Characterization of Iprodione-Resistant Isolates of *Alternaria brassicicola*

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ABSTRACT

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The resistance of isolates of *Alternaria brassicicola* to iprodione exhibited a polymodal distribution. Four distinct groups were detected demonstrating ED_{50}s of 0 to 7, 10 to 40, 70 to 80, and 300 to 516 µg a.i. per ml. Most resistant isolates produced smaller colonies on unamended potato-dextrose agar. Fewer conidia were produced per colony for some resistant isolates than for their corresponding wild types, while other resistant isolates produced more conidia per unit of colony area. The resistant isolates produced either smaller lesions or sporulated less, or both, than the sensitive isolates on untreated broccoli leaf disks. Only resistant isolates were able to produce lesions on leaf disks sprayed with 50 µg a.i. of iprodione per ml. Conidia of resistant isolates germinated in distilled water containing iprodione at a concentration as high as 250 µg a.i. per ml, while the germination of sensitive isolates was greatly inhibited in distilled water containing only 5 µg a.i. of iprodione per ml. Iprodione resistant isolates were cross resistant to vinclozolin and dichloran. Resistant isolates exhibited a greater osmotic sensitivity than sensitive isolates; however, osmotic sensitivity was independent of the degree of resistance.

Alternaria brassicicola (Schwein.) Wiltshire is the most important pathogen of black spots in brassicaceous crops (e.g., broccoli, cauliflower, and cabbage) in Israel (12) and worldwide (6,9). Currently, fungicide applications are the main measure employed for controlling black spots in brassicaceous vegetables. The fungicide iprodione effectively controls *A. brassicicola* by either seed dressing (19,20) or foliar applications in the field (13). Resistance to iprodione has been well documented in many plant-pathogenic fungi, including some *Alternaria* species (1,7,21, 25,26,28). Monitoring iprodione resistance in *A. brassicicola* and characterizing resistant isolates are necessary for managing iprodione resistance in this fungus. When evaluating the effect of iprodione applications on the buildup of iprodione resistance in populations of *A. brassicicola* under field conditions, no indication of the development of iprodione resistance was found after seven weekly iprodione sprays (R. Huang and Y. Levy, *unpublished*). However, under laboratory conditions, resistant isolates arose frequently in iprodione-amended culture dishes.

Mathematical and experimental studies have shown that the parasitic and saprophytic fitness of a resistant subpopulation is crucial for its competition with other subpopulations (16,18). Only highly fit, resistant individuals can be maintained in or even dominate the population, causing loss of control of the corresponding fungicides. It has been frequently observed that iprodione-resistant isolates of different plant-pathogenic fungi have higher osmotic sensitivity than do sensitive isolates (1,2,14,15). This high osmotic sensitivity is considered to be responsible for the lower pathogenicity and lower fitness of the resistant isolates. In the present study we investigated the parasitic and saprophytic fitness, the resistance to other fungicides, and the osmotic sensitivity of 25 laboratory mutant isolates of *A. brassicicola* exhibiting iprodione resistance and the corresponding wild types from which they were isolated.

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MATERIALS AND METHODS

Isolation of resistant isolates. During routine ED_{50} value determinations of *A. brassicicola* isolates from broccoli plots of a field experiment, all isolates collected from both the control plots and plots with seven weekly sprays of iprodione (250 g a.i. per ha) were not able to grow on potato-dextrose agar (PDA) dishes amended with 10 or 50 µg a.i. of iprodione per ml. However, resistant sections frequently appeared 1 to 2 weeks later. Twenty-five resistant isolates were obtained by transferring mycelial tips from these fast-growing (resistant) sections. In most cases only one randomly selected resistant section was transferred from each field isolate, though more such sections arose from a single mycelium disk. The extension "R" or "W" was added to the code of each isolate in order to designate the resistant isolate and its corresponding wild type.

Determination of ED_{50} value. Iprodione resistance was measured using the poison plate method based on the inhibition of mycelial growth. Three-millimeter disks of mycelia from the growing edge of a 7-day-old colony were placed on PDA culture dishes amended with 0.1, 1, 10, or 50 µg a.i. of iprodione per ml (Rovral 50WP) and a control dish (unamended PDA), and incubated at 23°C with 12 h light (70 $\mu E \cdot m^{-2} \cdot s^{-1}$). In each case, 1-ml aliquots of fungicide suspension containing the appropriate concentration (aqueous suspension using sterile distilled water) were added to 200 ml of molten PDA (approximately 50°C) after autoclaving. Measurements of colony size were taken on the seventh day by measuring two diameters of the growing colony at right angles to each other. Isolates exhibiting more than 50% growth on dishes containing 50 µg a.i. of iprodione per ml relative to the control (unamended PDA), were further tested on PDA dishes amended with 100 and 500 µg a.i. of iprodione per ml. The experiment was conducted twice.

The ED_{50} value for each isolate, which was defined as the concentration of iprodione causing a 50% inhibition of mycelial growth, was determined using the PROBIT procedure of the SAS statistical package (SAS Institute Inc., Cary, N.C.).

Mycelial growth and sporulation on PDA. Three-millimeter disks of mycelia were cut from the growing edge of a 7-day-old colony, placed on PDA dishes, and incubated under the same conditions as described above. Measurements of colony diameter were taken on the seventh day as described previously. Three dishes were used for each isolate. After the measurement of colony size (on the seventh day), five resistant isolates (R1-R, R8-R, S2-R, S3a-R, and S5-R) together with their corresponding wild types were randomly chosen for evaluation of sporulation. Conidia were washed off with 40 ml of distilled water containing 0.1% Tween 20 (0.1% Tween 20) using a camel-hair brush. The conidial suspension was allowed to sediment for 30 min. The supernatant was discarded and the sediment was resuspended with 0.1% Tween 20 to a total volume of 40 ml. The absorption value for each suspension was measured at a wave length of 600 nm using a spectrophotometer. The concentration of the conidial suspension was estimated using the following regression equation developed by Huang and Levy (12): $Y = 1.498 + 156.734 \times D$ ($R^2 = 0.99$, $s^2 = 4.770$), where Y is the conidial concentration ($\times 10^4$/ml) and D the absorption value of the suspension. The experiment was conducted twice. In the first experiment S2-R was not included, while in the second S8-R was not included, because of bacterial contamination.

Conidial germination. Three resistant isolates (R1-R, S3a-R, and S6b-R) and their corresponding wild types were selected according to their ED_{50} values in order to test the effect of iprodione on conidial germination. Five 10-µl droplets of conidial suspension (10^4 conidia per ml) containing 0, 5, 50, and 250 µg a.i. of iprodione per ml (as suspension of Rovral 50 WP) were placed on glass slides. The slides were incubated in the dark on two layers of filter paper saturated with distilled water in a plastic tray (25 × 25 × 2 cm) at 23°C. The percentage of spore germination was determined by scoring 100 conidia for each isolate and treatment after a 22-h incubation. A conidium was scored as germinated, if the germ tube length was at least twice the length of the conidium itself. The length of the germ tubes was also determined by measuring 10 selected germ tubes (only the longest germ tube was measured for each germinated conidium). The experiment was conducted twice, but in the first experiment germ tube length was not measured and the concentration of 250 µg a.i. per ml was not included.

Parasitic fitness. In the first experiment, four resistant isolates (R1-R, R8-R, Ck3-R, and Ck10-R) and their wild types were selected for parasitic fitness experiments. Twenty-five-millimeter disks of fully expanded leaves of broccoli (cv. Shugon) plants at approximately the 10-leaf stage were cut and placed with the adaxial side up on one layer of filter paper wetted with 4 ml of distilled water in a 9-cm petri dish (six disks per dish). A 10-µl droplet of a conidial suspension (10^5 conidia per ml) prepared from a 7-day-old colony was placed in the center of each leaf disk. Three dishes were used for each isolate. After an incubation period of 24 h at 23°C in the dark, the dishes were transferred to a bench at 25°C with 12 h light per day (70 $\mu E \cdot m^{-2} \cdot s^{-1}$). Water was added every 2 days to prevent drying of leaf disks. Ten days after inoculation, the number of disks exhibiting *Alternaria* symptoms was counted and the diameter of each lesion was measured. The infection frequency was defined as the percentage of leaf disks exhibiting visible *Alternaria* symptoms. The conidia on the leaf disks in each dish were then washed off with 40 ml of 0.1% Tween 20, and the conidial concentration was determined for each dish using the method described above, only the process of sediment-supernatant discarding was conducted twice.

In the second experiment, six resistant isolates (R1-R, R8-R, S5-R , S3a-R, Ck10-R and Ck15-R) and their corresponding wild types were selected according to their ED_{50} values. Disks were cut from broccoli leaves sprayed either with 50 µg a.i. of iprodione per ml in 0.1% Tween 20 or 0.1% Tween 20 alone. Three petri dishes, each with seven leaf disks, were used for each isolate and iprodione treatment combination. Both experiments were conducted twice.

Sporulation and diseased area data were subjected to analysis of variance (ANOVA), with resistance, parental isolate, and repeat (block) as main effects in a randomized complete block design. In order to stabilize the variance among isolates and treatments, the square root transformation was performed for the diseased area data before ANOVA. The two-tailed *t* test was used to test the significance of the difference between a resistant isolate and its corresponding wild type.

Resistance to other fungicides and osmotic motility. Twenty-three isolates with ED_{50} values ranging from 0.6 to 443 µg a.i. per ml were selected for testing their mycelial growth on PDA dishes amended with 100 µg a.i. of vinclozolin per ml (Ronilan 50WP), 50 µg a.i. of dichloran per ml (Allisan 50WP), 0.26 M and 0.45 M NaCl, and unamended PDA. Colony diameter was recorded on the seventh day as described above. The experiment was conducted twice. Correlation analysis was performed using logarithm transformed ED_{50} of iprodione and relative mycelial growth (RMG) on PDA amended with either each of the both fungicides or each concentration of NaCl for each isolate. The RMG on unamended PDA was set to 1.00.

RESULTS

Colony morphology of the resistant isolates and their ED_{50} value distribution. The general morphological characteristics of the resistant isolates were not distinguishable from those of their corresponding wild types when grown on PDA. Only a few resistant isolates exhibited some aerial mycelia, which were generally

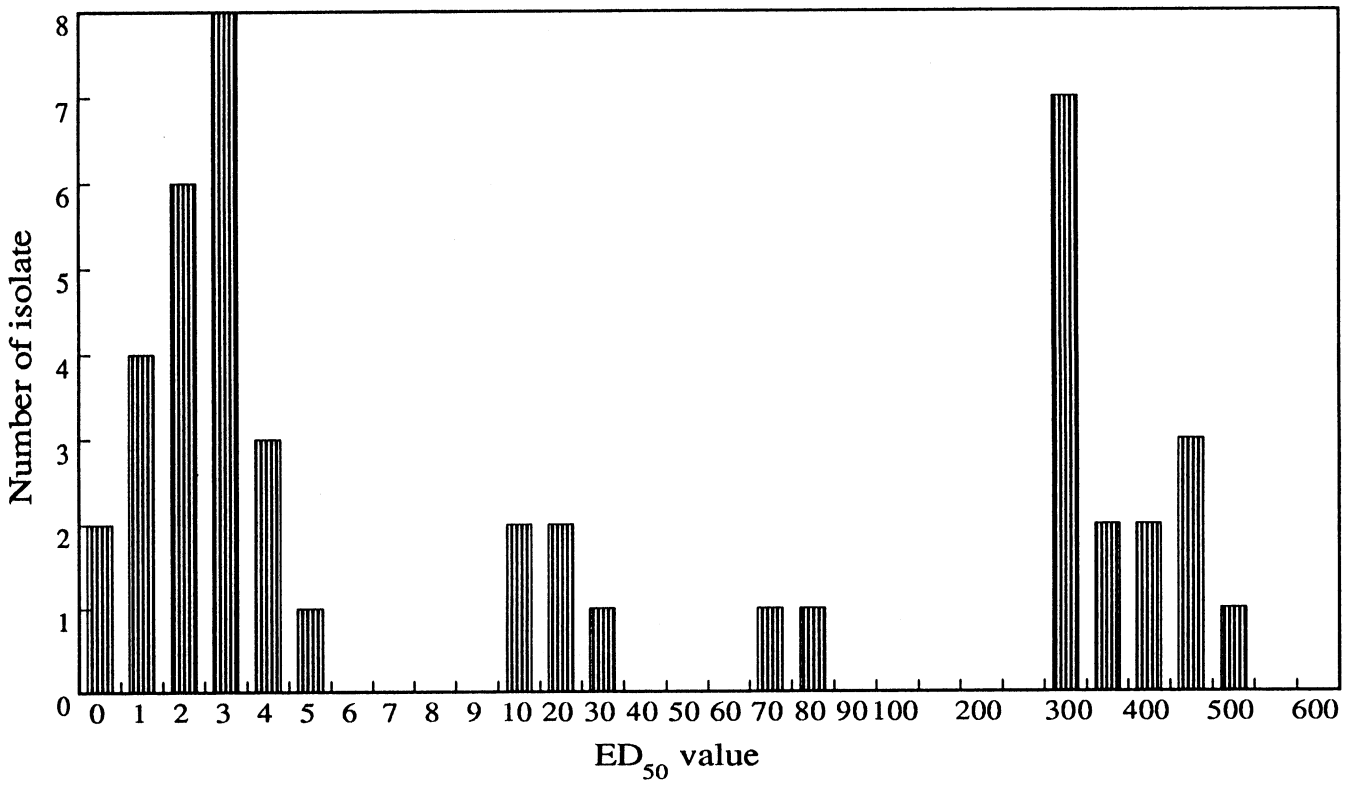

Fig. 1. Distribution of ED_{50} values (µg a.i. of iprodione per ml) of isolates of *Alternaria brassicicola*.

absent from the wild type colonies. The ED_{50} values of the 25 resistant isolates ranged from 4.0 to 516 µg a.i. per ml, while that of the 21 wild types ranged from 0.65 to 3.70 µg a.i. per ml. A polymodal distribution of their ED_{50} values is presented in Figure 1. Four distinct resistance categories were found based on the ED_{50} values: 0 to 7 (24 isolates); 10 to 40 (5 isolates); 70 to 90 (2 isolates); and 300 to 516 µg a.i. per ml (15 isolates).

Mycelial growth. Colony size of most of the resistant isolates was smaller than that of their respective wild types (Fig. 2); only three of the 25 resistant isolates were larger than those of their corresponding wild types. No relationship was found between the relative mycelial growth of the resistant isolates (in proportion to their corresponding wild types) and their ED_{50} values.

Sporulation on PDA. The results of ANOVA (with repeats as blocks) of sporulation data (conidia per mm^2) of the five resistant isolates tested and their corresponding wild types showed that the effect of resistance on sporulation per unit area was highly significant ($P = 0.002$). R1-R and R8-R sporulated significantly more per unit area than did their corresponding wild types (Table 1). In some cases, the difference in the sporulation of the wild-type and resistant isolates could be detected visually. Colonies of the resistant isolates were usually darker (stronger sporulation) than their corresponding wild types.

The data of sporulation per colony was also analyzed the same way as above. The effect of resistance was highly significant ($P = 0.006$); S3a-R and S5-R produced significantly less conidia per colony than did their corresponding wild types (Table 1).

Infection frequency, lesion size, and sporulation on broccoli leaf disks. Six of the seven resistant isolates tested caused significantly less disease than their corresponding wild types on untreated broccoli leaf disks (Tables 2 and 3). Disease caused by other isolates, except R8-R, was also less severe than that caused by their corresponding wild types, although the difference was not statistically significant. Sporulation of all resistant isolates was significantly lower than that of their corresponding wild types (Table 2). However, there was no difference between the infection frequency of the resistant isolates and that of their corresponding wild types. In most cases, the resistant isolates were capable of penetrating the leaf tissue and the inoculated leaf disk exhibited symptoms. However, lesions of the resistant isolates were smaller.

On leaf disks sprayed with 50 µg a.i. of iprodione per ml, none of the wild types were able to cause any visible symptoms, whereas all the resistant isolates caused visible symptoms, although the disease was less severe than observed on leaf disks without iprodione (Table 3). Iprodione treatment of leaf disks reduced both infection frequency and lesion size. This reduction in lesion size was more pronounced for isolates with lower ED_{50} values (e.g., S3a-R and S5-R) than for those with relatively higher ED_{50}s (e.g., R1-R and Ck10-R).

Effect of iprodione on germination. There was no difference in germination rate and germ tube length between the resistant and the wild types in distilled water (Table 4). Germination of most wild types was inhibited at 5 µg a.i. of iprodione per ml, while resistant isolates were able to germinate in the highest concentration tested (250 µg a.i. per ml). For example, 250 µg a.i. of iprodione per ml had almost no effect on the germination of R1-R, while germination of R1-W was totally inhibited at a concentration of 5 µg a.i. per ml. The effect of iprodione on germination was related to the corresponding ED_{50} value. For example, the germination rates of R1-R, S6b-R, and S3a-R were 87, 55, and 4% in 50 µg a.i. per ml, respectively, while their ED_{50} values were 443, 83, and 23 µg a.i. per ml respectively. Germ tube growth was more sensitive than germination was to iprodione. For instance, germination of R1-R in concentrations between 0 and 250 µg a.i. per ml were not affected by the concentration of iprodione, while the germ tube length decreased with increased iprodione concentration.

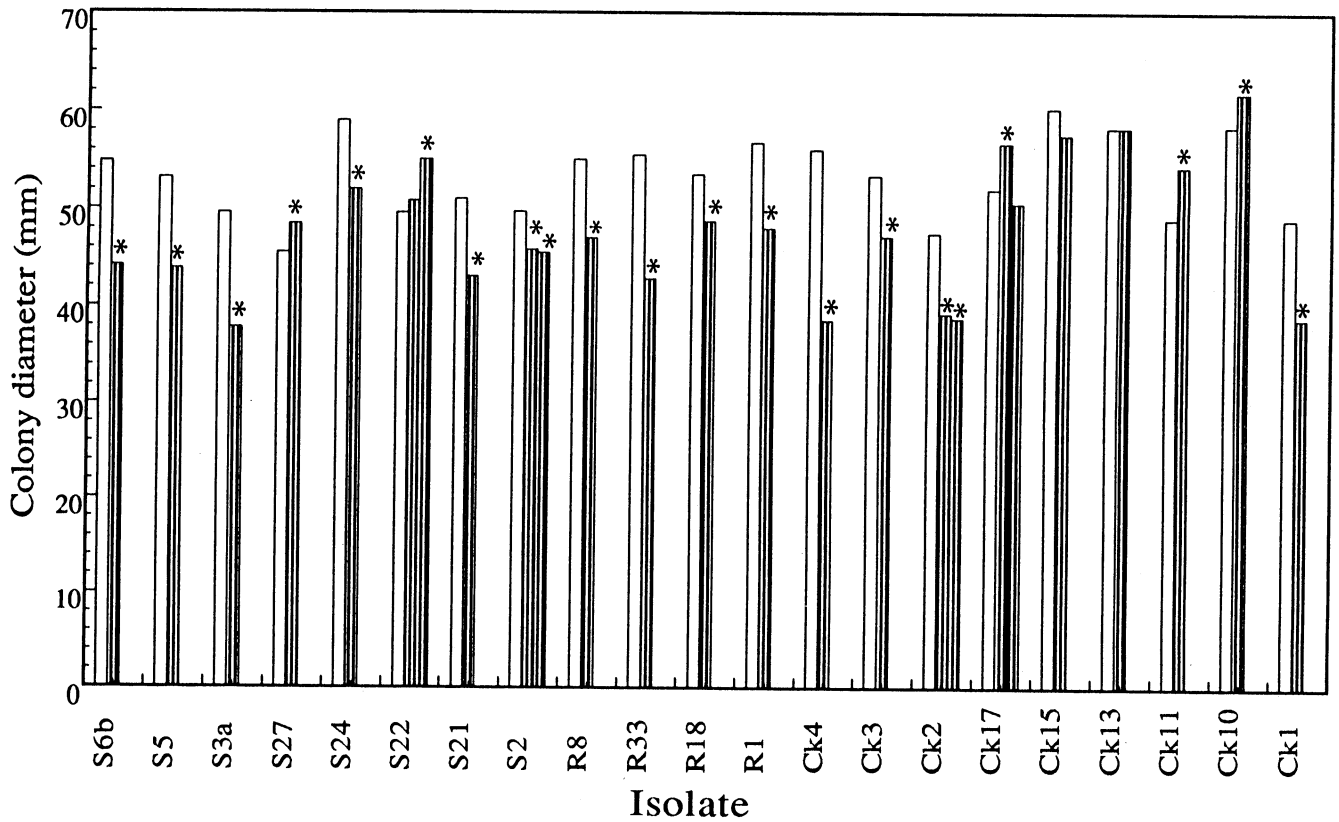

Fig. 2. Mycelial growth (colony diameter after 7-day incubation) of resistant isolates (filled) of *Alternaria brassicicola* and their wild types (empty). Note that in four cases two resistant isolates were isolated from a single wild-type isolate. Asterisk (*) indicates that the growth of the resistant isolate is significantly different from that of its (their) wild type ($P = 0.05$, two-tailed t test).

Osmotic resistance. There was no significant difference ($P > 0.05$) in the relative growth on PDA amended with 0.26 M NaCl (compared with unamended PDA) between resistant (mean = 0.43) and wild types (mean = 0.45). However, on PDA dishes amended with 0.45 M NaCl, the relative growth of resistant isolates (mean = 0.38) was significantly lower than that of the wild types (mean = 0.44). Thus, the resistant types were more sensitive to osmoticity than the wild types, but no relationship was found between ED_{50} value and the osmotic sensitivity ($r = -0.14$, $P = 0.59$).

Cross-resistance. Correlation analysis revealed significant positive correlation between resistance to iprodione, vinclozolin, and dichloran. Correlation coefficients between resistance to iprodione and vinclozolin, iprodione and dichloran, and vinclozolin and dichloran were 0.704 ($P = 0.0002$), 0.634 ($P = 0.0012$), and 0.956 ($P = 0.0001$), respectively, indicating that cross-resistance exists among these fungicides.

Table 1. Sporulation ($\times 10^4$) on potato-dextrose agar of resistant isolates of *Alternaria brassicicola* and their corresponding wild types (combined data from two repeated experiments)

Isolate	ED_{50}	Per colony	Per mm²
R1-W	3.6	6,542	2.621
R1-R	443.3	7,184	3.921*[a]
R8-W[b]	2.4	4,732	1.900
R8-R[b]	4.7	5,018	2.851*
S3a-W	0.7	6,034	2.955
S3a-R	23.0	3,468**	2.837
S5-W	1.7	5,382	2.236
S5-R	13.3	3,856*	2.348
S2-W[b]	3.7	6,222	3.082
S2-R[b]	341.7	4,904	3.540

[a] * and ** indicate a significant difference from its wild type at $P < 0.05$ and $P < 0.01$, respectively, based on a two-tailed t test.
[b] Isolate was tested only in one repeat.

Table 2. Diseased area per leaf disk (25 mm in diameter) and sporulation (per mm² diseased area) of four resistant isolates of *Alternaria brassicicola* and their corresponding wild types (combined data from two repeated experiments)

Isolate	Infection frequency (%)	Diseased area per leaf disk (mm²)	Sporulation ($\times 10^3$ per mm²)
R1-W	100	170	1.60
R1-R	100	71**[a]	0.54**
R8-W	97	182	2.13
R8-R	88	193	0.38**
Ck3-W[b]	100	270	1.15
Ck3-R[b]	100	143**	0.52*
Ck10-W[b]	100	123	0.99
Ck10-R[b]	94	94	0.38*

[a] * and ** indicate significant difference between the resistant isolate and its corresponding wild type at $P < 0.05$ and $P < 0.01$, respectively, based on a two-tailed t test.
[b] Isolate was tested only in one repeated experiment.

DISCUSSION

The results presented in this paper demonstrate that resistance of *A. brassicicola* to iprodione is associated with slow mycelial growth on PDA dishes, low virulence, and weak sporulation on broccoli leaves in the absence of iprodione. This is in agreement with earlier observations on *A. dauci* (8), *Botrytis cinerea* (4,11), *Penicillium expansum* (28), and *Monilinia fructicola* (26). Beever et al. (3), Pak (22), and Pak et al. (23) also observed a decline in the frequency of iprodione-resistant isolates of *B. cinerea* on grapevines in the absence of dicarboximide fungicides. This decline in the frequency of resistant isolates may be caused by low fitness of resistant isolates. More directly, Pak (22) demonstrated that, on grape berries, absolute fitness (lesion size, infection incidence, and sporulation) of *B. cinerea* isolates collected from vineyard decreased with increased ED_{50} values. Based on available data, it seems likely that if resistant isolates of *A. brassicicola* appeared, they would most likely not increase rapidly to a dominant level in the population. Furthermore, since sporulation and pathogenicity of resistant isolates were reduced in the absence of iprodione, resistant populations should be diluted rapidly.

The resistance of *A. brassicicola* isolates to iprodione exhibited a polymodal

Table 3. Infection frequency (%) and diseased area per leaf disk (25 mm in diameter) of six resistant isolates of *Alternaria brassicicola* and their corresponding wild types on broccoli leaf sprayed with either 50 µg of iprodione per liter or water (control)

		Control		50 µg per liter	
Isolate	ED_{50}	Infection frequency (%)	Diseased area per disk (mm²)	Infection frequency (%)	Diseased area per disk (mm²)
R1-W	3.6	90	196	0	0
R1-R	443	93	110*[a]	88	79**
R8-W[b]	2.4	100	168	0	0
R8-R[b]	4.7	93	136*	76	11
S5-W[b]	1.7	95	176	0	0
S5-R[b]	13.3	90	83*	52	7
S3a-W	0.7	90	122	0	0
S3a-R	23.0	95	43*	100	13*
Ck10-W	3.5	95	147	0	0
Ck10-R	332.9	98	99	93	52**
Ck15-W	2.9	98	193	0	0
Ck15-R	349.7	100	95*	90	68**

[a] *, ** indicate significant difference from its wild type at $P < 0.05$ and $P < 0.01$, respectively, based on a two-tailed t test.
[b] Isolate was tested only in one repeated experiment.

Table 4. Effect of iprodione on germination (%) and germ tube length (GTL, in micrometers) of isolates of *Alternaria brassicicola* after 22 h of incubation

Isolate (ED_{50})	Iprodione (µg a.i./ml)[a]	Germination[b]	GTL[c]
S6b-R (83)	0	99	166±46[d]
	5	90	112±70
	50	55	72±31
	250	58	64±19
S6b-W (1.7)	0	99	191±45
	5	19	30±7
	50	0	0
	250	0	0
S3a-R (23)	0	100	300±105
	5	77	30±8
	50	4	21±4
	250	0	0
Ssa-W (0.7)	0	100	270±96
	5	1	0
	50	0	0
	250	0	0
R1-R (443)	0	96	262±102
	5	87	203±89
	50	87	96±24
	250	92	82±28
R1-W (3.6)	0	97	260±97
	5	0	0
	50	0	0
	250	0	0

[a] The concentration 250 µg a.i./ml was not tested in the first repeat.
[b] 100 conidia were scored in each of the two repeats.
[c] 10 germ tubes were measured in the second repeat.
[d] Mean and standard deviation.

distribution. This is in agreement with observations of *B. cinerea* (22,23). Based on the data presented in this paper and those of Pak (22) and Pak et al. (23), resistance to iprodione in *A. brassicicola* and other pathogenic fungi such as *B. cinerea* is likely to be controlled by different major genes, though data from more detailed genetic experiments are still needed to test this hypothesis.

Although some concentrations of iprodione used in this research exceeded its solubility in water (about 12 µg/ml), we observed clear differences in mycelium growth of some isolates among the concentrations of 50, 100, and 500 µg a.i. of iprodione per ml. Beever and Brien (2) compared the effect of acetone solubilized iprodione (technical grade, 97%) with that of suspended commercial Rovral (50 WP) on mycelium growth of *B. cinerea,* and found no differences between the two forms in all concentrations tested, including 20 and 100 µg a.i. per ml.

There are only five and two isolates with ED_{50} values within the ranges of 10 to 30, and 70 to 80 µg a.i. per ml, respectively (Fig. 2). Thus, the separation of these isolates into groups is not certain. To clarify this point, more isolates with ED_{50} values within these ranges need to be tested.

Development of fungicide-resistant isolates is a great matter only if the new isolates are capable of causing destructive disease and have sufficient parasitic fitness to compete with the sensitive isolates of the population. Nearly all resistant isolates tested were less virulent and sporulated less on broccoli leaf disks than did their wild types. However, it is still not known whether other resistant isolates may also have low virulence and may sporulate weakly. Based on the fact that resistant isolates are easily isolated in the laboratory, it is likely that they can also develop in the field. If this happens readily, then the selection pressure (iprodione application) may not only select for resistant individuals but also may enhance their parasitic fitness.

The increased osmotic sensitivity of resistant isolates is frequently cited as an explanation for their reduced pathogenicity and lower fitness (1,2,14,15). The relationship between dicarboximide resistance and osmotic sensitivity is still controversial. Leroux and Fritz (15) were able to find a direct correlation between the degree of dicarboximide resistance and osmotic sensitivity in their *B. cinerea* isolates. However, Beever and Brien (2) observed that dicarboximide-resistant isolates of *B. cinerea* were more sensitive to increased osmotic values than were sensitive isolates. This was found to be independent of the degree of resistance. No difference in osmotic sensitivity was found between dicarboximide-resistant and sensitive isolates of *Neurospora crassa* (10) and *B. cinerea* (18). Our observations are similar to those of Beever and Brien (2); dicarboximide-resistant isolates exhibited greater osmotic sensitivity than the sensitive isolates. However, this osmotic sensitivity was independent of the degree of dicarboximide resistance. It is not known whether this increase in osmotic sensitivity is associated with the mutation itself or with the gene(s) responsible for iprodione resistance. The same is also true for the reduced mycelial growth of the resistant isolates.

It is interesting that some iprodione-resistant isolates of *A. brassicicola* sporulated more per unit area than their corresponding wild types on unamended PDA. However, two of the five resistant isolates tested sporulated less per colony than their corresponding wild types. The high sporulation per unit area may be a compensation, at least in part, for slow mycelial growth. Though the number of the isolates tested is very small, visual examination of other isolates (data not presented) also revealed that the colonies of resistant isolates were usually darker than their corresponding wild types, an indication of greater sporulation. Studies on *B. cinerea* (24) and *Neurospora crassa* (10) demonstrate that dicarboximide-resistant isolates usually have lower sporulation than the sensitive isolates.

Iprodione treatment of leaf disks reduced both infection frequency and lesion size (Table 3). This implies that iprodione can not only inhibit penetration but may also restrict the further development of the fungus after penetration. Reduced lesion size might also be the result of reduced number of conidia, which were able to germinate and successfully penetrate in the presence of iprodione. There was a positive relationship between inoculum density and lesion size with both leaves of intact plant and leaf disks (R. Huang, *unpublished data*).

Highly significant correlation coefficients between resistance to iprodione and vinclozolin, iprodione and dichloran, and vinclozolin and dichloran imply that these fungicides are cross resistant to one another. Cross-resistance among these fungicides was also observed in *B. cinerea* (5), *Penicillium expansum* (28), and *Monilinia fructicola* (27). These fungicides apparently have at least one common site of action in the fungus.

It is reasonable to assume that resistant isolates, obtained by transferring mycelial tips from the resistant sections of a sensitive isolate grown on PDA amended with iprodione, arose from mutation of the sensitive (wild) isolates, rather than from a mixture of sensitive and resistant isolates, since resistant sections appeared 5 to 7 days (some even as long as 2 weeks) after the inoculation of the mycelial disks on PDA dishes containing iprodione. If a mycelial disk consists of both resistant and sensitive isolates, the resistant isolate should be detected within 2 to 3 days, or should even overgrow the sensitive one, since most resistant isolates can grow more than 5 mm within 3 days on PDA dishes amended with 10 µg a.i. of iprodione per ml. The fact that resistant mutants arose from single spore isolates (R. Huang, *unpublished*) also supports this hypothesis. Besides mutation, sorting of the nuclei of multinucleate fungal cells might also be a possible cause of the development of resistance. However, we don't believe this is important for the development of the resistant isolates reported here. The ED_{50} value of 15 (60%) resistant isolates fell into the range of 300 to 516 µg a.i. per ml, while in the range of 4 to 7, 10 to 40, and 70 to 90 µg a.i. per ml there were only 2, 5, and 2 isolates respectively. If sorting of nuclei had been an important factor in the development of resistance in these isolates, there would have been more isolates with lower ED_{50} values, e.g., 4 to 7 and 10 to 40 µg a.i. per ml, because the chance of developing multinucleate cells with less resistant nuclei should be greater than that with more resistant nuclei.

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