MICROFLORA OF BUCKWHEAT SEED, CHANGES IN STORAGE AND EFFECT OF SEED TREATMENTS ON SEEDLING EMERGENCE[1]

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Abstract

The microfloral components of 37 lots of buckwheat (Fagopyrum sagittatum) seed harvested in Manitoba were determined after 7 days incubation. Field fungi predominated, with a high incidence of Botrytis sp. Storage of 11 tough and damp lots of buckwheat for 390 days in sealed jars in an unheated storage shed resulted in decreased germination, decreased infection with field fungi, including Botrytis, increased infection with storage molds, and increased moisture content. In contrast, the one dry sample stored for the same period showed increased germination, reduced Botrytis, increased Cladosporium, no storage molds, and decreased moisture content. Emergence was significantly decreased for seed with high Botrytis levels at Brandon, Manitoba, and was not improved with seed treatment. No phytotoxic symptoms were observed with 14 seed treatment chemicals in 1970 field trials and, with the possible exception of Manzate 200 at 2.60 and 5.20 g/kg, emergence was not reduced by any of the 10 treatments used in 1971.

Introduction

Buckwheat, Fagopyrum sagittatum Gilib., is an important special crop in Western Canada. The number of hectares sown to buckwheat in Manitoba has increased from 20,250 (50,000 acres) in 1968 to 32,400 (80,000 acres) in 1970 (6), constituting over 50% of tne total Canadian crop. Also in 1370, for the first time Saskatchewan and Alberta both grew over 8,130 ha (20,000 acres). Most of the grain is exported to Japan where the flour is used for making noodles, pancakes, and other edible products (1). The hulls are used in the packing' industry and for filling pillows. Diseases of buckwheat have not been reported commonly (4,10) and the seed is not treated in Canada. However, reports of wilting in buckwheat at Morden, Manitoba, in 1969 and requests from farmers for suitable fungicides prompted a study of the seed microflora of buckwheat with particular reference to possible pathogens and efficacy of seed treatments. The interrelationships between the microfloral components, storability, and germination was also studied by using naturally damp buckwheat seed.

Materials and methods

Thirty seven lots of buckwheat seed produced in 1969 were received from southern Manitoba in January 1970 (Tables 1,2, and 4). Twelve lots (Table 1, nos. 1 to 12) grown at Carman, Morden, and Winkler were received from Federal Grain Co., and the remainder were from the CDA Research Station, Morden. Lots 1 to 3 and 6 to 10 were of common buckwheat; lots 4, 5, and 11 to 20 were of the cultivar Tokyo, and the remainder of other cultivars (Table 2). Lot 4 had been dried on the farm and lots 17 to 19 were harvested from wilted plots. Moisture contents were determined by AACC method 44-18(2) on duplicate 10-g samples of lots 1 to 12 (Table 1) on arrival. Lots 1 to 12 were then stored in tightly sealed 0.5-liter glass jars, 225 g per jar, in an unheated storage shed; moisture contents were again determined after 390 days. The range in official temperatures, over the storage period 7 January 1970 to 1 February 1971 was -38 to +36 C. Microfloral components present on seed of lots 1 to 37 were determined on receipt (0 days) and for lots 1 to 12 again after 390 days storage. To determine the microflora on the seed a No. 3 Whatman filter paper disc (9 cm) in a petri dish was moistened with 5 ml distilled water and 25 seeds placed on it in a circular pattern near the periphery. There were four replicates each of 25 seeds. The plates were exposed to daylight for 7 days at room temperature (17-24 C) after which the microflora of each seed was examined microscopically. Germination was determined from the same plates after 7 days and the results subjected to an analysis of variance.

The source, formulation, and composition of the 14 seed treatment chemicals used in 1970 are given in Table 3. Each chemical was

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applied to 200 g of seed at the indicated dosage and shaken well in a 1-liter glass jar. The jars were kept sealed for 2 days to allow the vapor, if any, to act and then lots of 120 seeds were package in envelopes. Envelopes that contained seed from the same treatment were then placed in polyethylene bags and stored at 15 C until seeding 7-8 days later. There were two field tests in 1970 with treated seed; one was to determine possible phytotoxicity (Table 3) by using seed with low (0-3%) Botrytis, content, and the other was to measure control of seed-borne Botrytis sp. infection (Table 4) by using seed with high and low levels of the pathogen. Both tests were sown at Brandon on 5 June 1970. The single-row plots were 3.66 m (12 ft) long, 22.8 cm (9 inches) apart and were replicated four times. One hundred and twenty seeds were sown in each row. The plants were pulled 7 days after seeding, emergence was recorded and results from all replicates were subjected to analysis of variance.

In 1971 there was one field test with treated diseased seed. The variety used, CD 7274, from Morden, had 15% Botrytis infection. The source, formulation and composition of the 10 chemicals used are given in Table 5. Twenty-two days after treatment four replicates of each treatment (200 seeds per row) were sown at Morden and Brandon on 19 and 20 May, respectively, and emergence was determined after 25 days.

Table 1. Microflora, germination, moisture content, and grade of 12 lots of buckwheat seed after 0 and 390 days storage

	% microfloral components arranged in groups**																	
	Field					Harvest		storage					Other					
Lot no.	Alt. sp.	Bot. sp.	Clad. sp.	Epi. sp.	Fus. sp.	Gon. sp.	Ceph. sp.	Strep. spp.	ASP. cand.	Asp. vers.	Asp. other	Pen. blue	Pen. other	Rhiz. sp.	Bact.	Germination (%)	Moisture content (%)	Grade†
0 days storage																		
1	89	37	31	3	0	0	0		0	0	0	0	0	0	0	82		
2	92	5	15	5	0	1	0		0	0	0	0	0	0	0	92		
3	95	8	19	9	0	0	0		0	0	0	0	1	0	0	89		
4	7 3 5	2 1 4	1	1	0	3	1	0	0	0	0	0	0	0	0	86	11.2	S
5	8 5 1	4 3 1	3	1	1	3	9	0	0	0	0	0	0	0	0	74	16.8	T
6	9 3	1 1 7	1	0	0	0	8	0	0	0	0	0	0	0	0	91	16.1	T
7	93	22	42	3	0	5	6	31	0	0	0	0	0	0	0	68	16.5	T
8	92	30	20	5	0	2	1	8	0	0	0	0	0	0	0	78	18.6	D
9	96	2	24	1	0	0	0	3	0	0	0	0	0	0	0	73	17.9	D
10	95	13	15	0	1	3	0	12	0	0	0	0	0	0	8	0	16.4	T
11	89	34	25	4	0	4	4	6	0	0	0	0	0	0	0	82	16.2	T
12	93	1	23	0	1	1	0	12	0	0	0	0	0	0	7	7	17.0	T
390 *days storage*																		
1	0-††	0-	0-	0	0	0	1	13+	0	0	0	0	8+	0	20+	26-	17.3	D
2	0-	0	0-	0	0	0	0	2	1	0	0	0	61+	0	1	78	15.7	T
3	0-	0-	0-	0-	0	0	0	67+	1	0	3	0	4	1	17+	24-	17.7	D
4	71	13-	54+	1	0	0	0	2	0	0	0	0	0	0	0	92	9.8	S
5	0-	0-	3-	0	1	0	2	18	0	0	0	1	5	0	0	17-	18.0	D
6	1-	0	0-	0	0	0	0	7	0	0	0	0	1	0	0	69	16.8	T
7	9-	0-	3-	0	6+	0	9	79+	7+	13+	0	0	62+	0	0	0-	17.1	D
8	25-	0-	0-	0	3	0	3	5	69+	35+	0	53+	34+	0	25+	0-	19.4	D
9	0-	0	0-	0	1	0	0	7	0	0	0	0	0	1	0	44-	18.1	D
10	0-	0-	45+	0	0	0	0	34+	1	0	0	1	11+	0	0	55-	16.1	T
11	0-	0-	0-	0	0	0	0	11	0	0	8+	0	7+	2	0	53-	18.2	D
12	0-	0	0-	0	0	0	0	28+	0	0	8+	0	4	0	2	63	18.0	D

Based on four replicates each of 25 seeds: Alt. = *Alternaria*; Bot. = Botrytis; Clad. = *Cladosporium*; Epi. = *Epicoccum*; Fus. = *Fusarium*; Gon. = *Gonatobotrys*; Ceph. = Cephalosporium; Strep. = *Streptomyces*; Asp. cand. = Aspergillus *candidus*; Asp. vers. = A. *versicolor*; Asp. other = Aspergillus, other species; Pen. blue = Penicillium, tall blue species; Pen. other = Penicillium, other species; Rhiz. = Rhinopus; Bact. = bacteria.

** See text for definitions of groupings of microorganism associated with seed at various stages

† Grade: S = standard (<14.8% moisture); T = tough (14.9-17.0% moisture); D = damp (>17.0% moisture).

†† + and - indicates an increase and a decrease ($P < 0.05$), respectively, compared with the corresponding value at 0 days.

Results and discussion

Microflora, germination, and moisture content of untreated seed

Components of the microflora present on untreated seed lots (Table 1 and 2, nos. 1 to 12 and 17 to 37) are listed in groups, namely: "field", "harvest", "storage", and "other". The "field" group includes those fungi that appear whilst the crop is developing in the field (3), and the "storage" groups include those fungi and bacteria that normally become apparent during storage (3). The "harvest" group includes those fungi and bacteria that generally appear before or at time of harvest in the period between the occurrence of "field" and "storage" fungi (7). "Other" microflora are those that are not in the field, harvest, or storage groups but appear when seed is plated on moist filter paper (7). In all lots at 0 days field fungi predominated, particularly Alternaria, Botrytis, and Cladosporium. The harvest microflora consisted of Cephalosporium and Streptomyces, but representatives of storage and other groups were almost entirely absent. Germination on filter paper ranged from 21 to 98% (Tables 1 and 2). After 390 days storage most field fungi had disappeared from all tough and damp samples, but not from the dry sample (lot 4). Streptomyces spp. (harvest), Penicillium spp. (storage), and bacteria (other) greatly increased, except in lots 4, 6, and 9 (Table

Table 2. Microflora and germination of 21 lots of buckwheat from CDA Research Station, Morden, Manitoba

		% microfloral components* arranged in groups**													
		Field							Harvest				Other		
Lot no.	Cultivar	Alt. sp.	Bot. sp.	Clad. sp.	Epi. sp.	Fus. sp.	Gon. sp.	Paec. sp. 1	Paec. sp. 2	Ceph. sp.	Pap. sp.	Strep. spp.	Trich. sp.	Rhiz. sp.	Germination (%)
17	Tokyo	90	1	2 9	1	2	2	1	1	1	0	16	12	0	82
18	Tokyo	90	1	2 1	1	0	2	0	0	5	0	11	3	0	79
19	Tokyo	88	1	3 2	3	0	0	0	0	0	2	55	4	0	75
20	Tokyo	93	4	2 5	0	0	2	0	0	0	0	9	1	0	94
21	CD 1356-40-3	98	3	3 3	1	0	1	0	0	3	6	15	2	0	85
22	CD 1356-42-3	97	1	1 8	2	0	2	0	0	0	1	21	0	0	92
23	CD 1370-61-4	93	0	2 0	0	0	5	0	0	2	2	12	0	0	87
24	CD 5852	92	1	1 3	2	1	5	3	1	4	0	21	0	0	78
25	CD 6183	94	4	22	4	0	12	1	0	4	0	15	0	0	82
26	CD 7269	92	1	2 2	7	0	0	1	0	0	0	3	1	0	93
27	CD 7271	94	0	2 8	1	0	5	0	0	1	3	20	0	0	80
28	CD 7272	93	12	28	2	0	8	0	2	0	0	37	4	0	88
29	CD 7274	98	0	3 3	1	0	3	0	0	0	0	10	0	1	89
30	CD 7464	89	7	1 7	1	0	7	3	2	6	3	28	2	0	85
31	CD 8217	97	1	3 3	4	0	3	0	0	4	0	15	8	0	85
32	Jap. B + 0 61-7	93	1	27	1	0	8	0	0	1	3	8	2	0	94
33	Jap. B + 0 61-15	85	3	25	3	0	5	1	1	5	5	45	1	1	21
34	Jap. B + 01 R-5	88	5	31	5	0	4	0	0	1	1	8	4	0	93
35	Jap. B + 01 R-13	92	1	15	1	0	9	0	0	3	1	21	5	0	88
36	Pennquad	95	3	46	1	0	11	1	3	6	2	19	21	0	64
37	Silverhall 24	95	4	35	2	0	7	0	1	4	3	22	1	0	58

* Based on four replicates each of 25 seeds; Paec. sp. 1 = Paecilomyces (large spores); Paec. sp. 2 = Paecilomyces (small spores); Pap. = *Papularia;* for other abbreviations see footnote to Table 1.

** See text for definitions.

1). The exceptions were apparently due to dry seed (lot 4), to a low proportion of unsplit hulls (lot 6), or to unknown factors (lot 9). Germination of lots 1 to 12 after 390 days storage ranged from 0 to 92%; with the exception of lot 4, percentage germination had decreased from the values at 0 days. In lots 7 and 8 the decrease was from 68% and 78% to 0, respectively. These were the only samples infested with Aspergillus candidus Link and A. versicolor (Vuill.) Tiraboschi. Most split hulls occurred in lot 1 and the least in lot 6. Over the 390-day period moisture contents in most lots increased, probably due to respiration of the microflora and of the grain itself; exceptions were lots 4 and 10. The maximum moisture increase, 2%, occurred in lot 11.

Efficacy of seed treatments on diseased seed

At Brandon in 1970, emergence of untreated seed with low levels of Botrytis sp. ranged from 76 to 82%; for seed with high levels of Botrytis emergence ranged from 67% to 76% (Table 4.) Emergence of the four lots of lightly infested seed was significantly greater than that of infested lots, but it was not increased by seed treatment. Emergence in the test using lightly (3%) infested treated seed (Table 3) was 87% in the untreated control and among the fungicide treated lots ranged from 78 to 92%. There were no differences in emergence between treatments ($P < 0.05$) and no phytotoxicity was apparent. Germination of seed treated with Panogen PX, Manzate D, or Arasan 75 was not reduced in the laboratory on filter paper (8).

Emergence in the 1971 field experiment from seed with medium (15%) infection was 53% in the control and according to treatment ranged from 48 to 56% (Table 5). No phytotoxic symptoms were apparent even at the rate of 8.60 g/kg of Benlate T. Manzate 200 at both 2.60 and 5.20 g/kg rates was associated with reduced emergence ($P < 0.05$).

Microfloral components present on freshly harvested buckwheat are predominantly field fungi, similar to those found on wheat, barley, and oats of the same age. However, Botrytis sp., a possible pathogen, occurs on buckwheat but rarely on cereals (5). Botrytis sp. has not been recorded on buckwheat previously (4, 10) in Canada or the U.S.A. Damp and tough buckwheat in sealed storage deteriorates, moisture and storage fungi increase, and, as with cereals, viability falls (9). If the buckwheat is dried to a lower moisture level, as the 11.2% in lot 4, viability is maintained and storage fungi are not apparent. In field tests, compared with lightly infested seed, heavy infestation with Botrytis sp. reduced emergence ($P < 0.05$), and emergence was not improved by seed treatment. The fungicides, with the possible exception of Manzate 200 in the 1971 trial, showed no evidence of adversely affecting emergence of buckwheat.

Table 3. Seed treatment materials, dosages, and emergence of buckwheat in 1970 field trial

Product name	Source*	Formulation**	Chemical name	Dosage (g product/kg)	Mean emergence (%)
Untreated					86.9
Arasan 42-S	Dupont	SL	thiram 42%	2.60	91.5
Arasan 70-S	Dupont	SL	thiram 70.0% + methoxychlor 2.0%	1.69	83.4
Arasan 75	Dupont	D	thiram 75.0%	1.69	89.6
Ceresan M	Dupont	D	ethyl mercury p-toluene sulfon-	0.65	89.8
Ceresan M	Dupont	D	anilide 7.7%	1.30	84.8
Manzate D	Dupont	D	maneb 80.0%	1.30	84.8
Manzate D	Dupont	D	maneb 80.0%	2.60	87.3
Manzate 200	Dupont	D	mancozeb (coordination product of zinc ion and maneb) 80.0%	2.60	84.7
Res-Q	Green Cross	D	hexachlorobenzene 20.0% + captan 20% + maneb 15.0%	1.30	88.6
Hoe 2981	Hoechst	WP	identity not available	1.30	86.1
Hoe 2981	Hoechst	WP	identity not available	2.60	79.8
TCMTB	Interprov.	D	2-(thiocyanomethylthio) benzo- thiazole 10.0%	3.12	84.7
Polyram 53.5	Niagara	D	zinc activated polyethylene thiuram	1.30	88.6
Polyram 53.5	Niagara	D	disulfide 80.0%	2.60	86.3
BEJ 15	Niagara	L	identity not available	2.60	78.4
Panogen PX	Nor-Am	D	methylmercuric dicyandiamide 0.9%	2.60	88.4
Panogen 15B	Nor-Am	L	methylmercuric dicyandiamide 3.7 oz/gal	0.98	86.5
Captan 50WP	Stauffer	WP	captan 50.0%	1.30	87.2
Captan 50WP	Stauffer	WP	captan 50.0%	2.60	87.2

* E. I. Dupont de Nemours & Co., Inc., Wilmington, Delaware; Green Cross Products, Division of CIBA Co. Ltd., Montréal, Quebec; Hoechst Chemical Co., Montreal, Quebec; Interprovincial Cooperatives Ltd., Winnipeg, Manitoba; Niagara Brand Chemicals, Burlington, Ontario; Nor-Am Agricultural Products Ltd., Woodstock, Illinois; Stauffer Chemical Co. of Canada Ltd., Montreal, Québec.

** D = dust, WP = wettable powder, L = liquid, SL = slurry.

Table 4. Effect of seed treatments on emergence* of buckwheat from seed infested with low and high levels of Botrytis sp.; 1970 field trial

Sample no.**	Botrytis (%)	Treatment†				Sample mean	Group mean††
		Check	Res-Q	Panogen PX	Arasan 75		
14	1	79.4	77.2	74.8	72.6	76.0	
13	2	75.9	75.5	80.1	76.5	77.0	
15	3	75.9	80.1	78.0	81.1	78.8	
16	3	81.9	77.8	80.2	74.0	78.5	77.6
8	30	71.1	69.9	71.5	74.0	71.6	
11	34	71.4	74.7	76.1	75.5	74.4	
1	37	67.4	69.7	72.2	62.2	67.9	
4	52	75.5	71.8	72.2	76.3	73.9	72.0

* Means of 4 replicates.

** Sample nos. 4, 11, and 13 to 16 were of the cultivar Tokyo; nos 1 and 8, common buckwheat.

† Dosages as Table 3.

†† Avg of sample means for samples having low and high levels of Botrytis infestation.

Table 5. Seed treatment materials, dosages, and emergence of buckwheat in the 1971 field trial

Product name	Source *	Formulation **	Chemical name	Dosage (g product/kg)	Mean † emergence (%)
Untreated					52.8
Agrox NM	Chipman	D	37.5% maneb + 10.0% hexachlorobenzene	2.60	50.8
Agrox NM	Chipman	D	37.5% maneb + 10.0% hexachlorobenzene	5.20	50.6
Arasan 75	Dupont	D	75.0% thiram	1.70	54.5
Arasan 75	Dupont	D	75.0% thiram	3.40	53.7
Benlate	Dupont	D	50.0% methyl 1-(butylcarbamoyl)-2-	2.60	53.5
Benlate	Dupont	D	benzimidazole carbamate	5.20	52.4
Benlate T	Dupont	D	Benlate + thiram	4.30	55.1
Benlate T	Dupont	D	Benlate + thiram	8.60	51.4
Manzate D	Dupont	D	80.0% maneb	2.60	52.5
Manzate D	Dupont	D	80.0% maneb	5.20	54.9
Manzate 200	Dupont	D	80.0% mancozeb (coordination product	2.60	48.4-
Manzate 200	Dupont	D	of zinc ion and maneb)	5.20	48.9-
Panogen PX	Nor-Am	D	0.9% methylmercuric dicyandiamide	2.60	56.3
Panogen PX	Nor-Am	D	0.9% methylmercuric dicyandiamide	5.20	54.0
Captan 50	Stauffer	WP	50.0% captan	2.60	53.1
Vitavax 75	Uniroyal	D	75.0% 5,6-dihydro-2-methyl-1,4-oxathiin-	1.75	54.5
Vitavax 75	Uniroyal	D	3-carboxanilide	3.50	51.0
Vitaflo DB	Uniroyal	D	Vitavax 40%W/W + thiram 40%W/W	3.30	51.6
Vitaflo DB	Uniroyal	D	Vitavax 40%W/W + thiram 40%W/W	6.60	52.5
LSD (0.05)					3.7

* Chipman Chemicals Ltd., Hamilton, Ontario; E. I. Dupont de Nemours & Co., Inc., Wilmington, Delaware; Nor-Am Agricultural Products Ltd., Woodstock, Illinois; Stauffer Chemical Co. of Canada Ltd., Montréal, Québec; Uniroyal Ltd., Elmira, Ontario.

** D = dust, WP = wettable powder.

† - indicates a significant decrease compared with the control at the 0.05 level.

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