Prevalence of oospores of Albugo cruciferarum in Brassica seed samples from western Canada, 1967-73[1]

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Oospores of *Albugo cruciferarum (A. candida),* the white rust fungus, were found in 468 of 585 seed samples of *Brassica campestris* (turnip rape) and in 20 of 25 samples of *B. napus* (rape) produced in western Canada between 1967 and 1973. The latter species is completely resistant to the disease. Turnip rape samples from Alberta generally had the heaviest infestations and those from Manitoba, the lightest. The **most** heavily infested sample (Falher. Alberta, 1969) had over 1500 oospores per gram of seed. With one exception, yearly provincial averages ranged from 6 to 41 spores per gram of seed; in 1969 the average for Alberta samples was 92 spores per gram. Although samples from the moister parts of the Prairies usually had higher infestation rates, samples from more southerly regions were also consistently infested.

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On a trouve des oospores du champignon de la rouille blanche, *Albugo cruciferarum (Brassica campestris),* dans 468 des 585 echantillons de semences de navette *(Brassica campestris)* et dans 20 des 25 echantillons de colza *(B. napus)* produits dans l'ouest du Canada de 1967 a 1973'. La derniere espece est parfaitement resistante a la maladie. Les echantillons de navette de l'Alberta ont generalement eu les taux d'infection les plus eleves et ceux du Manitoba, les plus faibles. L'echantillons le plus gravement infecte (Falher, Alberta, 1969) contenai plus de 1500 oospores par gramme de semences. Sauf une exception, les moyennes provinciales annuelles ont varié de 6 a 41 spores par gramme de semences; en 1969, cette moyenne a ete de 92 spores par gramme pour les echantillons de l'Alberta. Bien que les échantillons provenant de parties plus humides des Prairies ont presente des taux d'infection plus eleves, ceux des regions situées plus au sud ont egalement ete infectés

Staghead (white rust) caused by *Albugo cruciferarum* S. **F.** Gray *[A. candida* (Pers. ex Lev.) Ktze.] has appeared dramatically in turnip rape *(Brassica campestris* L.) on relatively weed-free land on which the crop had never been grown previously and which was many miles from other *Brassica* fields. Oospores sown with the seed would appear to be the most likely source of infection. **A** recent study (7) has shown that oospores of this fungus will germinate in large numbers following a period of washing in water. This lends weight to the idea that oospores are the important primary inoculum source. Even a small number of oospores in a seed sample might be sufficient to establish significant numbers of infections in a crop. These considerations prompted an investigation of the prevalence of oospores of *Albugo* in samples of *Brassica* seed, the results of which are presented in this paper.

Methods

De Tempe (2) in comparing different means of recovering spores from seed samples concluded that filtration techniques were the most accurate, those involving centrifugation being difficult to standardize. Therefore, although attempts were made initially to use a centrifugation method similar to that of Cherewick (1), the technique outlined below was also developed. Ultimately this second method was the one employed in the processing of 610 *Brassica* seed lots (Table 1) which were a representative sampling of those used in a previous study of seed-borne fungi (5, 6).

Table 1. No. and source of *Brassica campestris* seed samples* washed for oospores of *Albugo* 1967-73

Year	Alberta	Saskatchewan	Manitoba	Total
1967	2	2	1	5
1968	24	32	5	61
1969	38	82	1	121
1970	54	98	23	175
1971	31	95	23	149
1972	6	18	2	26
1973	13	29	6	48
Total	168	356	61	585

* Twenty-five ***B. napus*** samples were also washed, giving a total of 610 samples.

For the common *B. carnpestris* cultivars, a 5 g subsample from each lot of seed was washed briefly in 10 ml of

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Table 2. A comparison of the results of washing three subsamples from each of 17 seedlots

Sample no.	Avg no. oospores per microscope field for replicate no. 1	2	3
193	10.1	18.2	9.8
522	1.2	2.2	1.5
536	1.1	8.5	0.8
545	0.6	0.4	0.4
547	2.5	1.7	2.9
1501	4.7	5.2	5.2
1544	0.5	0.8	0.7
1689	3.4	3.0	2.9
1737	1.1	0.7	0.9
1796	1.8	1.4	16.7
2062S	5.3	5.6	5.1
2062A	0.2	0.1	
2063A	0.5	0.5	0.5
2064S	0.9	0.4	0.5
2067A	tr	0.4	
2067S	1.4	0,8	0.3
2041A	1.2	0.4	0.3

water containing a drop of the wetting agent Tween 20. To compensate for greater seed size, 7.5 g subsamples of *B. campestris* 'Yellow Sarson' and 6 g subsamples of *B. napus* L. were taken. The seeds were caught in a sieve as the wash water was poured into a Millipore filtering apparatus. The spores were collected by suction on a 2.5 cm filter disc which was then dried and cleared with mineral oil. The numbers of spores in 10 microscope fields (magnification 80 X) were recorded and the averaged counts multiplied by 30.5 to convert them to numbers of spores per g of seed. To express the results on an oospore per seed basis the number of spores per g of seed was divided by 430, the average number of seeds per g in several 1 g subsamples from several lots. A number of seed lots representing a range of infestation rates were sampled a second and a third time to obtain an indication of the variation to be expected among

Table 3. Distribution of oospores in washings of 15 *Brassica campestris* seed samples; spores in 10 microscope fields counted following deposition on filter paper

Sample no.	Rep. no.	No. of oospores per microscope field Range	Mean	S.D.
39		3 - 12	6.4	2.72
77		2 - 14	6.3	3.53
193	1	6 - 14	10.1	2.47
	2	5 - 12	9.8	2.20
	3	13-26	18.2	3.79
257		2 - 7	4.4	1.64
374		5 - 20	8.7	5.06
530		18-46	28.2	9.95
536		3 - 14	8.5	4.30
555		11-23	15.7	6.55
1501	1	2 - 9	4.7	2.00
	2	3 - 9	5.2	1.87
	3	2 - 10	5.2	2.66
1689	1	2 - 6	3.4	1.35
	2	1 - 7	3.0	1.63
	3	1 - 6	2.9	1.52
1713		6 - 18	12.0	3.22
1715		2 - 8	4.3	1.77
2062S	1	2 - 9	5.3	2.16
	2	3 - 8	5.6	1.78
	3	3 - 10	5.1	2.38
R-500S		3 - 9	4.4	1.78
2069		2 - 6	3.6	1.43

subsamples. Standard deviations were calculated for many of the more heavily infested samples to give an indication of the variation in spore numbers between microscope fields.

Results

The filtration technique was generally reliable for rapid quantitative screening of large numbers *of Brassica* seed samples for the presence of oospores (Tables 2 & 3). Without exception much poorer recovery of oospores was obtained using centrifugation. Starting from the same artificially introduced spore load, it was shown that the recovery of oospores by the latter technique was

Table 4. Oospores (no./g seed) detected in seed samples of *Brassica campestris* grown in Saskatchewan Crop Districts 1-9, 1968-73

Crop District	1968 Avg	1968 Max.*	1969 Avg	1969 Max.	1970 Avg	1970 Max.	1971 Avg	1971 Max.	1972 Avg	1972 Max.	1973 Avg	1973 Max.	2- to 6-year avg
1-4					5	6	9	3 4			1	1	7
5	24	40	25	265	7	62	14	55	68	162	4	12	24
6			9	24	4	12	4	12			50	134	17
7			15	49	4	12	6	18			6	12	8
8A	28	101	2	15	14	143	12	34	3	3	7	18	11
8B	140	308	11	31	10	31	9	24	19	43	7	21	33
9	26	76	51	668	5	21	11	40	4	6	6	15	17
Provincial average	41		22		8		10		19		12		19

* Max. = Highest no. oospores in any sample.

greatly influenced by the amount of dirt naturally present in a seed sample; the more grit present the higher the spore counts. When the filtration method was employed, more extraneous matter could be present before results were affected through the spores being hidden from view. The variability noted in some of the results when filtration was used (Tables 2 & 3) appeared to be due in large part to inability to adequately disperse clumps of spores during washing of the seeds.

Albugo spores were detected in approximately 77% of the 356 ***B.*** *campestris* samples from Saskatchewan. The highest average infestation rate, 41 spores per g of seed, occurred in 1968 (Table 4). Thereafter, the averages ranged from 8 per g in 1970 to 22 per g in 1969. The highest spore load found in any Saskatchewan sample, 668, occurred in 1969. This seed lot was from Leask in Crop District 9. The 2- to 6-year averages for crop districts reveal somewhat lower spore loads in samples from the southern districts (Nos. 1-4) and the west central district (No. 7) than in those from other districts (Table 4). However, 8 of 10 1970 and 1971 turnip rape samples from Crop Districts 1-4 contained oospores, the highest level being 34 per g. It is perhaps surprising that Crop District 8A in the northeast had a relatively low average. [The Canadian prairie crop districts and their subdivisions were illustrated by Williams (8)]

Oospores were detected in 20 of 25 *B. napus* seed samples tested, all of which were from Saskatchewan. Infestation ranged from a trace to 12 spores per g, with an average of 3 per g. This is of interest, as the author has never observed white rust infections on *B. napus* cultivars in the field and they have been immune upon inoculation in the greenhouse.

Oospores were found in 87% of the 168 Alberta samples. Those from 1969 were among the most heavily infested of any examined in the course of the study (Table 5). Over 1500 spores per g occurred in a sample from Falher and 860 per g were found in one from Beaverlodge. Both localities are in Alberta Agricultural Reporting Area (ARA) 7. In ARA 4, a sample from Viking had 479 spores per g. The highest infestation in other years was 366 spores per g in a 1970 sample from Bon Accord in ARA 5. The 3- and 4-year averages for ARAs 2-7 indicate a considerable increase in the average number of spores per sample going from south to north (Table 5).

Infestation of Manitoba samples never exceeded 50 per g (Table 6). Slightly in excess of 72% of the 61 Manitoba seed lots had detectable oospore infestation.

In Tables 7 to 9 the samples from Alberta, Saskatchewan, and Manitoba, have been grouped in a number of infestation severity categories. Infestations heavier than one spore per 10 seeds occurred in 13.1% of the total Alberta samples, 6.5% of those from Saskatchewan, and 3.3% of those from Manitoba. In 1968, 31.2% of the Saskatchewan samples had in excess of this level of

Table 5. Oospores (no./g seed) detected in seed samples of *Brassica campestris* grown in Alberta, 1968-71

ARA*	1968 Avg	1968 Max.†	1969 Avg	1969 Max.	1970 Avg	1970 Max.	1971 Avg	1971 Max.	4-Y avg
1					3	9	12	12	
2	6	12	19	73	6	34	8	15	10
3	34	82	6	18	5	9	21	49	17
4	9	9	104	479	11	37	32	64	49§
5	14	49	20	73	62	366	38	70	34
6	101	195	23	76	25	104	19	46	42
7	1	1	318	1577	26	64	7	21	117§
Provincial average	23		92		24		18		39

* ARA =Agricultural Reporting Area.

† Max. = Highest no. oospores in any sample.

§ 3-y average.

infestation. The percentage of heavily infested samples declined to 1.0 in 1971, increasing only slightly thereafter. Yearly fluctuations of this magnitude did not occur in the Alberta and Manitoba samples.

Samples of *B. campestris* cultivars Echo, Span, and R-500 (Yellow Sarson) from the 1973 cooperative rapeseed varietal tests were compared for levels of oospore infestation. R-500 has generally been the most susceptible to infection in the greenhouse and field; Echo and Span were usually similar in reaction. In the present experiment, seed from 10 locations across the Prairies was sampled. Averages for spores per g were 20 for R-500, 14 for Span, and 8 for Echo. However, relative amounts of infestation in samples of the three cultivars from different stations varied considerably.

Alternaria spores and spore fragments occurred in extremely large numbers in the washings of several of the 610 samples. Many belonged to *Alternaria brassicae* (Berk.) Sacc. Conidia of *Helminthosporium* and *Curvularia* were among the more easily recognizable ones that occurred less frequently.

Table 6. Oospores (no./g seed) detected in seed samples of *Brassica campestris* grown in Manitoba, 1970-71

Crop District	1970 Avg	1970 Max.*	1971 Avg	1971 Max.
10, 11	3	9	20	34
13, 14	12	49	5	15
Others	4	9	14	37
Provincial avg	6		14	

* Max. = highest no. oospores in any sample.

Discussion

It is evident that *Albugo* oospores occur commonly in *Brassica* seed samples throughout the Prairies. The actual inoculum levels may be considerably above the apparent levels, for upon germination a single oospore may release in the order of 40 to 60 zoospores. In addition, pieces of staghead material (hypertrophied inflorescence) up to several mm across have been observed in seed lots. Such pieces would not be accounted for by the washing technique as all but the smallest would be screened out with the seed. Their presence reemphasizes the need for proper cleaning of seed. Infection in the form of mycelium within the seed coat might also be of some importance. In sections of pods bearing oospore-containing blisters, hyphae have been seen within the cotyledons of embryos (unpub-

Table 7. Percentage of 356 Saskatchewan samples of *Brassica campestris* seed of eight infestation severity categories

Year	No. of oospores per g of seed: 0	Tr-3	4-11	12-43	44-86	87-129	130-323	Over 323
1968	18.8	12.5	12.5	25.0	21.9	3.1	6.2	0
1969	30.5	29.3	12.2	19.5	4.9	0	2.4	1.2
1970	23.5	32.6	23.5	18.4	1.0	0	1.0	0
1971	17.9	22.1	22.1	37.9	1.0	0	0	0
1972	22.2	22.2	16.7	33.3	0	0	5.6	0
1973	17.3	41.4	6.9	27.6	3.4	0	3.4	0
1967-73	22.8	27.2	17.7	25.8	3.9	0.3	2.0	0.3

Table 8. Percentage of 168 Alberta samples of *Brassica campestris* seed in each of 10 infestation severity categories

Year	No. of oospores per g of seed: 0	Tr-3	4-11	12-43	44-86	87-129	130-323	324-645	646-1290	over 1290
1968	0	29.2	25.0	33.3	8.3	0	4.2	0	0	0
1969	13.2	18.5	23.7	26.3	10.5	0	0	2.6	2.6	2.6
1970	20.4	22.2	16.6	27.7	7.4	1.9	1.9	1.9	0	0
1971	6.5	16.1	12.9	48.4	16.1	0	0	0	0	0
1972	33.3	33.3	16.7	16.7	0	0	0	0	0	0
1973	15.4	46.2	23.0	15.4	0	0	0	0	0	0
1968-73	13.1	23.8	19.0	31.0	8.9	0.6	1.2	1.2	0.6	0.6

lished data). Although this could represent any of a number of fungi (4), the likelihood of it being *Albugo* infection is great.

In surveys conducted between 1970 and 1972 (3), it was found that up to 46% of the plants in fields of turnip rape had systemically infected branches. There is a possibility that many of these infections originated with oospores sown with the seed. The oospore load in the seed should now be related to the production of stagheads and other symptoms in the field. Soil-borne oospores could also add to the inoculum potential. The prevalence of these in fields following turnip rape has yet to be investigated.

In some instances the oospores found in samples of the resistant host, *B. napus* might have come from infected *B. campestris* plants occurring in fields of the former. Such mixtures have frequently been observed. Alternatively spores might have been introduced in handling and cleaning seed prior to receipt of the samples. It is thought that careful washing of the filtration apparatus between samples minimized contamination during processing in the laboratory.

Rapeseed is grown much more intensively in central and northern prairie crop districts than in the drier southern ones. However seed from southern areas was frequently infested, and *Albugo* would appear to be more prevalent

Table 9. Percentage of 61 Manitoba samples of ***Brassica campestris*** seed in each of six infestation severity categories

Year	No. of oospores per g of seed: 0	Tr-3	4-11	12-43	44-86	Over86
1970'	43.5	21.7	26.0	4.4	4.4	0.0
1971"	8.7	21.8	26.0	43.5	0.0	0.0
1967-73	27.8	24.6	21.3	23.0	3.3	0.0

* 23 samples,

in samples from these regions than are pathogenic *Alternaria* spp. (5). Therefore, there do not appear to be areas on the Prairies where seed free of *Albugo* infestation might be consistently produced. The effectiveness of seed treatment chemicals in reducing the incidence of *Albugo* is being investigated.

The results presented strongly support the view that in western Canada seed-borne oospores are an important source of primary infection for the white rust fungus *A. cruciferarum*. A research effort directed to substantially lowering the levels of this form of inoculum might be rewarded by significantly improved turnip rape seed yields.

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