Effectiveness of Chemical Seed Treatments in Controlling Karnal Bunt Disease of Wheat

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ABSTRACT

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Forty-seven chemical products recommended for the control of bunts and smuts were screened for their effectiveness as wheat seed treatments to control *Tilletia indica*. Chemicals that reduced teliospore germination appeared to be limited in the length of their activity. Most were effective for up to 6 mo, but only a few for longer periods of time. Of those with a longer period of activity, triphenyltin hydroxide, methoxyethylmercury acetate, and ethylmercury chloride were effective for up to 18 mo. However, with the possible exception of the mercurial compounds, none was capable of killing *T. indica* teliospores when applied to infected seeds. There is currently no chemical seed treatment that can guarantee that wheat seed is not carrying viable *T. indica* teliospores.

Karnal bunt is caused by *Tilletia indica* Mitra (syn. *Neovossia indica* (Mitra) Mundkur), a flower-infecting organism that partially infects the seed of bread wheat (8), durum wheat, and triticale (1). The teliospores of *T. indica* may be deposited on the soil at harvesting and threshing, or they may become attached to the surface of the seed as an external contaminant (4). Upon germination at the soil surface, each teliospore gives rise to a promycelium, which bears filiform primary sporidia at its tip (11). These primary sporidia and secondary sporidia, which develop subsequently, are carried to the wheat spike either by air currents or by splashing water (12).

The effectiveness of different compounds as seed treatments for wheat seed infected with Karnal bunt has been evaluated by assessing their effect on teliospore germination. In these studies (2,3,9,10,13,14) the following treatments were found to reduce teliospore germination: butrizol (Indar 70LC), 2.5 g/kg; copper carbonate, 2 g/kg; ethylmercury chloride (Ceresan), 2 g/kg; formalin, 0.25%, for 10 min; methoxyethylmercury chloride (Uspulun), 0.25%, for 30 min; phenylmercury acetate (Agrosan GN), 2.5 g/kg; thiram, 3 g/kg; triphenyltin acetate (Brestan 60WP), 2.5 g/kg; and triphenyltin hydroxide (Du-Ter 20WP), 2.5 g/kg.

Fuentes et al (5) found methoxyethylmercury acetate (Panogen 15) at 0.7 ml/kg and pentachloronitrobenzene (Terrazan 75PH) at 1 g/kg to be the most effective fungicides. Hexachlorobenzene (Anticarie 40) at 2.1 g/kg gave moderate control. Ethylmercury chloride (Ceresan) and phenylmercury acetate (in two forms, Agrosan GN and Mist-O-Matic) offered less satisfactory control at the doses evaluated. Carboxin plus captan (Vitavax 300) and oxycarboxin (Plantvax 75) had little inhibitory effect. Benomyl (Benlate), propiconazole (Tilt), fenpropimorph (Corbel), and thiabendazole (Mertect) allowed teliospore germination equal to or better than that of the control.

The aim of this study was to screen chemicals recommended for the control of bunts and smuts, to evaluate their efficacy as wheat seed treatments to control *T. indica*. Several compounds have previously been screened, but with no indication of the length of their activity or whether they are fungistatic or fungitoxic, i.e., whether they kill teliospores or merely inhibit the germination of teliospores in their presence.

MATERIALS AND METHODS

Forty-seven chemical products were screened. Two samples of wheat (cultivar Ciano 79) were treated with each product, at the application rate to be tested. The first sample consisted of 300 g of healthy seed, and the second consisted of 25 g of seed naturally infected with Karnal bunt mixed with 75 g of disease-free seed. The infected seed was harvested from farmers' fields in the Yaqui Valley, Sonora, Mexico. The chemical was placed in a conical glass flask, the seed was added, and the flask was shaken by hand until the seed was uniformly covered by the chemical. The amount of each chemical added to the flask was 10% more than the required application rate, to allow for the quantity that adhered to the sides of the flask during the treatment process. Also, in slurry treatments the solid materials were dissolved in distilled water to a total volume equivalent to 15 ml/kg of seed.

After 24 hr, 1 wk, and 1, 2, 4, 6, 9, 11, 12, 14, and 18 mo, 400 seeds were taken from each 300-g seed lot for seed germination tests in rolled paper towels, according to the rules of the International Seed Testing Association (7).

After the same periods of time, 50 seeds infected with Karnal bunt were taken from each 100-g seed lot for teliospore germination tests. Seeds were placed in a sterile screw-cap vial with approximately 10 ml of sterile distilled water and agitated on a vortex mixer until a large number of teliospores were shaken out from the bunted grains. The teliospore suspension was then passed through a 60-μm-mesh sieve and placed in a 15-ml sterile centrifuge tube. Sterile distilled water was added to bring the total volume to 10 ml. The tube was centrifuged to 2,500 rpm, the supernatant was decanted, and 7 ml of a 5% solution of household bleach (7% sodium hypochlorite) was added to the teliospores retained in the tube. The bleach was added to prevent fungal or bacterial contamination of germinating teliospores on agar plates. The tube was shaken well and then centrifuged as described above. The teliospores were only immersed in the sodium hypochlorite solution for 1–2 min, since it reduces the germination of teliospores if left in contact with them too long (Warham, *unpublished*). The supernatant was then decanted, 10 ml of sterile distilled water was added, and the tube was centrifuged again. This rinse with distilled water was repeated a second time, the supernatant was decanted, and 7 ml of sterile distilled water was added.

A 0.5-ml aliquot of the teliospore suspension was then placed on each of five water agar plates. The plates were incubated at 15 C with 12-hr alternating periods of light and dark. The photosynthetically active radiation was 27 $\mu E \cdot m^{-2} \cdot sec^{-1}$ (400–700 nm).

The percentage of germinated teliospores was recorded after 21 days. On each of the five replicate plates, 20 fields of view (magnified 100×) with approximately 50–75 teliospores were scored.

If less than 1% of teliospores germinated in 4 mo after treatment with

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a chemical product, it was retested at a wider range of concentrations. The two most promising products, maneb (Manzate D) and triphenyltin hydroxide (Du-Ter), were examined further with a Gustafson batch laboratory seed treater, to eliminate any irregularities that might be caused by treating the seed in flasks.

As the vortex mixer might not shake out the teliospores from the center of an infected grain or from point infections where the pericarp had not ruptured, a sample treated with a chemical at the highest application rate was milled in a coffee grinder, set on a coarse grind, before the teliospores were separated. This was carried out to determine whether the chemical penetrated the seed to inhibit all teliospore germination.

In addition, a sample treated with a chemical at the highest application rate was washed with a solvent of that chemical, to determine if the chemical is fungistatic or fungitoxic, i.e., whether it only inhibits the germination of teliospores when in contact with them or whether it kills them.

No statistical comparisons of compounds or rates of compounds were made, since the objective of this study was to identify seed treatments capable of killing all of the teliospores present.

RESULTS

Of the 47 chemical products tested, 34 failed to reduce teliospore germination to less than 1% after 4 mo (Table 1). Each of the remaining 13 products was retested at a wider range of concentrations (Table 2). Those that reduced teliospore germination appeared to be limited in their length of activity; most were effective for up to 6 mo, and only a few were effective for 18 mo. The most promising of those effective for the longer period, as judged on consistency of performance and minimal effects on wheat seed germination, were maneb (Manzate D) and triphenyltin hydroxide (Du-Ter). These two products were examined further. Maneb proved disappointing (Table 3), but triphenyltin hydroxide at 2–5 g/kg of seed achieved complete inhibition of teliospore germination after 2 mo (Table 4).

Milling the samples before separating the teliospores showed that triphenyltin hydroxide did not penetrate the infected portions of the seed (Table 5). Also, washing the samples with the solvent acetone showed that triphenyltin hydroxide was fungistatic and not fungitoxic (Table 5).

Apart from the mercurial compounds, none of the chemicals tested had a significant effect on seed germination. Results of the seed germination tests for triphenyltin hydroxide and maneb are given in Tables 3 and 4.

DISCUSSION

The only chemical that induced complete inhibition of teliospore germination of *T. indica*, apart from the mercurials (ethylmercury chloride and methoxyethylmercury chloride), was triphenyltin hydroxide. However, triphenyltin hydroxide neither penetrated to all infected parts of the seed nor killed the teliospores. The mercurial seed treatments were not retested, since they are banned in most countries and therefore not a viable solution for quarantine purposes. They were initially included in the experiments for a comparison with the other chemicals under test.

Therefore, even fungicides that achieve a substantial reduction in teliospore germination of *T. indica* may not

Table 1. Seed treatments that failed to reduce teliospore germination of *Tilletia indica*

Common name	Trade name	Formulation[a]	Company	Application[a] D	S	L	Rate (per kg of seed)
Bitertanol	Bay KWG 0599	50WP	Mobay	*	*		3 g
Captan	Vitizan 50	50WP	Stauffer		*		8 g
Captan + maneb	Granox P-F-M	30 + 30D	Chipman	*	*		8 g
Cupric hydroxide	Kocide 101	50WP	Kocide	*	*		8 g
Dihaloalkyl arylsulfone	GUS 4002-2FL	32.4F	Gustafson			*	2 g
Etaconazole	Vangard	10WP	Ciba-Geigy	*	*		6 g
Formaldehyde (formalin)		37.5L	Sigma			*	1% (10 min)
Furmecyclox	EPIC 500FF	47.9F	Gustafson			*	2 g
Hexachlorobenzene	Anticarie	40WP	A. P. Rossinger	*	*		6 g
Imazalil		10L	Wilbur-Ellis			*	2 g
	CPO-AGRO II/36/A2	25WP	Wilbur-Ellis	*	*		8 g
3-Iodo-2-propynyl butylcarbamate	Guardsan 388	35L	Troy			*	15 ml
	Guardsan 389	40WP	Troy	*	*		10 g
IPTFB[b]	MV 785 1.5D	1.5D	Stauffer	*	*		3.5 g
Mancozeb	Dithane M-45	80WP	Rohm and Haas	*	*		8 g
	Manzate 200	80WP	Du Pont	*	*		10 g
Maneb	Delsene M	64WP	Du Pont	*	*		10 g
	TF 3090	50WP	Chipman	*	*		10 g
Maneb + hexachlorobenzene	Granox F	25 + 5L	Chipman			*	5 g
	Granox NM	50 + 10D	Chipman	*	*		10 g
Mercury phenyl acetate	Tillantina	2D	Bayer	*	*		2 g
Nuarimol	EL-228	70WP	Eli Lilly	*	*		4 g
	TF 3611	10WP	Chipman	*	*		4 g
	TF 3682	7.5L	Chipman			*	1 ml
Pentachloronitrobenzene	Trigan F	45L	Cydsa			*	15 ml
Sodium hypochlorite	Cloralex	6L	Allen			*	0.7% (1 hr)
TCMTB	Busan 30A	30L	Buckman Labs.			*	10 ml
Thiabendazole	Mertect LSP	30F	Gustafson			*	4 g
Thiophanate-methyl	Topsin 30	30F	Gustafson			*	4 g
Thiram	Thiram 75PH	75WP	Birgam	*	*		8 g
Triadimefon	Bay MEB 6447	25WP	Mobay	*	*		4 g
Triadimenol	Baytan + Baysas 9244	20 + 2.7WP	Mobay	*	*		2 g
Zineb	Zineb 80	80WP	Vimsa	*	*		8 g
UBI-A1055		50WP	Uniroyal	*	*		1 g

[a] D = dust; F = flowable; L = liquid; S = slurry; WP = wettable powder.
[b] *N*-3-isopropoxyphenyl 1,2-trifluoromethyl benzamide.

Table 2. Seed treatments that reduced teliospore germination of *Tilletia indica*

Common name	Trade name	Formulation[a]	Company	Application Type[a]	Application Rate (per kg of seed)	Length of activity[b] (mo)
Bitertanol + fuberidazole	Sibutol	37.5 + 2.3L	Bayer	L	9–10 ml	18
Chloroneb	Demosan	65WP	Du Pont	S	6 g	6
Copper carbonate		Light D	Tennessee	S	6–7 g	6
Copper sulfate		Tri-Basic D	Tennessee	S	6–7 g	6
DCNA	Botran	75WP	Upjohn	D	6–10 g	6
				S	6–10 g	18
Ethylmercury chloride	Ceresan	15.4D	Excel	D	0.4–2 g	6
				S	0.4–2 g	18
Maneb	Manzate D	80D	Du Pont	D	6–10 g	6
				S	6–10 g	18
Methoxyethylmercury acetate	Panogen	15L	KenoGard	L	2.5–15 ml	18
Pentachloronitrobenzene	Terrazan F	45L	Cydsa	L	3–12 ml	6
	Terrazan 75PH	75WP	Cydsa	D	2–4 g	6
				S	2–4 g	6
	Trigan S	25EC	Cydsa	L	2–5 ml	2
Thiram	Thylate	65WP	ICI	S	8–10 g	2
Triphenyltin hydroxide	Du-Ter	47.5WP	Uniroyal	D	1–5 g	18
				S	1–5 g	18

[a] D = dust; EC = emulsifiable concentrate; L = liquid; S = slurry; WP = wettable powder.
[b] Germination of less than 1% of teliospores.

Table 3. Effect of maneb seed treatment on teliospore germination of *Tilletia indica* and wheat seed germination

Treatment	Rate (g/kg of seed)	Teliospore germination (%) Time after application 24 hr	1 wk	1 mo	Seed germination (%) Time after application 24 hr	1 wk	1 mo
Maneb dust	2	25.1	35.6	20.0	98.0	96.8	98.5
	4	31.6	26.1	24.7	97.8	97.5	98.0
	6	22.5	15.8	11.0	96.5	97.3	96.5
	8	26.9	18.4	7.2	95.8	97.3	96.5
	10	6.6	4.3	6.1	97.5	97.0	97.3
Maneb slurry	2	25.6	10.8	14.0	98.3	97.3	96.5
	4	6.4	7.7	1.4	97.8	97.5	98.5
	6	14.3	25.1	9.3	96.0	96.0	98.3
	8	5.3	3.2	4.2	97.8	98.5	97.3
	10	4.9	1.6	2.4	96.5	97.0	96.3
Control		46.6	33.3	49.5	95.8	96.5	95.5

Table 4. Effect of triphenyltin hydroxide seed treatment on teliospore germination of *Tilletia indica* and wheat seed germination

Treatment	Rate (g/kg of seed)	Teliospore germination (%) Time after application 24 hr	1 wk	1 mo	2 mo	Seed germination (%) Time after application 24 hr	1 wk	1 mo
Triphenyltin hydroxide dust	1	0	0	0.2	2.8	98.5	98.8	98.5
	2	0	0	3.5	0	97.5	98.3	98.3
	3	0.3	0	1.1	0	98.3	97.8	97.8
	4	0	0	0.2	0	97.3	99.3	96.0
	5	0	0	0	0	98.5	97.5	97.5
Control		35.3	39.3	47.5	33.1	98.5	97.8	98.8

Table 5. Effect of triphenyltin hydroxide on teliospore germination of *Tilletia indica* in wheat seed milled or washed with a solvent after treatment

Treatment	Teliospore germination (%) Normal[a] 24 hr	1 wk	1 mo	Milling[b] 24 hr	1 wk	1 mo	Solvent[c] 24 hr	1 wk	1 mo
Triphenyltin hydroxide[d]	0	0	0	14.1	13.0	14.8	5.8	3.3	39.1
Control	35.3	39.3	47.5	25.2	22.4	16.0	21.1	29.3	37.6
Control[e]				30.7	37.0	31.5			

[a] Normal separation of teliospores from the sample.
[b] Teliospores separated after the sample was milled.
[c] Teliospores separated after the sample was washed with acetone.
[d] Dust, 5 g/kg of seed.
[e] Milled healthy grains were added to teliospores separated from the control sample.

eradicate the pathogen when applied to infected seed. Mitra (9) believed the reason to be that the teliospores in point infections are well protected by the pericarp, and therefore the fungicide cannot reach them. If this is correct, volatile compounds with strong fumigant action should be more effective. However, Smilanick et al (15) found that fumigations with chloropicrin, sulfur dioxide, and methyl bromide do not reduce teliospore germination more than 75% and are phytotoxic to seeds at rates lower than those at which teliospore germination is inhibited.

This study compared a large number of fungicides that have been recommended for other bunts and smuts, but very few of them were effective against *T. indica*. For example, fungicides shown to be effective against *T. caries* by Hoffmann and Walder (6) include benomyl (Benlate), carboxin (Vitavax), chloroneb (Demosan), hexachlorobenzene (Anticarie), thiabendazole (Mertect), and triadimefon (Bayleton). With the exception of carboxin, these were investigated in this study and found to have little or no effect on *T. indica*. Fuentes et al (5) also found carboxin to have little inhibitory effect on teliospore germination of *T. indica*. The reason for this difference may be that when a fungicide is tested against *T. caries*, the usual procedure is to apply the fungicide to seed contaminated with teliospores and measure the proportion of plants that become infected. In this case the fungicide has the opportunity to act on germination and on the delicate fungal structures involved in infection. The ability to kill dormant teliospores is not a necessary criterion of effectiveness. This is the requirement imposed by these tests for *T. indica* and, in relation to the problem, the correct one.

Finally it should be noted that even

if seed treatments were to achieve complete eradication of *T. indica* on infected seed samples, they would not by themselves provide control of the disease, since teliospores may survive for 4 yr in the soil.

With the possible exception of mercurial compounds, none of the available fungicides are capable of killing teliospores of *T. indica* when applied to infected seed. This means that there is currently no chemical seed treatment that can guarantee that wheat seed is not carrying viable teliospores of *T. indica*.

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