Tolerance of Venturia inaequalis to dodine in Nova Scotia[1]

R.G. Ross and R.J. Newbery

Isolates of ***Venturia inaequalis*** tolerant to dodine were detected following the failure of dodine to control scab in apple orchards in which it had been used successfully for many years; tolerance was confirmed by the response to dodine in artifical media of isolates of ***V. inaequalis*** from orchards in which dodine gave poor control of scab. The average tolerance of isolates from dodine-sprayed orchards was greater than that of isolates from orchards never exposed to dodine. The level of tolerance of isolates to dodine varied within and among orchards and appeared to be shifting towards increased dodine tolerance.

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On a decouvert des isolats de ***Venturia inaequalis*** tolerants a la dodine dans des vergers de pomme ou le produit avait ete efficacement utilisé contre la tavelure pendant de nombreuses annees. Cette tolerance a ete confirmee par la reaction a la dodine de certains isolats de ***V. inaequalis*** cultives sur substrats artificiels et provenant des vergers où le produit avait donne de mauvais resultats. La tolerance moyenne des isolats provenant des vergers pulverises était plus grande que celle des isolats issus des vergers temoins. Le degre de tolerance des isolats a varie au sein d'un même verger et d'un verger a l'autre, et semblait afficher une tendance a la hausse.

Tolerance of the apple scab fungus ***Venturia inaequalis*** (Cke.) Wint. to the fungicide dodine was first reported in western New York State in 1969 (8). Dodine failed to control apple scab in orchards in which it had previously given excellent control and these failures were most evident where dodine had been used regularly for 5-10 years. Subsequent reports (1, 10) verified that tolerance was related to intensive use of dodine and showed that isolates of the fungus from dodine tolerant orchards were less sensitive to dodine that those from nontolerant sources. Tolerance of ***V. inaequalis*** to dodine has recently been reported in Michigan (2) and the inheritance of dodine tolerance has been studied (1, 5).

In Nova Scotia dodine has been widely used for apple scab control since it was first recommended for us8 in 1961. Following the development of tolerance in other areas, apple growers in Nova Scotia were advised to report any failures of dodine to control apple scab that did not appear to be due to poor application or adverse weather. In 1973 and 1974, several growers reported poor scab control with dodine. Tests were done to determine the dodine tolerance level of isolates of ***V. inaequalis*** from their apple orchards and the results are reported in this paper.

Materials and methods

In 1974, isolates of ***V. inaequalis*** were obtained from leaves and/or fruit in orchards where dodine failed to control apple scab. Response of the isolates to dodine was determined in potato-dextrose agar which had been autoclaved, cooled to 45°C, and amended by adding a series of concentrations of dodine in ethanol. Each amended medium was poured into 6cm plastic petri dishes each containing 1 ml of the appropriate inoculum prepared by comminuting an agar slant of the isolate of ***V. inaequalis*** in 100 ml sterile water in a Waring Blendor. After thorough agitation the medium was allowed to solidify and the dishes were incubated at 18°C for 2 weeks. Controls consisted of media containing the same quantity of ethanol as that in which dodine was added. The concentrations of dodine in the media were approximate since in preparing the amended media no allowance was made to compensate for the 1 ml of inoculum and the quantity of amended media added to each dish was estimated by depth to be 9 ml. The dodine used was extracted from commercial grade material and recrystallized to melting point. Its potency was checked periodically by comparing it in an assay with commercial grade dodine. After 2 weeks at 18°C, growth in each dish was rated on a numerical scale of 0 to 10, 0 being no growth, 1 being dishes containing two or three isolated colonies, and 10 being maximum growth like that of the controls.

The isolates were assayed in groups of five and a Race 1 isolate of ***V. inaequalis*** used in previous work (6) served as a standard and was assayed each time a group was done. Assays were also done on isolates obtained from orchards in 1957 (7). prior to the introduction of dodine for the control of apple scab.

A comparison of the tolerance of isolates to dodine was also done using the agar diffusion technique (3). Filter paper (Whatman No. 1) disks 6 mm in diameter were dipped in ethanol solutions of dodine, drained uniformly, and placed on solidified potato-dextrose agar in large

[1] ***Contribution No. 1618, Research Station, Agriculture Canada, Kentville, Nova Scotia B4N 1J5***

Table 1. Growth of isolates of *Venturia inaequalis* obtained from Nova Scotia orchards in 1974 and 1957 in potato–dextrose agar containing different concentrations of dodine

Isolate	Origin		Growth of mycelium at the following concentrations of dodine (ppm):				
	Orchard	Cultivar	0.25	0.50	1.0	1.5	2.0
1974 isolates							
1-74	A	King	10.0*	10.0	3.5	0.5	0
2-74	B	McIntosh	10.0	10.0	10.0	7.0	2.5
3-74	C	Red Delicious	9.0	8.5	3.0	2.0	1.0
4-74	C	McIntosh	10.0	10.0	3.0	1.5	1.0
5-74	C	Gravenstein	10.0	9.5	3.0	0	0
6-74	D	Gravenstein	9.0	7.0	2.5	0	0
7-74	D	Cortland	9.5	7.5	3.5	0	0
8-74	E	McIntosh	10.0	10.0	9.0	5.0	2.5
9-74	E	Red Delicious	10.0	10.0	2.5	0	0
10-74	F	McIntosh	10.0	5.0	2.0	0	0
11-74	G	McIntosh	10.0	10.0	8.0	3.0	2.0
12-74	H	Red Delicious	9.0	5.0	2.0	1.0	0
13-74	H	Gravenstein	10.0	9.0	6.5	3.5	2.5
14-74	H	McIntosh	10.0	10.0	10.0	10.0	10.0
15-74	I	Red Delicious	10.0	10.0	7.0	3.0	0.5
16-74	I	Cortland	9.0	6.0	1.5	0.5	0
17-74	I	Cortland	10.0	9.0	2.0	1.0	0.5
18-74	I	McIntosh	6.5	4.0	2.0	1.0	1.0
19-74	I	McIntosh	10.0	10.0	10.0	8.0	3.0
20-74	J	Gravenstein	10.0	10.0	8.0	4.0	1.0
21-74	J	McIntosh	10.0	3.0	1.0	0.5	0.5
22-74	K	McIntosh	10.0	9.0	5.0	0	0
23-74	L	McIntosh	10.0	10.0	6.0	2.0	2.0
24-74	L	Red Delicious	10.0	9.0	2.0	1.0	0.5
Avg 1974 isolates			9.7	8.4	4.7	2.3	1.3
1957 isolates							
1-57			8.0	5.0	1.0	1.0	0.5
3-57			9.0	7.0	2.0	1.0	1.0
4-57			0	0	0	0	0
5-57			5.0	3.5	1.0	0.5	0
6-57			9.0	5.0	1.0	1.0	1.0
7-57			1.0	0.5	0.5	0	0
8-57			10.0	10.0	6.5	3.0	2.5
10-57			10.0	5.5	3.0	1.0	1.0
12-57			2.5	1.5	0.5	0.5	0
16-57			1.0	0	0	0	0
Avg 1957 isolates			5.6	3.8	1.6	0.8	0.6
Race 1**			10.0	8.7	2.6	0.5	0

* 0, no growth; 10, maximum growth

** Average of 18 assays

glass trays. Before pouring, the agar was seeded with inoculum from an agar slant culture of the isolate under test. After 10-12 days at 18°C the diameters of the clear zones around the disks were measured.

In greenhouse tests actively growing apple seedlings (cv. Beautiful Arcade) in pots were sprayed with different concentrations of dodine up to 15 ppm. Soon after the trees had dried, they were sprayed with suspensions of *V. inaequalis* containing about 5 x 10^4 conidia/ml and held in a moist chamber for 48 h.

Results and discussion

The growth ratings (Table 1) for *V. inaequalis* in

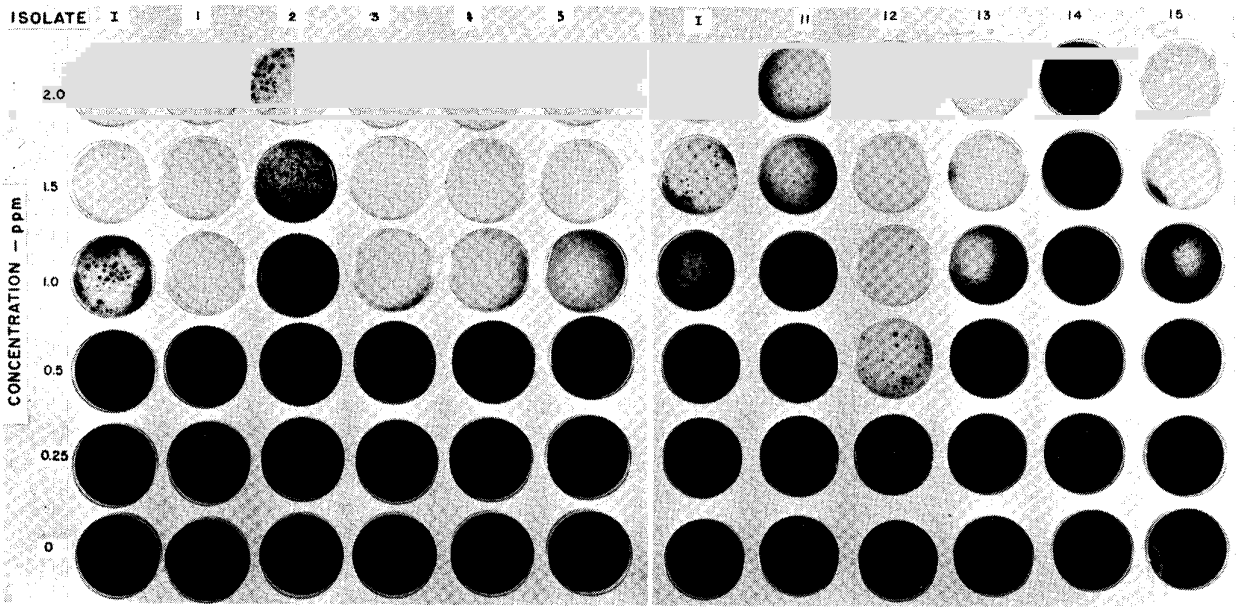

Figure 1. Growth of Race 1(I) and of 10 isolates of *V. inaequalis* obtained from Nova Scotia orchards in 1974 in media containing different concentrations of dodine.

different concentrations of dodine are the average of at least two assays for each isolate. Among both the 1974 group of isolates, from apple orchards in which dodine was not giving satisfactory control of scab, and the 1957 group, which were isolated prior to dodine use, there was considerable variation in sensitivity to dodine. On average the 1957 isolates were more sensitive to dodine than the 1974 isolates and among the latter there were isolates such as 2-74, 14-74, and 19-74 that appeared to be unusually tolerant to dodine. In the 1957 group sensitivity ranged from that of isolate 4-57 where all concentrations of dodine inhibited growth to that of isolate 8-57 which might be suspected of having developed tolerance to dodine if it had been exposed to it.

Orchards H and I were particularly suspect of having lines of *V. inaequalis* tolerant to dodine. This fungicide had been used almost exclusively for the control of apple scab for many years in both orchards and prior to 1974 had always given excellent control. Despite repeated applications of dodine in 1974, scab was abundant on the foliage and fruit and subsequent dodine sprays failed to halt its development. Isolate 14-74 from Orchard H and 19-74 from Orchard I were both tolerant to dodine in culture (Table 1). Isolate 2-74 was also tolerant in culture (Fig. 1) but poor scab control in orchard B was thought to have been due to poor timing of a spray in relation to an infection period. In Michigan, Yoder and Klos (11) obtained the most tolerant isolates from orchards in which dodine was used extensively but not necessarily from orchards with poor scab control.

The growth of isolates 2-74 and 14-74 and of Race 1 of *V. inaequalis* was compared at dodine concentrations up to 10 ppm and the results in Table 2 are the average of the assay repeated four times. A further comparison was done by the agar diffusion technique using disks dipped in dodine, and the results given in Fig. 2 are the average of three assays done at different times. Isolate 2-74 grew in media containing up to 4 ppm dodine and isolate 14-74 up to at least 10 ppm, whereas Race 1 was inhibited by 3 ppm. The disk assay gave the same contrast between the orchard isolates and Race 1 but did not distinguish the same degree of tolerance between isolates 2-74 and 14-74.

The inoculation experiment in the greenhouse did not give conclusive results. Isolate 14-74 did not infect sprayed or unsprayed apple seedlings. In a comparison of Race 1, isolate 2-74, and isolate 19-74 with concentrations of dodine up to 15 ppm, there were no significant differences in control and all lines caused infection at 15 ppm. Szkolnik and Gilpatrick (9) differentiated between sensitive and tolerant isolates at this level of dodine.

It seems unlikely that the sensitivity of field isolates of *V inaequalis* to dodine in culture can be used to determine whether dodine will fail in the orchard because of tolerance. Yoder and Klos (11) obtained tolerant isolates in orchards with good scab control, and in Nova Scotia there was considerable variation in sensitivity among isolates from the same orchard (Table 1). By taking sufficient isolates the 14-74 level of tolerance to dodine

Table 2. Growth of three isolates of ***Venturia inaequalis*** in media containing different concentrations **of** dodine

	Concentration of dodine (ppm):									
Isolate	1	2	3	4	5	6	7	8	9	10
2–74	10.0*	8.2	3.7	1.2	0	0	0	0	0	0
14–74	10.0	10.0	10.0	7.2	5.0	3.5	2.5	2.2	1.5	0.5
Race 1	6.0	0.5	0	0	0	0	0	0	0	0

* 0, no growth; 10, maximum growth

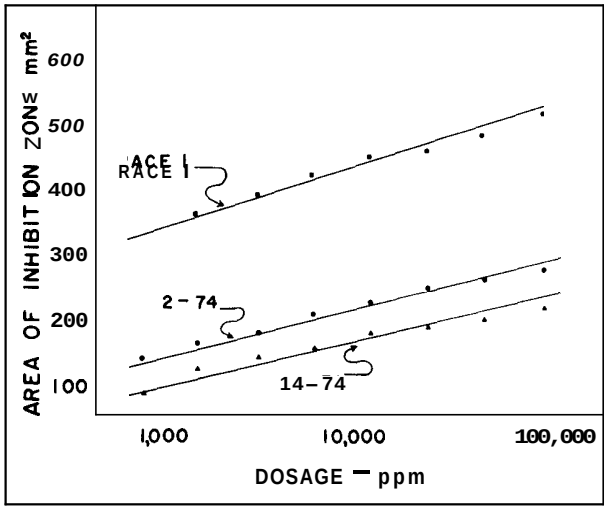

Figure 2. Dosage-response curves for dodine with three isolates of ***V.*** inaequalis.

might have been obtained in any of the orchards. According to MacNeill and Schooley (4) tolerance in *V. **inaequalis*** may evolve by adaptation and/or mutation. In culture they obtained a mutation rate of about 1 in 10^6. The results reported here (Table 1) suggest that the average tolerance to dodine has increased with the use of the fungicide in Nova Scotia; this supports the prediction of MacNeill and Schooley that the ecological balance might shift in favor of dodine tolerant strains within populations of ***V. inaequalis***. The differences in the two groups of isolates (1957 and 1974) were not as decisive as those Polach (5) reported for isolates from dodine sprayed orchards and isolates from abandoned orchards.

The presence in Nova Scotia of tolerance in *V. **inaequalis*** to dodine poses a problem for apple growers. In addition to being a good protectant and eradicant or after-rain fungicide for apple scab control, dodine has been used to burn out established scab lesions. The effect of tolerance on its eradicative and burning out properties is not known. The owner of orchard A (Table 1) considered that dodine was no longer effective for the latter purpose. The incidence of tolerance to dodine in Nova Scotia orchards may never be known because growers are changing to other scab fungicides rather than waiting for tolerance to become evident.

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