Photosynthesis and Yield of Wheat (*Triticum aestivum*) Treated with Fungicides in a Disease-Free Environment

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ABSTRACT

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Fungicidal growth-regulating effects of a tank mixture of triadimefon (Bayleton 50WP) and zinc ion + manganese ethylene bisthiocarbamate) (Dithane M-45) were evaluated on the soft red winter wheat cultivar Coker 762. Analysis of photosynthesis, leaf conductance, and transpiration 2 and 6 days after foliar application indicated no significant differences between fungicide-treated plants and nontreated controls. Fungicide treatments caused significant ($P = 0.05$) reduction in germination for seed harvested from plants sprayed at Feekes growth stages 9.0+ 10.3 and 10.3 but not at stage 9.0. Carryover plant growth-regulating effect from fungicide application increased chlorophyll content from 4 to 40% over controls for dark-grown seedlings after exposure to light. This research indicates that any benefit of increased grain yield from foliar-applied fungicides other than by the control of pathogens is unlikely to occur from improved leaf gas exchange and that a carryover effect from fungicides does influence subsequent germination.

Application of fungicides to small cereal grains to suppress disease-causing organisms is well documented (5,6,16, 23,25). In many cases, effective control of disease in the period between flag leaf appearance and milk stage of grain development has resulted in significant yield increases compared with nonsprayed plots. Fungicides are usually applied to protect host plant tissue from parasitic organisms that may utilize photosynthetically derived carbohydrates otherwise translocated to developing kernels.

In addition to controlling disease-causing organisms, fungicides may have beneficial yield effects that are not directly attributable to the control of recorded disease. King et al (18) reported that wheat sprayed with three fungicides—benomyl, dichlofluanid, and zineb—showed a decrease in percent senescence when compared with nonsprayed plants. When senescence is delayed, the flag leaf may remain green for a longer period, thus lengthening the grain fill period (21). This was reported by Spiertz (23), who showed that growth rate of grains during the phase from the milk-ripe stage to the dough-ripe stage was raised from 204 to 230 kg/ha per day. It seems probable that such a yield enhancement effect could be detected by measuring photosynthesis following the application of fungicide.

The triazole derivative triadimefon is a systemic fungicide used routinely on wheat (*Triticum aestivum* L.) to suppress leaf rust (*Puccinia recondita* Rob. ex Desm. f. sp. *tritici*) and powdery mildew (*Erysiphe graminis* DC. ex Merat f. sp. *tritici*). This fungicide has also been reported to inhibit shoot and root growth of wheat (3) and barley (12) and shows cytokinin-like antisenescent activity in detached barley leaves (3,13). Cytokinins are reported to have a variety of effects on chloroplast development. Benzyladenine and kinetin stimulate division of mature chloroplasts in tobacco (19), and kinetin is required for development of functional chloroplasts in growing tobacco callus (20). Activities of photosynthetic enzymes also are stimulated by cytokinins (14). Kane and Smiley (17) indicated that chlorophyll content of Kentucky bluegrass was not increased by triazole fungicides, although treated plants sometimes appeared darker green. Buchenauer and Grossman (3) reported that seed treatment with triadimefon retarded growth of coleoptiles, primary leaves, and roots of wheat seedlings. Similar findings were reported for barley seedlings (4). With increasing seedling age, the primary leaves of plants treated with triadimefon contained a significantly higher content of chlorophylls, carotenoids, xanthophylls, and nucleic acids than control leaves (11). In other investigations, systemic (2,8,9) and nonsystemic (22) fungicides have been shown to cause large reductions in photosynthesis in apple and in pecan (26).

In the present study, the effects of a conventional fungicide tank mix on photosynthesis, leaf conductance, transpiration, and yield of wheat were evaluated. The purpose was to elucidate what role a commonly used tank mixture of fungicides may play on grain fill when disease is not a limiting factor to yield potential. The plant growth-regulating properties that subsequent progeny plants may have also were studied. Growth-regulating effects may influence quality of carryover seed or seedling physiology. Seedling parameters studied on harvested seed were percent germination, shoot and root lengths, and total chlorophyll content.

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MATERIALS AND METHODS

The soft red winter wheat cultivar Coker 762 was grown in the field in a clay-loam mix textured soil over two seasons (1985–1987). Each season, plants were grown until Feekes growth stage 5.0, then transplanted into 1-gal plastic containers and transported to an environmental growth chamber. Transplants were carefully inspected to ensure they were disease-free. Environmental conditions for subsequent growth were set at 25 C continuous temperature with a light/dark period of 14/10 hr. During the course of the study, chamber-grown plants were watered daily and received Hoagland's nutrient solution twice a week. Before being transplanted, plants were fertilized at Feekes growth stage 3.0 with ammonium nitrate at a rate of 50.5 kg/ha of nitrogen.

Fungicide spray. A tank mixture of triadimefon (Bayleton 50WP), 141 g a.i./ha (2 oz a.i./acre), and zinc ion + manganese ethylene bisthiocarbamate) (Dithane M-45), 2.2 kg/ha (2 lb/acre), was applied at an output of 188 L/ha (20 gal/acre). Foliar applications were made with a CO_2 pressurized backpack sprayer equipped with 8003LP tips at 1.38×10^5 Pa (20 lb $in.^{-2}$).

Three spray treatments and a control group were evaluated in a four-replicate randomized complete block design. The experiment was conducted twice. Fungicides were applied at Feekes growth stages 9.0, 10.3, and 9.0+ 10.3. Stages 9.0 and 10.3 represent emergence of flag leaf and extension of heads halfway out of the culm (heading), respectively. To avoid contamination of control plants, plants to be sprayed were removed from the growth chamber and placed outdoors for application of fungicides.

Leaf gas exchange. Photosynthesis (PS), leaf conductance (CS), and transpiration (TR) were measured simultaneously with a Li-Cor 6000 portable photosynthetic unit. Three (1986) and four (1987) representative flag

leaves attached to the main stem were measured in each replicate of all four treatments. At ambient CO_2 levels and with growth chamber light intensity at 370 ± 37 $\mu E \cdot m^{-2} \cdot s^{-1}$, PS, CS, and TR measurements were taken 2 and 6 days after each fungicide application.

Yield and seed quality. Plants were harvested at maturity, and grain was threshed with a single-head thresher for analysis of yield component data. After yield components were determined, harvested seed was placed in coin envelopes and stored in the laboratory at 25 C for 1 mo.

Only seed harvested in 1987 was used for seed quality determinations; 1986 seed was discarded accidentally. Twenty-five seeds from each replicated treatment were placed in 100 × 15 mm plastic disposable petri dishes lined with one Whatman No. 2 filter paper and irrigated with 8 ml of water containing 0.01% of captan 50WP to control growth of any seedborne pathogens that may have occurred during germination. Seeds were incubated at 25 C in the dark, and seedlings were evaluated after 7 days for percent germination and shoot and root lengths. Lengths were measured with a metric rule. Root measurements entailed main plus seminal root lengths. This study consisted of eight replicates for each treatment in a randomized complete block design.

Leaf chlorophyll. Seed harvested from plants sprayed with fungicides and from the control group were planted in a potting soil and allowed to germinate under continuous dark or dark/light (10/14 hr daily) at 25 C. Light intensity in a controlled-climate chamber was 400 $\mu E \cdot m^{-2} \cdot s^{-1}$ derived from cool-white fluorescent and incandescent bulbs.

Planted seeds were watered daily for 7 days, then given half-strength Hoagland's nutrient solution daily for the next 7 days. On the 15th day, plants grown in the dark were exposed to the same lighting regime used for the dark/light-grown plants. Plants from both environments were evaluated for total chlorophyll content after 14-, 28-, and 42-hr exposure to light. Twelve plants (four replicates of three samples each) were used for chlorophyll determination at each exposure period. For determination of chlorophyll content, leaves of approximately uniform age were excised, weighed (fresh), and placed in test tubes containing 10 ml of dimethylsulfoxide at 65 C (15). After 6 hr, tubes were air-cooled and the absorbance of the supernatant at 645 and 663 nm was measured with a Sargent/Welch spectrophotometer. Chlorophyll content ($\mu g\ mg^{-1}$ fresh weight) was calculated using the equations of Arnon (1).

Table 1. Flag leaf gas exchange measurements for wheat taken 2 and 6 days after foliar fungicide application at flag leaf emergence (Feekes growth stage 9.0)

Treatment	Day	Leaf gas exchange[a] CS	PS	TR
Control	2	0.77	0.52	85.1
Fungicide	2	0.76	0.50	87.7
Control	6	2.47	0.50	164.1
Fungicide	6	2.28	0.51	161.5
	C.V.	51.7	18.1	20.3

[a] CS = conductance (cm s^{-1}), PS = photosynthesis (mg $CO_2\ s^{-1}\ m^{-2}$), TR = transpiration (mg $H_2O\ m^{-2}\ s^{-1}$). Data are means over 2 yr. Column values within days are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Flag leaf gas exchange measurements for wheat taken 2 and 6 days after foliar fungicide application at heading (Feekes growth stage 10.3)

Growth stage at fungicide application	Day	Leaf gas exchange[a] CS	PS	TR
Control	2	0.79	0.45	97.1
9.0 + 10.3	2	0.75	0.43	90.3
9.0	2	0.81	0.45	96.7
10.3	2	0.67	0.43	83.3
Control	6	0.68	0.41	91.1
9.0 + 10.3	6	0.66	0.39	90.0
9.0	6	0.79	0.44	100.1
10.3	6	0.59	0.41	82.2
	C.V.	28.1	15.1	19.2

[a] CS = conductance (cm s^{-1}), PS = photosynthesis (mg $CO_2\ s^{-1}\ m^{-2}$), TR = transpiration (mg $H_2O\ m^{-2}\ s^{-1}$). Data are means over 2 yr. Column values within days are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 3. Grain yield components[a] of wheat for fungicide-treated and control (nonsprayed) plants grown at 25 C

Growth stage[b] at fungicide application	Number of seed/head[c]	Thousand kernel weight[c] (g/1,000 seed)
Control	27	23.5
9.0 ± 10.3	25	22.4
9.0	23	23.1
10.3	23	22.8
	C.V. 21.0	8.8

[a] Yield component data were pooled over years because the fungicide × year interaction was not significant.
[b] Feekes growth stages 9.0 (flag leaf emergence) and 10.3 (heading).
[c] Column values are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Wheat seedling growth parameters measured after 7 days of growth in the dark at 25 C

Growth stage[x] at fungicide application	Growth parameters[y] Shoot length (cm)	Root length (cm)	Germination (%)
Control	3.0 a[z]	7.0 a	93 a
9.0 + 10.3	3.2 a	6.4 a	83 b
9.0	3.2 a	6.8 a	90 ab
10.3	3.0 a	5.5 a	82 b
	C.V. 16.9	33.7	9.7

[x] Feekes growth stages 9.0 (flag leaf emergence) and 10.3 (heading).
[y] Data are for seed harvested from fungicide-treated and nontreated control plants from the 1987 harvest.
[z] Column values followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

RESULTS

Leaf gas exchange. Analysis of photosynthetic parameters CS, PS, and TR taken 2 and 6 days after fungicide applications showed no significant differences due to fungicide treatments (Tables 1 and 2). Two-way interactions, fungicide × day and fungicide × year, also were nonsignificant. Tables 1 and 2 depict data taken 2 and 6 days after fungicide application at Feekes growth stages 9.0 and 10.3, respectively, compared with nonsprayed plants.

Yield and seed quality. Yield component data were pooled over years, since the fungicide × year interaction was not significant. Data indicated no significant differences in number of seeds per head and thousand kernel weight resulting from foliar fungicide treatment (Table 3). No detectable differences in PS between fungicide-treated wheat plants and control plants supported our findings of no significant yield differences (Table 3). No delays in leaf senescence from foliar application of fungicide were noted, suggesting that durations of PS were similar. Therefore, a difference in the rate of PS is not expected where differences in grain yield are not observed.

Seed quality studies of harvested seed from the 1987 trial indicated no significant effects on either shoot length or total root length from fungicide applications (Table 4). Although seedling growth parameters appear unaffected by foliar fungicide treatment to parent plants, there was a significant ($P = 0.05$) reduction in percent germination of seed harvested from plants sprayed at stage 9.0 + 10.3 and stage 10.3. Results of a field study (*unpublished*) support these seed quality findings.

Leaf chlorophyll. There was interest in determining whether precursor chlorophyll constituents were synthesized in seedlings grown in the dark (7,10,24). Although the main factor (fungicide) and the fungicide × time (14-, 28-, and 42-hr exposure to light after the dark period) interaction were not significant, data tended to indicate evidence of increased chlorophyll synthesis in dark-grown seedlings derived from fungicide-treated plants. Eight of the nine chlorophyll measurements from fungicide treatments were numerically greater than control measurements (Table 5). Data for dark/light-grown seedlings showed no

significant differences in total chlorophyll when measured at the same time periods shown in Table 5.

DISCUSSION

In these experiments, no effect was detected from foliar applications of Bayleton plus Dithane M-45 on flag leaf gas exchange characteristics of wheat. Further, in neither year was any prolonged greening effect of intact tissue observed in treated plants. These findings support the nonsignificant yield differences resulting from fungicide application shown in Table 3.

These results differ from those of field studies indicating yield increases from foliar fungicides in the absence of recorded disease. It may be that these field studies failed to account for the control of secondary pathogens. The application of a foliar fungicide is usually seen as a control measure to protect plant tissue from a specific pathogen. Yet this cultural practice may simultaneously control unnoticed pathogens or pathogens of minor significance that may otherwise contribute to an overall reduction in crop yield.

The significant reduction in percent germination observed in the present study indicates that potential carryover effects for wheat from the foliar fungicides used in this study may influence the results of certain laboratory studies where control seed are assumed to be void of plant growth-regulating compounds. Also, a reduction in seed germination could have a considerable impact on producers growing certified seed. Although a tank mixture was used, Bayleton, because of its systemic activity, probably caused the reduction in seed germination; Dithane M-45, a nonsystemic fungicide, would not likely have reduced seed germination.

The results of the leaf chlorophyll content assays suggest that Bayleton, with its systemic activity, may translocate to the developing grain, with residual effect on chlorophyll synthesis in dark-grown seedlings but not in dark/light-grown seedlings. Harvey et al (14) reported that chloroplasts of etiolated cucumber cotyledons had crystalline prolamellar bodies, whereas those treated with benzyladenine had a well-developed plastid membrane system.

Table 5. Total leaf chlorophyll content of wheat seedlings grown in the dark after 14-, 28-, and 42-hr exposure to light at 25 C

Growth stage[a] at fungicide application	Chlorophyll content[b] (μg mg^{-1}, fresh weight)		
	14 hr	28 hr	42 hr
Control	0.148	0.217	0.518
9.0 + 10.3	0.139	0.247	0.552
9.0	0.241	0.353	0.537
10.3	0.221	0.351	0.632
C.V. 32.3			

[a] Feekes growth stages 9.0 (flag leaf emergence) and 10.3 (heading.)

[b] Differences are not significant within each time period at $P = 0.05$ according to Duncan's multiple range test.

In summary, the results indicate that Bayleton and Dithane M-45, sprayed in a tank mix, do not increase yield potential for wheat in the absence of disease. Their role in increasing yield levels appears to be exclusively as control agents of foliar diseases in wheat and not as plant-growth regulants. Our study did show, however, that the application of fungicides resulted in a plant growth-regulator effect for seed harvested from fungicide-treated plants.

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