# RACES OF PLASMODIOPHORA BRASSICAE INFECTING CRUCIFER CROPS IN CANADA'

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## **Abstract**

The screening of isolates of <u>Plasmodiophora brassicae</u> Wor. has indicated that at least six races of the organism are present in the soils of Canada. Differential distinction of clubroot inocula showed that races 2 and 3 were the most common variants in isolates from the Maritime provinces and that race 2 was the most prevalent biotype in diseased tissue samples from Quebec. Race 6 was the only pathogen variant in two spore samples from British Columbia and in one sample from Ontario. Localized infestations of races 1, 6, and 6A were established for Quebec. Scattered infestations of race 1 were demonstrated for the Maritime Provinces while race 4 was found in two clubroot tissue samples from Prince Edward Island. The sole distinction of race 7 was in a spore sample from Wisconsin, U. S. A. The rutabaga variety York proved resistant to races 2, 3, 6, 6A, and 7 and for this reason is considered relatively secure from clubroot infection when planted in most crucifer growing areas of Canada. The prevalence of race 2 in Quebec and the Maritime Provinces presents a challenge to cole crop breeders as no genes for resistance to this race have been found in cole crop lines bred €or resistance to other races of <u>P. brassicae</u>.

## Introduction

Evidence of physiologic specialization in the clubroot organism Plasmodiophora brassicae Wor. was advanced by Honig (2) in 1931, and since that time other workers throughout the world have demonstrated variation in this pathogen by means of reactions obtained on various stocks of differential crucifers. Walker (6) found that two turnip varieties had different clubroot reactions in England than when grown in Wisconsin, U.S.A. Lammerink (3) distinguished six races of the organism occurring in New Zealand, and Seaman, Walker, and Larson (5) contributed to knowledge on pathogen variation in Wisconsin, U.S.A. In studies reported in this paper a more exact method of identifying races of the clubroot organism has been developed than that described earlier (1). This has been achieved largely by careful selection and propagation of differential seed stocks for homozygosity in disease reaction when exposed to what are considered to be specific races of the organism. The method used to assess variation in the pathogen is essentially similar to that described by Williams (7), who used four differentials and classified 16 possible host reactions. The author has added an additional differential host to facilitate distinction of certain differences in inocula obtained in the Maritime Provinces of Canada. Data assembled by the author were

based on an assessment of variation in inocula gathered over the period 1959-71. More than 160 spore samples were screened for variation, and the reactions of 68 representative samples are reported in this paper.

## Materials and methods

Clubroot tissue samples for studies on pathogen variation were obtained from various crucifer growing areas throughout Canada with the major portion of samples collected in the Maritime Provinces and Quebec. Spores were water extracted from infected root tissues using a Waring Blendor. This procedure was followed by coarse filtering and centrifuging to remove host tissues and soil particles to the extent that spores could be readily detected upon microscopic examination. Spore counts were made with a bright line haemocytometer, and inoculum was introduced and thoroughly mixed with sandy loam clubroot-free soil at the rate of 6.1 x 10' spores per cm of soil medium. These artificially infested soil samples were stored at 38° F(3.3°C), until screened for pathogen variation. Differentials used in race studies were cabbage (Brassica oleracea L. var. capitata L. 'Damish Ballhead' and 'Badger Shipper') and rutabaga (Brassica napobrassica Mill. 'Laurentiam', wilhelmsburger', and 'Ditmars S2'). Pathogen variation studies were conducted in the greenhouse using 2-inch earthenware pots partially filled with infested soil. For

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each infested soil sample, differentials were planted at the rate of 12 to 16 seeds per pot with four pots per crucifer. Pots were sunk in sphagnum moss, regulated in moisture content to allow sufficient penetration of capillary water for germination of seed. At the two-leaf stage of seedling development soil moisture was raised to above saturation levels for a period of 48 hr, following which the contents of each 2-inch pot were embedded in clubroot-free soil in a 4-inch pot. Temperatures were maintained at 70°F (21°C) during the course of the experiments and plants were scored for clubbing 30 days after seeding. Forty plants of each differential from each inoculum series were assessed as follows: (1) no symptoms, (2) nodulation, (3) restricted sphaeroid clubbing, (4) unrestricted clubbing. The first two categories were rated zero for disease index since nodulation caused little or no plant damage. Plants showing no restriction in the development of typical clubroot distortion were given a rating of 100, irrespective of whether symptoms were slight, moderate, or severe. This latter classification was based on the author's experience that all such plants lack genes for resistance.

The system of classification of races shown in Table 1 is at variance with that of Williams (7) in that an additional differential, Ditmars S2 rutabaga, was used to distinguish reactions of mixtures of races 2 and 3 from those of race 2 alone. Seven main differential reactions are shown out of

a possible 16 listed by Williams. Race 5 and races 8 to 16 were not encountered by the author in the course of studies on pathogen variation.

Table 1. Differential reactions to infection by races of Plasmodiophora brassicae

	Reaction* to race**						
Differential	1	2	3	4	5	6	7
Cabbage							
Danish Ballhead	+	+	+	+	-	+	+
Badger Shipper	-	+	-	+	-	-	+
Rutabaga							
Laurentian	+	+	+	+	-	-	_
Wilhelmsburger	+	-	-	+	-	-	_
Ditmars \$2	+	-	+	+	-	-	-

 $\boldsymbol{+}$  indicates susceptibility,  $\boldsymbol{\bar{\phantom{a}}}$  indicates resistance.

Races 4 and 5 according to Ayers (1) have been redesignated according to Williams (7). Races 1, 2, 3, and 6 were classified similarly by both authors. Race 7 was designated by Seaman et al. (5).

 $^\dagger$  Race 6A is distinguished from races 6 and 7 by a restricted sphaeroid clubbing of roots of Badger shipper. This cultivar is resistant to race 6 and fully susceptible to race 7.

Table 2. Race Classification of isolates of Plasmodiophora brassicae on five differential crucifers

				Percentage infection <sup>t</sup> on differential hosts					
Isolate	source of isolates			Cabbage		Rutabaga			
	Province and district	Crucifer	Variety (where known)	Danish Ballhead	Badger Shipper	Lauren- tian	Wilhelms- burger	Ditmars \$2	Race
	Prince Edward Island								
1	Winslos	Rutabaga	Wilhelmsburger	98	100	100	100	100	4
2	Greenfield	Rutabaga	Laurentian		94	100	0	100	2+3
3	Mermaid	Rutabaga	Laurentian	72	100	90	0	0	2
4	South Melville	Rutabaga	Laurentian	86	88	88	0	0	2
5	Powna1	Rutabaga	Laurentian	98	98	100	0	0	2
6	Murray Harbor	Rutabaga	Laurentian	100	100	100	0	100	2+3
7	Waterside	Rutabaga	Laurentian	100	100	100	0	2	2
8	Kingston	Rutabaga	Laurentian	100	100	100	0	0	2
9	Summerside	Rutabaga	Laurentian	100	100	100	0	0	2
10	Montague	Turnip	Greystone	100	100	100	0	100	2+3
11	Upton	Rutabaga	Ditmars 52	100	0	100	0	100	3
12	Souris	Cabbage		100	14	100	0	100	3+2*
13	Riverdale	Rutabaga	Laurentian	98	100	100	2	0	2
14	Argyle shore	Rutabaga	Laurentian	100	100	100	0	0	2
15	Uigg	Rutabaga	Laurentian	100	100	100	0	0	2
16	Morell	Rutabaga	Laurentian	100	88	100	0	100	3+2
17	Tracadie	Rutabaga	Laurentian	100	80	100	0	0	2
18	Rollo Bay	Rutabaga	Laurentian	100	100	100	0	0	2
19	Vernon River	Rutabaga	Laurentian	100	100	100	0	100	2+3
20	York	Rutabaga	Laurentian	100	100	70	0	0	2
21	Loyalist	Rutabaga	Wilhelmsburger	100	91	100	100	100	4
22	Fredericton	Rutabaga	Laurentian	95	83	100	0	0	2
23	Clyde River	Rutabaga	Laurentian	100	94	100	0	90	2t3
24	Souris	Rutabaga	York	100	0	100	58	100	1
25	Cornwall	Cabbage		95	0	100	90	100	1
	Nova Scotia								
26	Nappan	Cabbage	Badger Shipper	93	88	100	0	0	2
27	Nappan	Rutabaga	Wilhelmsburger	100	o	100	100	100	1
28	Nappan	Rutabaga	Laurentian	100	22	100	100	100	1+2*
29	Port Howe	Rutabaga	Laurentian	100	65	100	100	100	1+2
30	Linden	cabbage		100	96	100	0	75	2+3

Darcantaga	infaction?	on differential	hoete

	Source of isolates			Cabbage		Rutabaga			
Isolate NO.	Province and district	Crucifer	Variety (where known)	Danish Ballhead	Badger Shipper	Lauren- tian	Wilhelms- burger	Ditmars \$2	Race
31	Marshville	Rutabaga	Laurentian	100	48	100	2	100	3+2*
32	Truro	Rutabaga	Laurentian	80	58	100	0	0	2
33	Parrsboro	Rutabaga	Laurentian	100	10	100	100	100	1
34	Cole Harbor	Cabbage		82	0	100	0	100	3
35	Wallace	Rutabaga	Laurentian	100	0	100	0	100	3
36	Hastings	Rutabaga	Laurentian	100	100	100	0	0	2
37	Brentwood	Rutabaga	Laurentian	100	98	100	0	0	2
38	Bras D'Or	Rutabaga	Laurentian	100	80	100	0	100	3+2
39	Leicester	Rutabaga	Laurentian	100	100	100	0	42	2+3*
40	Berwick	Rutabaga	Laurentian	100	8	100	O	100	3
41	Heatherton	Cabbage		100	100	100	O	2	2
42	St. Joseph New <i>Brunswick</i>	Cabbage		100	100	100	0	2	2
43	Upper Burton	Rutabaga	Laurentian	59	40	70	0	22	2+3
44	Naskwaaksis	Cabbage		100	100	100	0	0	2
45	Moncton	Brussels sprouts		100	20	100	0	100	3+2*
46	Moncton	Cabbage		100	42	100	0	100	3+2*
47	Sussex	Rutabaga	Laurentian	100	21	100	70	82	1+2*
48	Marysville	Rutabaga	Laurentian	100	78	100	0	6	2
	Quebec						·	o o	_
49	St. Martin	Cabbage		100	6	0	0	0	6
50	Ste. Clotilde	Cabbage		96	50-r**	ō	ō	0	6A
5 1	St. Rémi	Cauliflower		96	40-r**	0	ō	ō	6A
52	Plessisville	Cabbage		100	92	100	0	o	2
53	Beauport	Cabbage		86	100	100	o	o	2
54	Neuville	Cabbage		100	100	100	0	0	2
55	Ste. Edwidge	Rutabaga	Laurentian	100	100	100	52	•	2+1*
56	Ile O'Orleans	Cabbage		100	100	95	0	0	2
57	Baie St. Paul	Cabbage		100	100	100	0	0	2
58	Levis	Cabbage		100	65	100	ō	ō	2
59	Duvernay	Rutabaga	Laurentian	100	98	100	ō	ō	2
60	Mascouche	Rutabaga	Lawentian	92	62	100	2	6	2
61	Rivière Ouelle	Rutabaga	Laurentian	100	100	100	0	0	2
62	Ste-Fov	Cabbage		98	95	100	30	8	2+1*
63	St. Nicoles	Cabbage		100	65	100	0	o	2
64	Ontario Bradford British Columbia	Cabbage		100	10	0	0	o	6
65	Keating	Cabbage		98	2	0	0	0	6
66	Cordova Bay	Cabbage		98	4	0	0	0	6
	U.S.A.								
67	Wisconsin	Cabbage		100	90-r**	0	0	0	6A
68	Wisconsin	Cabbage	Badger Shipper	100	100	0	0	0	7

<sup>†</sup> Infections of 10% and under were not classified.

# Results

Clubroot differential reactions obtained with 68 representative isolates of the organism are presented in Table 2. Race 2 proved to be the most prevalent pathogen variant in isolates from the Maritime Provinces while race 3 was also shown to be common to this area of Canada. In Quebec race 2 was also the most common variant, while races 1, 6, and 6A were present to a lesser extent. Race 6 was the only variant identified in spores samples from British Columbia and Ontario. Scattered infestations of race 1 were indicated for the Maritime Provinces. Race 4 was found in two isolates from Prince Edward Island, while race 7 was identified in a spore sample from Wisconsin, U. S. A. Difference in identity between races 6A and 7 was based on symptom expression on Badger Shipper cabbage. Race 7 caused unrestricted clubbing while race 6A caused a restricted sphaeroid distortion of roots at ground level.

Without the use of Ditmars \$2 as a differential, mixtures of races 2 and 3 would probably have been classified as race 2. The author was able to isolate race 2 from representative mixtures of races 2 and 3 using Badger Shipper cabbage; similarly, race 3 was isolated from race 2 using Ditmars \$2 rutabaga. Further evidence that mixtures of races 2 and 3 are so constituted is apparent in reactions obtained with York, a rutabaga variety selected at Charlottetown in 1964 for resistance to races 2 and 3. This variety was exposed to all inocula under test between 1964 and 1971 and complete resistance to clubbing was shown where inocula were classified as races 2 or 3 or as mixtures of these races (Table 3).

In tests with samples designated as mixtures of races 2 and 3, moderate to low infection percentages were encountered frequently in either the Badger Shipper or Ditmars S2 differentials, thus indicating that two races were present and that the spore load of one was insufficient to cause

<sup>\*</sup> Indicates lower spore load of component in racial mixture.

<sup>\*\*</sup> r indicates restricted sphaeroid type of clubbing.

Table 3. Clubroot susceptibility in York rutabaga exposed to classified representative isolates listed in Table 2

Race	Reaction*
1	+
1 + 2	+
2	•••
2 + 3	-
3	-
4	+
6	_
6A	-
7	_

+ indicates susceptibility,

full infection in one or other of these crucifers. A parallel situation was encountered with mixtures of races 1 and 2 in a small plot area at the Experimental Farm, Nappan, N.S. Badger Shipper cabbage grown in this land segregated race 2 (Isolate 26, Table 2), while Wilhelmsburger rutabaga grown in close proximity segregated race 1 (Isolate 27), and Laurentian rutabaga became infected with both races (Isolate 28). However, isolate reactions indicated that race 2 spore load was minimal in comparison with that of race 1.

Attempts to segregate possible components in Isolates 1 and 21 were not successful. Inoculum from Badger Shipper and Wilhelmsburger grown under exposure to these spore samples caused heavy infection in all differentials. These isolates were therefore classified as race 4. No further occurrences

of this race have been demonstrated in studies conducted at Charlottetown.

Gene pools for resistance to races 1, 2, 3, and 4 were demonstrated in four turnip (B. rapa L.) varieties, and clubroot reaction data are presented in Table 4 together with reactions of six additional crucifers. The relatively broad type of resistance of turnip varieties under test indicates that such stocks would be potentially useful in cross breeding with York rutabaga to obtain progeny resistant to all races classified in this paper. The broccoli selection B-0-23 (N.Y.) showed resistance to races 1 and 3 and complete susceptibility to race 2. The writer has tested numerous lines of cole crops and has not encountered any genes for resistance to race 2.

# Discussion

The identification and distribution of races of the clubroot organism as determined in studies conducted by the author should provide a useful guide to those engaged in breeding for clubroot resistance in rutabagas and cole crops. York, a purple top rutabaga selected for clubroot resistance at Charlottetown and registered for commercial production in 1964, has proven resistant to races 2, 3, 6, 6A, and 7. It is now extensively planted in areas of Eastern Canada where races 2 and 3 are the prevalent biotypes of the pathogen. The York variety has thus replaced Laurentian because of the high susceptibility of the latter to races 2 and 3. Wilhelmsburger rutabaga, although possessing similar resistance to that of York, is not in commercial table stock production, largely because of its green top and heavy root system. York is susceptible to races 1 and 4 but these pathogen variants are not of widespread occurrence; if the York variety is planted in a rotation of 5 or more

Table 4. Reaction to clubroot races 1, 2, 3, and 4 in turnips and other crucifers

Crucifer		Percentage clubbing*						
	Variety	Race 1	Race 2	Race 3	Race 4			
Turnip	Meetjeslander	2.5	0.0	0.0	10.0			
Turnip	Halflange 70K	30.0	0.0	7.5	6.7			
Turnip	Halflange Gele	10.0	5.0	7.5	0.0			
Turnip	Novitas	5.0	0.0	0.0	12.5			
Broccoli	B-0-23 (N.Y.)	17.5	100.0	20.0				
Cabbage	Badger Shipper	0.0	100.0	0.0	97.5			
Rutabaga	Wilhelmsburger	95.0	0.0	0.0	100.0			
Rutabaga	Laurentian	95.0	100.0	100.0	100.0			
Rutabaga	York	77.5	0.0	0.0	100.0			
Rutabaga	Ditmars \$2	80.0	0.0	100.0	100.0			

Based on numbers of plants clubbed of 40 examined per crucifer-race exposure.

<sup>-</sup> indicates resistance.

years resistance should be maintained in most areas for many years.

Badger Shipper cabbage was officially named and released in 1959 by the University of Wisconsin. In studies reported herein, this variety proved resistant to unrestricted clubbing when exposed to races 1, 3, 6, and 6A. A broccoli line showed resistance to races 1 and 3. No cole crop lines, including stocks of cabbage, cauliflower, and broccoli obtained from breeding stations, have shown any resistance to race 2 in screening trials conducted at Charlottetown. The importance of finding cole crop resistance to this race is apparent in results tabulated in Table 2, wherein 49 out of 68 isolates of P. brassicae under test were found to harbor race 2 incole crops, breeders must continually search for resistant germ plasm in these crops or possibly rely on interspecies crosses to effect a transfer of race 2 resistant genes from turnip and rutabaga.

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In limited screening of isolates from Ontario and British Columbia, race 2 was not encountered and the spore samples tested were classified as race 6. In Ontario Reyes (4) identified four isolates from cabbage and cauliflower as race 6 and one from rutabaga as race 2. The presence of race 6 in British Columbia was established by Williams and Walker (8) and Williams (7). As the common rutabaga varieties are resistant to race 6, clubroot would not appear to be as serious a rutabaga production problem in British Columbia as in areas of Canada where races 2 or 3 are prevalent.

With the exception of isolates designated as racial mixtures, most isolates showed marked individual homogeneity when subjected to race differentiation. In the extensive testing of isolates from Prince Edward Island over the period 1959-71, the author found little change in patterns of race infestation irrespective of whether isolates were obtained early or late during this period. Extensive plantings of resistant York rutabagas in Prince Edward Island may, in time, result in changes in racial patterns of infestation as the fungus adapts to this variety.

# **Acknowledgments**

I am grateful to those who forwarded samples of clubroot infected root tissue, and to Mr. J. N. Richard for technical service rendered during the course of this investigation.

## Literature cited

- 1. Ayers, G. W. 1957. Races of Plasmodiophora brassicae. Can. J. Bot. 35:923-932.
- Honig, F. 1931. Der Kohlkropferreger (Plasmodiophora brassicae Wor.). Gartenbauwissenschaft 5:116-225.
- 3. Lammerink, J. 1965. Six races of Plasmodiophora brassicae Wor. in New Zealand J. Agr. Res. 8:156-164.
- 4. Reyes, A. A. 1969. Detection of Plasmodiophora brassicae races 2 and 6 in Ontario. Plant Dis. Rep. 53:223-225.
- Seaman, W. L., J. C. Walker, and R. H. Larson.
  Plasmodiophora brassicae affecting
  Badger Shipper cabbage.
  Phytopathology
  53:1426-1429.
- 6. Walker, J. C. 1942. Physiologic races of <u>Plasmodiophora</u> <u>brassicae</u>. Phytopathology 32:18.
- 7. Williams, P. H. 1966. A system for the determination of races of Plasmodiophora brassicae that infect cabbage and rutabaga. Phytopathology 56:624-626.
- 8. Williams, P. H., and J. C. Walker. 1963. Races of clubroot in North America. Plant Dis. Rep. 47:608-611.