Specific Tolerance of *Sclerotium cepivorum* to Dicarboximide Fungicides

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ABSTRACT

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Five isolates of *Sclerotium cepivorum* tolerant to one dicarboximide fungicide showed cross-tolerance to the dicarboximides iprodione, myclozolin, and vinclozolin and to dicloran and PCNB but not to benomyl, captan, or thiram. The dicarboximides dicloran and PCNB share a common structural subunit. EC_{90} values for most tolerant isolates were >1,000 times those of the parent isolates. Sensitivity to benomyl was unchanged by tolerance to dicarboximides. Wide variations in sensitivity of the parent isolates to PCNB and thiram were noted. Frequency of occurrence of dicarboximide tolerance was too low and variable to obtain a reliable estimate.

Tolerance of plant pathogens to synthetic fungicides is becoming a significant problem. In a 1967 review (9), fungicide tolerance was not considered important, but since then, the number of reports of fungicide tolerance has risen sharply and the problem has been the subject of numerous reviews (2,3,4,8). Although strategies of using mixtures or alternating fungicides with different modes of action have been studied on a theoretical basis (4,10,19), most authors stress the need for assessing the potential for tolerance in the laboratory and monitoring for its appearance in the field (2,3,8).

Control of white rot of *Allium* spp. caused by *Sclerotium cepivorum* Berk. using the dicarboximide group of fungicides (notably iprodione and vinclozolin) has been reported from various parts of the world (7,14,22). Although these fungicides show real promise for control of this disease, they also have a propensity for selecting tolerant strains of other pathogenic fungi (1,13,18). The purpose of this study was to assess the potential for development of tolerance to dicarboximide fungicides in *S. cepivorum* and to evaluate the in vitro sensitivity of different isolates of *S. cepivorum* to dicarboximide and other fungicides.

MATERIALS AND METHODS

All concentrations of fungicides in this report refer to active ingredients only. All fungicides were added as aqueous suspensions to cooled, autoclaved Difco potato-dextrose agar (PDA). Fourteen isolates of *S. cepivorum* were tested for tolerance to dicarboximide fungicides by sprinkling 100–500 sclerotia of each source (S) isolate onto PDA containing 100 μg/ml of either iprodione (Rovral 50W) or vinclozolin (Ronilan 50W), using two plates per isolate. Mycelium from germinating sclerotia was transferred to unamended PDA. Mycelial plugs (5 mm in diameter) from the subcultures were placed on PDA amended with 100 μg/ml of the same chemical from which the subculture was originally obtained. Those showing radial growth exceeding 15-mm total colony diameter after 4 days of incubation at 22–24 C were transferred onto unamended PDA. These subcultures were subjected to at least 10 transfers (each lasting at least 10 days) on unamended PDA, then transferred onto PDA containing 100 μg/ml iprodione or vinclozolin, as appropriate, to test for stability of tolerance. Those showing radial growth exceeding 15 mm within 4 days at 22–24 C were designated as tolerant (T) isolates.

All S and T isolates were tested to determine their levels of sensitivity to iprodione, vinclozolin, myclozolin (BCI-100F 50W), dicloran (Botran 75W), PCNB (Terraclor 75W), benomyl (Benlate 50W), captan (Orthocide 50W), and thiram (Arasan 75W), each at 100, 10, 1, 0.1, and 0.01 μg/ml in PDA. Any isolates that grew at 100 μg/ml of any active ingredient were also tested at 1,000 μg/ml of that chemical. Radial growth was recorded after 4 days of incubation at 22–24 C. The four parent S isolates (those that yielded T isolates) were tested on vinclozolin-amended PDA at 0.02, 0.04, 0.06, 0.08, 0.10, 0.30, 0.50, 0.70, 0.90, and 1.10 μg/ml with 5-mm-diameter plugs for 4 days as described before. They were also tested on iprodione-amended PDA at 0.06, 0.08, 0.10, 0.30, 0.50, and 0.70 μg/ml as before. Appropriate data transformation was determined using the P6D program of BMDP (5), and transformed data were subjected to linear regression by the P7D program of BMDP (5). Significance and r^2 values were derived by the method given by Zar (23). EC_{90} values (concentration that inhibits growth by 90%) were obtained from the regression equations. Where growth occurred at 1,000 μg/ml, the EC_{90} value was recorded as >1,000 μg/ml.

The frequency of occurrence of tolerance in the four parent S isolates was estimated in populations of sclerotia produced on PDA and collected after the medium had completely dehydrated. Sclerotia were scattered on PDA plates amended with 100 μg/ml of vinclozolin or iprodione, counted, incubated for 14 days at 22–24 C, and the resulting colonies counted. Identity of the colonies was confirmed by plating onto unamended PDA. Viability of the sclerotia was estimated by plating them onto unamended PDA and counting germination over 14 days. Each viability test was replicated at least 19 times for each isolate.

RESULTS

Five colonies able to grow on PDA amended with either 100 μg/ml vinclozolin (four colonies) or iprodione (one colony) were recovered from four of the 14 S isolates tested. These five isolates all grew to more than 15 mm in diameter within 4 days on 100 μg/ml vinclozolin or iprodione, as appropriate, when retested after passage through unamended PDA. After 10 or more subsequent transfers on unamended PDA, all five isolates grew well on vinclozolin- or iprodione-amended PDA and were designated as T isolates. None of the parent S isolates grew on these media.

Cross-tolerance to iprodione was observed for the T isolates recovered from vinclozolin-amended PDA and vice versa. At this point, the study of cross-tolerance was enlarged to include another dicarboximide fungicide, myclozolin; two other structurally related fungicides, dicloran and PCNB; and three unrelated fungicides, benomyl, thiram, and captan (Table 1).

One of the four T isolates obtained originally from vinclozolin-amended

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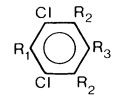
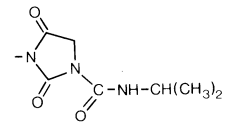
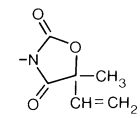
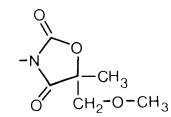
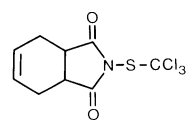
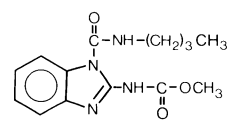
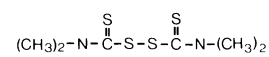
Table 1. Structural relationships among eight fungicides tested for toxicity to *Sclerotium cepivorum*

Fungicides containing the structural subunit	 N-substituted 3,5-dichlorophenyl		
	R_1	R_2	R_3
PCNB	−Cl	−Cl	
Dicloran	$-NH_2$	−H	
Iprodione	−H	−H	
Vinclozolin	−H	−H	
Myclozolin	−H	−H	
Fungicides structurally unrelated			
Captan			
Benomyl			
Thiram			

PDA (J191V) and the single isolate obtained from iprodione-amended PDA (VDMI) showed complete cross-tolerance to all five fungicides containing the common N-substituted dichlorophenyl structural subunit (Table 2). Mycelial growth of these two isolates occurred on media amended with 1,000 μg/ml of vinclozolin, iprodione, myclozolin, dicloran, or PCNB. The remaining three isolates originally recovered from vinclozolin-amended PDA also grew on PDA containing 1,000 μg/ml of dicloran or PCNB. They were less tolerant to iprodione and myclozolin, but were nevertheless more tolerant to these fungicides than were their respective parent S isolates (Table 2). Isolate S187bVa reverted to sensitivity comparable to that of its parent S isolate S187b after 25 transfers on unamended PDA.

The tolerances of the five T isolates to benomyl were completely unaltered from those of the parent S isolates (Table 2). Tolerances of four of the five T isolates to thiram were increased 10-fold over the tolerances of their respective S isolate parents and tolerance of the remaining T isolate (S187bV) decreased 10-fold (Table 2). Tolerances of T and parent S isolates to captan remained the same for three of the five T isolates and increased 10-fold over those of parent S isolates for the remaining two T isolates.

The 14 S isolates were highly sensitive to vinclozolin, iprodione, myclozolin, dicloran, and benomyl and highly tolerant to captan. Marked variations (10,000-fold differences) in the sensitivity of S isolates to PCNB were observed, and substantial variation in sensitivity to thiram (100-fold differences) was also noted (Table 2).

With one exception, EC_{90} values for vinclozolin and iprodione against the

Table 2. Highest concentrations (μg/ml) of various fungicides in potato-dextrose agar allowing detectable growth of various isolates of *Sclerotium cepivorum*

Isolate	Source	Vinclozolin	Iprodione	Myclozolin	Dichloran	PCNB	Benomyl	Thiram	Captan
				Source isolates					
J191	Hull, U.K.	0.1	0.1	0.1	0.1	0.01	1.0	10	1,000
NZ32	Aukland, New Zealand	1.0	1.0	0.1	0.1	0.01	1.0	100	1,000
BBY	Burnaby, BC	0.1	0.1	0.1	0.1	0.01	1.0	1,000	1,000
ELS	Fairview, Alberta	0.1	0.1	0.1	0.1	0.01	1.0	1,000	1,000
GF	Grand Forks, BC	1.0	1.0	0.1	0.1	0.01	1.0	1,000	1,000
NZ37	Aukland, New Zealand	0.1	0.1	0.1	0.1	1.00	1.0	10	1,000
S187a	Australia	0.1	0.1	0.1	0.1	100.00	1.0	10	1,000
S197a	Australia	0.1	0.1	0.1	0.1	100.00	1.0	10	1,000
S197b	Australia	0.1	0.1	0.1	0.1	100.00	1.0	10	1,000
S201a	Australia	0.1	0.1	0.1	0.1	100.00	1.0	10	1,000
J192	Hull, U.K.	0.1	0.1	0.1	0.1	100.00	1.0	100	1,000
VDM	Wageningen, Holland	0.1	0.1	0.1	0.1	100.00	0.1	100	1,000
S187b	Australia	1.0	0.1	0.1	0.1	100.00	1.0	100	1,000
S201b	Australia	0.1	0.1	0.1	0.1	100.00	1.0	100	1,000
				Tolerant isolates[a]					
S201bV	S201b	1,000.0	1.0	10.0	1,000.0	1,000.00	1.0	1,000	1,000
S187bVa[a]	S187b	100.0	10.0	10.0	1,000.0	1,000.00	1.0	1,000	1,000
S187bV	S187b	1,000.0	1,000.0	10.0	1,000.0	1,000.00	1.0	10	1,000
J191V	J191	1,000.0	1,000.0	1,000.0	1,000.0	1,000.00	1.0	100	1,000
VDMI	VDM	1,000.0	1,000.0	1,000.0	1,000.0	1,000.00	0.1	1,000	1,000

[a] Reverted to sensitivity after 25 transfers on unamended PDA.

four stable T isolates of *S. cepivorum* were >1,000 μg/ml (Table 3). A determination of a precise EC_{90} value for iprodione against T isolate S201bV was inadvertently omitted. Data in Table 2 show that S201bV was substantially more sensitive to iprodione than the other T isolates, and that the EC_{90} value for iprodione against this isolate was between 1 and 10 μg/ml. The EC_{90} values for vinclozolin and iprodione against the four parent S isolates were remarkably similar and ranged from 0.35 to 0.52 μg a.i./ml in PDA.

Even though almost 85,000 sclerotia were screened, the numbers of tolerant sclerotia detected were extremely low and variable. This precluded the calculation of a precise and reliable frequency estimate. Despite this, the four parent S isolates showed similar ranking of frequencies on either iprodione- or vinclozolin-amended PDA (Table 4). Isolates VDM, S187b, S201b and J191 yielded 1.90×10^{-1}, 4.8×10^{-2}, 2.7×10^{-2}, and 1.8×10^{-2}% tolerant sclerotia, respectively, averaged over both fungicides.

Table 3. Comparative[a] EC_{90} values for vinclozolin and iprodione against radial growth of tolerant (T) and source (S) isolates of *Sclerotium cepivorum* on fungicide-amended potato-dextrose agar

Isolate	Status	EC_{90} (μg/ml)	Isolate	Status	EC_{90} (μg/ml)
		Vinclozolin			
S187b	S	0.39	S187bV	T	>1,000
S201b	S	0.38	S201bV	T	>1,000
J191	S	0.38	J191V	T	>1,000
VDM	S	0.43	VDMI	T	>1,000
		Iprodione			
S187b	S	0.52	S187bV	T	>1,000
S201b	S	0.35	S201bV	T	>10[b]
J191	S	0.44	J191	T	>1,000
VDM	S	0.40	VDMI	T	>1,000

[a] $r^2 > 0.933$ For all regressions; $P \leq 0.025$.
[b] Precise value not determined.

Table 4. Frequency of occurrence of tolerant sclerotia of *Sclerotium cepivorum*

	Isolate			
	VDM	**S187b**	**S201b**	**J191**
Vinclozolin				
Replicates	10	12	15	14
No. tested	9,828	9,040	14,497	15,059
No. germinated	21	6	4	4
Mean frequency (%)	0.214	0.066	0.028	0.027
Iprodione				
Replicates	7	9	10	11
No. tested	4,877	7,466	11,643	12,084
No. germinated	7	2	3	1
Mean frequency (%)	0.143	0.027	0.026	0.008
Untreated				
Replicates	21	23	25	19
Mean germination (%)	88.1	70.7	89.0	96.1

DISCUSSION

It is clear that *S. cepivorum* has substantial biological potential for developing tolerance to the dicarboximide fungicides. Our data show that even at or above the limits of solubility for vinclozolin or iprodione (20), significant growth of the T isolates occurs, even though this represents concentrations >1,000-fold higher than the EC_{90} values for these chemicals of the parent S isolates. The phenomenon of cross-tolerance among the dicarboximides and dicloran and PCNB fungicides noted by other authors for other organisms (8,11,13) clearly occurs in *S. cepivorum*.

Although the mode of action of the dicarboximides is still unclear (6,15,16), the widespread occurrence of cross-tolerance within this group (1,11, 13,17,18,21) and between the dicarboximides and the other fungicides containing the N-substituted 3,5-dichlorophenyl structural subunit (8,11,13) indicates that tolerance to these chemicals may have its basis in this structural feature they share (Table 1). Note that although dicloran would properly be called an N-substituted 2,6-dichlorophenyl compound because of the nomenclatural primacy of the amine group at position R1, its structure fits the same basic pattern as the rest of the N-substituted 3,5-dichlorophenyl compounds shown in Table 1. In the interest of simplicity, we have grouped it as an N-substituted 3,5-dichlorophenyl compound for this discussion, realizing that this is not strictly correct. This cross-tolerance is of particular interest in the control of onion white rot because tolerance to dicloran has been reported for this pathogen (12).

Benomyl appears to be a good candidate for use in a mixed or alternating chemical approach to control of onion white rot because it was the only fungicide tested to which sensitivity remained unchanged between the T and S isolates, and all isolates were sensitive to low concentrations (Table 1). This lack of correlation in tolerance between benomyl and the dicarboximides and N-substituted 3,5-dichlorophenyl-containing fungicides has been noted in other organisms (6,21), although multiple resistance is possible (21).

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