Effects of Seed Treatment with Thiabendazole on Septoria Leaf Blotch and Growth of Wheat

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ABSTRACT

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Seed treatment with thiabendazole did not suppress development of Septoria leaf blotch (caused by *Mycosphaerella graminicola*) in wheat in two field trials, but yields were increased by 11.2 and 5.9%. In a third trial, no disease was detected and yields were increased by 24.4%. Plant density, number of heads per plant, and foliar growth of the plants were unaffected by the treatment. However, significantly more ($P \leq 0.05$) roots were observed in plants from seed treated with thiabendazole than in plants from nontreated seed (5.2 more roots per plant, 32.5% increase). In controlled environment trials, thiabendazole application at the rate of 0.9 kg a.i./t decreased the number of primary roots per plant by 1.1 to 1.4 (20–23%), while increasing the number of adventitious roots per plant by 1.3 to 1.9 (31–39%).

Septoria leaf blotch, caused by *Mycosphaerella graminicola* (Fuckel) Schröt. in Cohn (anamorph: *Septoria tritici* Roberge in Desmaz.), is a major disease of wheat (*Triticum aestivum* L.) in Israel. During severe epidemics, vulnerable wheat cultivars may suffer yield losses of 30–50% and produce shriveled grains unfit for milling (7). Wheat seedlings may become infected soon after emergence. Possible sources of primary inoculum include viable pycnidia and perithecia on crop residues from the previous seasons, infected volunteer wheat plants, and alternative hosts (7). Under favorable weather conditions (frequent rains together with temperatures of 12–25 C), the disease spreads vertically to the upper plant parts. Vertical spread of the disease is mainly facilitated by splash dispersal of spores by rain or by droplets during overhead irrigations (2,7). Even though plants may be infected at an early stage of development, yield losses are induced only once the disease infects the upper two leaves (15,21) because these are the main source of carbohydrates for grain filling (1).

When yield losses attributable to Septoria leaf blotch are considered likely, fungicides should be applied to the foliage. Growers determine the likelihood of loss by comparing disease incidence in the field to an action threshold level while also taking into account relevant agronomic and climatic factors (14). In any growing season, the later the disease attains the action threshold level, the less likely the need for fungicide application.

Protection of seedlings against foliar diseases by treatment of seed with systemic fungicides is a common practice in cereals (3,4,12). Preliminary work done in Israel showed that seed treatment with thiabendazole reduced Septoria leaf blotch infections on the first wheat leaves and that plots planted with treated seed yielded significantly more than control plots (6). In this study, we examined the possibility of delaying the development of Septoria leaf blotch in wheat crops by treating the seed with thiabendazole. We also examined the effect of thiabendazole on wheat growth.

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MATERIALS AND METHODS

Field trials. Three field trials were conducted in the northern Negev region of Israel. The local wheat cultivar Lakhish was sown in 1981 (trial 1) and the cultivar Barkai in 1982 (trials 2 and 3). Seeds were sown in the second week of November each year at a rate of 130 kg/ha. The previous crop was wheat. Methods of sowing, fertilizing, irrigation, and other cultural practices were as recommended to wheat growers in Israel. Lakhish and Barkai are semidwarf and dwarf spring wheat cultivars, respectively; both were susceptible to Septoria leaf blotch. Experimental plots in each trial were arranged in a randomized complete block design with six replicates. The size of each replicate was 40 × 60 m (trial 1) and 5.3 × 12.5 m (trials 2 and 3). Seed (obtained from Hazera, Brurim, Israel) was treated with thiabendazole (Tectoril, 45% a.i.) at the rate of 0.9 kg a.i./t. Plants from nontreated seed (of the same seed lot) were used as controls.

Natural epidemics of Septoria leaf blotch developed in trial 1. In trial 2, infested wheat straw from the previous season was spread before plant emergence to ensure disease development. In trial 3, disease did not develop throughout the entire growing season. Disease incidence (proportion of infected leaves or plants) and disease severity (proportion of leaf area with disease symptoms) were recorded every 7–10 days on 20 randomly chosen main tillers per replicate. Disease severity was assessed using a disease assessment scale, specifying seven levels of disease severity—0, 1, 5, 10, 20, 40, and 60% (9).

In all three trials, plant density was assessed at the five-leaf stage and the number of heads per plant was recorded at the kernel filling stage (growth stages [GS] 15 and 70, respectively) (20) by counting two 1-m randomly chosen sample rows per replicate. Counts were averaged and expressed as number per square meter. After the crop had matured, areas of 60–100 m^2 in trial 1, two randomly chosen samples each of 1 m^2 in trial 2, or 25–30 m^2 in trial 3 were harvested from each replicate. Grain yield was recorded and expressed as tons per hectare.

The effect of thiabendazole seed treatment on the host in the absence of disease was evaluated in trial 3. Random samples of 10 plants per replicate were taken 11, 21, and 35 days after emergence. The growth stage, length of the main tiller, and weight were recorded for each plant. After oven-drying (70 C for 48 hr), the sample dry weight was recorded. Twenty

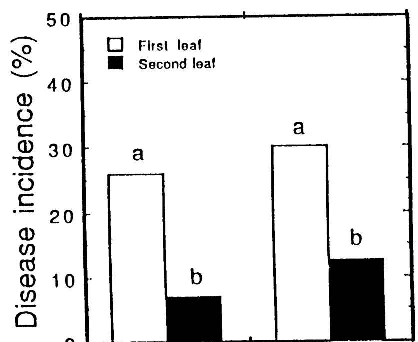

Fig. 1. Effect of thiabendazole seed treatment on the incidence of Septoria leaf blotch (trial 2) at the tillering stage. Bars followed by different letters differ significantly ($P \leq 0.05$) as determined according to the *F* test.

plants, each with two tillers, were sampled 28, 49, 84, and 96 days after emergence. Plants were dug with part of the root system still attached. The remaining soil was washed from the roots and the number of roots per plant was recorded. At the milky-ripe stage (GS 75), 117 days after emergence, half of each plot was harvested for silage and the yield was expressed as tons per hectare.

Soil and root samples from trial 3 were taken several times during the growing season and tested for the presence of soilborne pathogens (by J. Katan, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot) and pathogenic nematodes (by D. Orion, ARO, The Volcani Center, Bet-Dagan). The roots were examined visually for crown rot symptoms. Then root segments were sampled at random and plated in petri dishes containing potato-dextrose agar (PDA) and on sucrose-asparagine medium selective to *Pythium* spp. (13). *Pythium* development was examined after 3 days of incubation at 28 C. Nematodes were extracted from the soil samples using the Baermann funnel technique and from the roots using the Young incubation method (16).

Controlled environment trials. The effect of thiabendazole seed treatment on the root system in wheat was examined in controlled environment trials with thiabendazole-treated seed. Nontreated seed was used as a control. The effect of thiabendazole on the rate of appearance of primary roots (roots initiated directly from the seed) was examined by means of a blotting paper test. Four seeds of Lakhish or Barkai were placed in 9-cm-diameter petri dishes, covered with wet blotting paper, and transferred to a growth chamber with 12-hr photoperiod at 18 C and light intensity of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$. The number of primary roots longer than 5 mm was recorded every 1 or 2 days. There were five replicates per treatment, and the experiment was repeated once.

In another experiment, different rates of thiabendazole were applied to seed and the effect on the final number of primary roots per seedling was examined by means of the blotting paper test described earlier. Thiabendazole rates ranged from 0.00017 to 3.4 kg a.i./t. Water solutions of each of the thiabendazole rates were mixed with seed of cv. Barkai (1.5 ml of water solution per 20 g of seeds) in Erlenmeyer flasks. The number of primary roots for each fungicide rate was recorded 10 days after the start of the experiment. The experiment was repeated twice. Results presented here are for one experiment, but the other results were similar.

The effect of thiabendazole on the wheat root systems was also examined in growth chamber trials. Seed of Lakhish was planted in 0.75-L pots. Trials consisted of 10 replicates of each treatment in a randomized complete block design with four seeds per pot. At tillering (GS 25), plants were removed from the pots and the soil was washed from roots. The numbers of primary and adventitious roots (roots originating from the first internode of the stem) per plant were recorded.

Seed of Barkai were planted in a growing tank 50 cm in height, 50 cm in width, and 5 cm in depth. Plants from treated and nontreated seed (10 replicates per treatment) were separated in the growing tank by wooden barriers. One wall of the tank was glass to enable observation of the rooting system in situ during the plant's growth. The glass wall was covered with a light-impermeable cover to avoid light interference with root growth. Plants were grown until the root tips reached the bottom of the growing tank at heading stage (GS 59). They were then removed, the soil was washed from roots, and the numbers of primary and adventitious roots, the lengths of the roots, and the number of tillers per plant were recorded. All data were subjected to analyses of variance and the means were compared by the *F* test.

RESULTS

Field trials. In trials 1 and 2, a large proportion of plants were infected with Septoria leaf blotch. However, the incidence of disease in seedlings grown from treated seed did not differ significantly from that in seedlings from nontreated seed (Fig. 1). Disease progress (both incidence and severity) in older plants was similarly unaffected by the seed treatment (Fig. 2). Other foliar diseases were not noticed in the experimental plots.

Plant density and the number of heads per plant were unaffected by seed treat-

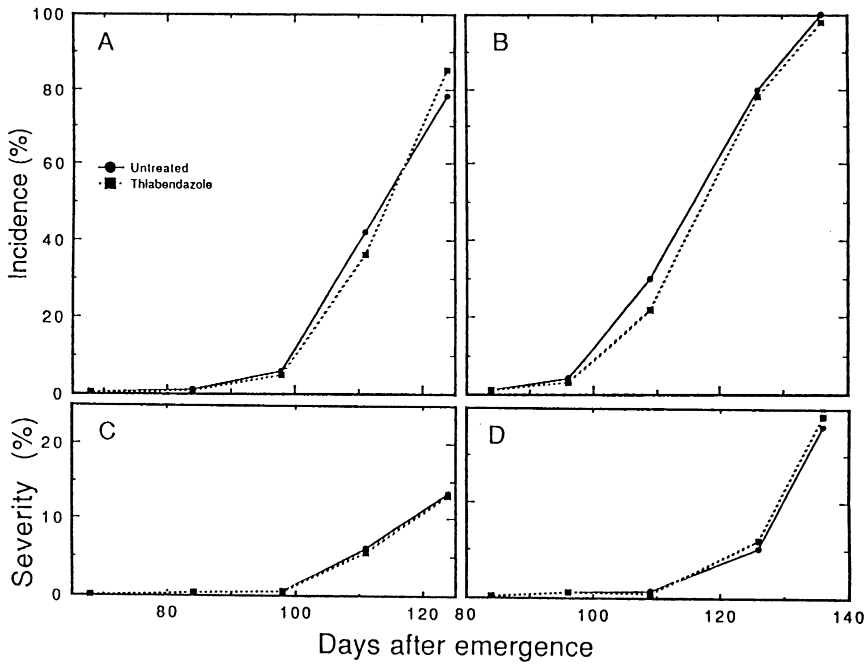

Fig. 2. Effect of thiabendazole seed treatment on the incidence and severity of Septoria leaf blotch on flag leaves. **(A and C)** Trial 1; **(B and D)** trial 2. Values for leaves of plants from treated and nontreated seed at each evaluation time do not differ significantly ($P \leq 0.05$) as determined according to the *F* test.

Table 1. Effect of thiabendazole seed treatment on plant density, the number of heads per plant, and harvested grain yields in three field trials

	Trial 1			Trial 2			Trial 3		
Treatment	Plant density[x]	Heads/ plant	Grain yield (t/ha)	Plant density	Heads/ plant	Grain yield (t/ha)	Plant density	Heads/ plant	Grain yield (t/ha)
Thiabendazole[y]	246 a[z]	2.14 a	6.88 a	300 a	1.69 a	4.99 a	206 a	1.71 a	4.18 a
Nontreated	239 a	2.08 a	6.18 b	265 a	1.72 a	4.71 a	197 a	1.88 a	3.36 b

[x] Number of plants per square meter.
[y] Seed was treated with thiabendazole at a rate of 0.9 kg a.i./t.
[z] Numbers in each column followed by different letters differ significantly ($P \leq 0.05$) as determined according to the *F* test.

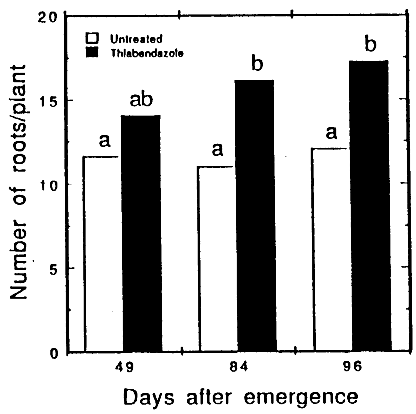

Fig. 3. Effect of thiabendazole seed treatment on the number of roots per plant at different times after emergence. Results were recorded in trial 3 in the absence of disease. Bars followed by different letters differ significantly ($P \leq 0.05$) as determined according to the *F* test.

ment with thiabendazole in all three field trials (Table 1). Seed treatment had no significant effect on foliar growth: plants from treated and nontreated seed did not differ significantly with respect to the length of the main tiller, dry weight, and harvested silage yield (13.6 vs. 13.4 t/ha, respectively). Root growth, however, was increased significantly by the treatment. By 28 days after emergence, adventitious roots had been initiated in 64% of plants growing from thiabendazole-treated seed but in only 15% of plants from nontreated seed. By 49 days after emergence, the number of roots of plants from treated and nontreated seed did not differ significantly (Fig. 3), but 84 and 96 days after emergence, there were significantly ($P \leq 0.05$) more roots in plants from treated than from nontreated seed (5.2 more roots per plant, 32.5% increase). Because it was difficult to distinguish between primary and adventitious roots in some cases, root counts on each sampling date represented the total number of roots per plant. It should be noted, however, that most of the counted roots were part of the adventitious system, because the number of primary roots in Barkai was no more than six. Harvested grain yield was affected by thiabendazole seed treatment. Plots planted with treated seed yielded 11.2% more than plots planted with nontreated seed in trial 1, 5.9% more in trial 2, and 24.4% more in trial 3 (Table 1). In trials 1 and 3, these differences were significant ($P \leq 0.05$).

Soil and root samples taken from trial 3 were tested for the presence of soilborne pathogens and pathogenic nematodes. No significant findings were obtained in all samples. In addition, significant root diseases in cereals were not reported in the experimental region (J. Katan, *personal communication*).

Table 2. Effects of thiabendazole seed treatment on the number of primary and adventitious roots of plants grown in pots (cv. Lakhish) or in a growing tank (cv. Barkai)

	Lakhish		Barkai	
Treatment	**Primary roots/plant**	**Adventitious roots/plant**	**Primary roots/plant**	**Adventitious roots/plant**
Thiabendazole[y]	4.2 b[z]	5.2 a	4.6 b	3.3 a
Nontreated	5.3 a	3.3 b	6.0 a	2.0 b

[y] Seed were treated with thiabendazole at a rate of 0.9 kg a.i./t.
[z] Numbers in each column followed by different letters differ significantly ($P \leq 0.05$) as determined according to the *F* test.

Controlled environment trials. The rate of primary root appearance was lower and the final number of roots was significantly lower for plants grown in petri dishes from seed treated with thiabendazole than for plants from nontreated seed (Fig. 4). In another experiment, a parabolic relationship was found between the rate of thiabendazole applied to the seed and the number of primary roots initiated per plant (Fig. 5). Low rates of application (<0.00034 kg a.i./t) had no significant effect. Above that rate, the number of primary roots increased with increasing rates of fungicide application up to 0.034 kg a.i./t, whereas higher rates induced a gradual decrease in the number of primary roots (Fig. 5). High rates of thiabendazole application (>1.7 kg a.i./t) completely prevented germination.

The effects of thiabendazole seed treatment on the primary roots of plants grown in pots or in the growing tank were similar to those described earlier for the petri dishes, i.e., the number of primary roots was significantly lower (1.1–1.4 less roots per plant, 20–23% decrease) in plants growing from treated than from nontreated seed (Table 2). Plants in pots and in the growing tank also developed adventitious roots, significantly more (1.3–1.9 roots per plants, 31–39% increase) of which were seen in plants growing from treated than from nontreated seed (Table 2). Plants from treated and nontreated seed did not differ significantly with respect to the number of tillers per plant (1.50 vs. 1.37, respectively) or in the length of the roots (52 vs. 55 cm, respectively).

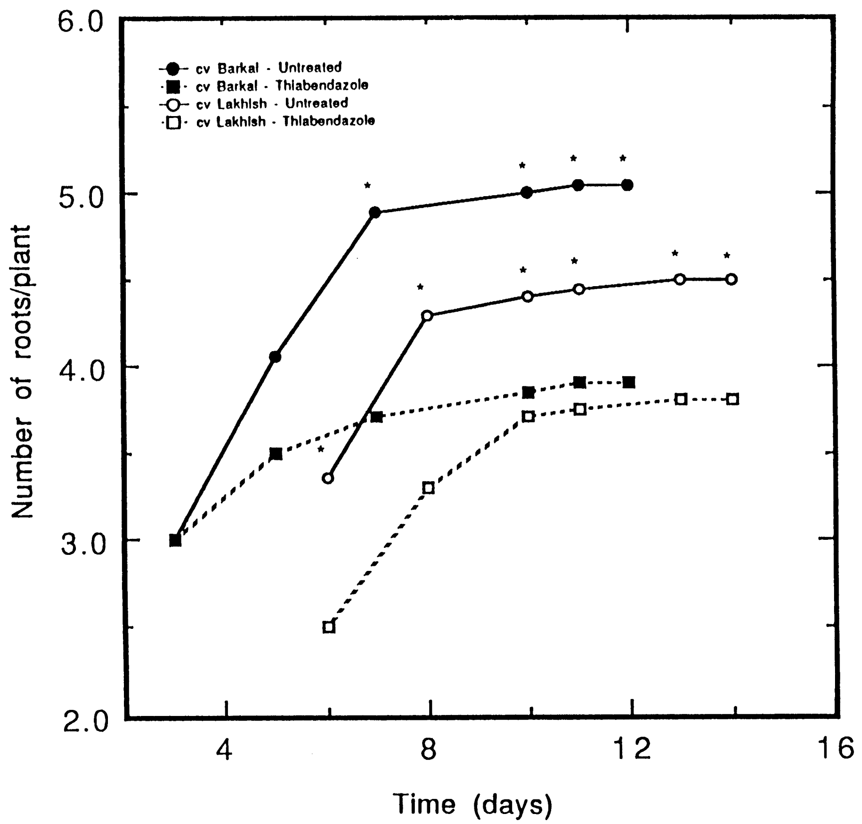

Fig. 4. Effect of thiabendazole seed treatment on the initiation of primary roots in two wheat cultivars. Asterisks indicate a significant difference ($P \leq 0.05$) between the number of roots in plants from treated and nontreated seed of the same cultivar, at the same evaluation date, as determined according to the *F* test.

DISCUSSION

Treatment of seed with thiabendazole did not affect seed germination or the top growth of wheat plants (Table 1). It had a pronounced effect, however, on the root system of the plants and on

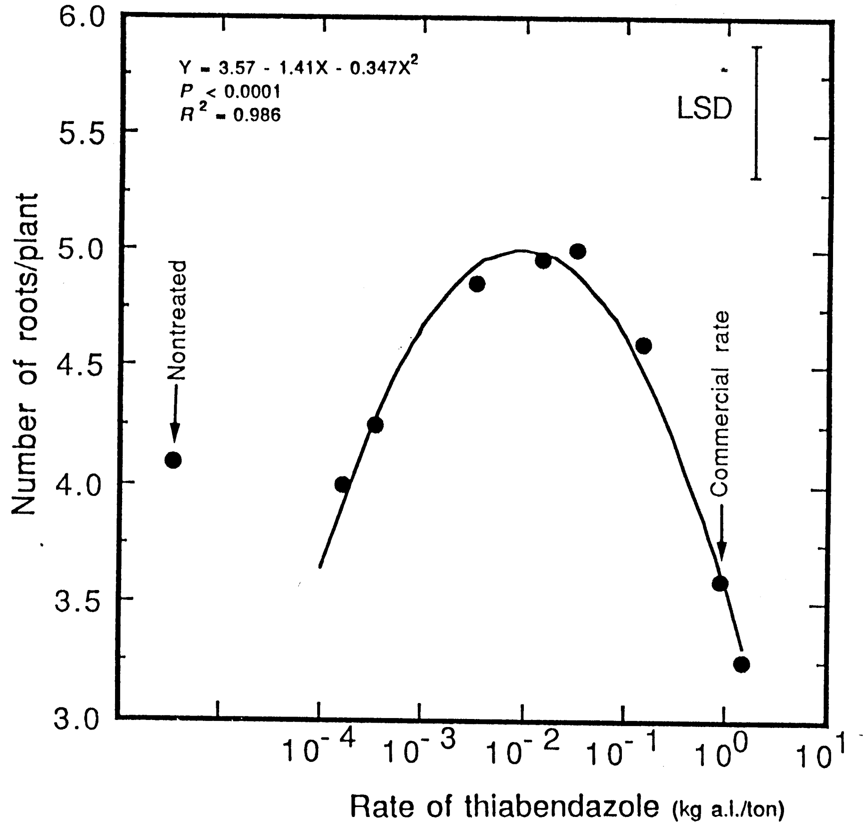

Fig. 5. Effect of the rate of thiabendazole seed treatment on the number of primary roots in wheat (cv. Barkai). Number of primary roots of plants from nontreated seed is indicated for comparison. Bar indicates the LSD.

the harvested yields: plants grown from treated seed produced significantly more roots and significantly higher yields (in two trials) than plants grown from nontreated seed (Fig. 3 and Table 1). It seems most unlikely, however, that these increases were attributable to suppression of Septoria leaf blotch, because disease development was not affected by thiabendazole (Figs. 1 and 2) and because these effects also occurred in the absence of the disease (trial 3). Our results are in line with previous field trials conducted in Israel, in which thiabendazole seed treatment resulted in significant yield increases of 23% (6) and 5% (A. Dinoor, *unpublished*). Other foliar diseases were not noticed in all trials and soilborne pathogens or pathogenic nematodes were not detected in soil samples taken from trial 3. However, the possibility that undetected diseases are involved should not be excluded.

The response of roots to thiabendazole was related to the concentration of fungicide applied to the seed: rates lower than 0.00034 kg a.i./t had no significant effect, whereas intermediate rates (0.0034–0.034 kg a.i./t) significantly increased the number of primary roots, and at high rates, the number of primary roots declined (Fig. 5). At 0.9 kg a.i./t, there was also an increase in the number of adventitious roots (Fig. 3 and Table 2). Inhibition of host growth as a result of treatment of seed with pesticides is well known and may be attributable to the phytotoxic effects of the compounds (5,12). Stimulatory effects, however, are less common but may occur if the pesticide directly or indirectly affects the phytohormonal system. Several lines of evidence indicate that benzimidazole fungicides may affect plant hormones. Application of benzothiazole and benzotriazole at high concentrations reduced the numbers of primary roots of wheat and beans, but the latter compound increased adventitious root initiation (10). Thiabendazole belongs to the group of benzimidazole fungicides, and part of its chemical structure resembles that of kinetin, a phytohormone (18). One of the functions of kinetin in the plant is the regulation of root initiation and growth: low kinetin concentrations induced initiation of adventitious roots in peas and beans, whereas high kinetin concentrations inhibited the formation of both primary and adventitious roots (8,11,17). Therefore, it seems possible that thiabendazole applied to seed may interact with the phytohormonal system and thus affect the regulation of root initiation and growth.

Under Israeli conditions, spring wheat is sown in November and harvested in May. The main rainy season extends from late November to late March. Wheat crops are subjected to water shortages often during the grain-filling stage. Water stress alters photosynthetic activity as well as carbohydrate translocation to the developing kernels, thus interfering with carbohydrate accumulation (19). The main route of water and nutrient uptake at that period is via the adventitious roots. In this study, it was found that plants grown from treated seed possessed significantly more roots than plants from nontreated seed (Fig. 3). It is reasonable to assume that the more roots a plant possesses, the better its water status. It is possible, therefore, that the larger number of adventitious roots was related to the higher yields. This conclusion appears to be supported by the failure to isolate any soilborne pathogens or pathogenic nematodes from the soil at the experimental sites.

Thiabendazole seed treatment, applied as described in this report, may increase wheat yields in other semiarid regions of the world. The associated investments and risks are relatively low, whereas the potential benefits are high. However, because thiabendazole was found to be phytotoxic if applied at high rates, the susceptibility of cultivars to the compound should be tested before this procedure is used commercially.

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