Relative Fitness of Benzimidazole- and Cadmium-tolerant Populations of Sclerotinia homoeocarpa in the Absence and Presence of Fungicides

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ABSTRACT

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To evaluate the relative potential for survival of fungicide-tolerant isolates of *Sclerotinia homoeocarpa* in nature, equal populations of tolerant and sensitive isolates were introduced into a disease-free, geographically isolated turf plot area in the fall of 1973 and the populations were allowed to increase naturally. Dollar spot leaf lesions were sampled from infection centers monthly from the following July through November, and 1,411 isolates obtained subsequently were tested for fungicide tolerance on cadmium succinate- and benomyl-amended potato-dextrose agar. In the absence of fungicides, benomyl-tolerant isolates dropped to a small percentage of the total *S. homoeocarpa* population, whereas sensitive and cadmium succinate tolerant isolates increased to approximately equal high frequencies. In 1975, the plot again was sampled on a regular basis. In August 1975, when infection centers were very numerous, the area was sprayed with the fungicides, cadmium succinate and benomyl, in an alternating criss-cross pattern to exert a selection pressure on the extant fungus population. In the presence of selection pressure from fungicide, the population was predominantly benomyl-tolerant in areas where benomyl was applied, and predominantly cadmium-tolerant where cadmium was applied. Six weeks after the fungicide applications were stopped, the benomyl-tolerant population rapidly decreased, whereas the cadmium-tolerant population remained high. These results indicate that even though benomyl-tolerant populations were low in the absence of benomyl, the application of benomyl resulted in a rapid resurgence of benomyl-tolerant population. With cadmium-tolerant isolates, the use of cadmium fungicides resulted in an almost complete predominance of the cadmium-tolerant population, which persisted even when cadmium sprays were curtailed.

Additional key works: turfgrass, dollar spot.

Results have been published regarding the relationship between the tolerance of plant pathogenic fungi to benzimidazole fungicides and fungal pathogenicity (1, 2, 3, 5, 9, 14, 15, 16, 18, 20). In some studies, tolerance of the isolates was induced artificially (1, 2), and these tolerant strains often were nonpathogenic or less virulent than their wild-type parents. In other studies, the isolates were naturally tolerant (3, 9, 14, 15, 16, 20) and virulence was equal to or greater than that of the corresponding natural strains which are normally sensitive to the fungicide. In these instances, the tolerant strains were isolated from infected plants after fungicide failures were noted.

Wolfe (18, 19) concluded that virulence and fungicide-tolerance of isolates is important, but laboratory and greenhouse evaluations of these qualities do not always provide sufficient information for evaluation of the survival capability of tolerant isolates under field conditions.

Few investigators have evaluated the survival potential and pathogenicity of fungicide-tolerant isolates under field conditions. MacKenzie (10, 11) reported that tolerance to cadmium compounds and cycloheximide (Actidione® Upjohn Co., Kalamazoo, MI 49001) existed in the natural population of *Cochliobolus carbonum*. However, when corn was inoculated with isolates tolerant and sensitive to both compounds and plot areas were sprayed with cadmium or cycloheximide, the sensitive isolate predominated despite selection pressure by the fungicide (12). In contrast, Lambert (9) showed that a benomyl-tolerant isolate of *Verticillium malthousei* predominated when benomyl was used for control of Verticillium spot in mushrooms. The proportion of the fungicide-tolerant fungus strain within the population tended to increase in the presence of fungicide.

The two major factors which affect fungal population composition appear to be fungicide selection pressure favoring fungicide-tolerant strains and the potential for survival of the tolerant strain in the presence or absence of fungicide selection pressure. Studies are lacking on the population trends of tolerant and nontolerant strains in the presence and absence of systemic fungicides with highly specific modes of action (4) which are widely used in turfgrass disease control.

Tolerance, both individual and combined, to cadmium and to the benzimidazole fungicides has been detected in populations of *Sclerotinia homoeocarpa* Bennett (7, 16), the causal agent of dollarspot disease of turfgrasses. This study is an assessment of population fluctuations over time of cadmium succinate- and benzimidazole-tolerant isolates of *S. homoeocarpa* in the presence and absence of fungicide selection pressure.

METHODS AND MATERIALS

The relative survival potential of fungicide-tolerant and nontolerant populations was evaluated in a geographically isolated area at the Joseph Valentine Turfgrass Research Center, University Park, PA 16802. This area was seeded in the fall of 1972 with a mixture of 60% Kentucky bluegrass (*Poa pratensis* L. 'Merion'), 10% Chewings fescue (*Festuca rubra* var. *commutata* Gaud.), and 30% perennial ryegrass (*Lolium perenne* L.). Grass was maintained throughout the experiment by use of standard golf fairway management practices. At the start of the experiment, the turfgrass exhibited no symptoms of dollar spot disease and none were observed during the 1973 growing season.

In the fall of 1973, four fungal isolates (S-9, sensitive to both cadmium and benomyl; S-24, cadmium succinate-tolerant; S-50, benomyl-tolerant; and S-62, both cadmium succinate- and benomyl-tolerant) were selected, based on data from a previous laboratory and greenhouse study (16). Isolates of *S. homoeocarpa* tolerant to cadmium succinate have been shown to be tolerant to all cadmium fungicides (H. Cole, *unpublished*) thus, the cadmium succinate-tolerant isolate will be designated as cadmium-tolerant in all subsequent references. Isolates similar in virulence and symptom production were selected. Inoculum was prepared by growing the fungus on autoclaved rye grain. Kentucky bluegrass (*Poa pratensis* L. 'Merion') growing in 10-cm diameter pots was inoculated when 8 wk old by placing a few kernels of infested rye grain on the foliage in the center of each pot. The pots of grass then were placed beneath individual transparent plastic covers and incubated for 5 days in the greenhouse under partial shade. Temperatures beneath the covers were 24-30 C.

In late September 1973, 50 pots of grass with equal amounts of disease incited by each of the four isolates were transplanted into the field test plot area, and allowed to overwinter. The location and isolate identification of each "plug" was recorded (Fig. 1).

The *S. homoeocarpa* population was monitored during 1974 by dividing the field into 504 (1.2 × 1.8 m) sampling plots (Fig. 1). The first sampling was made in July 1974, by collecting newly diseased leaves immediately adjacent to the original inoculum "plug" in the 200 inoculated

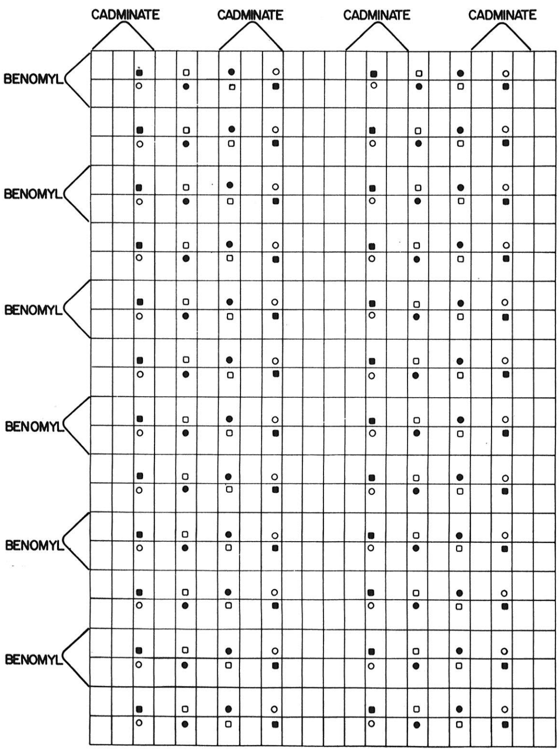

Fig. 1. Field plan (27 m × 44 m) of inoculation with *Sclerotinia homoeocarpa* and fungicide spray pattern:
Legend: o = Benomyl/cadmium-sensitive isolate S-9; ● = Benomyl-tolerant isolate S-50; □ = Cadmium-tolerant isolate S-24; and ■ = Cadmium/benomyl-tolerant isolate S-62.

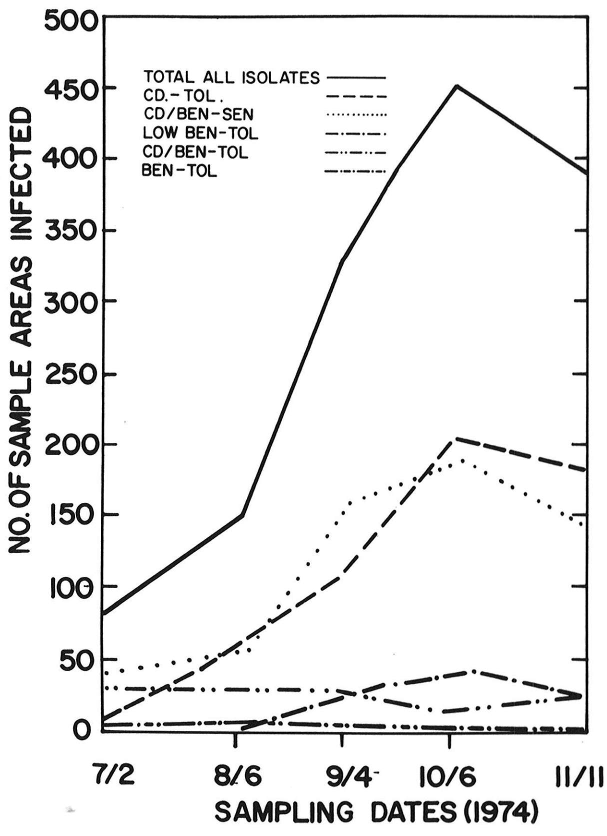

Fig. 2. Disease development and spread in 1974 as measured by *Sclerotinia homoeocarpa* isolated from 504 sampling plots (1.2 m × 1.8 m) in the absence of fungicide selection pressure.

plots. This sampling regime was designed to provide a measure of capability of each isolate for overwinter survival. In addition, each noninoculated plot was sampled randomly by taking leaves from infection centers or from single leaves that contained bleached, straw-colored lesions suggestive of dollar spot symptoms. A total of 504 samples (one from each plot) was taken each month from July through November, and cultured for *S. homoeocarpa* on acidified Difco potato-dextrose agar (PDA). The isolates were tested for fungicide tolerance on cadmium succinate- and benomyl-amended PDA (16). The location and tolerance of each recovered isolate was recorded to determine the population dynamics of the introduced isolates within the plot area.

In June and July 1975, the area was sampled again in a manner similar to the 1974 sampling. This was done to determine whether the population had stabilized. Beginning in July 1975, when infection centers were very numerous, the plot area was sprayed with fungicides to exert a selection pressure on the extant population. On 24 July, 1 August, and 13 August, cadmium succinate [60%, wettable powder (wp)] and benomyl (methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate) (50 wp) were sprayed on the plot with a boom sprayer at the rate of 28.2 g of formulated product per 93 centares in 8 liters of water. The plot was sprayed in a 3.7 m-wide criss-cross strip pattern, with alternating untreated checks (Fig. 1). The plot was sampled 2 and 6 wk after the last fungicide application to determine the effect of fungicide selection pressure and the stability of such effect.

RESULTS

Prior to fungicide application, 1,411 isolates of *Sclerotinia homoeocarpa* were obtained from 2,500 samples collected during 1974. During July of 1974, when the first samples were taken adjacent to the 200 original inoculation sites (50 per isolate), fungicide-sensitive isolates were recovered from 37 (76%) of the sites inoculated with this isolate and the cadmium benomyl-tolerant isolate was recovered from 29 (58%) of the sites where it had been introduced. Benomyl-tolerant and cadmium-tolerant isolates were recovered from 5 (10%) and 7 (14%), respectively, of their original inoculation sites. The pathogen was recovered from only two of the 304 noninoculated plots. Soon after, however, the pathogen began to spread. When the total number of monthly isolates were plotted against time (Fig. 2), disease development, as measured by number of successful isolations, followed a sigmoid curve. The populations of sensitive and cadmium-tolerant isolates, when plotted against time, resulted in a similar curve. After the initial overwinter drop, the benomyl-tolerant and cadmium/benomyl-tolerant populations remained at approximately the same low proportion of the total population throughout the ensuing summer and fall.

Viewing the data from sampling prior to fungicide application as percent of total population (Table 1), the individual components of the *S. homoeocarpa* population in the plot appeared to have stabilized by the fall of 1974. At this time, the originally introduced benomyl-tolerant and cadmium/benomyl-tolerant isolates comprised a very small percentage of the total population, whereas, sensitive and cadmium-tolerant isolates occurred at approximately equal high frequencies. Sampling in June and July of the following year (1975), before the application of fungicides, indicated that the individual populations continued over winter and into the next growing season in a relatively stable state.

In the August sampling in 1974, an isolate which was determined to be tolerant to a low level of benomyl was recovered from the plot. In laboratory tests, 10 μg benomyl/ml PDA were required to completely inhibit its growth, whereas sensitive isolates were completely inhibited by 1 μg/ml, and the introduced benomyl-tolerant isolate required 500 μg/ml for complete growth suppression. This isolate increased in frequency and stabilized at approximately the same level as the benomyl-tolerant and cadmium/benomyl-tolerant isolates that had been intentionally introduced (Fig. 2). These two benomyl-tolerant isolates were considered together in tabulations of the fungicide spray experiment (Table 2).

Two wk after the last application of fungicides, the populations had shifted to predominantly benomyl-tolerant isolates (93%) in areas where benomyl had been applied, and to cadmium-tolerant isolates (96%) where cadminate had been applied (Table 2). In the cadminate-benomyl sprayed areas, cadmium-tolerant and benomyl-tolerant isolates were recovered in approximately equal frequency, 49% and 41%,respectively. Areas treated with both cadminate and benomyl were the only ones from which the cadmium/benomyl-tolerant strain (10%) was isolated.

The plot again was sampled 6 wk after the last fungicide application (Table 2). The population in the benomyl-sprayed areas already had begun to shift from

TABLE 1. Population composition of isolates of *Sclerotinia homoeocarpa* recovered from 504 monthly samples in the absence of fungicide selection pressure over the period from July 1974 to July 1975

Isolate chemical resistance status	Population composition (%) during:						
	7/74	8/74	9/74	10/74	11/74	6/75	7/75
Cadmium/benomyl-sensitive	48	39	46	41	38	47	39
Benomyl-tolerant	6	3	1	1	1	1	1
Cadmium-tolerant	9	39	36	46	49	45	51
Cadmium/benomyl-tolerant	37	18	9	2	6	1	1
Low benomyl-tolerant	...	1	8	10	6	6	8
Total isolates recovered (no.)	79	152	331	457	392	392	457

TABLE 2. Population of *Sclerotinia homoeocarpa* isolates from different fungicide treatments, 2 and 6 wk after the last fungicide application

	Population 2 and 6 wk after the last fungicide application							
	Benomyl-sprayed area		Cadmium-sprayed area		Cd/benomyl-sprayed area		Unsprayed control area	
Isolate chemical resistance status	2 wk (%)	6 wk (%)	2 wk (%)	6 wk (%)	2 wk (%)	6 wk (%)	2 wk (%)	6 wk (%)
Benomyl-tolerant	93	55	2	7	41	20	30	27
Cadmium-tolerant	5	36	96	91	49	75	50	55
Cadmium/benomyl-tolerant	0	1	0	1	10	1	0	2
Cadmium/benomyl-sensitive	2	8	2	1	0	4	20	16
Isolates recovered (no.)	73	108	146	137	41	118	128	135

predominately benomyl-tolerant isolates toward cadmium-tolerant and sensitive strains. Frequency of recovery of the benomyl-tolerant strain had dropped from 93% at 2 wk after the last fungicide application, to 55% after 6 wk (Table 2). This relative decrease in the frequency of recovery of benomyl-tolerant strains resulted from a marked increase in the number of cadmium-tolerant and -sensitive strains recovered, rather than from a drop in the number of benomyl-tolerant strains. In the areas sprayed with cadminate, the number of cadmium-tolerant isolates recovered remained relatively constant at 2 and 6 wk after the last fungicide spray. In the cadminate/benomyl-sprayed areas, recovery of the benomyl-tolerant strain dropped from 41% to 20% and the cadmium/benomyl-tolerant strain dropped from 10% to 1%, whereas, recovery of the cadmium-tolerant and -sensitive strains increased. The individual populations in the unsprayed control areas had remained stable.

DISCUSSION

The data show that the benomyl-tolerant strains of *S. homoeocarpa* did not become dominant over other populations in the absence of fungicide selection pressure, but did so in the presence of benomyl application. In contrast, the cadmium-tolerant strains did become the predominate isolate in the presence and absence of cadminate. Further evidence for its fitness for survival is the fact that the predominant strain of *S. homoeocarpa* for the past 7 yr at the Joseph Valentine Turfgrass Research Center [approximately 2.83 hectares (7 acres) of turfgrass] has been a cadmium-tolerant strain which was introduced into a small plot area in 1967 (13). This isolate has spread rapidly over the entire area occupied by the Center.

Wolfe (18, 19) theorized that a strain's competitive ability is the major factor in determining whether it becomes dominant in the population and suggested that, after the application of a fungicide, the effectiveness of the compound gradually declines and critical competition occurs within the pathogen population. Regardless of whether Wolfe's competition theory is accepted, it does seem clear that rapid change in the strain composition of the *S. homoeocarpa* population did occur soon after fungicide treatment. If the tolerant strains that are favored by fungicide application are more fit to survive, they may become persistently dominant, or, at least, permanently increase in frequency in the population. The cadmium-tolerant isolate included in this investigation exhibited such behavior. If strains are not fit, they may quickly decline to their original frequency until the fungicide is used again. Both the naturally and artificially introduced benomyl-tolerant strains in the study exhibited lack of fitness. The use of fungicides to which tolerance may occur exerts a high and continuous selection pressure favoring the proliferation of the tolerant population.

It is important to determine whether discontinuing the use of a fungicide to which tolerance has developed in a fungus will reduce the tolerant fungal population to a level low enough to allow reuse of the fungicide. In partial answer to this question, the results obtained in experiments with insects that have become tolerant to organic insecticides may apply (6, 8). In these studies, tolerant individuals persisted for long periods, at varying levels in the absence of selection pressure, and with resumption of the use of the selective compounds, they reappeared in increased frequency in the population. This indicates that tolerance, at least to insecticides, is persistent in populations. Results from this study and work done by Lambert (9), suggest strongly that the same is true for fungicide tolerance. The level at which tolerant individuals persist in populations in the absence of pesticide selection pressure varies with the tolerant species and the pesticide. In contrast to the results from this study, Wicks (17) has found a high incidence of benomyl-tolerant individuals persisting in a population of *Venturia inaequalis* in an orchard 2 yr following removal of the benomyl selection pressure. Continuing field studies are needed to determine the long-term effects of various fungicide regimes on fungicide-tolerant fungal populations. Increased knowledge of pathogen variation and population dynamics, coupled with epidemiological studies and monitoring for fungicide tolerance are needed to determine the epidemiological importance of newly-appearing tolerant strains.

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