Races, Pathogenicity and Chemical Control of Plasmodiophora brassicae in Ontario

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ABSTRACT

Two races of *Plasmodiophora brassicae* were identified from 20 isolates in Ontario. Most race 2 isolates were recovered from rutabaga in sandy soil and race 6 isolates from other crucifers in loam and muck soils. All cultivars of cabbage, cauliflower, broccoli, and brussels sprouts recommended for commercial production in Ontario were susceptible to races 2 and 6, whereas all radish cultivars resisted infection. The following cruciferous weeds, *Brassica kaber, Camelina microcarpa, Capsella bursa-pastoris, Descurainia sophia, Erucastrum gallicum, Erysimum cheiranthoides, Lepidium ruderale, Sisymbrium officinale* and *Thlaspi arvense* were susceptible to race 6. Benlate was the most effective of the chemicals studied for control of *P. brassicae* and provided good control in the field when drenched at transplanting and again one mo later.

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Additional key word: clubroot.

Plasmodiophora brassicae Wor., the pathogenic agent of clubroot in crucifers, was first reported in Ontario in 1923 affecting rutabaga, *Brassica napobrassicae* (L.) Mill. (9). In 1930, it attacked 15-20% of cauliflower, *B. oleracea* L. var. *botrytis* L., in Lincoln County (10). In 1956, some fields of cabbage, *B. oleracea* L. var. *capitata* L., in the Hamilton-Toronto area were totally destroyed by clubroot and the whole Bradford Marsh appeared to be infested (5).

Races 2 and 6 of the organism were identified in Ontario in 1967 (15). Most cultivars recommended in Ontario have not been tested for susceptibility against these races (2, 7, 8, 14). Furthermore, despite the prevalence of clubroot, chemicals were not recommended for its control in Ontario (14).

The objectives of this work were to identify the races of *P. brassicae* found in Ontario, demonstrate the susceptibility of commercial cultivars and cruciferous weeds to these races, and compare the efficacy of fungicides for the control of clubroot.

MATERIALS AND METHODS.—*Collecting and identifying races of isolates.*— Fresh, firm, clubbed roots containing mature sporangia of *P. brassicae* were collected from the fields visited by Reyes et al. (16). Each collection was assigned an isolate number. Roots were washed thoroughly, sealed in plastic bags, and stored at −10 C.

Inoculum was prepared and races were determined by the procedures of Williams (18). The roots of 18-day-old seedlings of cabbage 'Jersey Queen' and 'Badger Shipper' and rutabaga 'Laurentian' and 'Wilhelmsburger' were dipped in the inoculum (10^8 spores/cc water) before transplanting into muck soil (pH 6.0) in 32×37×14 cm plastic pans. Previously, the muck soil had been screened (3.96-mm opening No. 5 mesh) and steamed at 105 C for 1.5 h. Preliminary tests indicated that this steaming was adequate for controlling clubroot. Five plants of each of the four cultivars were grown in single pans in the greenhouse (24±2 C, 65-75% relative humidity). Plants were watered as required and each wk 400 cc/pan of 20-20-20 fertilizer solution (5 g/liter) were applied. Four wk after transplanting, the roots were removed from the soil and rated for degree of clubbing. Each isolate was tested three times and the results were averaged.

Preparing, infesting, and planting microplots.— To demonstrate susceptibility of commercial cultivars and the efficacy of fungicides under field conditions, 48 microplots (1.2×6.1 m each) were set up in each of two isolated fields (Vineland silt loam, pH 5.9) at Jordan, Ontario. The microplots were separated by 1.2 m sod strips along the sides and 1.8 m strips at the ends. In early April 1969, roots of 6-wk-old seedlings of Laurentian rutabaga were dipped in the race 2 inoculum (mixture of three isolates). Twenty of these seedlings were planted in each plot in Field I. Similarly, 20 seedlings of Jersey Queen cabbage were inoculated with race 6 (mixture of six isolates) and planted in each plot in Field II. After 75 days, the clubbed roots were chopped and mixed thoroughly into the soil. This procedure was repeated in June 1969 and twice in 1970 to ensure a high level of *P. brassicae* inoculum in the two fields.

On 27-28 April 1971, four cultivars of early-season crops (14) were planted in each microplot. Five, 6-wk-old seedlings of cabbage and cauliflower were transplanted at 60-cm spacing in the microplot. Three seeds of each radish (*Raphanus sativus* L.) cultivar were direct-seeded in each of five hills to correspond to the spacing of the five transplants. Jersey Queen cabbage and Laurentian rutabaga seedlings served as checks. Radish cultivars were harvested six wk after seeding, and the cabbage, cauliflower, and rutabaga 10 wk after transplanting.

Mid-season crops (14) were all direct-seeded on 31 May and 1 June, 1971 in the same manner as for early-season radish. Four wk later cabbage and rutabaga were thinned to a single plant/hill. Radish cultivars were harvested five wk after seeding, and cabbage and rutabaga 13 wk after

TABLE 1. The reaction (clubroot index[a]) of cruciferous weeds artificially inoculated with race 6 isolates of *Plasmodiophora brassicae*

Weed host	*P. brassicae* isolate					
	67E4-348[b]	68D6-10	68D9-18	68D9-19	69S19-1	69S19-2
Barbarea vulgaris R. Br.	0		0		0	
Brassica kaber (DC.) L.C. Wheeler	1.7			2.0	2.7	2.7
Camelina microcarpa Andrz.	2.5	3.2	2.2	2.5	3.7	3.0
Capsella bursa-pastoris (L.) Medic.	2.0	1.0	2.3	2.7	1.7	2.3
Descurainia sophia (L.) Webb	1.3				2.3	2.3
Erucastrum gallicum (Willd.) O.E. Schulz				2.0		3.0
Erysimum cheiranthoides L.	1.4	1.5	1.2	2.3	2.2	3.0
Hesperis matronalis L.	0	0	0	0	0	0
Lepidium campestre (L.) R. Br.	0	0	0	0	0	0
L. ruderale L.	1.7	0.5		2.7	1.5	1.7
Raphanus raphanistrum L.	0	0	0	0	0	0
Sisymbrium altissimum L.		0	0	0.8	0.5	0.6
S. officinale (L.) Scop.	3.3	1.3	2.0	3.0	3.5	1.8
Thlaspi arvense L.	1.3	2.0	1.0	0.7	1.0	2.7
Check, rutabaga 'Laurentian'	0	0	0	0	0	0
Check, cabbage 'Jersey Queen'	2.7	2.8	2.3	2.3	2.2	2.2

[a] Disease index is calculated from ratings (0=no club, 1=very small clubs on primary or secondary roots, 2=slight clubbing on primary and secondary roots, 3=moderate clubbing on these roots, 4=severe clubbing, 5=decayed roots) summed and averaged for the no. of plants observed.

[b] Isolate collected in 1967 by Reyes (15).

seeding. Late-season crops (14) were also direct-seeded as above on 15-16 June, 1971 and all crops except radish were thinned as for the mid-season crops. Radish cultivars were harvested six wk after seeding, and cabbage, cauliflower, broccoli (*B. oleracea* L. var. *italica* Plenck), brussels sprouts (*B. oleracea* L. var. *gemmifera* Zenker) and rutabaga were harvested 14 wk after seeding.

Each cultivar was replicated four times at each planting. Recommended cultural practices (14) were used throughout the study. At each harvest, the roots of the plants were carefully removed from the soil and indexed for clubroot.

Growing and inoculating weeds.— Fresh seeds of cruciferous weeds (Table 1) collected from the field during summer were air-dried and stored at room temp. The seeds were germinated on moist filter paper at room temp and were then transferred to 12-cm diam plastic cups filled with steamed-muck soil. When the seedlings were 3-5 cm tall, the roots were inoculated by dipping. Six race 6 isolates of *P. brassicae* were used and to test each isolate six plants of each species were used. Because the weed species grew at different rates, seeding time was adjusted so that all plants were approximately of equal height at inoculation. Inoculated seedlings were transplanted into 7-cm diam paper cups filled with a steamed-soil mixture of peat moss, sandy loam, and sand (1:3:1, v/v, pH 7.3). The cups were arranged at random on a greenhouse bench. Eighteen-day-old seedlings of Jersey Queen cabbage and Laurentian rutabaga were used as susceptible and nonsusceptible checks, respectively. The plants received a 20-20-20 fertilizer solution (5 g/liter) at the rate of ca. 30 cc/cup. The roots were rated for degree of clubbing four wk after inoculation. Each isolate was tested three times and the results averaged.

Greenhouse testing of fungicides.— In greenhouse experiments 15 fungicides were tested for control of clubroot (Table 2). In Experiment I, two 6-wk-old seedlings of Jersey Queen cabbage were transplanted into each 31-cm diam clay pot that contained a steamed-soil mixture. Immediately, 400 cc of spore suspension of *P. brassicae* (race 6 from Field II microplots) was poured into each pot. The next day, 400 cc of each fungicide suspension were added; the application was repeated one mo later. The following chemicals were used: Benlate [methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate], Bravo (tetrachloroisophthalonitrile), Chloroneb (1, 4-dichloro-2, 5-dimethoxybenzene), Dexon [*p*-(dimethylamino) benzenediazo sodium sulfonate], Dithane Z-78 (zinc ethylene bisdithiocarbamate), Terraclor (pentachloronitrobenzene) and Terraclor Super X (10% pentachloronitrobenzene + 2.5% 5-ethoxy-3-trichloromethyl-1, 2-4-thiadiazole). Each treatment was replicated five times and the whole experiment was set up in a randomized block design on a greenhouse bench. Plants were watered and fertilized as required and the roots were indexed for clubroot 12 wk after transplanting.

In Experiment II, the following materials were compared with Benlate (Table 2): Bay 33172 [2-(2-furyl)-benzimidazole], Bay 78175 [*N, N'*-dipropyl-*N, N'* (dichlorofluoromethylthio)-sulfamide], Chemagro 4497 [bis (1,2,2-trichloroethyl) sulfoxide], Chemagro 5506 [2-(1,2,2,-trichloroethyl)dithiopropionamide], Thiophanate [1, 2-bis (3-ethoxycarbonyl-2-thioureido) benzene], Thiophanate Methyl [1,2-bis(3-methoxycarbonyl-2-thioureido) benzene], Sclex [3-(3,5-dichlorophenyl)-5, 5-dimethyl oxazolidine-dione-2, 4]. The procedure described above was used except each treatment was replicated six times. Phytotoxic symptoms were recorded, cabbage heads were weighed, and the roots were indexed for clubroot after 10 wk.

Field testing of fungicides.— Experiment I: Benlate was tested in the race 6 infested microplots (Field II). On July 23, 1969, 10, 6-wk-old Jersey Queen cabbage seedlings were planted in each of two rows/plot at 60 cm spacing in the row. Each plant was drenched with 400 cc [0.28 g active ingredient (a.i.)] of Benlate suspension at transplanting and again one month later. Check plants received 400 cc of water. Three plots were used for Benlate treatment and three plots for the check plants. These plots were randomized. Standard cultural practices were used. Cabbage heads were weighed and the roots were indexed for clubroot after nine wk.

Experiment II: Because Vorlex (80% 1, 2-dichloropropane, 1, 3-dichloropropene + 20% methyl isothiocyanate) is being used on vegetable crops for control of soil-borne diseases (1), Benlate and Vorlex were compared for the control of clubroot in the field.

In previous experiments 0.28 g a.i. Benlate in 400 cc of suspension was applied to each plant, but in field practice 177 cc (6 oz) starter solution (14) is applied at transplanting.

TABLE 2. The effect of chemicals on *Plasmodiophora brassicae* on cabbage in the greenhouse

Chemical	Rate (g a.i./400 cc suspension per pot[w])	Clubroot index[x]	wt/head (g)
Experiment I[y]			
Benlate	0.48	1.3 c[z]	
Bravo	2.88	3.2 b	
Chloroneb	1.06	3.9 a b	
Dexon	0.48	3.9 a b	
Dithane Z-78	1.06	3.7 a b	
Terraclor	3.59	3.8 a b	
Terraclor Super X	3.59	4.0 a b	
Check (water)	0	4.1 a	
Experiment II			
Bay 33172	0.48	1.2 b	23 c
Bay 78175	0.48	1.5 b	119 b c
Chemagro 4497	0.72	0 b	0 c
Chemagro 5506	0.48	1.1 b	8 c
Thiophanate	1.06	0.8 b	157 b
Thiophanate Methyl	0.72	1.0 b	92 b c
Sclex	1.06	3.8 a	55 b c
Tecto	0.48	0.9 b	0 c
Benlate	0.24	0.8 b	302 a
Check (water)	0	4.0 a	17 c

[w] Drenched at transplanting and again one mo later. Abbreviation g a.i. = grams active ingredient.

[x] Disease index is calculated from ratings (0 = no club, 1 = very small clubs on primary or secondary roots, 2 = slight clubbing on primary and secondary roots, 3 = moderate clubbing on these roots, 4 = severe clubbing, 5 = decayed roots) summed and averaged for the no. of plants observed.

[y] Experiment I data are means of five replicates, Experiment II six replicates.

[z] Any two figures labelled with a common letter are not significantly different at 5% level.

TABLE 3. The effect of Benlate and Vorlex on *Plasmodiophora brassicae* on cabbage and rutabaga in the field

Chemical	Clubroot index[w]		Cabbage g wt/head	Rutabaga g wt/root
	Cabbage	Rutabaga		
Experiment I[x]				
Benlate (0.28 g a.i./400 cc per plant)	2.8 b[y]		875 a	
Check (water)	4.1 a		97 b	
Experiment II				
Benlate 1 (0.28 g a.i./177 cc per plant)[z]	2.6 b	3.3 a	431 a	167 b
Benlate 2 (0.28 g a.i./177 cc per plant)	1.8 c	1.8 b	503 a	408 a
Vorlex (461 liters/hectare)	2.8 b	3.0 a	481 a	242 b
Check (water)	3.2 a	3.3 a	388 a	123 b

[w] Disease index is calculated from ratings (0 = no club, 1 = very small clubs on primary or secondary roots, 2 = slight clubbing on primary and secondary roots, 3 = moderate clubbing on these roots, 4 = severe clubbing, 5 = decayed roots) summed and averaged for the no. of plants observed.

[x] Experiment I data are means of three replicates, Experiment II four replicates.

[y] Any two figures labelled with a common letter are not significantly different at 5% level.

[z] Benlate 1 = Benlate applied at transplanting, Benlate 2 = Benlate applied at transplanting and again one mo later.

Greenhouse studies had shown that 0.28 g a.i. Benlate in 177 cc water was not phytotoxic to 6-wk-old cabbage 'Early Greenball' seedlings. Therefore, field treatments were as follows: (i) Benlate 1 = 177 cc suspension (0.28 g a.i.)/plant at transplanting, (ii) Benlate 2 = Benlate as in (i) and repeated one mo later, (iii) Vorlex = 461 liters/ha and (iv) Check = 177 cc water/plant.

Experimental sites were located in a field, artificially infested with race 6, at Jordan, Ont., and in a field naturally infested with an unidentified race (Isolate 70D4-37), at Tavistock. Rows, 15 m long and 71 cm apart, were prepared in each field. At Tavistock, Vorlex was injected 18 cm deep in a 2.4-m band by a spring tooth fumigation rig with chisels 20 cm apart. The soil surface was sealed by pulling a heavy plank float behind the fumigation rig. At Jordan, the chemical was injected in single rows with a hand gun. Two wk after fumigation, the soil was shallow-cultivated to allow aeration and break the soil crust before transplanting or seeding.

At Jordan, 25 6-wk-old cabbage 'Eastern Ballhead' seedlings were transplanted 60 cm apart in the row on July 4, 1972. At Tavistock, the field was seeded with rutabaga on June 30, 1972, and 4 wk later plants were thinned to 15 cm spacing. Standard cultural practices were used. Cabbage plants were harvested 8 wk after transplanting and rutabagas were harvested 15 wk after seeding. Cabbage heads and rutabaga roots were weighed at harvest and roots of all plants were indexed for clubroot. Treatment plots consisting of 15 m rows were replicated four times and randomized.

Indexing.— The degree of root clubbing was rated as follows: 0 = no club, 1 = very small clubs on primary or secondary roots, 2 = slight clubbing on primary and secondary roots, 3 = moderate clubbing on these roots, 4 = severe clubbing, 5 = decayed roots. The ratings for all plants/cultivar were totalled and divided by the number of plants to give the disease index.

RESULTS AND DISCUSSION.—*Races.*—Twenty isolates of *P. brassicae* were obtained from 10 counties, three soil types, and five different hosts. Four isolates were identified as race 2, 15 isolates as race 6, and one was unclassified. Race 2 isolates were generally collected from sandy soil, whereas the race 6 isolates were usually from loam and muck soils. In addition, most race 2 isolates were recovered from rutabaga; race 6 isolates were generally from broccoli, cabbage, cauliflower, and pak-choi [*B. campestris* L. var. *chinensis* (L.) Makino].

The unidentified isolate (67E9-204) reported by Reyes (15) in 1969 was confirmed as race 2. In Canada, race 2 has also been reported in New Brunswick, Nova Scotia, Prince Edward Island, and Quebec; race 6 has been reported in British Columbia and Quebec (3, 18). Race 2 has been detected in Finland, Japan, New Zealand, Sweden, and the USSR; race 6 in Australia, Czechoslovakia, Germany, Sweden, and the USA (13, 18, 19).

Pathogenicity on cultivated crucifers.— All cultivated crucifers recommended in Ontario (14), except radish, were susceptible to races 2 and 6 of *P. brassicae* in the field. Early-season cultivars of cabbage and cauliflower were slightly to moderately susceptible but the mid- and late-season cultivars were highly susceptible. In contrast, all cultivars of broccoli and brussels sprouts were highly susceptible to the organism. Radish cultivars were generally resistant to infection.

Wherever clubroot is a problem, cultivars should be checked for susceptibility to the race of the organism involved. Some crucifer cultivars resistant to race 6 in other localities were susceptible in Ontario. For example, cabbage 'Bonanza' resistant to race 6 in Quebec (7) was susceptible to this race in Ontario. Factors such as seed source of the plant, method and time of planting and the inoculum involved may have contributed to this discrepancy. On the other hand, we found in preliminary tests that cabbage line 8-41 resistant to race 6 and susceptible to race 2 in Quebec (6) and rutabaga 'York' resistant to both races in Prince Edward Island (2) reacted in the same manner to these races in Ontario.

Pathogenicity on cruciferous weeds.— Race 6 isolates differed in pathogenicity on cruciferous weeds (Table 1). All isolates tested were pathogenic to *B. kaber, C. microcarpa, C. bursa-pastoris, D. sophia, E. gallicum, E. cheiranthoides, L. ruderale, S. officinale*, and *T. arvense*. Isolates 69D9-19, 69S19-1, and 69S19-2 infected *S. altissimum* whereas isolates 68D6-10 and 68D9-18 did not. None of the isolates tested attacked *B. vulgaris, H. matronalis, L. campestre*, or *R. raphanistrum*.

Ideally, no cruciferous weeds should be allowed to grow in fields intended for a commercial cruciferous crop because they perpetuate *P. brassicae* in the soil. Wherever possible, herbicides and cultural practices should be used to eliminate these weed hosts.

Chemical control in the greenhouse.— The greenhouse experiment showed that, of all the chemicals tested for control of *P. brassicae* on cabbage plants, only Benlate and Bravo reduced the disease significantly below the level found in the check plants (Table 2, experiment I). The Bravo-treated plants were clubbed significantly more than those treated with Benlate. Plants treated with Benlate at 0.48 g a.i./400 cc suspension per pot showed marginal chlorosis and savoying of the leaves indicating phytotoxicity. Our results with Benlate confirmed earlier reports (4, 11, 12, 17).

At 0.24 g a.i./400 cc suspension per pot Benlate was not phytotoxic, gave good control of clubroot, and cabbage head weight was significantly higher than of any other treatments (Table 2, Experiment II). Thiophanate also controlled *P. brassicae* without phytotoxicity, whereas Bay 33172, Bay 78175, Thiophanate Methyl, Tecto, Chemagro 4497, and Chemagro 5506 provided control but all were phytotoxic. Sclex did not control the organism.

Chemical control in the field.— Benlate (0.28 g a.i./plant in 400 cc) drenched at transplanting and again one mo later controlled *P. brassicae* on cabbage in the field (Table 3, Experiment I). The head weight of Benlate-treated plants was nine times that of the check plants. The same amount of Benlate applied similarly in 177 cc (the volume of starter solution usually applied by machine) also gave adequate control of *P. brassicae* on cabbage and rutabaga in the field (Table 3, Experiment II). Head weights of Benlate-treated cabbages tended to be heavier than those of the check plants. Root weight of Benlate-treated rutabaga was greater than the checks. However, Benlate did not give adequate control of *P. brassicae* on either plant in the field when applied only once. Vorlex was not practical for controlling this organism on cabbage and rutabaga in the field.

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