Effect of Benomyl on Eradication of Ustilago striiformis from Agrostis palustris and on Plant Growth

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ABSTRACT

Benomyl eradicated *Ustilago striiformis* from *Agrostis palustris* in pot culture after 4 weeks' exposure to 700 mg/liter active ingredient per pot. Low fungicide dosages failed to eradicate *U. striiformis* after 5 weeks. Histopathological examination of nodes of stolons produced on treated plants confirmed eradication of the pathogen. Increasing rates of benomyl caused a decrease in dry weight of all plants; stripe-smutted plants, however, had higher dry weights than healthy plants, indicative of a temporary stimulatory effect between benomyl and *U. striiformis*. Healthy and stripe-smutted plants exposed to several rates of benomyl displayed abnormal stolon proliferation which was interpreted as a phytotoxic response.

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Several cultivars of *Agrostis palustris* Huds. are perennially infected by *Ustilago striiformis* (West.) Niessl var. *agrostidis* (Davis) Thir. & Dick. (3, 9, 11). The fungus is perpetuated within stolons by mycelium that proliferates within nodes and grows with stolons produced from adjoining axillary buds (10). Development of mycelium within stolons also is influenced by temperature (12).

Numerous attempts have been made, with varying degrees of success, to control *U. striiformis* with fungicides (4, 7, 17, 18) and fertilization and cultural practices (6, 16). Control of *U. striiformis* in *A. palustris* and *Poa pratensis* with benomyl has been demonstrated (2, 5, 8, 13). Although control of the pathogen appears promising in field studies, it is still not known if benomyl eradicates the pathogen in diseased plants or the effect of benomyl on plant growth. The purpose of this study was to determine if benomyl would eradicate *U. striiformis* from stolons of *A. palustris*, and to determine its effect on growth and development of stolons.

MATERIALS AND METHODS.—*Agrostis palustris* 'Arlington' was used for all studies. All healthy and diseased plants were propagated from 2.5-cm stolon lengths, each with an axillary node bud, in a steamed 2:1 soil-peat mixture in 3-inch clay pots. Benomyl was applied to the soil as a drench at 300, 700, and 2,100 mg/liter.

The influence of benomyl on development of *U. striiformis* in *A. palustris* was determined by collecting treated plants at weekly intervals for a period of 10 weeks and transplanting them into soil free of benomyl. The first 5 weeks, 10 whole plants (shoots and roots) were collected from each treatment at weekly intervals, and the second 5 weeks, 5 stolons only were collected from each treatment at weekly intervals. Whole plants were washed and transplanted; stolons were washed and cut into lengths, each with an axillary node bud, and the node buds were serially propagated. All plants were observed 15 weeks for development of stripe smut. Healthy and stripe-smutted control plants were collected and propagated in the same way.

Whole plants and stolons from each treatment also were examined histologically at weekly intervals to check for the presence of *U. striiformis*. All plants were fixed in Formalin-acetic acid alcohol and dehydrated in an ethanol-tertiary butyl alcohol series (14). Plants were placed under vacuum for 4 hr in molten 61-C Tissuemat and embedded. All sections were cut 8 to 10 μ thick and mounted on slides with Haupt's adhesive (14). Sections were stained for 15 min in 1% chlorazal black E in 70% ethanol (ETOH) adjusted to pH 4.0. Sections were washed in 70% ETOH and counterstained in 0.1% erythrosin in 70% ETOH. Cover slips were mounted with Harleco synthetic resin in xylene.

Influence of benomyl on growth of treated plants was determined by counting stolons and determining dry weight of 35 plants of each treatment. Dry weight was determined after drying for 48 hr at 50 C.

RESULTS.—*Progressive eradication of U. striiformis.*—The number of stripe smut sori in all treated whole plants decreased progressively as exposure to the various rates of benomyl increased from 1 to 5 weeks (Table 1). Number of diseased plants also decreased more rapidly as the rate of benomyl was increased. Eradication of *U. striiformis* occurred in whole plants after 4 weeks' exposure to 700 mg/liter active benomyl (Table 1). No stripe smut sori developed in plants propagated from stolon

TABLE 1. Number of transplanted whole plants of *Agrostis palustris* showing stripe smut symptoms 15 weeks after 1 to 5 weeks' exposure to several rates of benomyl[a]

Benomyl treatments, mg/liter	Weeks of exposure to benomyl before transplanting				
	1	2	3	4	5
Healthy					
Control	0	0	0	0	0
Stripe smut					
Control	10	10	10	10	10
300	9	9	4	2	1
700	10	6	2	0	0
2,100	9	4	1	0	0

[a]Ten plants/week per treatment.

pieces exposed to the various rates of benomyl for 6 to 10 weeks.

Histopathology.—Mycelium of *U. striiformis* was present in nodes of all stripe-smutted control plants sectioned. Presence of mycelium in stolons of treated plants decreased in each younger node as time and rate of benomyl increased. After 5 weeks' exposure to benomyl at 700 and 2,100 mg/liter, *U. striiformis* was eradicated from stolon nodes of whole plants. Mycelium was absent in all nodes of stripe-smutted plants exposed to all rates of benomyl for 6 to 10 weeks.

Stolon growth and dry weight production.—No significant differences occurred in stolon production between healthy and stripe-smutted plants. No significant differences occurred in stolon production among untreated and treated stripe-smutted stolons except at 2,100 mg/liter (Table 2). Production of stolons among untreated healthy control plants and healthy plants exposed to benomyl was erratic; maximum stolon production occurred after exposure to benomyl at 300 mg and 2,100 mg/liter.

Dry-weight production of all treated plants decreased with increasing rates of benomyl, except for stripe-smutted plants at 300 mg/liter (Table 2). Stripe-smutted plants produced more dry matter than did healthy plants in all treatments; however, differences were significant only at rates of 300 and 700 mg/liter.

DISCUSSION.—Systemic activity of benomyl is subject to concentration and time. Numbers of stripe-smutted plants were significantly reduced after 2 weeks' exposure to all rates of benomyl, and eradication occurred after 4 weeks' exposure to 700 mg/liter (Table 1). The progressive reduction and eventual eradication of stripe-smutted plants indicates that uptake of benomyl and subsequent inhibition of *U. striiformis* are relatively rapid. It should be pointed out, however, that eradication of *U. striiformis* in pot culture probably has little relationship to eradication in the field. Very high concentrations of benomyl were employed in this study, and were confined to small soil areas (3-inch pot); the rates of benomyl employed would be impractical for field applications, and it is improbable that each plant in a turf would be exposed to the

TABLE 2. Stolon production and dry weight of healthy and stripe-smutted plants of *Agrostis palustris* exposed to several rates of benomyl for 15 weeks

Benomyl treatments, mg/liter	Healthy plants[a]		Stripe-smutted plants[a]	
	No. stolons[b]	Dry weight[b]	No. stolons[b]	Dry weight[b]
Control	15.3 a	10.2 a	15.1 a	11.5 a
300	17.0 b	6.2 b*	15.9 b	13.9 b*
700	14.5 c	4.8 c*	15.4 ab	7.6 c*
2,100	16.8 b	5.1 c	17.5 c	6.9 d

[a]35 plants/treatment.

[b]Means followed by same letter not significantly different at 5% (Duncan's multiple range test); * = significantly different (5%) mean dry weight values between healthy and stripe-smutted plants of same treatment ("Student" t-test). There were no significant differences between stolon numbers produced by healthy and stripe-smutted plants.

fungicide. Field studies with benomyl indicate that multiple applications would probably be necessary to control *U. striiformis* in *A. palustris* and *Poa pratensis* (2, 5, 8, 13). Eradication in the field is improbable; however, it has been reported in one field study that *U. striiformis* was apparently eradicated from *P. pratensis* (8).

It is clear that high concentrations of benomyl can influence growth of *A. palustris*. At the highest benomyl rate (2,100 mg/liter), there was an increase in stolon proliferation among healthy and stripe-smutted plants (Table 2). At the same concentrations, dry weight of healthy control plants and stripe-smutted plants decreased significantly (Table 2). Thus, total growth was inhibited while numerous short stolons were proliferated at the base of each plant. These growth characteristics are interpreted as a phytotoxic reaction. No immediate explanation can be provided for such stolon proliferation; however, it has been observed that benomyl is translocated to the growing points of *A. palustris* (19). Also, it has been suggested that benzimidazole might serve as a precursor to kinetinlike compounds in plants (24).

With the exception of stripe-smutted plants exposed to benomyl at 300 mg/liter, dry weight decreased among all plants as the rate of fungicide was increased (Table 2). This reduction in dry weight is believed to be more complex than direct fungicidal inhibition of plant growth. Among untreated healthy and stripe-smutted control plants, there was no significant difference in dry weights; dry weight of stripe-smutted plants exposed to benomyl at 300 and 700 mg/liter, however, was significantly greater than that of healthy plants of the same treatment (Table 2). These results may indicate a temporary synergistic relationship between benomyl and *U. striiformis* at some time between initial uptake of the fungicide by the plant and eradication of the pathogen. *Ustilago striiformis* can cause proliferation of stolons in *A. palustris* (12). Also, benomyl breaks down to methyl 2-benzimidazolecarbamate (MBC) in aqueous solution

and in plants (1, 20). There is also limited evidence that MBC may break down further in plants (21). The fate of MBC in plants is of interest because the benzimidazole portion of the compound has been shown to increase protein synthesis (15) and to maintain chlorophyll biosynthesis (22, 23) in detached wheat leaves. It is possible that in this complex of characteristics exhibited by *U. striiformis* and benomyl there may be an explanation for the increased dry-weight production among stripe-smutted plants.

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