Some Characteristics of *Alternaria alternata* Strains Resistant to Iprodione

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ABSTRACT

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An isolate of *Alternaria alternata* was recovered from sweet cherries in storage, and variants within the wild population of the fungus were resistant to the fungicide iprodione. These resistant strains remained stable after a series of transfers in the absence of the fungicide and responded similarly to the wild type strain when tested against other chemicals. The iprodione-resistant strains were tolerant of DCNA; the wild type was sensitive. The capacity of these resistant isolates to cause rotting of cherries in storage was demonstrated.

Alternaria spp. are the cause of green mold on cherries, which is becoming a common disorder on harvested fruit in the Okanagan Valley. The incidence of rots due to *Alternaria* spp. also is increasing in storage fruits on the U.S. West Coast (I. MacSwan, *personal communication*).

Alternaria spp. are not sensitive to benomyl, which is commonly used to prevent brown rot infections caused by *Monilinia* spp. In evaluating other fungicides for efficacy against *Alternaria* and *Monilinia*, it was noticed that, although the fungicide iprodione was very effective against *Alternaria*, resistant strains arose frequently.

This study compares the growth, pathogenicity, and fungicidal sensitivity of *Alternaria alternata* (Fr.) Keissler isolates sensitive and resistant to iprodione.

MATERIALS AND METHODS

The wild type *A. alternata* used in this study was recovered from sweet cherry fruit several days after harvest. Seven resistant strains were isolated during tests to determine the effect of iprodione on the germination of spores from susceptible isolates of *A. alternata*. The sample of iprodione (RP-26019, 50% WP) was obtained from May & Baker (Canada) Ltd. and was used at the 100 ppm level for tests.

The iprodione-resistant isolates obtained, designated as R_1 to R_n, were also tested for their response to benomyl (50% WP), thiabendazole (60% WP), thiophanate methyl (70% WP), chlorothalonil (75% WP), polyoxin AL WP, 2,6-dichloro-4-nitroaniline (DCNA 75% WP), and captafol (80% WP). All isolates were maintained on potato-dextrose agar (PDA) at 20–25 C, and all fungicides were incorporated into the agar at 45 C as suspensions.

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For linear growth experiments, 3-mm square blocks were cut from the growing edge of a 2- to 4-day-old colony and inverted on the surface of amended or unamended PDA. Linear measurements were taken at intervals by measuring two diameters of the growing colony at right angles to one another. Three plates were used for each concentration. The percent spore germination was determined on PDA at 25 C by scoring 100 spores for each isolate.

The generation of iprodione-resistant strains is of serious concern only if the new strains can cause the destructive green mold of cherries characteristic of the original wild type isolate. To check the virulence of the R_1, R_2, and R_3 isolates, approximately 0.1 kg of cherries was placed in a plastic bag, sprayed with a spore suspension (1×10^5 spores/ml) by using a hand atomizer, and stored at room temperature for 7 days. At this point, green mold was visible, and the cherries were removed and examined. Isolations were made from the infected fruit and the resulting colonies checked for iprodione resistance.

RESULTS

Resistant colonies of *Alternaria* grew on agar plates containing iprodione at 100 and 25 ppm. Eighteen plates had been inoculated with 2×10^4 spores per plate, and three resistant colonies were found. A repeat experiment generated four resistant colonies on 24 plates, or one resistant colony from 120,000 spores. These resistant strains were designated R_1 to R_7 in order of isolation.

The linear growth and spore germination of wild type and resistant strains on PDA alone and on PDA containing 100 ppm iprodione are shown in Table 1. The original isolate failed to grow on PDA plates impregnated with the fungicide at concentrations as low as 5 ppm. The linear growth responses of five of the seven resistant isolates were very similar to those of the wild type. R_7 grew slightly more slowly than the other isolates on PDA plus iprodione and on PDA alone.

The original isolate failed to germinate normally at 1 ppm iprodione, but at 100 ppm, all resistant isolates germinated >90%. Germ tubes from R_7 spores germinated in the presence of iprodione but were noticeably shorter than those of wild type spores on unamended PDA or of other resistant strains on PDA containing iprodione.

Differences in the colony morphology of resistant isolates and wild type were evident but not striking. Resistant strains were darker with less aerial mycelium and usually were without the scattered tufts of aerial mycelium characteristic of wild type cultures. The R_3 isolate was very similar to the wild type, however.

When five successive transfers of R_1, R_2, and R_3 and three of R_4 to R_7 were made on PDA followed by transfer to PDA plus iprodione, growth responses were unchanged from those recorded in Table 1, indicating the stability of the variants. Single spore isolates from the original isolates responded identically to the mycelial transfers shown in Table 1.

Table 1. Growth of wild type and resistant strains on potato-dextrose agar (PDA) and PDA plus 100 ppm iprodione at 25 C

	Linear growth (mm) after 50-hr incubation		Germination after 22-hr incubation (%)	
Strain	PDA alone	PDA plus 100 ppm iprodione	PDA alone	PDA plus 100 ppm iprodione
Wild	25.3	0	100	0
R_1	25.5	19.8	96	100
R_2	26.2	19.8	97	99
R_3	23.2	20	100	98
R_4	23.3	17.5	95	96
R_5	22.8	17.8	100	94
R_6	23.5	17.5	100	95
R_7	16.5	15.5	100	97[a]

[a] Germination was 97%, but germ tubes were stunted.

The isolates R_1, R_2, and R_3 were grown on agar containing various other fungicides in an attempt to establish the specificity of resistance (Table 2). The response of the iprodione-resistant strains R_1, R_2, and R_3 was similar to wild type in the presence of benomyl, thiabendazole, thiophanate-methyl, chlorothalonil, and polyoxin AL WP; that is, all strains tolerated these fungicides. Captafol was effective against both the wild type and the iprodione-resistant strains and therefore its mode of action is independent of both the iprodione and benomyl resistance mechanisms. The iprodione strains were resistant to DCNA; the wild type strain was sensitive.

Germination of the wild type isolate in the presence of iprodione results in protoplastlike buds that eventually form into a mass of spherical pseudocells (Fig. 1A) instead of the usual threadlike germ tube characteristic of normal germination (Fig. 1C). This is similar to the response of the wild type *Alternaria* to polyoxin AL (Fig. 1B).

Strains differed in the amount of green mold produced when cherries were inoculated by applying a spore suspension (Table 3). Reisolation and transfer to PDA plus iprodione revealed that R_1, R_2, and R_3 retained their resistance, and the wild strain remained sensitive.

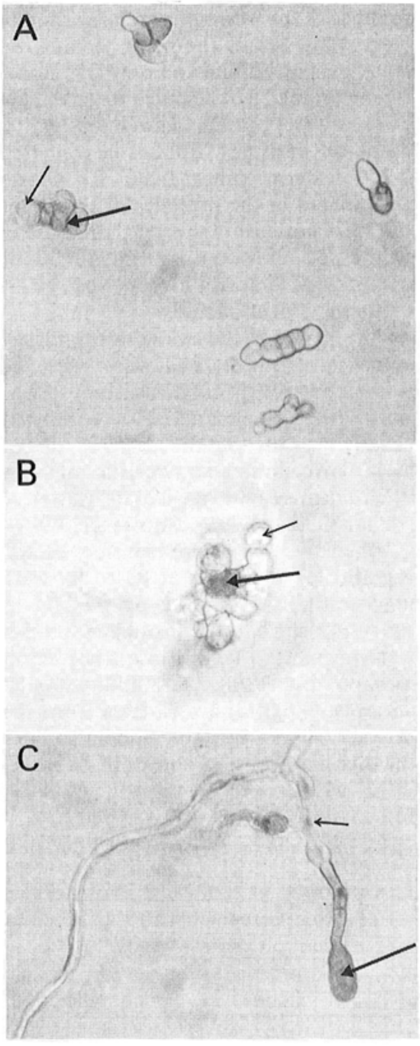

Fig. 1A–C. *Alternaria alternata* spores germinating on: **(A)** potato-dextrose agar (PDA) plus 100 ppm iprodione 18 hr at 25 C, **(B)** PDA plus 100 ppm polyoxin AL for 18 hr at 25 C, and **(C)** PDA after 18 hr at 25 C. (Large arrow indicates the spore. Small arrow indicates the spherical pseudocells and, in 1 C, the normal germ tube.)

DISCUSSION

Iprodione-resistant fungi have been reported previously. Leroux et al (4) demonstrated *Botrytis cinerea* resistance to 30 ppm, and Sztejnberg and Jones (7) reported iprodione-resistant *Monilinia fructicola* isolates that grew and sporulated on PDA containing up to 3,000 μg a.i./ml.

This study demonstrates the high frequency of iprodione-resistant strains within a population of *Alternaria* commonly infecting cherries. The frequency is approximately 20 times that of iprodione-resistant *M. fructicola* (7). These iprodione-resistant strains R_1 to R_7 were stable physiological isolates that maintained a high degree of resistance even after five consecutive transfers. Induced tolerance of some fungal species has been shown and the phenomenon explained as adaptive enzyme formation (6), but Dekker pointed out (1) that this type of tolerance seldom reaches a high level and is usually rapidly lost after transfer to a fungicide-free medium.

The phenotypic similarity between isolates R_1, R_2, R_4, R_5, and R_6 does not necessarily indicate that they are

Table 2. Response of wild *Alternaria alternata* and iprodione-resistant strains R_1, R_2, and R_3 to other fungicides (24 C incubation)

Fungicide (100 ppm)	Strain	Colony diameter after 45 hr (mm)	Germination after 22 hr (%)
Benomyl	Wild type	17	
	R_1	24	
	R_2	24	
	R_3	22	
Thiabendazole	Wild type	30	98
	R_1	31	98
	R_2	31	100
	R_3	29	97
Thiophanate methyl	Wild type	31	100
	R_1	31.5	98
	R_2	33	98
	R_3	29.5	96
Chlorothalonil	Wild type	22	
	R_1	25	
	R_2	24.5	
	R_3	23	
Polyoxin AL	Wild type	23.5	
	R_1	20	
	R_2	23	
	R_3	21	
DCNA	Wild type	5.5	
	R_1	21	
	R_2	22.5	
	R_3	21	
Captafol	Wild type	0	0[a]
	R_1	0	2
	R_2	0	2
	R_3	0	0

[a]Less than 4% germination after 120-hr incubation. Colony diameters are the mean of four replicates.

Table 3. Incidence of rot on cherry fruits inoculated with wild type and iprodione-resistant strains of *Alternaria alternata* incubated at 22–24 C for 7 days

Inoculated strain	Cherries with Alternaria rot (%)	Resistance of isolates from fruit to 100 ppm iprodione
R_1	12	Retained same level of resistance
R_2	42	Retained same level of resistance
R_3	51	Retained same level of resistance
Wild type	53	Sensitive to iprodione

genetically equivalent (2,8). The abnormal response of spores of *A. alternata* to iprodione and polyoxin AL may result from the inhibition of normal chitin metabolism resulting in the lack of a rigid cell wall. A similar abnormal germination of an *Alternaria* sp. (5) has been shown to be caused by polyoxin D, which interferes with normal chitin synthesis.

Resistance in the field has now become a major problem as a result of extended use of fungicides (3,9). Close monitoring of pathogens for development of resistance to frequently used fungicides and also screening of new formulations for their capacity to generate resistant populations should be routine.

The apparent high frequency at which resistant strains of *Alternaria* sp. are isolated in the presence of iprodione may indicate the potential for a rapid population shift from sensitivity to resistance. This may have unfortunate consequences if this compound is used regularly to suppress *Alternaria* infection of cherries. In the future, testing for induced population shifts should be routine in evaluating new fungicides in an attempt to minimize the chance of a disaster in the field.

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