

# Resistance of *Venturia inaequalis* to Benomyl and Dodine in British Columbia, Canada

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## ABSTRACT

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An initial survey of 28 orchards in British Columbia in 1986 identified resistance of *Venturia inaequalis* to benomyl in nine orchards and also indicated some resistance to dodine in eight orchards. In 1987 samples of the apple scab fungus were collected from 145 orchards and tested for resistance to benomyl and dodine. Conidia from 54.3% of all 173 orchards were resistant to dodine at 2.0  $\mu\text{g/ml}$ , conidia from 25.4% of the orchards were resistant to benomyl at 1.0  $\mu\text{g/ml}$ , and conidia from 20.8% were resistant to both fungicides. The geographic distribution of resistance was consistent with pesticide use patterns. Resistance was most common in the wetter northern area of the Okanagan Valley, where benomyl has been used since 1973 and dodine since 1961. No resistance was evident in southern areas of the Okanagan Valley, where the climate is drier and apple scab is of much less economic importance. On the other hand, the four samples from the Victoria area of Vancouver Island, which has a wet, coastal climate, were all resistant to benomyl, and three samples were also resistant to dodine. Benomyl has been widely used to control apple scab in this area, but dodine has had minimal use there in recent years because of problems with fruit russetting.

Apple scab, caused by *Venturia inaequalis* (Cke.) Wint., is an important disease in the northern Okanagan Valley (from Kelowna north to Salmon Arm), Kootenays (Creston), and coastal areas (Vancouver Island and the lower mainland) of British Columbia, especially during years of above-average rainfall. Dodine has been recommended for apple scab control in British Columbia since 1961 (1) and benomyl since 1973 (2), although in subsequent years benomyl was not always recommended because of concerns about its effect on predatory mites. Thiophanate-methyl has been recommended since 1976 (3).

Dodine resistance was reported for the first time in 1969, after 9–10 yr of use, in the western fruit-growing counties of New York State, where an average of eight to 12 fungicide applications are made each year (13). Similarly, resistance to dodine developed after 10 yr of regular use in Michigan (6). Dodine resistance was reported in Canada in the early 1970s from both Nova Scotia (9) and Ontario (7).

In general, resistance of *V. inaequalis* to benomyl has developed within a shorter period of time than resistance to dodine. It was first reported in Australia in 1974 (15), followed by Michigan in

1975 (6), after less than 3 yr of regular use of benomyl. In Canada benomyl resistance was first discovered in Ontario in 1978, 5 yr after benomyl was introduced (8), and was reported in Nova Scotia 8 yr after it was first recommended (11). Documented reports of *V. inaequalis* resistance to both dodine and benomyl are rare, but it has occurred where these fungicides were used in combination following many years of exclusive dodine use (5).

In 1986 a grower in the Vernon area of the Okanagan Valley reported that he was unable to control apple scab with benomyl, although he had sprayed the fungicide at the appropriate times and at the recommended concentration. Subsequent tests by the manufacturers of benomyl and thiophanate-methyl showed that benzimidazole-resistant *V. inaequalis* was present in this orchard. In response to this information, we tested samples of the apple scab fungus from 28 orchards in 1986 and from 145 orchards in 1987 for resistance to benomyl and dodine. We did not test for resistance to thiophanate-methyl because previous studies have shown that benomyl and thiophanate-methyl break down into the same fungitoxic product (14).

## MATERIALS AND METHODS

**Sampling procedure.** Twenty apples or 50 leaves with freshly sporulating lesions were randomly selected from each orchard. Fruits were preferred to leaves because fruit lesions invariably yielded more conidia for testing. Twenty-eight orchards experiencing poor control of

apple scab were sampled in July and August of 1986, and 145 orchards were sampled in June and July of 1987.

**Sensitivity tests.** The methods we used for testing for benzimidazole resistance in strains of *V. inaequalis* were adapted from Northover (8) and McKay and MacNeill (7). Conidia were scraped with a sterile needle from several lesions on each fruit or leaf into 10 ml of sterile water in test tubes. Enough conidia were scraped from the infected fruit or leaves to discolor the water.

Concentrations of 0.6, 1.0, and 2.0  $\mu\text{g}$  of dodine per milliliter were prepared by incorporating predetermined volumes of a water suspension of dodine (Cyprex 65 WP, Cyanamid Canada Inc.) into autoclaved 1.5% water agar held at 50 C. Benomyl at 1  $\mu\text{g/ml}$  was prepared by incorporating a water suspension of benomyl (Benlate 50 WP, Dupont Canada Inc.) into the water agar medium before autoclaving. The fungicide-amended media were poured into polystyrene petri dishes 100 mm in diameter. Four drops of conidial suspension adjusted to contain 50–100 conidia per drop were placed on the agar and incubated for 24 hr at 20 C. Each test had four replicates.

Conidia were considered resistant to dodine when the germ tube was more than twice as long as the conidium; they were considered resistant to benomyl, which affects growth more than germination, when the germ tube was more than four times as long as the conidium. The percentage of germination was calculated based on the number of conidia that germinated on unamended water agar.

## RESULTS AND DISCUSSION

Benomyl-resistant *V. inaequalis* conidia that germinated and grew on 1  $\mu\text{g/ml}$  benomyl were detected in samples from 25.4% of the orchards surveyed (Table 1). The highest proportion of orchards with benomyl-resistant strains (4/4) was found in Victoria. This probably reflects the fact that benomyl or thiophanate-methyl is now used exclusively on Vancouver Island for apple scab control because dodine is more likely to russet fruit in cool, slow-drying weather. The most orchards at one location with benomyl-resistant strains (20/42) were in Vernon. Several

other locations in the province also had benomyl-resistant strains, indicating that resistance to benomyl was widespread.

Northover (8) showed that *V. inaequalis* conidia that germinated on water agar amended with benomyl at 0.5 µg/ml would infect apple plants sprayed with a 300 µg/ml dilute solution of benomyl. The registered dilution rate for benomyl is 250 µg/ml (4). In commercial practice, fungicides are normally applied with a concentrate sprayer at comparable rates per unit area. Therefore, resistance of the pathogen explains the poor field control reported by orchardists who used benomyl or thiophanate-methyl.

Dodine-resistant *V. inaequalis* conidia that germinated and grew on 2 µg/ml dodine were detected in 54.3% of the orchards (Table 1). Twenty percent or more of the conidia in samples from 81 of 142 orchards in the north end of the Okanagan Valley (from Kelowna to Vernon and in Salmon Arm, where scab has been a recurring problem) were resistant to dodine at 2 µg/ml. Conidia from even more orchards (66.5%), also primarily from the north Okanagan Valley, were resistant to dodine at 1 µg/ml.

McKay and MacNeill (7) found that apple scab may occur at significant levels in orchards sprayed with dodine when the *V. inaequalis* population attains an ED<sub>50</sub> value of approximately 0.7 µg/ml dodine. Thus we expect that if the majority of conidia from a sample will germinate on water agar amended with 1 µg/ml dodine, then field control with this fungicide will be less than adequate. We believe this is the case in British Columbia because if control had been

adequate in orchards where dodine was used at the reasonable management levels observed, we would not have been able to collect scab-infected leaves and fruit with such ease.

Conidia from 36 samples, primarily from the north Okanagan Valley, were resistant to both benomyl and dodine when the criterion for resistance was the ability of at least 20% of the conidia from a sample to germinate on agar amended with both benomyl and dodine at 1 µg/ml (Table 1). Resistance could have developed to both fungicides in these orchards because the initial use of dodine for several years allowed the buildup of dodine-resistant *V. inaequalis*, which remained in the population and later developed benomyl resistance when benomyl and thiophanate-methyl were introduced and used extensively for powdery mildew and apple scab control. Ross and Newberry (10) reported little change in the sensitivity to dodine of isolates from an orchard 4 yr after use of the fungicide was discontinued because resistance had developed.

Resistance to both dodine and benomyl is especially serious because growers must rely solely on a protectant control program except early in the growing season, when dichlone can be used as an eradicant. At present no sterol-inhibiting fungicides are registered in Canada for the control of apple scab. They are being proposed as alternatives to dodine and benomyl where strains of *V. inaequalis* resistant to one or both fungicides have developed (12).

This is the first documented report of *V. inaequalis* resistant to benomyl and/or dodine in British Columbia. The

resistance is widespread and occurs in the chief apple-growing regions of the province. Possible reasons for the delayed development of resistance in British Columbia compared to eastern Canada include lower inoculum levels, fewer annual infection periods (two to four), and fewer scab sprays (one to three). Resistance to benomyl took 8 yr longer to develop in British Columbia than in Ontario, and resistance to dodine took 13 yr longer to develop than in Nova Scotia. The use of higher eradicant rates of dodine probably gave control for several years after resistance to lower protectant rates had developed.

We do not believe that benomyl resistance went undetected for more than one or two growing seasons, because only in 1986 did growers claim they were unable to adequately control scab with benomyl or thiophanate-methyl. On the other hand, dodine resistance could have gone undetected for several seasons, because loss of field control with this fungicide is gradual and orchards were not monitored for resistance to dodine before 1986.

To overcome resistance, growers are switching to protectant control programs using chemicals such as metiram, mancozeb, and captan. Dichlone is also used as an eradicant in the early part of the growing season before blossom.

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**Table 1.** Resistance to dodine and benomyl of *Venturia inaequalis* from orchards sampled in 1986 and 1987

Location of orchards	Number of orchards sampled	Orchards with strains resistant to				
		Dodine <sup>a</sup>			Benomyl <sup>b</sup>	Benomyl and dodine <sup>c</sup>
		0.6 µg/ml	1.0 µg/ml	2.0 µg/ml		
Creston	12	9	8	6	1	1
Kelowna	38	29	24	18	6	5
Langley	3	1	1	0	1	0
Oliver	3	1	1	0	0	0
Oyama	18	12	12	12	6	4
Penticton	2	0	0	0	0	0
Saanich	1	1	0	0	0	0
Salmon Arm	5	5	5	5	3	3
Summerland	1	0	0	0	0	0
Vernon	42	35	29	21	20	17
Victoria	4	3	3	2	4	3
Winfield	44	34	32	30	3	3
Total	173	130	115	94	44	36
Percentage of total		75.1	66.5	54.3	25.4	20.8

<sup>a</sup>Conidia from these samples were considered resistant when 20% or more germinated on water agar amended with dodine and the germ tube was more than twice as long as the conidium after incubation at 20 C for 24 hr.

<sup>b</sup>Conidia from these samples were considered resistant when 20% or more germinated on water agar amended with 1.0 µg/ml benomyl and the germ tube was more than four times as long as the conidium after incubation at 20 C for 24 hr.

<sup>c</sup>Both fungicides at 1.0 µg/ml.

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