Relation of Dosage Rates, Nutrition, Air Temperature, and Suscept Genotype to Side Effects of Systemic Fungicides on Turfgrasses

Bobby G. Joyner and Houston B. Couch

Respectively, formerly Graduate Assistant, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, now Plant Pathologist, Diagnostic Laboratory, Chem-Lawn Corp., 1052 Crupper Ave., Columbus, Ohio 43229, and Professor of Plant Pathology, Department of Plant Pathology and Physiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

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ABSTRACT

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Field trials and laboratory-based experiments were conducted to determine the effects of benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate], thiophanate-methyl [dimethyl 4,4-o-phenylenebis-(3thiophanate-methyl [dimethyl thiophanate-ethyl [diethyl thioallophanate)], phenylenebis-(3-thioallophanate)], and thiabendazole [2-(4thiazolyl)benzimidazole] on seven nontarget diseases of turfgrass. Also studied was the relation of dosage rates, air temperature, nutrition, and suscept genotype to the phytotoxicity of these compounds to turfgrasses. The fungicide treatments did not affect the incidence of zonate evespot (caused by Helminthosporium giganteum) of hairy crabgrass (Digitaria sanguinalis), Helminthosporium leaf spot (caused by H. sorokinianum) of Seaside bentgrass (Agrostis palustris), or Pythium blight (caused by P. ultimum) of Manhattan ryegrass (Lolium perenne). However, the levels of red leaf spot (caused by Helminthosporium erythrospilum) of Seaside bentgrass, Helminthosporium leaf spot of Merion and common Kentucky bluegrass (Poa pratensis), and Helminthosporium blight (caused by H. dictyoides) of K-31 tall fescue (Festuca arundinacea) were reduced. Benomyl, thiophanate-methyl, and thiophanate-ethyl reduced the severity of Pythium blight of Seaside bentgrass. The incidence of rust (caused by Puccinia coronata) on Manhattan ryegrass increased after applications of thiabendazole and thiophanate-methyl. Single applications of 279 mg active ingredient/m² of either benomyl, thiophanate-methyl, or thiophanate-ethyl, induced chlorosis of field-grown Penncross bentgrass 36-72 hours from the time of treatment. The treated areas were also characterized by light yellow rings 30-60 cm in diameter which persisted for approximately 21 days. In the laboratorybased studies, symptoms of benomyl-induced phytotoxicity developed sooner, were more severe, and persisted longer in bentgrass, Kentucky bluegrass, and perennial ryegrass grown under low nitrogen nutrition. Thiabendazole was more phytotoxic to bentgrass grown under high nitrogen nutrition. Bentgrass and Kentucky bluegrass grown at 35 C were more severely injured by benomyl than plants produced at 22 C. In descending order of susceptibility to benomyl-induced phytotoxicity, certain cultivars were ranked as follows: Merion Kentucky bluegrass > Manhattan ryegrass > Highland bentgrass > Pennfine ryegrass > Penncross bentgrass > common Kentucky bluegrass.

Additional key words: benzimidazole fungicides, phytotoxicity, turfgrass diseases, disease control, disease susceptibility.

The usefulness of benomyl [methyl 1-(butyl-carbamoyl)-2-benzimidazolecarbamate] and related systemic fungicides in plant disease control is well documented. However, their widespread use has also led to reports of deleterious side effects. With plant species other than turfgrass these have included (i) phytotoxic and hormonal effects (11, 14, 19, 26), (ii) changes in resistance of target microorganisms to the fungicide (2, 3, 12, 17, 27), and (iii) alterations of population levels and/or ratios of species of ancillary microorganisms (1, 13, 20, 25).

Certain systemic fungicides have also been reported to be phytotoxic to turfgrasses. Robinson and Hodges (15) observed growth inhibition and stolon proliferation in creeping bentgrass after the use of benomyl at the rate of 2,100 mg/liter. These symptoms occurred in both healthy and Ustilago striiformis-colonized plants. Vargas (21) reported a tip dieback in Merion Kentucky bluegrass after two applications of either benomyl or thiophanatemethyl [dimethyl 4,4-o-phenylenebis-(3-thioallophanate] at the rate of 1,000 μ g/ml. In the same study, thiabendazole [2-(4-thiazolyl)-benzimidazole] was shown to cause destruction of the top growth after one 1,000 μ g/ml application. Triarimol [α (2,4-dichlorophenyl)- α -phen-

yl-t-pyrimidinemethanol], at 1,000 μ g/ml, caused black spots to develop on the leaf blades. Dale (5) found that the systemic fungicide carboxin (5, 6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide) caused a discoloration of Penncross bentgrass when used as a drench at the rate of 1,600 and 2,000 μ g/ml.

Warren et al. (22) found that soil ammendments of either benomyl or thiabendazole resulted in an increase in the dry weight of the foliage of Seaside bentgrass and Merion Kentucky bluegrass. With bentgrass, the benomyl ammendment increased foliar potassium and decreased phosphorus, calcium, magnesium, boron, and sodium. The thiabendazole ammendment increased potassium and decreased magnesium and sodium.

Concerning the development of resistance to systemic fungicides by fungi pathogenic to turfgrasses, in 1973, Goldenberg and Cole (6) reported the lack of control of Sclerotinia dollar spot (caused by S. homoeocarpa) with benomyl. Later, isolates taken from areas where benomyl was not controlling Sclerotinia dollar spot were studied and compared to nontolerant isolates (23). In this work, it was shown that S. homoeocarpa isolates tolerant to benomyl were tolerant to other benzimidazole configuration fungicides. Robinson and Hodges (16)

reported benomyl-induced stimulation of the rate of growth of an isolate of *Ustilago striiformis* in vitro. Vargas (21) reported a strain of *Erysiphe graminis* colonizing Merion Kentucky bluegrass resistant to benomyl, thiabendazole, and thiophanate-methyl at application rates of 1,000 μ g/ml, respectively.

Instances have been reported in which nontarget pathogenic fungi have increased in turfgrass stands after the use of systemic fungicides. Jackson (9) has noted that in studies directed at the control of stripe smut on Kentucky bluegrass with benomyl there was a resultant increase in the severity of melting-out (caused by Helminthosporium vagans). Later Jackson Fenstermacher (10) recommended the use of pentachloronitrobenzene with benomyl to control both stripe smut and melting-out. Warren et al. (24) reported an increase in the severity of Pythium blight on greenhousegrown Penncross bentgrass treated with benomyl at an equivalent of 23 mg active ingredient/m2. Smith et al. (18) observed the development of a brown patch, caused by a Basidiomycete, on bentgrass that had been sprayed with benomyl to control Sclerotinia dollar spot. This Basidiomycete tolerated benomyl at 25 µg/ml and was stimulated by concentrations of 0.5 to 1.0 μ g/ml.

Benomyl, thiophanate, and thiabendazole are very effective in the control of certain turfgrass diseases, and are used extensively in turfgrass culture (4). The possibility that their use might result in an increase in the incidence and severity of nontarget diseases is important in the development of balanced turfgrass disease control programs. In these instances, emphasis would have to be placed on spray schedules that integrate closely the systemic fungicide in question with contact fungicides highly efficient in their control of the latter group of diseases. Also, while it has been generally recognized that systemic fungicides may be phytotoxic to turfgrasses, there has been a general lack of unanimity on either the rates of active ingredient required to produce these responses or the length of time such side effects will persist. The purpose of the present investigation, then, was to (i) study the effects of rates of application of systemic fungicides on the incidence and severity of certain nontarget diseases of turfgrasses, and (ii) determine the relation of dosage, air temperature, suscept nutrition, and suscept genotype to the injury of turfgrasses by systemic fungicides.

MATERIALS AND METHODS

During a period of six consecutive years, field trials and laboratory-based experiments were conducted to determine the effects of benomyl, thiophanate, and thiabendazole on the incidence and severity of seven nontarget diseases of turfgrasses. The diseases studied in the field tests were: red leaf spot (caused by Helminthosporium erythrospilum Drechsler) of Seaside bentgrass (Agrostis palustris Huds.), cut at 1.3 cm and Penncross bentgrass (A. palustris) under putting green management, zonate eye spot (caused by H. giganteum Heald and Wolf) of hairy crabgrass [Digitaria sanguinalis (L.) Scop.], Helminthosporium leaf spot (caused by H. sorokinianum Sacc. ex Sorokin) of Kentucky bluegrass (Poa pratensis L.), Helminthosporium blight (caused by H. dictyoides Drechsler) of tall fescue (Festuca

arundinacea Schreb.), melting-out (caused by H. vagans Drechsler) of Kentucky bluegrass, and rust (caused by Puccinia coronata Corda) of perennial ryegrass (Lolium perenne L.). Benomyl, thiophanate-methyl, and thiophanate-ethyl were applied at 93 mg and 279 mg and thiabendazole at 56 mg and 233 mg active ingredients respectively in 62 ml water/m². The applications were made with a custom-built sprayer equipped with a boom with Tee-Jet flat spray tip nozzles and delivering a pressure of 2.1 kg/cm² at the nozzles.

In all trials, the individual plots were 14 m² and the treatments were randomized through four replications. Two applications of each treatment were made at 7- to 10-day intervals. Seven days after the second application, leaf samples were collected from each plot and stored at -10 C. A total of 100 leaves was then examined from each plot and rated on the basis of the presence or absence of lesions or pustules incited by the pathogen in question. Evaluation of level of disease control, then, was based on percentage of diseased leaves per plot.

All data were subjected to analysis of variance and then the individual treatments were compared by means of Duncan's multiple range test.

Greenhouse and laboratory tests also were conducted to determine the effects of benomyl, thiophanate-methyl, and thiophanate-ethyl on the susceptibility of Pennfine ryegrass and Seaside bentgrass to Pythium blight (caused by *P. ultimum* Trow.), and Seaside bentgrass and Merion Kentucky bluegrass to Helminthosporium leaf spot. Plants were grown in plastic containers measuring 12 × 8 cm with Weblite (a granular, heat-expanded shale manufactured by the Webster Brick Co., Salem, Virginia) serving as the support medium. After seed germination, irrigation was accomplished with a normal-strength, balanced Hoagland's solution (8).

With the Pythium blight series, plants were cut to a height of 2.5 cm 42 days after seed germination and sprayed with benomyl, thiophanate-ethyl, or thiophanate-methyl at rates equivalent to 610 mg, 2,441 mg and 3,662 mg active ingredient in 62 ml water/m², respectively. Ninety-six hours later, plants of the individual treatment groups were inoculated in the following manner: circular plugs 10 mm in diameter were cut from cultures of *P. ultimum* growing on potato-dextrose agar (PDA) in petri dishes and a single unit placed on the foliage in the center of each container. The plants then were stored in a moisture-saturated atmosphere at 30 C. After 120 hours, they were rated for disease incidence by visually estimating the percentage of foliage blighted in each container.

With the Helminthosporium leaf spot group, the individual treatments listed above were made 21 days from the time of seed germination. Ninety-six hours later, the foliage was sprayed to run-off with aqueous conidial suspensions of *H. sorokinianum* from cultures grown on PDA for 14 days at 21 C. After this, they were stored in a moisture-saturated atmosphere at 27 C. Ratings for disease incidence were performed on the bentgrass plants 72 hours from the time of inoculation, whereas the total incubation period for the Kentucky bluegrass plants was 168 hours.

All data in the experiments described above were subjected to analysis of variance and then comparisons were made by Duncan's multiple range test.

The growth responses of bentgrass, Kentucky bluegrass, and ryegrass to varying rates of benomyl and thiophanate also were studied in field and laboratory experiments. In the field trials benomyl, thiophanateethyl, and thiophanate-methyl were tested as 50 percent wettable powders applied in 62 ml water/m². The trials were conducted on Penncross bentgrass mowed at 0.5 cm and under low nitrogen fertilization. The fungicides were applied through a sequence of individual 279-mg rates to provide within a 28-day period a series of plots that had received the equivalent of 279 mg, 558 mg, 837 mg, and 1,116 mg active ingredient/m2, respectively. All applications were made with the custom-built sprayer described above delivering 2.1 kg/cm² pressure at the nozzles. The individual plots comprised 14 m² and the fungicide treatments and nonsprayed controls were randomized through four replications.

Tests were conducted in the laboratory to determine the relationships between plant nutrition, air temperature, turfgrass species and cultivars, and fungicide dosage levels on phytotoxicity. Turfgrasses used were Merion and common Kentucky bluegrass, Highland and Penncross bentgrass, and Manhattan and Pennfine perennial ryegrass. The plants were grown in styrofoam cups measuring 7 × 8 cm with Weblite as the support medium. After seed germination, two nitrogen nutrition level groups were established by irrigating the plants with 0.1 × and 3.0 × nitrogen level nutrient solutions respectively. These solutions were prepared by adjusting the nitrogen component only of the formula for a normal, balanced Hoagland's solution (8). Twenty-one days from the time of initiation of the nitrogen nutrition, each group was subdivided into three sections and these were treated with foliar applications of benomyl at the equivalent rates of 610 mg, 1,221 mg, and 3,662 mg active ingredient in 62 ml water/m², respectively. These then further were subdivided into two subunits and each placed in environment control chambers at 22 C and 35 C with 12 hours of light per day, respectively. Seven days from the time of treatment, all plants were rated for phytotoxic response. These data were subjected to analysis of variance and then compared by means of Duncan's multiple range test.

A greenhouse test also was performed to determine the relation between plant nutrition and dosage rate to the phytotoxicity of thiabendazole to Penncross bentgrass. The plants were grown in the manner outlined above with the exception that the standard $(1.0\times)$ Hoagland's basal solution was modified to provide two additional nutritional regimes of $0.5\times$ nitrogen and $1.5\times$ nitrogen, respectively. Throughout the test, the air temperature of the greenhouse was maintained at 27 ± 5 C.

Twenty-eight days from the time of seed germination, the three nutrition groups were divided into three subgroups that received foliar applications of thiabendazole at the rates of 723 mg, 1,465 mg, and 2,928 mg active ingredient in 62 ml water/m². Ratings for phytotoxicity were made 5 days from the time of treatment. All data were subjected to analysis of variance and then compared by means of Duncan's multiple range test.

RESULTS

In the field trials, the incidence of zonate eyespot on

hairy crabgrass was not affected by any of the fungicidal treatments. However, the levels of red leaf spot of bentgrass, Helminthosporium blight of tall fescue, melting-out of Kentucky bluegrass, and Helminthosporium leaf spot of Kentucky bluegrass were reduced by applications of benomyl, thiophanate-ethyl, and thiophanate-methyl at 93 mg and thiabendazole at 56 mg active ingredient rates, respectively. These reductions were statistically significant from the nontreated controls at P = 0.01. Their efficacy, however, was not comparable to that provided by the contact fungicides recommended for control of these diseases (4). The incidence of rust on Manhattan ryegrass increased after applications of thiabendazole at the 56 mg and thiophanate-methyl at the 279 mg rates, respectively. These increases were of a magnitude of eight percent each and were statistically significant from the nonsprayed controls at P = 0.01.

The development of P. ultimum on Manhattan ryegrass was not affected by the fungicidal treatments. The incidence of infection and degree of colonization of Seaside bentgrass, however, was reduced by benomyl and thiophanate-methyl at the 610 mg rate and thiophanate-ethyl at the 3,662 mg rate. The magnitudes of these reductions were 18, 18, and 16 percent, respectively. These differences were statistically significant from the nontreated controls at P = 0.01.

In the field tests, single applications of 279 mg active ingredient/m² of either benomyl, thiophanate-methyl, or thiophanate-ethyl induced macroscopically discernible injury. The first symptoms appeared 36-72 hours from the time of fungicide application and lasted approximately 21 days. The symptom pattern on individual leaves was characterized by a tip die-back that began as a yellow discoloration and progressed to a light brown color. In overall view, the turf assumed a mottled chlorotic appearance, with lighter yellow rings 30-60 cm in diameter. Also, the growth rate of the grass in the treated areas was reduced significantly. This latter side effect was particularly evident at and above the 558 mg active ingredient accumulation level.

In the laboratory experiments, all turfgrass cultivars began to show symptoms of benomyl-induced phytotoxicity within 72-120 hours of the first fungicide application. The symptom pattern for individual leaves was identical to that described above for treated plants in the field tests. Phytotoxic symptoms developed sooner, and the effects of benomyl were more severe, on plants grown at 0.1 × nitrogen nutrition as compared with those receiving 3.0 × nitrogen. With ryegrass, there was no correlation between air temperature and the expression of phytotoxic symptoms. However, with the other species, the benomyl-induced symptoms appeared sooner and were more severe at 35 C.

With the thiabendazole treatment series, the relation between nitrogen nutrition and degree of phytotoxicity was the reverse of that observed in the benomyl tests. Bentgrass plants grown at $1.5 \times$ nitrogen were more severely damaged by thiabendazole than those maintained at $0.5 \times$ nitrogen.

There was a relationship between turfgrass genotype and relative susceptibility to benomyl phytotoxicity. Listed in descending order of susceptibility, the cultivars ranked as follows: Merion Kentucky bluegrass > Manhattan ryegrass > Highland bentgrass > Pennfine

ryegrass > Penncross bentgrass > common Kentucky bluegrass.

All differences listed above for relationships between nutrition, air temperature, and plant genotype and the severity of benomyl-induced phytotoxicity were statistically significant at P = 0.01.

DISCUSSION

The results of the present investigation demonstrate that while certain systemic fungicides may induce side effects in turfgrass ranging from leaf chlorosis to alteration in susceptibility to pathogenic fungi, the types and magnitudes of these responses may be determined by the interaction of such factors as fungicide formulation, air temperatures, suscept genotype, and suscept nutrition.

The effect of low nitrogen nutrition on predisposing turfgrass to injury by benomyl, thiophanate-methyl, and thiophanate-ethyl apparently is not subject to being offset by other attending factors. This is evidenced by the fact that with all species and cultivars tested, the phytotoxic effects of these compounds appeared sooner and persisted longer in the plants grown at $0.1 \times$ nitrogen than in those grown at $3.0 \times$ nitrogen. This positive correlation between nitrogen nutrition and phytotoxic symptom expression follows the pattern reported by Hartill and Campbell (7) for benomyl injury to tobacco.

The finding that thiabendazole was more phytotoxic to bentgrass grown under high nitrogen nutrition demonstrates that the direct effects of nutritional levels on plant response to systemic fungicides may vary significantly among compounds.

Although the relation between air temperature and the degree of phytotoxicity of benomyl was pronounced, the possibility does exist that its level of influence may vary with certain species and/or cultivars. This is borne out by the findings that injury to the two ryegrass cultivars was equally severe at 22 C and 35 C, whereas damage to the bentgrass and Kentucky bluegrass cultivars was more pronounced at 35 C.

Because bentgrass is more prone to injury by benomyl, thiophanate-methyl, and thiophanate-ethyl at low nitrogen nutrition, when the air temperatures are high, the use of these materials on bentgrass putting greens during the warm summer months should be limited to single applications of no more than 93 mg active ingredient/m² at 21-day intervals. Dosages above this level will progressively reduce the growth rate of the grass in proportion to the total amounts of active ingredient applied. If an application of either 279 mg or 332 mg active ingredient/m² is necessary for a disease control situation, then this should constitute the total amount of systemic fungicide to be applied to the area for a minimum period of 120 days.

Under certain turfgrass management situations, the levels of nitrogen fertilization could create a situation for the latent expression of benomyl or thiabendazole-induced phytotoxicity symptoms. As noted, it is possible for the macrosymptoms of benomyl phytotoxicity to be masked during periods of high nitrogen fertilization and low air temperatures. As a general rule, during the growing season, the application rates of nitrogen fertilizer to bentgrass putting greens is reduced as the daily mean air temperatures increase. With lower soil nitrogen levels

and higher air temperatures, it then becomes possible for the phytotoxic potential of a high level of benomyl that had been applied weeks earlier to be expressed. Also, as noted above, in the case of thiabendazole-induced phytotoxicity the active ingredient threshold for injury of bentgrass growing under high nitrogen fertilization is less than for plants at low nitrogen nutrition. In the event of a sudden increase in the level of available soil nitrogen, then, the capacity of a bentgrass putting green to accommodate a particular dosage of thiabendazole could be lowered to a point at which an application made weeks earlier could now become phytotoxic. In our judgement, the development of bentgrass putting green management programs should take these points into consideration to ensure that the systemic fungicide schedule is compatible with the schedule of nitrogen fertilization.

The diversity of effects of benomyl, thiophanatemethyl, and thiophanate-ethyl on nontarget turfgrass diseases noted in the present investigation further illustrates the principle that interacting factors may significantly affect the types of responses these compounds are capable of inciting. For example, of the five Helminthosporium-incited diseases studied, zonate eyespot on hairy crabgrass and Helminthosporium leaf spot on Seaside bentgrass was not altered by treatment of the suscepts with these materials. On the other hand, the level of Helminthosporium leaf spot on two cultivars of Kentucky bluegrass, and red leaf spot of bentgrass, melting-out of Kentucky bluegrass, and Helminthosporium blight of tall fescue were reduced by applications of these compounds.

Pythium blight also showed a suscept-species interaction. The disease was not affected by applications of fungicides to perennial ryegrass, whereas treatments of Seaside bentgrass with either benomyl, thiophanate-ethyl or thiophanate-methyl resulted in a reduction in infection and colonization.

The failure of the fungicides used in the present study to reduce the incidence of Helminthosporium leaf spot and Pythium blight of bentgrass and ryegrass, respectively, could be due to relatively higher degrees of susceptibility of these species to the two pathogens in question. The level of control of these diseases on the other turfgrass species was statistically significant, but not of the magnitude provided by contact fungicides now in general use. Therefore, their additional degree of inate disease susceptibility could have easily placed them in a threshold of disease control category outside the range of the efficiency of the fungicides in question.

As a general rule, in the field testing of fungicides for use in turfgrass culture, the probability of alteration of the incidence and severity of nontarget diseases by the compounds in question is not given primary consideration. In the present investigation, thiabendazole and thiophanate-methyl increased the incidence of rust on Manhattan ryegrass. In view of these findings, and the reports that applications of benomyl have resulted in an increase in the severity of melting-out of Kentucky bluegrass (9) and Pythium blight of bentgrass (24), it is important that the potential for this particular side effect be determined for all systemic fungicides before they are used in turfgrass management programs.

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