Electron Beam Irradiation Effects on Wheat Quality, Seed Vigor, and Viability and Pathogenicity of Teliospores of *Tilletia controversa* and *T. tritici*

J. W. SITTON, Seed Technology Laboratory, Department of Crop and Soil Sciences, Washington State University, Pullman 99164-6422, J. BORSA, AECL Research, Whiteshell Laboratories, Pinawa, Manitoba, Canada ROE 1LO, T. R. SCHULTZ, Cooperative Extension, 2525 Merryfield St., Friday Harbor, WA 99250, and J. D. MAGUIRE, Seed Technology Laboratory, Department of Crop and Soil Sciences, Washington State University, Pullman 99164-6422

ABSTRACT

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Wheat seed infested with sori or free teliospores of *Tilletia controversa* and *T. tritici*, respectively, were irradiated with high energy electrons at doses ranging from 0–10.2 kGy to determine the suitability of electron irradiation to disinfest wheat. The germination of spores was then assayed to determine the sensitivity of each smut species to irradiation. Data indicated that *T. controversa* teliospores were somewhat more resistant to electron beam radiation than were teliospores of *T. tritici*. For *T. tritici*, doses of 4.6 and 6.7 kGy completely eliminated germination of free teliospores and teliospores in intact sori. For *T. controversa*, 10.2 kGy was required to completely eliminate germination of both free teliospores and teliospores in sori. Substerilizing doses of radiation delayed germination of the teliospores of both species. There was no significant deleterious effect of irradiation on wheat quality measurements, except for reduced surface texture and water absorption. As expected, irradiation significantly reduced seed germination and seedling vigor. Irradiation dosages above 2.6 kGy on teliospores significantly eliminated *T. tritici* infection of wheat, while irradiation doses of 10.2 kGy on sori reduced infection from 75.5 to 0.08%. No *T. controversa* infection was observed in wheat seed inoculated with irradiated or nonirradiated teliospores.

Dwarf bunt of wheat (*Triticum aestivum* L.), caused by *Tilletia controversa* Kühn in Rabenh., sporadically infects winter wheat (11) in the Pacific Northwest of the United States. The Peoples Republic of China (PRC) has implemented a "zero tolerance" on importation of wheat infested with this fungus (11,23). Difenoconazole (Dividend 3FS, CIBA Corp., Greensboro, NC), the only effective chemical seed treatment (21), has been registered in the United States for control of dwarf bunt in winter wheat, but its ability to totally eliminate dwarf bunt is questionable. Common bunt of wheat, caused by *T. tritici* (Bjerk.) G. Wint. in Rabenh., can be controlled in the United States. There are resistant wheat varieties, effective chemical seed treatments, and crop rotations for control of this disease (11). The effectiveness of Dividend and dwarf bunt resistant wheat cultivars will not solve the export problem to the PRC. The need therefore exists for effective methods to treat grain infested with *T. controversa*, prior to loading into clean ships to satisfy the demands of the PRC quarantine inspectors.

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Chemical control methods that have been tried include use of sodium hypochlorite (8) and gaseous hydrogen peroxide (22), which kills teliospores of *T. controversa* (8). Sodium hypochlorite and hot water (70 C for 5 s) have been used effectively in an experimental treater to decontaminate grain infested with teliospores of *T. controversa* and *T. tritici* (R. P. Cavalieri, *unpublished*). The disadvantages of these methods are that liquid sodium hypochlorite and gaseous hydrogen peroxide are hazardous to worker health, the chemicals require special methods of disposal or decontamination, and there is an added cost of seed drying following treatment with these solutions.

Schultz and Maguire (19) used irradiation with cobalt-60 gamma rays, at 0–20 kGy, to effectively decontaminate wheat with *T. tritici* and *T. controversa* teliospores. There was no significant reduction of wheat quality with these treatments. Gamma irradiation has been successfully used throughout the world for disinfestation of many foods (7,9, 10,16,20). However, shippers in the Pacific Northwest of the United States are skeptical of using a radioactive source to irradiate *T. controversa*-contaminated grain (T. R. Schultz, *unpublished*).

Treatment with high-energy electrons from an electron accelerator can be used to decontaminate a variety of food products (4,5,13). This type of irradiator does not contain any radioactive materials, but instead converts electricity to ionizing energy. An electron beam facility has several advantages over gamma facilities. It is more suitable for on-line treatment of grain, it can be shut off when there is no grain to be treated, it has no long-term storage requirement for a potentially hazardous material, it is more easily transported, there is no perceived waste disposal problem, and it is more acceptable to the public. In this preliminary evaluation, we examined the effectiveness of various doses of electron beam irradiation to decontaminate wheat in which both sori and teliospores of *T. controversa* and *T. tritici* were present, and determined the changes in wheat quality, seed vigor, and teliospore pathogenicity and viability.

MATERIALS AND METHODS

Wheat heads infected with *T. controversa* were collected from a commercial wheat field in Cavendish, ID, in 1993, and heads infected with *T. tritici* were collected from an experimental plot at Pullman, WA, in 1990. The sori were removed from the wheat heads. Half of the sori of each pathogen were hand crushed by means of a mortar and pestle. Teliospores were sieved through a 150-μm screen, prior to use, to eliminate most material other than individual spores. Experiments were carried out in which the spores were irradiated as clean preparations, either as sori or teliospores, and also as contaminants on wheat, to simulate the expected commercial condition.

The teliospores, sori, and wheat cv. Stephens were shipped to AECL Research in Pinawa, Manitoba, Canada, for irraditation. Aliquots (ca. 0.3 g) of teliospores and sori, respectively, were transferred to small, screw-topped glass vials. Samples (ca. 750 g) of contaminated wheat were packaged in plastic bags prior to irradiation. Duplicate samples of teliospores, sori, and wheat seed were exposed to graded doses of 0–10.2 kGy of electron beam radiation in two tests in an AECL I10/1 linear accelerator (Fig. 1) supplying approximately 1 kW of 10 Mev electrons. Irradiation was at an ambient temperature of 22 C throughout the treatment. Following irradiation, the samples were shipped to Washington State University (WSU) for testing wheat quality, seed vigor, and pathogenicity and viability of *T. controversa* and *T. tritici* teliospores.

The viability of free teliospores and teliospores extracted from intact sori was assessed by suspending ca. 0.01 g of teliospores in 10 ml of sterile water. The spore suspensions were agitated on a vortex-type mixer prior to assay. For *T. controversa,* 0.5-ml aliquots of teliospore suspensions were spread on 2% soil extract agar and incubated in continuous light (95 lux) at 5 C for 1 mo (15). For *T. tritici,* the teliospores were spread on 2% water agar on a laboratory bench (301 lux) at ca. 23 C for 5 days. The percentage of spore germination was determined by examining 6–68 teliospores, for a total of nine microscope fields at 100× on three petri dishes.

Wheat quality was evaluated by analyzing triplicate samples at the USDA-ARS, Western Wheat Quality Laboratory, WSU, for test weight, flour yield, break flour yield, flour ash and protein, milling score, water absorption, surface texture, and cookie diameter (1). These tests were repeated twice.

Germination (3) and cold tests (2,17) were used to test the vigor of electron beam–treated seed. For standard germination, the seeds were placed on saturated germination papers (76 lb., 1,153 cm sq; Anchor Paper Co., St. Paul, MN), rolled into rag dolls, and incubated in the dark for 7 days at 20 C. In cold tests, the seeds were placed on saturated seed germination paper coated with Palouse Silt Loam soil, rolled into rag dolls, and incubated in the dark for 7 days at 5 C, then for 4 days at 20 C. Following incubation, the seeds were visually examined to determine percentage of seed germinated and germination abnormalities, if present. These tests were repeated twice.

Stephens wheat samples (ca. 750 g), sealed in plastic bags, were shipped (January 1994) to AECL Canada for electron beam irradiation (0 and 10.2 kGy) and to Battelle PNL (Richland, WA) for irradiation in a GB650 irradiator at doses of 0, 4, 6, 8, or 10 kGy. The electron beam treatments were repeated twice, while the gamma irradiation treatments were repeated once. On receipt at WSU, Pullman, WA, the seed was stored at ca. 23 C. The seed was planted at Pullman, WA, at 5 g/1.5-m-long row in randomized blocks, covered with 2.5 cm soil on 7 March 1994. There were 15 replications per treatment. Seedling emergence was assessed on 7 April 1994.

The pathogenicities of irradiated *T. controversa* and *T. tritici* were assessed on wheat by transferring eight sori or 0.1 g of free teliospores in three drops of a 0.5% methyl cellulose solution into a sterile 17-ml shell vial. The sori were crushed by means of a sterile glass rod to liberate the teliospores. An additional 27 drops of methyl cellulose solution were transferred to the spore solution, and the mixture was agitated for ca. 15 s on a vortex mixer. To eliminate possible contamination of seed with nonirradiated spores, seeds of wheat cvs. Orin and Lemhi were surface disinfested by immersion in a 3:1,000 formalin/water solution for 15 min and air dried on absorbent paper in a smut-free greenhouse (14). Six drops of the spore-methyl cellulose suspension were transferred to a sterile vial along with 5 g of surface-disinfested wheat seed. An additional 27 drops of methyl cellulose were transferred to the wheat-teliospore mixture and agitated for ca. 20 s. The seeds (5 g) were planted in 1.5-m-long open furrows, covered with 2.5 cm of soil, 40 cm apart in randomized blocks at Observatory Hill (site 1) and Plant Pathology Farm (site 2) near Pullman, WA, on 27 October 1993 (run 1 on Orin at site 1), 14 November 1993 (irradiation run 2 on Orin at both sites), and 11 March 1994 (irradiation run 2 on Lemhi at both sites). Disease (percentage of heads with smut) was assessed on 9 August 1994 after the wheat was mature. Each replication (five to six) consisted of at least 250 heads per plot.

Teliospore germination, seed vigor tests, wheat quality assessments, and pathogenicity data from test plots were analyzed with SAS-GLM and Fisher's protected least significant difference (18).

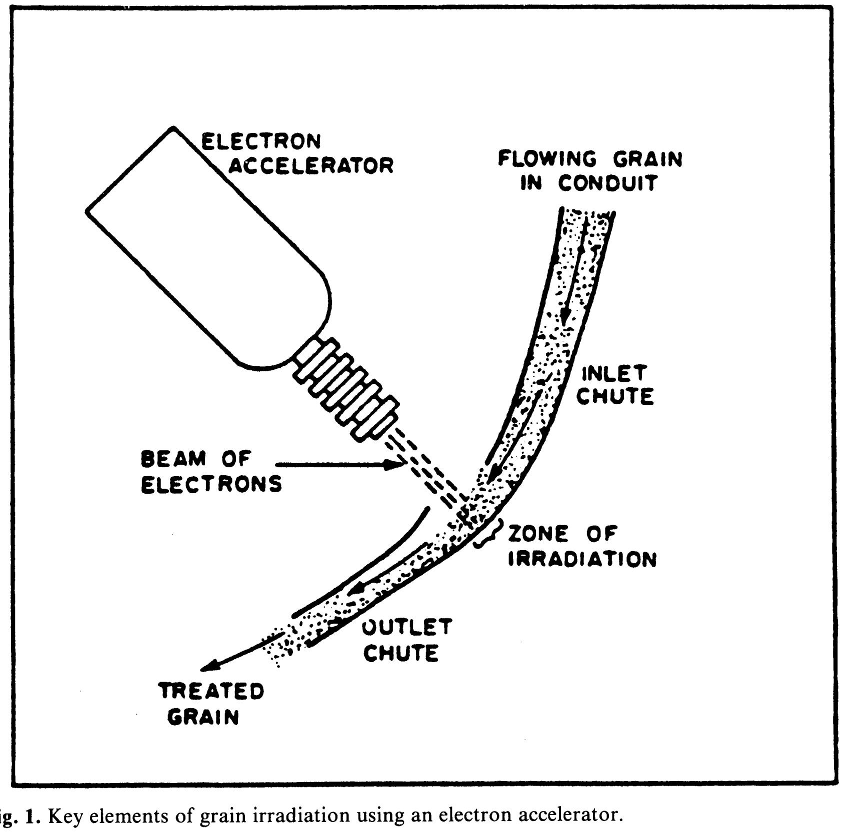

Fig. 1. Key elements of grain irradiation using an electron accelerator.

RESULTS AND DISCUSSION

Table 1 presents the effects of electron beam doses on teliospore germination. For *T. tritici,* the germination of teliospores was reduced to undetectable levels at 4.6 kGy for irradiated free spores, and at 6.7 kGy for irradiated sori. *Tilletia controversa* teliospores were resistant to radiation; 10.2 kGy were required to eliminate germination of either free-spores or spores in intact sori. With *T. controversa* teliospores irradiated to a dose of 6.7 kGy, only promycelia (noninfective for wheat) (11) were observed in such spores. Sporidial "H" structures (wheat infective) (11) were observed in samples irradiated at low doses (1.2 and 2.6 kGy) and in nontreated controls. Wheat is most susceptible to infection when it has 2–3 leaves, or 1–3 tillers, under snow cover (12,21). Since a dose of 6.7 kGy retards the rate of sporidial development, it is possible that a dose of 6.7 kGy would be just as effective as 10.2 kGy, because the delay in development would cause the spores to effectively miss the "window of infection" in the field.

There were no significant reductions in test weight, flour yield, break flour yield, flour ash, flour protein, milling score, or cookie diameter. There were significant differences in surface texture and water absorption (Table 2), which should not affect consumer acceptance of electron beam irradiated wheat.

Results of the seed germination and cold tests are presented in Table 3. Irradiation reduced the germination of seeds; even in the lowest dose tested (1.2 kGy) germination was near zero. In the cold test the effect was even more dramatic.

Table 1. Effect of electron beam doses on the germination of free teliospores or teliospores treated in sori of *Tilletia controversa* after 33 days at 5 C on 2% soil extract agar and of *T. tritici* after 5 days at 23 C on 2% water agar

	Germination (%)[a]			
	T. tritici		*T. controversa*	
Electron dose	Free spores	Spores from sori	Free spores	Spores from sori
kGy[b]				
0.0	89.5[c]	97.7	92.8	96.0
1.2	87.5	75.8	88.9	95.0
2.6	12.7	7.3	19.4	16.2
4.6	0.0	7.3	5.4	2.4
6.7	0.0	0.0	1.3	0.8
10.2	0.0	0.0	0.0	0.0
LSD ($P = 0.05$)	7.3	5.8	6.2	4.5

[a] Mean number of germinating spores with two irradiation replications in three microscope fields (6–68 teliospores per field).
[b] kiloGray (kGy) = Joules per cubic meter of material treated.
[c] Means within a column were compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

Table 2. Effect of electron beam irradiation on surface texture, water absorption, and cookie diameter

	Wheat quality assessments[a]		
Electron dose kGy[b]	Surface texture	Water absorption	Cookie diameter (cm)
0.0	7.0[c]	54.1	9.2
1.2	7.0	52.3	9.0
2.6	7.0	52.5	9.2
4.6	7.0	51.3	9.0
6.7	5.7	51.4	8.9
10.2	5.7	51.5	9.0
LSD ($P = 0.05$)	0.9	0.6	0.3

[a] Mean of three wheat samples of 200 g per sample.
[b] kiloGray (kGy) = Joules per cubic meter of material treated.
[c] Means within a column were compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

Table 3. Effects of electron beam treatments on germination (normal, weak or abnormal germination [Abn], and no germination [No Germ]) of cv. Stephens wheat[a]

	Seed germination %[b]					
	Germination test			Cold test		
Electron dose (kGy)[c]	Normal	Abn.	No Germ.	Normal	Abn.	No Germ.
0.0	94[d]	5.3	0.5	88	11.0	1.3
1.2	0	99.0	0.8	0	95.8	4.3
2.6	0	98.0	2.0	0	70.5	27.0
4.6	0	98.8	1.3	0	42.3	57.8
10.2	0	94.0	6.0	0	1.8	98.3
LSD ($P = 0.05$)	2	3.5	2.9	2	8.4	6.8

[a] In germination tests with rolled paper towels according to Association of Off. Seed Analyst (2) procedures at 20 C for 7 days and cold tests rolled paper coated with 28.3 g of saturated loam soil at 5 C for 7 days, then 20 C for 4 days.
[b] Mean percentage of germinating seeds representing 400 seeds (100 from each of four electron beam replications).
[c] kiloGray (kGy) = Joules per cubic meter of material treated.
[d] Means within a column were compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

In the standard germination test, the 10.2 kGy treatment showed 94% germination (albeit weak or abnormal) and 6% with no germination. In the cold test, the same treatment had only 1.8% germination (all weak or abnormal) and 98.3% no germination.

No wheat irradiated with 4 kGy and above emerged in test plots at Pullman, WA (Table 4).

None of the plants from seed inoculated (0–10.2 kGy) with *T. controversa* teliospores (free-teliospores or sori) at two locations became infected with dwarf bunt (Table 5). This agrees with previous work (11) that showed that dwarf bunt infection from seedborne teliospores is rare. Irradiated free-teliospores and sori of *T. tritici* lost pathogenicity in doses above 4.3 kGy, with the exception of one test in which a trace amount (0.08%) of common bunt occurred at 10.2 kGy. This trace amount of common smut may be due to natural, soilborne infection from inoculum from previous tests at this site. It is unlikely to represent trace survivors of the radiation process since there was no infection at 4.6 kGy. We believe that this minute level of infection is insignificant.

Table 4. Emergence of cv. Stephens wheat seed irradiated with electron beams and gamma rays (Co60) planted in 1.5-m rows, 2.5 cm deep in Palouse silt loam soil in plots at Pullman, WA, on 7 March 1994

	Emergence (no. coleoptile per row)[a]	
Dose (kGy)[b]	Electron beams	Gamma rays
0	14.7[c]	19.3
4	...	0
6	...	0
8	0	0
10	...	0
LSD ($P = 0.05$)	3.0	4.1

[a] Mean number of coleoptiles emerging from 5 g of seed per row in each of 15 replications determined on 7 April 1994.
[b] kiloGray (kGy) = Joules per cubic meter of material treated.
[c] Means within a column were compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

In summary, electron beam treatments of wheat seed infested with *T. controversa* and *T. tritici* were effective in eliminating the viability and pathogenicity of spores of both species. In addition, low doses (4 kGy) of electron beam irradiation prevented emergence, hence disease development, of wheat seed potentially infected with bunt fungi. At the required doses, there was only minimal reduction in wheat quality using a variety of indicators. The process itself is flexible, cost effective (6), and suitable for use in export terminals as a decontamination method for grain being loaded into ships. Based on these findings and considerations, we conclude that irradiation has the potential of solving the dwarf bunt problem in wheat designated for export to the PRC, and elsewhere where these microorganisms may be a problem.

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Table 5. Pathogenicity of free teliospores and teliospores in intact sori of *Tilletia tritici* treated with 0-10.2 kGy doses of electron beams[a]

Cultivar and dose (kGy)[c]	Infected heads (%)[b]			
	Site 1		Site 2	
	Free TS	TS in sori	Free TS	TS in sori
Lemhi				
0.0	98.4[d]	94.4	95.0	90.5
4.3	0.0	0.3	0.0	0.0
7.5	0.0	0.0	0.0	0.0
9.6	0.0	0.0	0.0	0.0
10.2	0.0	0.0	0.0	0.0
LSD ($P = 0.05$)	0.5	2.8	1.0	2.2
Orin				
0.0	80.8	75.5		
1.2	69.2	66.7		
2.6	3.2	0.6		
4.6	0.0	0.0		
6.7	0.0	0.1		
10.2	0.0	0.1		
LSD ($P = 0.05$)	14.1	10.7		

[a] Cultivars Orin and Lemhi wheat (*Triticum aestivum*) were inoculated with treated teliospores and planted at two sites near Pullman, WA on 27 October 1993 (Orin) and 11 March 1994 (Lemhi). The severity of wheat infection (% infected heads) was determined on 8 August 1994.

[b] Mean percentage of heads with common bunt assessed on a minimum of 250 heads per plot in each of five replications at the Plant Pathology Farm and six replications at Observatory Hill.

[c] kiloGray (kGy) = Joules per cubic meter of material treated.

[d] Means within a cultivar and column were compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

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