Pathotypes of *Plasmodiophora brassicae* in Washington, Oregon, and California

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ABSTRACT

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This study analyzes the pathotypes or races of *Plasmodiophora brassicae* occurring in crucifer-production areas of Washington, Oregon, and California. On the European Clubroot Differential set (ECD), all collections were similar in virulence. ECD code designations of the collections were either 16/02/31 or 16/03/31, both corresponding to race 7. Collecting spores from one ECD host with limited infection and reinoculating with these spores onto the same and other ECD host was important for verifying virulence genes present at low frequencies.

Clubroot is a serious crucifer disease that has been present for many years in British Columbia, Canada, and in Washington, Oregon, and California in the United States. Recently it has been found in the Salinas Valley of California, an important broccoli and cauliflower area.

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Programs to develop clubroot-resistant cole crops are in progress (2,5). It is important to define the pathotypes present in the pathogen populations to aid these breeding programs. Ayers (1) first developed a differential host series that was later modified by Williams (9), who identified race 7 (able to attack Badger Shipper cabbage) on the West Coast of the United States. The European Clubroot Differential set (ECD) represents an international attempt to standardize the race nomenclature for *Plasmodiophora brassicae* Wor. (3). This study was initiated to more thoroughly characterize pathotypes from cole crop-production areas in Washington, Oregon, and California, using the ECD set.

MATERIALS AND METHODS

Clubbed roots were collected from 13 locations in California, Oregon, and Washington. Samples were cleaned and frozen at 0 C for not longer than 8 mo. Spores were prepared as described by Williams (9), quantified with a hemacytometer, diluted to about 10^8 spores per milliliter, and refrigerated at 4 C until needed but never more than 4 wk.

The 15 differential hosts of the ECD set (five in the *Brassica campestris* group, five in the *B. napus* group, and five in the *B. oleracea* group) were used in each test. One milliliter of spore suspension was pipetted into each planting hole before seeding. Twenty-five seeds of each host, five seeds per pot, one per planting hole, were planted in 7-cm plastic pots filled with a 3:1 muck-soil:peat mix, pH 6.0–6.5, and placed in plastic flats of moist peat in the greenhouse at 20–25 C with 16 hr of daylight supplemented with cool-white fluorescent light. Each collection was evaluated in separate flats to prevent cross-contamination. The plants were well watered and were fertilized every 2 wk with a water-soluble 20-20-20 mix at 1 tablespoon per gallon. The peat bed was kept moist to prevent the soil mix from drying out. After 6–7 wk, clubroot symptoms were evaluated.

Each plant was assigned to a symtom category: 0 = no clubs, 1 = clubs on lateral roots, 2 = small clubs or clubs not girdling main taproot, and 3 = large club girdling the main taproot—a system similar to

Table 1. Results of typical European Clubroot Differential (ECD) test using a clubroot sample collected from broccoli at Mount Vernon, WA

ECD set		Symptom category[a] 0	1	2	3	Disease index[b]	Reaction type[c]
Brassica campestris	01	29	0	0	0	0	−
	02	30	0	0	0	0	−
	03	27	0	0	0	0	−
	04	29	0	0	0	0	−
	05	0	0	0	24	100	+
Brassica napus	06	23	2	1	0	5	?
	07	0	0	0	23	100	+
	08	24	1	0	1	5	?
	09	24	1	0	0	1	?
	10	20	3	0	0	4	?
Brassica oleracea	11	0	1	8	5	77	+
	12	0	0	0	22	100	+
	13	0	0	0	25	100	+
	14	0	0	0	20	100	+
	15	0	1	4	15	90	+

[a] 0 = No clubs; 1 = small clubs on lateral roots, 2 = small clubs on main root not girdling root, and 3 = large club girdling main taproot at or near soil level.

[b] $DI = \frac{\Sigma \text{ (no. of plants in each symptom category} \times \text{category no.)}}{\text{total no. plants}} \times \frac{100}{3}$.

[c] Reaction type based on DI cutoff point of 33:susceptible = "+" (DI ≥ 33); resistant = "−" (DI = 0) or "?" (0 < DI < 33).

Table 2. Verification of uncertain ("?") virulence genes by reinoculation of European Clubroot Differential (ECD) hosts with spores collected from specific ECD differential hosts

ECD set designation	Symptom category[a] 0	1	2	3	Disease index[b]	Reaction type[c]
Clubs from ECD cultivar 06 reinoculated on:						
06	0	0	7	12	87	+
05	0	0	0	23	100	+
Clubs from ECD cultivar 08 reinoculated on:						
08	8	0	0	2	20	?
05	0	0	0	10	100	+

[a] 0 = No clubs, 1 = small clubs on lateral roots, 2 = small clubs on main root not girdling root, and 3 = large club girdling main taproot at or near soil level.

[b] $DI = \frac{\Sigma \text{ (no. of plants in each symptom category} \times \text{category no.)}}{\text{total no. plants}} \times \frac{100}{3}$.

[c] Reaction type based on DI cutoff point of 33:susceptible = "+" (DI ≥ 33); resistant = "−" (DI = 0) or "?" (0 < DI < 33).

that described by Buczacki et al (3). A disease index (DI) was then calculated for each host from these categories using: DI = [Σ (no. of plants in each symptom category × category no.)/total no. of plants] × 100/3. This index was used to assign each ECD host to a reaction type as resistant (DI = 0 designated "−" or 0 < DI < 33 designated "?") or susceptible (DI ≥ 33 designated "+"). Where possible, spores were collected from "?" reaction hosts and reinoculated onto part or all of the ECD set. Finally, each collection of *P. brassicae*, representing a population of resting spores of one or more pathotypes, was assigned a numerical code designation based on susceptible ECD hosts as described by Buczacki et al (3).

RESULTS

Table 1 illustrates a typical ECD reaction to a clubroot sample (sample F) collected from broccoli in Washington state. Based on a DI cutoff point of 33, ECD 09 gives a resistant reaction indicating no virulence genes present in the pathogen, whereas ECD 07 exhibits a susceptible reaction showing the presence of virulence genes. When spores from clubs of an ECD host with an uncertain ("?") reaction, eg, ECD 06 and 08 in Table 1, were reinoculated onto appropriate ECD hosts, ECD 06 proved to be susceptible, whereas ECD 08 remained "uncertain" (Table 2).

Table 3 summarizes the virulence after reinoculation of the populations. Collections were remarkably homogeneous with respect to ECD virulence codes that were either 16/02/31 or 16/03/31.

DISCUSSION

Assigning each pathotype a coded response to the ECD hosts based on susceptible reactions requires a clear distinction between resistant (−, ?) and

Table 3. Virulent genes in collections of *Plasmodiophora brassicae* from West Coast states of the United States defined by the European Clubroot Differential set

Host plants and locations of *Plasmodiophora brassicae* collections[a]	Disease reaction types[b] *B. campestris* 01	02	03	04	05	*B. napus* 06	07	08	09	10	*B. oleracea* 11	12	13	14	15	Pathotype numerical designation[c]
Chinese cabbage, Puyallup, WA	−	−	?	−	+	+	+	?	−	?	+	+	+	+	+	16/3/31
Collard, Puyallup, WA	−	−	−	−	+	?	+	?	−	?	+	+	+	+	+	16/2/31
Mustard, Puyallup, WA	−	?	?	?	+	−	+	?	−	?	+	+	+	+	+	16/2/31
Cabbage, Puyallup, WA	−	?	?	−	+	−	+	?	−	−	+	+	+	+	+	16/2/31
Cauliflower, Mt. Vernon, WA	−	−	−	−	+	+	+	?	?	?	+	+	+	+	+	16/3/31
Broccoli, Mt. Vernon, WA	−	−	−	−	+	+	+	?	?	?	+	+	+	+	+	16/3/31
Cauliflower, Mt. Vernon, WA	−	?	−	−	+	?	+	−	−	−	+	+	+	+	+	16/2/31
Chinese cabbage, Fir Island, WA	−	−	?	?	+	+	+	?	−	−	+	+	+	+	+	16/3/31
Cauliflower, Gresham, OR	−	−	−	−	+	−	+	−	−	−	+	+	+	+	+	16/2/31
Broccoli, Corvallis, OR	−	−	−	−	+	?	+	−	−	?	+	+	+	+	+	16/2/31
Cauliflower, Brooks, OR	−	−	?	−	+	+	+	?	−	?	+	+	+	+	+	16/3/31
Brussels sprouts, Half Moon Bay, CA	−	−	−	−	+	+	+	−	−	−	+	+	+	+	+	16/3/31
Broccoli, Salinas, CA	−	−	?	−	+	+	+	?	−	?	+	+	+	+	+	16/3/31

[a] All *Brassica oleracea* L. except Chinese cabbage, *B. campestris* L., and *B. juncea* (L.) Coss.

[b] Reaction type based on DI cutoff point of 33: susceptible = "+" (DI ≥ 33); resistant = "−" (DI = 0) or "?" (0 = < DI < 33).

[c] ECD hosts in each *Brassica* spp. group were assigned values of 1, 2, 4, 8, and 16, respectively. By adding the values for each susceptible reaction, a unique virulence designation is obtained (3), eg, 16/3/31 represents positive reactions for 05, 06, 07, and 11-15. All uncertain reactions (?) were treated as resistant.

susceptible (+) reactions. This was often not clear-cut. A DI cutoff point was needed to permit one to establish arbitrarily whether a host was susceptible or resistant based on the disease data. Although no established cutoff point has been agreed upon, a DI of 33 has frequently been used by other workers and was followed in this work.

Numerical pathotype designations must be interpreted carefully. Field collections of resting spores may consist of mixtures of virulent pathotypes (4). One pathotype may be dominant and easily identified; another pathotype occurring at a very low frequency may infect only a few plants of a susceptible ECD host and be overlooked as insignificant. To avoid such a host being mislabeled as resistant, all plants showing questionable reactions were reinoculated with spores from the same infected ECD host so that the frequency of the pathotype or virulence gene was greatly increased to the point where a susceptible reaction could be seen (Tables 1 and 2, ECD 06). Williams (9) and Crute et al (4) have also stressed that any code can be misleading because hiding virulence genes can occur at low levels where infection of susceptible hosts is below the cutoff point, thus being rated resistant.

In cases where the virulence code did not change upon reinoculation, a lack of genetic homogeneity of certain ECD hosts would make adequate pathotype differentiation impossible in a single screening. Susceptible plants in genetically impure differential stocks may account for one or two severely infected plants even after reinoculation (Table 2, ECD 08).

Despite the lack of genotypic homogeneity of certain differentials, the populations of *P. brassicae* collected in California, Oregon, and Washington show considerable similarity in their virulences (Table 3). Generally, there was little or no virulence in the pathogen populations for the *B. campestris* group except for the universal suscept (05), more virulence for the *B. napus* group, and complete virulence for the *B. oleracea* group. In preliminary studies, one of us (J. Robak) obtained an ECD code 16/02/31 for spores collected from Chinese cabbage in Puyallup, WA, from a mustard species in Willamette Valley, OR, and from cabbage in Tillamook, OR, and in Mount Vernon, WA (6). A clubroot collection from broccoli grown in Corvallis, OR, initially showed low virulence to the *B. napus* group (ECD 16/0/31) but upon reinoculation, virulence was determined to be 16/02/31 (Table 3). ECD results with similar virulence patterns have also been reported with collections from British Columbia (8).

In terms of pathotypes or races, race 7 virulent on ECD 11 (Badger Shipper) is present as Williams (9) reported in 1966. This is in contrast to race 6, which is avirulent on Badger Shipper and which is predominant in the eastern United States (7,9). Race 6 may also be present in the western states but could not be detected because genes virulent to ECD 11 were present in all collections. To clearly separate mixtures of pathotypes, single spore isolates would be required.

LITERATURE CITED

1. Ayers, G. W. 1957. Races of *Plasmodiophora brassicae*. Can. J. Bot. 35:923-932.
2. Baggett, J. R. 1976. "Oregon CR-1" broccoli. HortScience 11:622-623.
3. Buczaki, S. T., Toxopeus, H., Mattusch, P., Johnston, T. D., Dixon, G. R., and Hobolth, L. A. 1975. Study of physiological specialization through an international approach. Trans. Br. Mycol. Soc. 65:295-303.
4. Crute, I. R., Gray, A. R., Crisp, P., and Buczacki, S. T. 1980. Variation in *Plasmodiophora brassicae* and resistance to clubroot disease in *Brassicas* and allied crops—A critical review. Plant Breed. Abstr. 50:91-104.
5. Gabrielson, R. L. 1977. Breeding for resistance to clubroot in broccoli (calabrese) and cauliflower in western Washington. Page 121 in: Proc. Woronin + 100 Conf., Univ. Wisc., Madison.
6. Robak, J. 1979. Clubroot Newsl. No. 8. Hürth, West Germany.
7. Rowe, R. C. 1980. Evaluation of radish cultivars for resistance to clubroot (*Plasmodiophora brassicae*) race 6 for midwestern United States. Plant Dis. 64:462-464.
8. Waring, M. Clubroot Newsletter No. 9. Hürth, West Germany.
9. Williams, P. H. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology 56:624-626.