Reduced-Rate Fungicide Mixtures to Delay Fungicide Resistance and to Control Selected Turfgrass Diseases

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ABSTRACT

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Metalaxyl was applied singly, in alternation, or in combination with mancozeb or propamocarb to greenhouse-grown perennial ryegrass inoculated with populations of *Pythium aphanidermatum* having known proportions of metalaxyl-resistant (R):metalaxyl-sensitive (S) individuals. The populations were cycled repeatedly through inoculation, fungicide application, incubation, and harvest/assay. In populations with R:S ratios of 1:10 and 1:1,000, reduced-rate mixtures of metalaxyl with mancozeb or propamocarb were most effective in delaying increases in the R proportion. Half-rate mixtures of metalaxyl with propamocarb, mancozeb, or fosetyl Al provided excellent control of Pythium blight (*P. aphanidermatum*) on field-grown perennial ryegrass. Excellent suppression of dollar spot (*Sclerotinia homoeocarpa*) on field-grown creeping bentgrass was provided by half-rate, two-component mixtures of benzimidazoles, dicarboximides, and sterol biosynthesis inhibitors.

Fungicide resistance in target pathogen populations is a continuing problem associated with the use of systemic fungicides. The broad-spectrum systemic fungicides presently in use include benzimidazoles, dicarboximides, and ergosterol biosynthesis inhibitors. Within these groups, there is a common mode of action and cross-resistance among the chemicals within each group (6). Disease control failures caused by benzimidazole resistance began shortly after intensive use of the fungicides started, and by 1976, Dekker (5) listed 37 reports of benzimidazole resistance in various pathogens on various crops. Dicarboximide control failures have been reported with *Botrytis cinerea* on cucumber, tomatoes, strawberries, and roses (14) and with *Sclerotinia homoeocarpa* (7) and *Fusarium nivale* (3) on turfgrasses. Wolfe (22) has reported insensitivity to the ergosterol biosynthesis inhibitor triadimefon in field populations of *Erysiphe graminis* f. sp. *hordei*. The narrow-spectrum acylalanines control diseases caused by species of *Pythium* and *Phytophthora*, and control failures are increasingly common with use of these fungicides (4,11,17,19). There is one report of induced resistance to the disease control agent fosetyl Al (1).

It has been suggested that alternations or mixtures of fungicides with different modes of action may delay or prevent control failures caused by fungicide resistance in pathogen populations (5,6,21). Observational evidence to support the use of fungicide mixtures for this purpose is cited by Delp (6) where benomyl resistance failed to develop in populations of *Botrytis* and *Cercospora* when the fungicide was applied in mixtures with captan or mancozeb. One of the earliest experimental studies with biocidal mixtures showed that the emergence of antibiotic resistance in populations of *Xanthomonas* and *Erwinia* was delayed with mixtures of streptomycin with terramycin (9). In a study with a mixed population of *Venturia inaequalis*, McGee and Zuck (15) reported that the benomyl-resistant proportion of the population increased when treated with benomyl alone or in alternation or combination with captan. The residual durations of these two fungicides are not well matched, since by the end of the 2-wk spray interval used, the efficacy of captan would have diminished, allowing selection pressure from the longer-residual benomyl. In a field comparison of a benomyl-sensitive and a benomyl-resistant isolate of *Cercospora beticola* (8), sprays of benomyl alone or in alternation or combination with triphenyltin hydroxide resulted in increases in the proportions of the resistant component. These two isolates, however, were not

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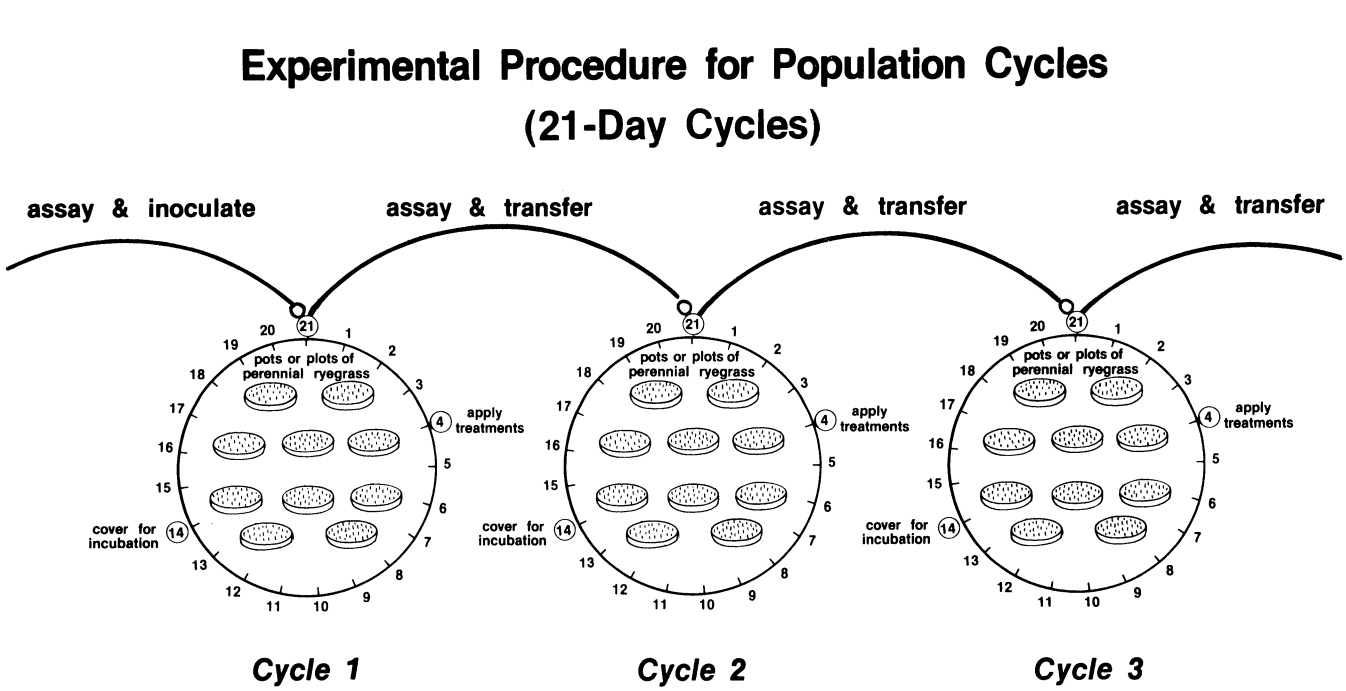

Fig. 1. Experimental procedure used in population cycling experiments.

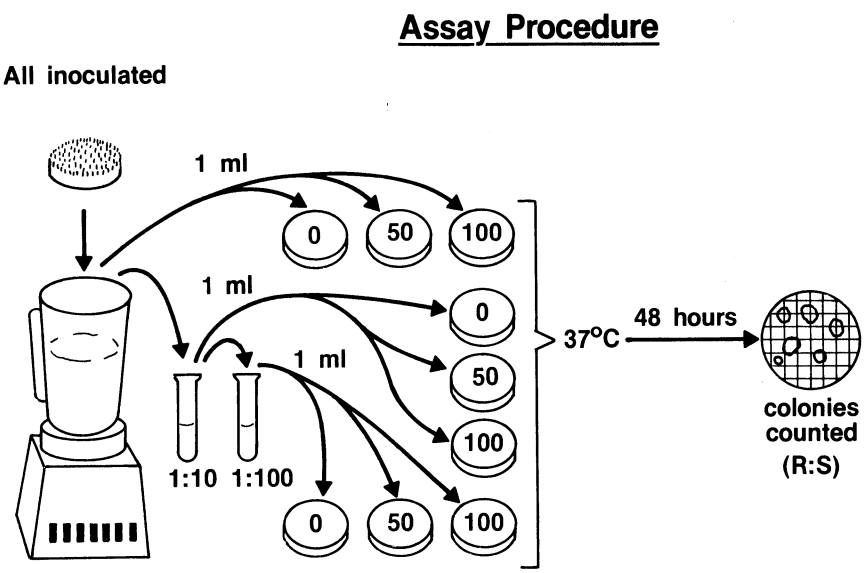

Fig. 2. Procedure for assaying *Pythium aphanidermatum* populations in population cycling experiments.

matched in fitness, since the resistant component also increased in the population treated with triphenyltin hydroxide alone and in the unsprayed control. In a growth-chamber study with a mixed population of *Septoria nodorum* on wheat seedlings, Horsten (12) reported that after nine passages, better disease control was provided by a half-rate mixture of carbendazim and edifenphos than with either chemical alone or a full-rate alternation of the two. Changes in the amount of leaf necrosis from passage to passage was used as a criterion for fungicide resistance. Valdes and Edgington (20) reported control failure caused by metalaxyl resistance in white rust (*Albugo candida*) on field-grown radish where metalaxyl was used alone. In contrast, season-long disease control was achieved in plots treated with metalaxyl in combination with mancozeb.

For fungicide mixtures to be of practical use for delaying or preventing disease control failures caused by fungicide resistance, such mixtures must also be economically feasible for commercial use. This requires the use of reduced-rate mixtures to avoid increased cost as well as unnecessary increases in amounts of pesticide applied. However, studies with reduced-rate mixtures are needed to demonstrate their efficacy. Jones and Dainello (13) have reported that a reduced-rate mixture of metalaxyl plus manganese ethylenebisdithiocarbamate was as effective in controlling *Albugo occidentalis* and *Peronospora effusa* on spinach as full rates of the individual compounds applied separately.

Our studies were undertaken to determine the effects of fungicide mixtures and alternations on the proportion of resistant propagules in populations of *Pythium aphanidermatum* and to determine the efficacy of half-rate fungicide mixtures in control of Pythium blight (*P. aphanidermatum*), large brown patch (*Rhizoctonia solani*), and dollar spot (*Sclerotinia homoeocarpa*) on field-grown turfgrasses.

MATERIALS AND METHODS

Population cycling experiments. These studies were designed to monitor the selection effects of various fungicide treatments on the frequency of the resistant component in experimental *P. aphanidermatum* populations. Perennial ryegrass was inoculated with *P. aphanidermatum* populations having known proportions of metalaxyl-resistant (R): metalaxyl-sensitive (S) individuals. Metalaxyl (Subdue 2E) was applied singly, in alternation, or in combination with mancozeb (Fore 80W) or propamocarb (Banol 6S). The populations were cycled repeatedly through inoculation, fungicide application, incubation, and harvest/assay as shown in Figure 1. Three separate cycling experiments were carried out, using *P. aphanidermatum* populations with 0, 0.1, and 10% R components.

Experiment 1. This study was carried out with a population that had no introduced R component. Six metalaxyl-sensitive isolates of *P. aphanidermatum* from turfgrass were grown separately on autoclaved rye grain and homogenized with sufficient sterile glass-distilled water to make a thin homogenate. Four 1-ml aliquots of this homogenate were removed and assayed as shown in Figure 2 on a modification (17) of a *P.*

aphanidermatum-selective medium described by Burr and Stanghellini (2). To partition the population into R and S components, the medium was used unamended and amended with 50 and 100 g of metalaxyl per milliliter.

The remaining homogenate was uniformly distributed over 70 pots of seedling, greenhouse-grown Pennfine perennial ryegrass. Four days after inoculum placement (Fig. 1), fungicide treatments were applied (rates given per 93 m^2) to 10 pots of grass per treatment as follows: 1) metalaxyl (M) at half rate (1/2M, 1.9 ml) applied singly; 2) M at full rate (3.7 ml) applied singly; 3) mancozeb (Ma) at full rate (227 g) applied in alternation with M (3.7 ml) as M,Ma,M, Ma; 4) 1/2M + 1/2Ma applied in combination (1.9 ml and 114 g, respectively); 5) Ma applied singly (227 g); and 6) untreated check.

These low rates of metalaxyl were necessary in the greenhouse because when the 1/2× and 1× field rates (14.8 and 29.6 ml of product per 93 m^2, respectively) were used, no disease developed in metalaxyl treatments after incubation. It was obvious that the greenhouse rates would have to be reduced or the cycle interval prolonged. Since the effect of either option is to reduce the level of metalaxyl in the grass to sublethal levels, the rate-reduction option was chosen in order to adhere to a 21-day cycle throughout. It was determined that at metalaxyl rates of 1.9 and 3.7 ml/93 m^2, disease breakthrough occurred in the greenhouse within the desired interval after fungicide application.

Ten days after fungicide application, all pots were covered with transparent plastic bags and incubated on a shaded greenhouse bench at 30–35 C. At the end of a 7-day incubation, the plastic covers were removed and grass foliage from each 10-pot treatment was pooled, homogenized in 750 ml of glass-distilled water, assayed (Fig. 2), and used to inoculate a new set of 10 pots of grass for each treatment. The population was cycled under the six treatment regimens through five 21-day cycles in the greenhouse as shown in Figure 1.

In early summer 1983, the six cycled populations were taken to the field, where five 21-day cycles were completed on plots of mature, field-grown Pennfine perennial ryegrass. In the field cycles, 1/2M and M rates used were 14.8 and 29.6 ml, respectively, of product per 93 m^2. Incubation environment in the field was provided by translucent-plastic-covered PVC-pipe-frame incubation chambers equipped with intermittent mist and an electric forced-hot-air heating system. At night, chamber ends were closed and no mist was applied. During the day, chamber ends were open and two 1-min mists were applied per hour. Supplemental heat was only used when needed to raise temperatures sufficiently for disease development. With these exceptions, field cycles were carried out in the same fashion as the greenhouse cycles (Fig. 1). At the end of the fifth field cycle, the *P. aphanidermatum* populations recovered from the six treatment regimens were returned to the greenhouse and six 21-day greenhouse cycles were completed, for a total of 16 cycles.

Experiment 2. In this study, eight population cycles were completed in the greenhouse as described. This experiment was begun with a 1:933 (R:S) *P. aphanidermatum* population, or a population with 0.1% R propagules of *P. aphanidermatum*. Inoculum to begin experiment 2 was obtained by inoculating 100 pots of seedling Pennfine perennial ryegrass with six S isolates of *P. aphanidermatum* as described. An additional 100 pots of seedling ryegrass were inoculated in the same manner with nine R isolates of *P. aphanidermatum* from a metalaxyl control failure location (17). Inoculated pots of grass were incubated under plastic covers as described. When all foliage was completely blighted, covers were removed and the blighted foliage was allowed to dry completely. Dried foliage from the two sets of pots was harvested separately and pulverized to a fine consistency by grinding dry in a Waring Blendor, then aliquots from both sets of inoculum were removed and assayed as described to determine the number of *P. aphanidermatum* propagules per gram dry weight. The test population (1R:933S) was obtained by mixing the two sets of dry inoculum in appropriate proportions. The R:S proportion was verified by assaying the prepared inoculum when it was applied to pots of grass to begin the first cycle of experiment 2. All other procedures were the same as those described for greenhouse cycles in experiment 1.

Experiment 3. Two greenhouse cycles were carried out as described previously. This experiment was begun with a *P. aphanidermatum* population having 1:8.5 R:S proportion or a 10% R component. The population was obtained by mixing the 15 previously described *P. aphanidermatum* isolates in required proportions. Three fungicide treatments, in addition to the six already described (for a total of nine treatments), were applied (rates given per 93 m^2) as follows: 1) M at full rate (3.7 ml) applied in alternation with propamocarb (P) at full rate (29.6 ml) as M,P,M,P; 2) 1/2M + 1/2P applied in combination (1.9 and 14.8 ml, respectively); and 3) P applied singly (29.6 ml).

Tests of half-rate fungicide mixtures for control of three turfgrass diseases. *Pythium blight.* Tests for control of Pythium blight were conducted in the summers of 1983 and 1984 on mature, field-grown Pennfine perennial ryegrass. Individual treatment plots were arranged in a randomized block design with three replicates. Fungicides (Tables 1 and 2) were applied with a CO_2-powered boom sprayer in water equivalent to 6 L/93 m^2. Two days after fungicide application, a 1-m-wide strip across all treatments was inoculated with a six-isolate pool of metalaxyl-sensitive *P. aphanidermatum* grown on autoclaved rye grain. The inoculated strip was immediately covered for incubation with the previously described incubation chambers. After 5–7 days of incubation, chambers were removed and the inoculated areas were visually rated, using a rating scale of 0–10, where 0 = no disease, 1 = 10% of plot area killed, and 10 = 100% of plot area killed. Data obtained were subjected to analysis of variance and Waller-Duncan *k*-ratio *t* test.

Dollar spot and large brown patch. Half-rate fungicide mixtures were tested for control of dollar spot and large brown patch in summer 1984 on mature, field-grown Penncross creeping bentgrass. Individual treatment plots were arranged in a randomized block design with three replicates. The experimental area was inoculated before fungicide application by hand-scattering *S. homoeocarpa*-infected rye grains over the entire test area at a density of 20–30/0.093 m^2. Fungicides (Table 3) were applied four times at 14-day intervals in water equivalent to 6.7 L/93 m^2, with the previously described equipment. Dollar

Table 1. Field control of Pythium blight with half-rate fungicide mixtures in 1983

Fungicide, formulation, and product rate/93 m^2	Disease severity (7 days)[a]
Check	7.3[b]
Metalaxyl (2E) 14.8 ml	2.7*[c]
Propamocarb (6S) 20.7 ml	2.0*
Metalaxyl (2E) 14.8 ml + propamocarb (6S) 20.7 ml	0.3*

[a] Days after fungicide application.
[b] Visual scale of 0–10, where 0 = no disease and 10 = complete infection of all grass in plot; mean of three replicates.
[c] * = Statistically different from the check.

Table 2. Field control of Pythium blight with half-rate fungicide mixtures in 1984

Fungicide, formulation, and product rate/93 m^2	Disease severity (9 days)[a]
Check	7.0[b]
Fosetyl Al (80W) 114 g	1.8*[c]
Mancozeb (80W) 114 g	0.7*
Metalaxyl (2E) 14.8 ml	0.5*
Metalaxyl (2E) 14.8 ml + fosetyl Al (80W) 114 g	0.5*
Metalaxyl (2E) 14.8 ml + mancozeb (80W) 114 g	0.3*

[a] Days after fungicide application.
[b] Visual scale of 0–10, where 0 = no disease and 10 = complete infection of all grass in plot; mean of three replicates.
[c] * = Statistically different from the check.

spot was evaluated visually 17 days after the last fungicide application by determining the number of infection centers per 0.093 m^2 of plot area. Brown patch determinations were made 21 days after the last application of fungicide using the previously described visual rating scale. Data obtained were subjected to analysis of variance and the Waller-Duncan *k*-ratio *t* test.

A second test of half-rate fungicide mixtures (Table 4) was conducted for control of dollar spot using the previously described host and methodology with the following exceptions: 1) Fungicides were applied once on a curative basis when there was 20–40% natural infection in the test area, and 2) disease severity was assessed 15 days after fungicide application, using the previously described visual rating scale.

Table 3. Field control of dollar spot and large brown patch with half-rate fungicide mixtures

Fungicide, formulation and product rate/93 m^2	Disease severity: Dollar spot (17 days)[a]	Disease severity: Brown patch (21 days)[a]
Check	12.1[b]	5.7[c]
Triadimefon (25DF) 14.2 g + anilazine (4F) 59 ml	0.0*[d]	0.5*
Triadimefon (25DF) 14.2 g + anilazine (4F) 124 ml	0.0*	1.8*
Iprodione (50W) 28.4 g + triadimefon (25DF) 14.2 g	0.0*	0.0*
Triadimefon (25DF) 14.2 g + benomyl (50W) 14.2 g	0.0*	0.0*
Vinclozolin (50W) 28.4 g + triadimefon (25DF) 14.2 g	0.0*	0.3*
Vinclozolin (50W) 28.4 g + benomyl (50W) 14.2 g	0.0*	0.2*
Triadimefon (25DF) 14.2 g	0.0*	1.3*
Anilazine (4F) 59 ml	3.0*	4.2*
Anilazine (4F) 124 ml	0.7*	1.0*
Benomyl (50W) 14.2 g	0.0*	0.3*
Iprodione (50W) 28.4 g	0.1*	0.2*
Vinclozolin (50W) 28.4 g	0.0*	0.0*

[a] Days after last fungicide application.
[b] Number of infection centers per 0.093 m^2; mean of three replicates.
[c] Visual scale of 0–10, where 0 = no disease and 10 = complete infection of all grass in plot; mean of three replicates.
[d] * = Statistically different from the check.

Table 4. Field control of dollar spot with half-rate fungicide mixtures

Fungicide, formulation, and product rate/93 m^2	Disease severity (15 days)[a]
Check	3.3[b]
Iprodione (50W) 28.4 g + fenarimol (50W) 4.3 g	1.7*[c]
Iprodione (50W) 28.4 g + prochloraz (40%EC) 47.4 ml	1.0*
Vinclozolin (50W) 14.2 g + prochloraz (40%EC) 47.4 ml	0.8*
Iprodione (50W) 28.4 g + propiconazole (1.1E) 14.8 ml	0.7*
Vinclozolin (50W) 14.2 g + fenarimol (50W) 4.3 g	0.7*
Benomyl (50W) 14.2 g + propiconazole (1.1E) 14.8 ml	0.7*
Benomyl (50W) 14.2 g + prochloraz (40%EC) 47.4 ml	0.3*
Vinclozolin (50W) 14.2 g + propiconazole (1.1E) 14.8 ml	0.0*
Iprodione (50W) 28.4 g + benomyl (50W) 14.2 g	0.0*

[a] Days after fungicide application.
[b] Visual scale of 0–10, where 0 = no disease and 10 = complete infection of all grass in plot; mean of three replicates.
[c] * = Statistically different from the check.

RESULTS AND DISCUSSION

Population cycling experiments. The population used in experiment 1 had no introduced R component and was cycled 16 times in field and greenhouse over a period of 18 mo. No R component was detected at any time in any treatment.

Population II (0.1% R) was cycled eight times in the greenhouse. Figure 3 shows the treatments under which R proportions changed. The population under treatment M was 100% resistant by cycle 3, under treatment 1/2M by cycle 5, and under treatment M,Ma,M,Ma by cycle 8. There was no detectable change in R proportion in the other three treatments.

Population III (10% R) was cycled only twice, but results were fairly definitive. Populations under treatments 1/2M; M; M,Ma,M,Ma; and M,P,M,P were 100% resistant by cycle 2, as shown in Figure 4. Populations under the remaining treatment regimens remained fairly stable, with the exception of the Ma treatment, which showed an increase in the R proportion.

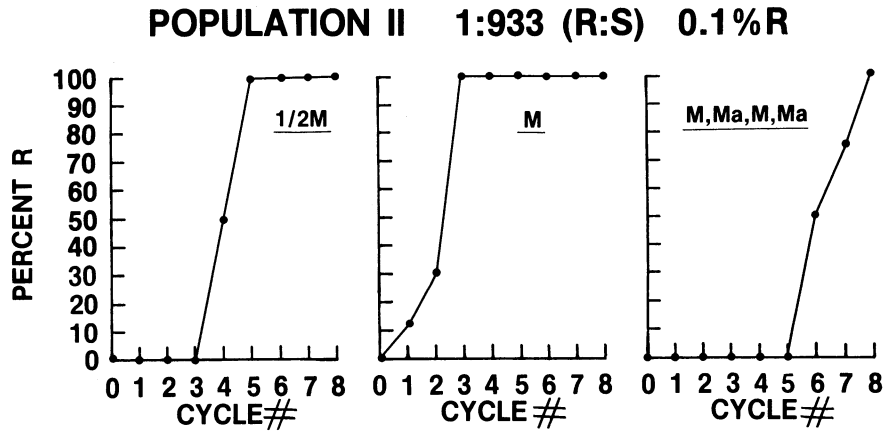

Fig. 3. Changes in resistant proportion in *Pythium aphanidermatum* populations in cycling experiment 2.

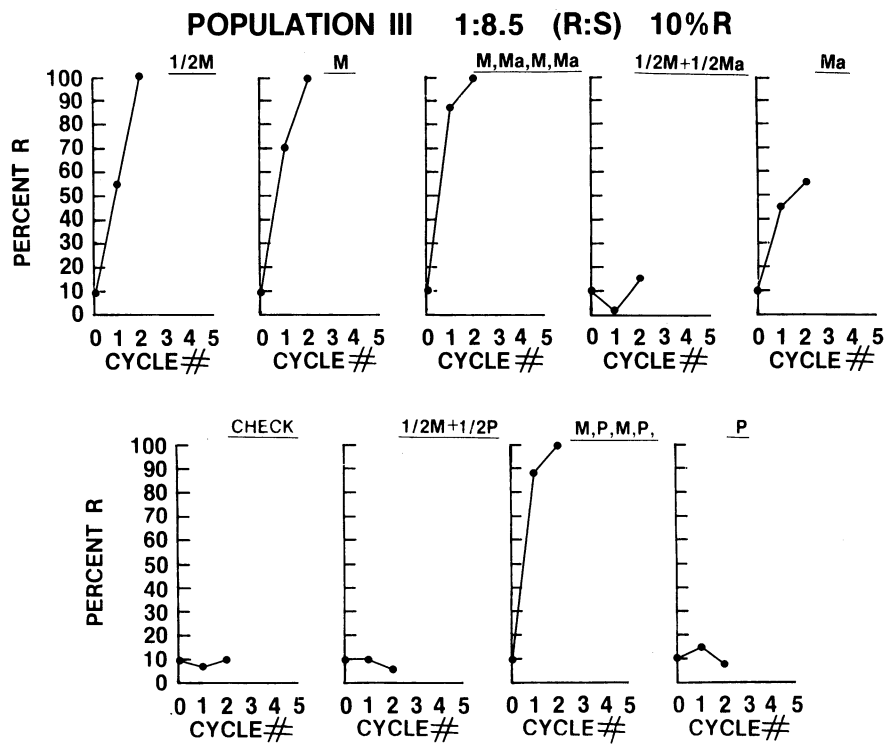

Fig. 4. Changes in resistant proportion in *Pythium aphanidermatum* populations in cycling experiment 3.

Since no detectable resistance developed in a sensitive *P. aphanidermatum* population after 16 cycles in the absence of an introduced R component (experiment 1), it appears that the genetic potential for resistance must be present in a population for selection by metalaxyl to high R proportion. For this reason, population cycling experiments for monitoring and predicting shifts in R proportion should make use of large numbers of isolates to incorporate as much genetic diversity as possible. Such experiments conducted with single paired R and S isolates are probably of very little value for predicting outcomes in field situations where large, diverse populations are under fungicide selection pressure.

The populations used in all three cycling experiments appear to be stable in the absence of fungicide selection pressure, since the untreated control populations remained stable throughout. It is important to use stable populations in such experiments, so that observed R changes can be associated with fungicide selection pressure rather than R and S population fitness factors. Within the experimental construct used for our experiments, R proportions increased to 100% only in populations treated with metalaxyl alone or in alternation with another chemical. This is not surprising, since whenever metalaxyl is applied alone, because it is in an alternating schedule, selection to increased R proportion is occurring.

The most promising spray protocols tested for delaying R increase in these experiments were the reduced-rate mixtures. There are several important considerations in selecting fungicides for use in reduced-rate mixtures. Only fungicides with different modes of action can be used in mixtures to delay or prevent control failures caused by fungicide resistance. The residual efficacies of the mixture components must be well matched to avoid directional selection to R. If a shorter-residual fungicide is included in a mixture for delaying resistance, an interspray of the short-residual component will probably be necessary.

The use pattern of fungicides and fungicide mixtures may also affect the speed with which fungicide resistance becomes a problem in the field. In our experiments, cycles were timed and fungicide rates selected so that the efficacy limits of the fungicides were reached during the incubation period, and disease breakthrough occurred. Such an experimental system allows the selected populations to increase and is analogous to a curative approach in the field, where fungicides are not applied until disease symptoms are noted. Preventive applications of fungicides may affect R:S population shifts differently than curative applications of the same fungicides.

The increased R proportion in populations treated with mancozeb alone was unexpected and unexplained but demonstrates the possibility of such unforeseen effects on R:S proportions in both experimental and natural fungal populations. Samoucha and Cohen (16) have reported differential sensitivity to mancozeb in metalaxyl-sensitive and metalaxyl-resistant isolates of *Pseudoperonospora cubensis*.

Sublethal fungicide rates were recently compared by Schein et al (18) with horizontal host resistance and were proposed as a strategy for avoiding fungicide resistance in pathogen populations. In our experiments, sublethal rates of metalaxyl alone were ineffective in avoiding the increase of the R component in the test populations.

Experiments with half-rate fungicide mixtures. Tables 1 and 2 show the excellent control of Pythium blight provided by the half-rate mixtures of metalaxyl with propamocarb, fosetyl Al, or mancozeb. The efficacies of these four fungicides, when applied alone, appear to be well matched at 7–9 days after application.

Tables 3 and 4 show the excellent control of dollar spot and large brown patch provided by the test mixtures. Although the mixtures of triadimefon at 14.2 g/93 m^2 with anilazine at 59 and 124 ml/93 m^2 gave good control of both diseases, data from areas treated with anilazine alone at 59 ml/93 m^2 indicate that the efficacies of these two fungicides are not well matched at 17 days after application; therefore, they should not be combined at this treatment interval unless the rate of anilazine is 124 ml/93 m^2 or higher. At 17 days after treatment, the mixture with lower rates of anilazine would exert selection pressure for triadimefon resistance on target populations.

Tests of reduced-rate mixtures and of individual components in particular use settings are important to determine disease control in these settings and to make appropriate matches for use in mixtures that can accomplish the dual goals of effective disease control and reduced risk of control failures caused by fungicide resistance. Additive, synergistic, or antagonistic effects may be possible with particular fungicide mixtures (10). It is therefore important that mixtures of various fungicides be tested, both for efficacy and for delay of R increases, within as many host/pathogen systems as possible.

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