance to dodine. They observed failures in orchards with a history of intensive and almost exclusive use of dodine for scab control for approximately 10 yr.

Ascospores of the fungus produced in perithecia in over-wintering infected leaves are the primary source of inoculum for this disease in New York. The dodine failures in 1969 reflected to a large degree the inability of this fungicide to prevent primary infections by ascospores. The germination studies reported herein measured the sensitivity to dodine of ascospores of this fungus collected at different locations in New York State from orchards with varying histories of dodine use and performance. The objective was to verify that dodine tolerance occurs in *V. inaequalis* in certain orchards of New York and that this accounts for the unsatisfactory control of apple scab with this fungicide in 1969. A preliminary report of this study has been published (2).

MATERIALS AND METHODS.—Overwintered, scab-infected apple leaves were collected from the ground of orchards in mid-May of 1970 during peak production of mature ascospores of *V. inaequalis*. These leaves were stored dry in plastic bags at 1 C and processed within 3 mo of collection. The techniques for obtaining ascospores from these leaves and conducting spore germination studies have been described (3).

Moistened leaves were placed in a wind-tower and the ejected ascospores allowed to impinge at selected sites on glass slides. The number of spores per site was regulated to about one hundred per low-power field of the microscope at ×100 magnification. One drop (12.5 μl) of freshly prepared dodine in water was placed at each spore site. The slides were then incubated at 100% relative humidity and 16.5 C. After 24 h, spore germination counts were made in four drops of each dosage for each ascospore source. Dosage-response curves were plotted on log-probability paper (Keuffel & Esser Co., No. 468049) and LD_{50}'s in μg/ml determined. Two standard sources of the fungus were used in all tests: NT which is

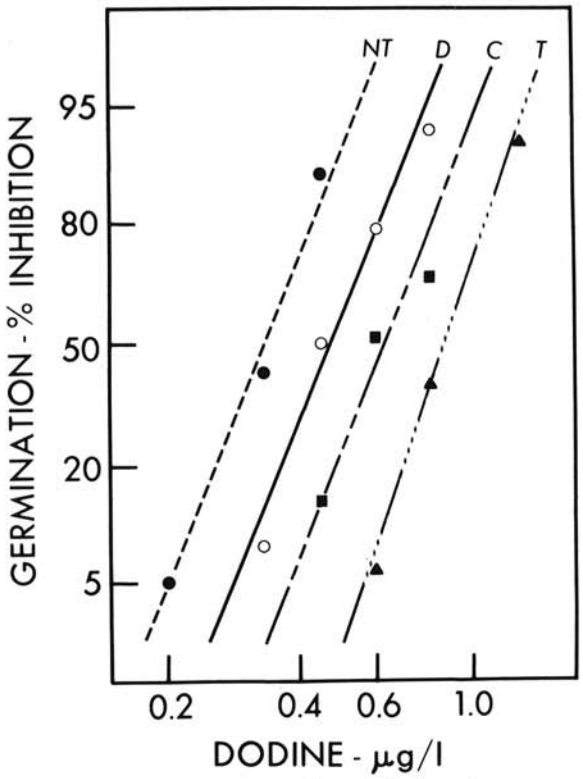

Fig. 1. Dosage-response curves for germination of ascospores of different collections of *Venturia inaequalis* in the presence of dodine on test date 8/7. NT is nontolerant to dodine; D, semitolerant; C and T, tolerant.

rated nontolerant of dodine by the authors and T which is rated tolerant. The dodine was technical grade of 98% purity obtained from the American Cyanamid Co., Princeton, New Jersey.

RESULTS.—Only a small portion of the total data collected in these studies is reported herein. It was difficult to obtain sufficient points for the preparation of dosage-response curves for some collections. For example, dosage levels providing less than 5% or more than 95% mortality were not included to avoid skewing of the normal distribution. In comparing the sensitivities of different collections such values, however, often were significant. The unreported data usually verified the conclusions drawn.

In these studies, ascospores of *V. inaequalis* were inhibited from germinating in the range of 0.2-2 µg/ml. Differences in sensitivity to dodine at this level are difficult to demonstrate in vitro because of the physical properties of dodine such as its tendency to sorb on glass and the slow growth of *V. inaequalis* on artificial media. Our technique (3), however, allowed us to develop a sufficient number of reproducible dosage-response curves so that comparative LD_{50}'s for inhibition of ascospore germination by dodine could be determined.

Our method is especially valid because it utilizes the ascospore which is the primary inoculum source for apple scab. Since an in vitro culture period between collection and testing was not required, the ascospores were assumed to be in a relatively normal physiological state at the time of the evaluation. This tended to simulate field conditions, even though actual spore germination occurred on a glass slide.

For ascospores collected from orchard NT where dodine controls scab, LD_{50}'s for inhibition of germination by dodine ranged from 0.3-0.65 µg/ml in seven tests (Table 1). For spores collected from orchard T where dodine has failed to control scab, LD_{50}'s ranged from 0.75-1.25 µg/ml. In each test, ascospores of the T source tolerated about 2-3 times more dodine than those from NT. Over a 14-yr period prior to 1969 dodine always gave excellent control of apple scab in orchard NT but failed to control this disease in orchard T in 1967-1969 (10). Thus the fungus collected from orchard NT was arbitrarily rated by the authors as nontolerant of dodine, whereas that from T was rated tolerant. Dosage-response curves illustrating the difference in sensitivity of the two sources on test date 8/7 are shown in Fig. 1.

Ascospores from orchards C, F, G, H, I, and J, where dodine failed to control scab satisfactorily in 1969, were also less sensitive to dodine than nontolerant source NT (Table 1 - test dates 7/30, 7/31, 8/5, 8/6, 8/7). All were classified as tolerant. Ascospores from C were more sensitive to dodine than those from the standard tolerant source T (Fig. 1); but because dodine has failed in C since 1969 (10), the fungus from this orchard is considered to be tolerant.

LD_{50}'s for ascospores from a wild source W and an abandoned orchard L, both of which had never been previously exposed to dodine, were identical to the LD_{50}'s for the ascospores of a nontolerant source NT (Table 1-test date 8/12). On any given date, ascospores from orchards D, E, and K which had had 3-5 yr dodine usage prior to 1969, were intermediate in sensitivity to dodine between those from sources NT and T (Table 1-test dates 8/5, 8/6, 8/7, 8/12, and Fig. 1). They were arbitrarily categorized semitolerant to dodine.

Difficulty was encountered in differentiating between the semitolerant collections D and E, and the tolerant collection C (Table 1-test date 8/5, 8/6, 8/7). Tolerance had not reached as high a level in C as in orchards such as T (Fig. 1). Although sources D, E, and K have developed some tolerance to dodine, that fungicide still controls scab satisfactorily in these orchards. In orchard C, tolerance had reached a sufficiently high level that dodine failed in practice (7, 10).

Although in the case of collections NT and T the dodine susceptibility of each population of ascospores varied from test to test, the position of the dosage-response curves of both collections shifted equally and their slopes remained constant. Thus their relative sensitivities to dodine also remained constant. The reason for this fluctuation of position of the curve is not known but the phenomenon has been reported in other dosage-response studies (1).

DISCUSSION.—These and other studies confirm the tentative conclusions of Szkolnik and Gilpatrick (7) that the failure of dodine to control scab in many orchards of western New York in 1969 was due to the tolerance of V. *inaequalis* to this fungicide. These authors (8, 9) found that conidia of the scab fungus from an orchard where dodine failed in 1967-69 were less sensitive to dodine than

TABLE 1. Sensitivity of isolates of *Venturia inaequalis* with different histories of exposure to dodine as determined by ascospore germination

Orchard		Previous dodine exposure (yr)[b]	Dodine efficacy 1969	LD_{50} - g dodine/ml for test data						
Source	Type[a]			7/30	7/31	8/5	8/6	8/7	8/12	8/20
NT	Exp.	14	S	0.65	0.45	0.3	0.3	0.35	0.6	0.6
T	Com.	10	U	1.25	0.75	0.85	0.95	0.9	1.0	1.1
C	Com.-Exp.	9	U	--	--	0.6	0.65	0.7	--	--
D	Com.	5	S	--	--	0.4	0.55	0.45	--	--
E	Com.	5	S	--	--	0.6	--	0.45	--	--
F	Com.	10	U	0.8	--	--	--	--	--	--
G	Com.	10	U	1.2	--	--	--	--	--	--
H	Com.	10	U	1.15	--	--	--	--	--	--
I	Com.	10	U	--	1.15	--	--	--	--	--
J	Com.	10	U	--	0.8	--	--	--	--	--
K	Com.	3	S	--	--	--	--	--	0.8	--
L	Ab	0	--	--	--	--	--	--	0.6	--
W	WT	0	--	--	--	--	--	--	0.6	--

[a]Exp. = experimental; Com. = commercial; Ab = abandoned; WT = wild trees.
[b]Years dodine used prior to 1969.
[c]S = orchard control of scab satisfactory; U = control unsatisfactory (dodine failure).

those from an orchard where dodine is still effective. MacNeill and Schooley (5) found lines of this fungus tolerant to dodine by a factor of 2 to 3 when conidia were seeded on agar containing this chemical. The genetic studies of Polach (6), and Yoder and Klos (11), demonstrated that certain lines of *V. inaequalis* exhibited this same level of tolerance to dodine and that this characteristic is heritable. Kappas and Georgopoulos (4) observed similar tolerance to dodine in *Nectria* haematococca.

The tolerance theory is logical, not only from the facts of in vitro and greenhouse evaluations, but also from a study of the history of dodine usage and performance in orchards of western New York, particularly in Wayne County. Here, apple culture is intensive and dodine became the preferred fungicide of many growers soon after its introduction in 1959 because of its low cost and efficiency of control. Apples in Wayne County are marketed primarily for processing, which requires less-rigid scab control than does fruit for fresh market. Wayne County growers during 1959-68 (especially in certain dry years of that period) reduced the dosage and number of applications of dodine recommended for near-complete scab control; consequently, moderate levels of scab often occurred on foliage even though scab on the fruit was at commercially acceptable levels. By 1969, which was a year highly favorable for apple scab, the fungus in many orchards of Wayne County had been exposed to approximately 80 applications of dodine involving at least nine sexual and more than 100 asexual generations. During 1959-1968 there was ample opportunity for the selection, survival, and dissemination of tolerant members of the scab fungus population in this area. To counteract the 1969 apple scab epidemic in Wayne County the growers returned to recommended practice for control with dodine, but found this to be inadequate to offset the tolerance in the scab fungus that had developed in the previous decade.

All of the tolerant sources evaluated in these studies came from orchards in Wayne County where growers had used dodine almost exclusively for the control of scab from 1959-1968 (10). Other fungicides were not used because scab is the only major disease. The one exception which we studied is orchard C. One half was sprayed with dodine each year between 1960 and 1968, the remainder with other scab fungicides. This may explain why isolates of *V. inaequalis* from this orchard are not as tolerant of dodine as those from other orchards of Wayne County.

The semitolerant and nontolerant collections came from areas outside Wayne County and had not been exposed to intensive dodine usage. The observations with sources NT and D are especially significant. Even though dodine was applied to 10-25% of the trees of orchard NT from 1955 to 1969, tolerance has not developed there (10). The remaining trees were sprayed each year with unrelated fungicides. Lines of *V. inaequalis* tolerant to dodine arising in one year were likely exposed to other fungicides in the following years and thus were eliminated before increasing extensively.

Such also appears to be the case in the Hudson Valley of eastern New York where apples are grown primarily for the fresh fruit market which requires more critical disease control than in the western area of the state where processing is more common. Here, several other scab fungicides are used in the program each year to control rusts and fruit rots. Using this practice, growers of the Hudson Valley still obtain satisfactory scab control with dodine. Some level of tolerance may exist in that area, however. Collection D, which had a low level of tolerance in our laboratory studies, came from an orchard in the Hudson Valley which had received dodine in a mixed fungicide program in the previous 5-yr period. However, scab control with dodine in this orchard is still excellent after 9 yr of use. These observations suggest that a program of efficient scab control using dodine in conjunction with other fungicides tends to suppress the

development of practical tolerance of the apple scab fungus to dodine.

Theories for mechanisms by which tolerant lines of *V. inaequalis* arose in western New York may be based on selection, adaptation, or genetic change. It is difficult to draw conclusions from field observations. However, MacNeill and Schooley (5) found that a few conidia of a large population of *V. inaequalis* were tolerant in vitro to dodine upon first exposure to a normally lethal dosage. Among these were lines which retained tolerance as a stable characteristic in repeated subculturing.

In nature, the sensitivity to dodine of individuals of any large population of *V. inaequalis* should follow a normal distribution. Under repeated exposure to dodine in western New York the mean sensitivity of populations in some orchards may have decreased by selection and eventually reached a level at which dodine no longer controlled scab satisfactorily. This change in sensitivity is only by a factor of 2 or 3 and there is an overlapping of the normal distribution of dodine sensitivity in both tolerant and nontolerant populations (Fig. 1) which could account for the difficulty of differentiating between tolerant and nontolerant lines in the small populations used in these studies.

These studies indicate the *V. inaequalis* must be exposed to dodine for a considerable time and that dodine must be used intensively as the principal fungicide if significant tolerance is to develop. Even through dodine has been used intensively in other apple-growing areas of the world for 10 yr or more, widespread failures have not yet been reported. The history of dodine usage in Wayne County involving long exclusive usage at low rates and with reduced application is probably unique and explains why tolerance is a practical problem in that region but not yet in other apple-growing areas.

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