Reaction of Lines of the Rapid Cycling Brassica Collection and *Arabidopsis thaliana* to Four Pathotypes of *Plasmodiophora brassicae*

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Abstract

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The clubroot reaction of five Rapid Cycling Brassica Collection (RCBC) lines (*Brassica carinata*, *B. juncea*, *B. napus*, *B. oleracea*, and *B. rapa*) and 84 lines of *Arabidopsis thaliana* to pathotypes 2, 3, 5, and 6 of *Plasmodiophora brassicae* (as classified on William's system) was assessed. Also, the reaction of the *Arabidopsis* lines to a single-spore isolate of each of pathotypes 3 and 6 was compared with that of a field isolate. Seedlings were inoculated with resting spores of *P. brassicae*, maintained at 25 and 20°C (day and night, respectively), and assessed for clubroot incidence and severity at 6 weeks after inoculation. Several lines of *A. thaliana* and RCBC exhibited a differential response to pathotype but none of the lines were immune. Among the RCBC lines, *B. napus* was resistant to all of the pathotypes; *B. oleracea* was resistant to pathotypes 2, 3, and 5; *B carinata* and *B. rapa* were resistant to pathotypes 2 and 5; and *B. juncea* was susceptible to pathotypes 5 and 6 and had an intermediate response to pathotypes 2 and 3. Line Ct-1 of *A. thaliana* was highly resistant to pathotype 2, Pu2-23 was highly resistant to pathotype 5, and Ws-2 and Sorbo were highly resistant to pathotype 6. These results indicate that the lines of RCBC and *A. thaliana* have potential for use as model crops for a wide range of studies on clubroot, and could be used to differentiate these four pathotypes of *P. brassicae*. The reaction of the RCBC lines to pathotype 6 was highly correlated with response under field conditions but the reaction to the single-spore isolates of pathotypes 3 and 6 was not strongly correlated with reaction to the field collections in the *Arabidopsis* lines.

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is an economically important disease of crops in the Brassicaceae family. The pathogen induces root malformations (clubs) on infected plants, reducing the capacity for water and nutrient uptake and transport (7). Plants become wilted and stunted, and yield can be reduced when symptoms are severe. Hundreds of millions of long-lived resting spores of *P. brassicae* are produced inside each clubbed root and are released into the soil when the clubs decay and disintegrate (32).

Historically, the impact of clubroot in Canada has been greatest on *Brassica* vegetables in British Columbia, Ontario, and Quebec (12). However, clubroot has recently become a major concern for canola (*Brassica napus* L.) producers in the Northern Great Plains, where it has spread rapidly since it was first reported on canola near Edmonton, AB in 2003 (12,13,30). Yield losses in canola can exceed 90% (12,13). As a result, *P. brassicae* is an emerging threat to the 4.7-million-ha canola industry on the Canadian prairies.

Various host differential sets, consisting of selected *Brassica* genotypes, have been suggested to differentiate the pathotypes of *P. brassicae* (5,15,27,33). The differential set of Williams is based on the reaction of cabbage (*B. oleracea* L. var. *capitata*) 'Jersey Queen' and 'Badger Shipper' and rutabaga (*B. napus* L. var. *napobrassica*) 'Laurentian' and 'Wilhelmsburger' (33). The European Clubroot Differential (ECD) set consists of five genotypes each of *B. napus*, *B. rapa*, and *B. oleracea* (5). These two differential sets have been used to characterize pathogen populations from Canada (3,22,29) and other regions of the world.

Pathotype 6 (ECD 16/02/30) is predominant in the Midwestern United States (23,33) and pathotype 7 (ECD 16/02/31 or ECD 16/03/31) in the eastern United States (8,33). The predominant pathotypes of *P. brassicae* in Canada are 2, 3, 5, and 6, based on the differential set of Williams and confirmed using the ECD set (28,29). Pathotype 6 (equivalent to ECD 16/0/14) is the predominant pathotype in Ontario and British Columbia (22,29). Pathotype 2 (ECD 16/15/31) is present in Quebec (29,31). Pathotype 3 (ECD 16/15/12) is dominant in Alberta but pathotype 5 (ECD 16/15/0) is also present in that province (29). Pathotype 3 is also the dominant pathotype in Nova Scotia (11). However, the pattern was found to be somewhat different when the pathotypes were assessed using single-spore isolates (35); pathotypes 2, 3, 6, and 8 were identified in Alberta; pathotypes 3, 5, and 8 in Ontario; and pathotype 6 in British Columbia. Previous studies indicate that there are potentially more pathotypes that are present at low frequency (6,12,28). Populations of *P. brassicae* in Canada are considered to be less diverse than those in Europe (9) but more diverse than those in the United States (8,23,33).

Arabidopsis thaliana is a host of *P. brassicae* and has been used as a model system for molecular studies on clubroot (2,14,18,20,26). The small, sequenced genome and the availability of many mutant lines of *A. thaliana*, together with its small stature and short life cycle, make it ideal for use as a model plant in molecular assessments. However, a model crop with a growth habit and architecture more similar to susceptible crop species would be useful for many other kinds of studies. The Rapid Cycling Brassica Collection (RCBC), also known as Wisconsin Fast Plants, consists of *Brassica* lines that are early flowering, with small stature and a short life cycle, and results from these lines could be obtained more quickly than with conventional crop cultivars (34). Their small size and rapid development make them excellent candidates

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for use as model crops in studies of clubroot under controlled conditions, where space is at a premium. Recently, the clubroot reactions of selected lines of *Brassica* vegetables, RCBC, and canola to pathotype 6 were assessed under field conditions (1). However, the reaction of RCBC lines to the other common pathotypes of *P. brassicae* is not known. Similarly, *A. thaliana* has been used for research on clubroot (2,14,18,20,26) but the reaction to the various pathotypes is mostly unknown.

The present study was undertaken to examine the reaction of selected lines of the RCBC and *A. thaliana* to pathotypes 2, 3, 5, and 6 of *P. brassicae*. Lines with a differential reaction to a pathotype may be useful as model crops for studies of clubroot on canola, vegetables, and other commercial crops. Any differential reaction to pathotypes among the lines might be used to study resistance reactions or, in the future, to develop additional methods to distinguish the pathotypes of *P. brassicae* in Canada.

Materials and Methods

Plant materials. The Wisconsin Fast Plants or RCBC lines *B. carinata* (L.) A. Braun (genome designation BCbbcc, haploid chromosome number for the b and c genomes, bc = 17), *B. juncea* (L.) Czern (ABaabb, ab = 18), *B. napus* (L.) (ACaacc, ac = 19), B. oleracea (Ccc, c = 9), and *B. rapa* (Aaa, a = 10, standard rapid cycling) were obtained from the RCBC, University of Wisconsin, Madison. Shanghai pak choy (*B. rapa* subsp. *Chinensis* (Rupr.) var. *communis* Tsen and Lee) 'Mei Qing Choy' and Chinese flowering cabbage (*B. rapa* subsp. *Chinensis* (Rupr.) var. *utilis* Tsen and Lee) 'Tsoi-sim' were obtained from Stokes Seeds Inc. In total, 84 lines of *A. thaliana* that readily produced viable seed were selected from a set of 96 lines at the NRC Plant Biotechnology Institute, SK, Canada. These stocks, originally obtained from the *Arabidopsis* Biological Resource Center, Columbus, OH, represent a breadth of geographical origin and genetic diversity (21,24).

Pathogen material. Four pathotypes of *P. brassicae* (pathotypes 2, 3, 5, and 6, William's system) collected from clubbed roots of canola grown in Quebec, central Alberta, southern Alberta, and Ontario, Canada, respectively, were used in this study. They were distinguished based on pathogenicity on the differential set of Williams or using the ECD set (28,29). In addition, one single-spore-derived isolate each of pathotypes 3 and 6 (35), from Napa cabbage (*B. rapa* subsp. *Pekinensis* (Lour.) Hanlet) 'Granaat', provided by the Strelkov lab, University of Alberta, AB, Canada, were also assessed. The single-spore-derived isolates were increased on the susceptible Shanghai pak choy Mei Qing Choy. Inoculum was prepared from clubbed roots that had been stored at –20°C. Spores were extracted by thawing the frozen clubs at room temperature, then homogenizing approximately 3 g in 100 ml of water at high speed for 2 min and straining the resulting spore suspension through eight layers of cheesecloth. The spore concentration was determined using a hemocytometer and adjusted to 3×10^6 resting spores ml^{-1}. Freshly prepared inoculum was used for each inoculation.

Cultivation and inoculation of plants. The RCBC lines were grown in tall plastic pots (21-by-3.8-cm Conetainers; Stuewe and Sons Inc.) filled with soil-less mix (Sunshine mix number 4; Sun Gro Horticulture Canada Ltd.). Seedlings were thinned to one per pot after 5 days. Plants were maintained in a growth room at 20 and 25°C (day and night, respectively) temperature with 75% relative humidity and a 16-h photoperiod, watered daily with demineralized water adjusted to pH 6.3 using commercial vinegar (5% acetic acid), and fertilized weekly with 20 ml of mineral fertilizer adjusted to pH 6.3 (80 g of 15:15:18 NPK fertilizer per liter stock solution, 5 ml stock solution per liter fertilizer solution; Plant Products Co. Ltd.). Each seedling was inoculated (field-collected isolates only) by pipetting 5 ml of resting spore suspension (3×10^6 spores/ml) at the base of the seedling. The control plants were mock-inoculated with deionized water. In addition, Shanghai pak choy Mei Qing Choy and Chinese flowering cabbage Tsoi-sim (both highly susceptible) were included as controls. The trial was laid out in a randomized complete block design with four replicates (10 plants per rep), and the entire trial was repeated.

The plants were harvested 6 weeks after inoculation. The roots were washed and assessed for clubroot incidence (CI) and severity, based on visual symptoms of root clubbing. Plants were separated into classes using a standard 0-to-3 scale, where 0 = no clubbing, 1 = less than one-third of root clubbed, 2 = one-third to two-thirds of root clubbed, and 3 = greater than two-thirds of roots clubbed (15,35). A disease severity index (DSI) was calculated using the following equation (29): $DSI = \{\Sigma[(\text{class number})(\text{number of plants in each class})]/[(\text{total number plants per sample})(\text{number of classes} - 1)]\} \times 100$.

The resistance response of the lines to *P. brassicae* was classified based on their mean DSI value, as follows: (i) resistant = 0 to 33 DSI, (ii) intermediate = 34 to 67 DSI, and (iii) susceptible = 68 to 100 DSI.

The clubroot reaction of lines of *A. thaliana* was evaluated in two phases. In the first phase, the 84 lines were evaluated for clubroot reaction using field-collected isolates of pathotypes 2, 3, 5, and 6 and single-spored isolates of pathotypes 3 and 6 (experiment 1). In the second phase, assessment of the 11 lines that displayed a resistant reaction to one or more pathotypes in experiment 1 was repeated (experiment 2). Each experiment was conducted and assessed using the same methods as for the RCBC lines, except as follows. The trial was laid out in a randomized complete block design with four replicates and eight plants per rep. The seed was stratified at 4°C in the dark for a week in damp soil-less mix; then, 14-day-old seedlings were transplanted individually into the cells of 96-cell plug trays (each cell = 3 by 2 by 6 cm) and inoculated with 3 ml of 3×10^6 spores/ml spore suspension. To avoid cross-contamination, the plants in each pathotype treatment (plus the noninoculated control) were grown in separate trays. Due to space and time constraints, only 11 lines of *A. thaliana* could be assessed at a time; therefore, Shanghai pak choy Mei Qing Choy was included in each set of inoculations as an internal control.

Data analysis. Treatment effects were assessed using the mixed-model analysis of variance of the data with the lines or species as the fixed effect and replication as a random effect (PROC MIXED, SAS software version 9.2; SAS Institute Inc.). The data set for each trial was tested for normality using the Shapiro-Wilk test of residuals, and checked for outliers using Lund's test of standardized residuals (19). No outliers were found in any data set. For the RCBC trial, there was no effect of repetition or repetition–treatment; therefore, the data were pooled across repetition for analysis. Means were separated using Tukey's test. The relationship between growth room assessments of the RCBC lines and previous assessments under field conditions (1) was examined using Pearson's correlation coefficient. For the assessment of *Arabidopsis* lines, the consistency of response in the 11 selected lines between the two repetitions was examined using Pearson's correlation coefficient. The relationship between the field pathotypes and single-spore isolates on the 84 *Arabidopsis* lines was also examined using Pearson's correlation coefficient.

Prior to analysis, percent data were arcsine-transformed when necessary to improve the normality and homogeneity of variance but nontransformed means are presented for uniformity of presentation. Differences were significant at $P \leq 0.05$ unless otherwise noted.

Results

In both repetitions of the assessment of RCBC lines, CI and DSI were 100% in the inoculated controls (Mei Qing Choy and Tsoi-sim) and no symptoms of clubroot developed in any noninoculated control (*data not shown*). Therefore, all of the control treatments were excluded from subsequent analyses. Also, there was no effect of repetition or repetition–treatment; therefore, the data from the two repetitions of the trial were combined for analysis. CI and DSI showed a similar pattern of response to treatment (Fig. 1); therefore, the focus in this presentation will be on DSI. The mean DSI of the pathotypes, listed in decreasing order, was pathotype 6 (DSI = 61%), pathotype 3 (32%), pathotype 5 (26%), and pathotype 2 (12%), with a standard error (SE) of 4.3%. The mean DSI for the

RBCB lines (SE = 4.1%), listed in decreasing order of susceptibility, was *B. juncea* (DSI = 59%), *B. carinata* (34%), *B. rapa* (24%), *B. napus* (12%), and *B. oleracea* (9%). However, because there was a line–pathotype interaction, the mean reaction (main effects) does not always reflect the important relationships, and reactions of single lines to individual pathotypes were investigated in more detail.

Some of the RCBC lines showed a differential response to pathotype (Fig. 1; Table 1). Four of five RCBC lines were resistant to pathotypes 2 and 5. The exception was *B. juncea*, which had an intermediate response to pathotype 2 (DSI = 55%) and was susceptible to pathotype 5 (68%). Similarly, two of five RCBC lines were resistant to pathotype 3 while *B. carinata*, *B. rapa*, and *B. napus* had an intermediate response (Fig. 1). *B. carinata* (91%) and *B. juncea* (100%) were highly susceptible to pathotype 6, *B. oleracea* (40%) and *B. rapa* (66%) had an intermediate response, and *B. napus* was resistant (2%) (Fig. 1).

There was a strong positive correlation ($r = 0.91$, $P < 0.0001$) when the DSI values of RCBC lines inoculated with pathotype 6 under controlled conditions in the current study were compared with results from a previous field trial conducted over several years at a site where pathotype 6 was dominant (1).

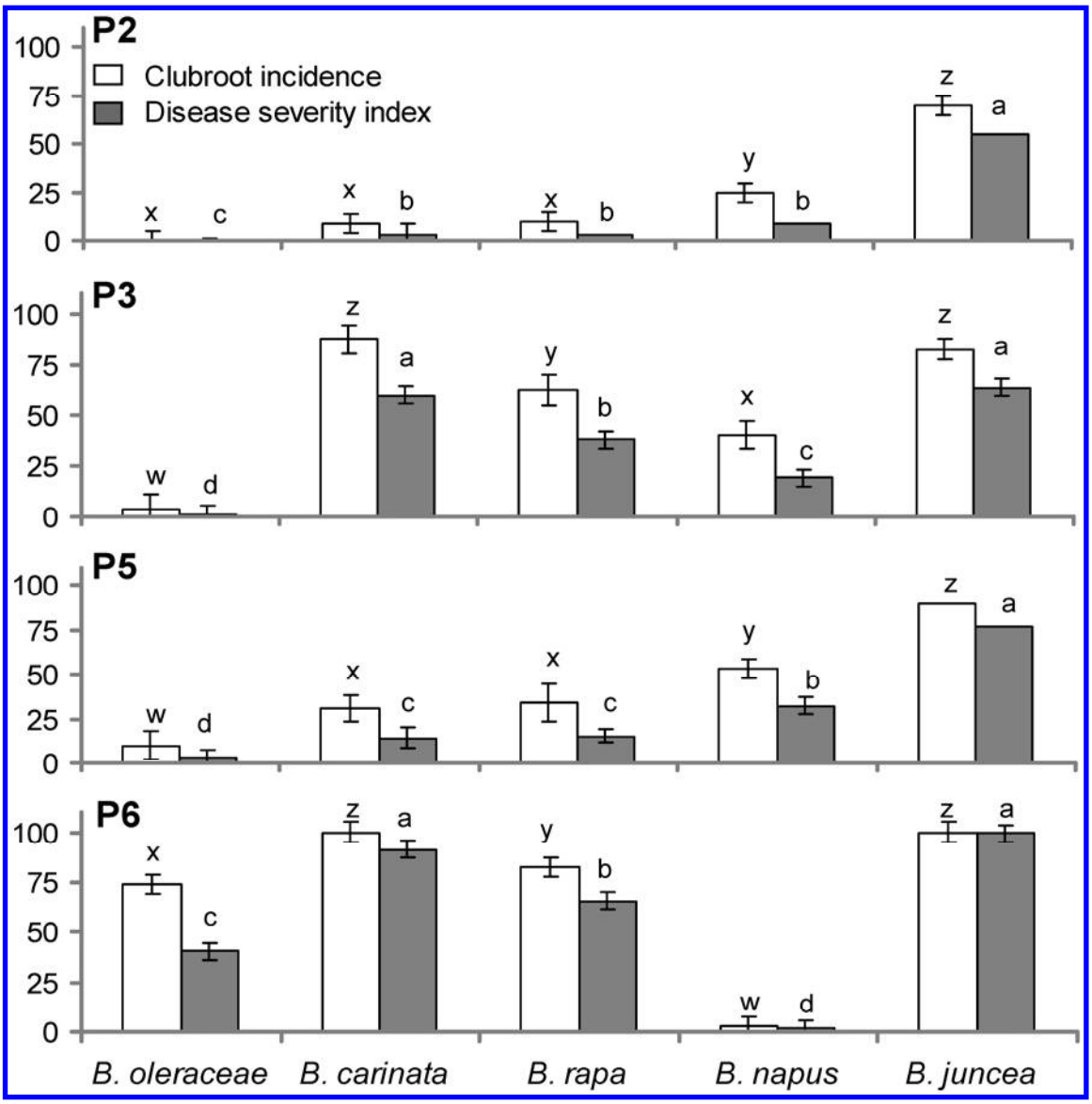

Fig. 1. Clubroot incidence (%) and severity (disease severity index) of the Rapid Cycling Brassica Collection lines inoculated with field-collected *Plasmodiophora brassicae* pathotypes 2, 3, 5 and 6. Bars with the same letter above do not differ based on Tukey's test at $P < 0.05$. Capped lines represent ± standard error.

Table 1. Analysis of variance of clubroot incidence (CI) and disease severity index (DSI) for the Rapid Cycling Brassica Collection lines inoculated with field-collected isolates of *Plasmodiophora brassicae* (data combined across two repetitions of the trial, each with four replicates)

		CI			DSI		
Source	**df**	**Mean square**	***F* value**	***P* value**	**Mean square**	***F* value**	***P* value**
Replication	7	132	2	0.21	57	1.7	0.12
Pathotype	3	17,303	307	0.0001	14,612	432	0.0001
Line	4	19,758	350	0.0001	20,027	592	0.0001
Pathotype × line	12	5,155	91	0.0001	3,113	92	0.0001
CV (%)[a]	…	…	16	…	…	17	…

[a] Coefficient of variation.

In experiment 1 of the assessment of *A. thaliana*, DSI in Shanghai pak choy Mei Qing Choy was 100% for each of the pathotypes in each run (*data not shown*). Based on this uniform response in the susceptible control, the data from the individual runs were pooled for analysis. Overall, pathotype 3 (mean DSI = 88%) and pathotype 6 (86%) produced the most severe symptoms, pathotype 5 was intermediate (79%), and pathotype 2 (69%) produced the least severe symptoms on the 84 lines assessed (SE = 0.46%). As for the RCBC lines, a strong line–pathotype interaction was found (Supplementary Table S1) and individual combinations were examined.

None of the *Arabidopsis* lines was resistant to all of the pathotypes, and the susceptible lines had various forms of galls compared with the resistant and noninoculated control lines (Fig. 2). Lines Ct-1(mean DSI = 0%), Hr-10 (15%), Mrk-0 (18%), Bay-0 (20%), Ler-1(20%), Col-0 (22%), Pro-0 (22%), Ed-1 (24%), Uod-1 (25%), Wa-1 (26%), Wt-0 (29%), and Uod-7 (31%) were resistant to pathotype 2; lines Pu2-23 (8%), Knox-10 (23%), C24 (25%), Uod-1 (26%), and Nok-3 (29%) were resistant to pathotype 5; and lines Sorbo (6%), Ws-2 (8%), Ct-1(18%), O-27(20%), and NFA-8 (22%) were resistant to pathotype 6. Only two lines, Nok-3 (29%) and Kz-9 (32%), were resistant to pathotype 3. Each of the other lines had a susceptible or intermediate reaction to all of the pathotypes.

In experiment 2, there was a strong line–pathotype interaction for both CI and DSI in analysis of variance. Overall, pathotype 3 produced the most severe symptoms (mean DSI = 92%), pathotype 5 (67%) and pathotype 6 (61%) were intermediate, and pathotype 2 produced the fewest symptoms (56%) (SE = 0.23%). Lines Ct-0 (DSI = 0%), Hr-10 (15%), Ler-1(17%), and Col-0 (18%) were resistant to pathotype 2 (SE = 1.4%); Nok-3 (20%) was resistant to pathotype 3 (SE = 1.9%); Pu2-23 (6%) and Nok-3 (31%) were resistant to pathotype 5 (SE = 2.6%); and Ws-2 (5%), Sorbo (6%), Ct-0 (16%), and NFA-8 (22%) were resistant to pathotype 6 (SE = 1.9%).

The pattern of response to pathotype of the 11 selected lines in experiments 1 and 2 was highly correlated (r = 0.98 for CI, r = 0.96 for DSI); therefore, the mean response for each line to pathotype across the two experiments is presented (Fig. 3). In contrast, the reaction of several of the 84 *Arabidopsis* lines to the single-spore isolates differed substantially from that of the field collections of pathotypes 3 (r = 0.08 for CI, r = 0.07 for DSI) and 6 (r = 0.09 for CI, r = 0.09 for DSI) (Fig. 4). There was a strong line–pathotype interaction for both CI and DSI in analysis of variance (Table 2). Overall, the field collections produced slightly more symptoms than the single-spore isolates (mean CI = 96 versus 90%, DSI = 88 versus 83% for pathotype 3; CI = 92 versus 83%, DSI = 86 versus 73% for pathotype 6; SE = 0.35% for CI, 0.47% for DSI). Of the 84 *Arabidopsis* lines, 62 lines had the same DSI reaction (susceptible, intermediate, and resistant) to the single-spore and field collection isolates of pathotype 3, and 46 lines had the same response to isolates of pathotype 6 (Fig. 4). For example, Hr-5, Hr-10, Kin-0, and Van-0 were susceptible to the field collection and resistant to single-spore isolates of pathotype 3 (Fig. 4).

Discussion

The RBCB lines in this study showed a differential reaction to the four pathotypes of *P. brassicae* that were assessed: *B. napus* was resistant to each of the pathotypes; *B. oleracea* was resistant to pathotypes 2, 3, and 5; *B. carinata* and *B. rapa* were resistant to pathotypes 2 and 5; and *B. juncea* had an intermediate response to pathotypes 2 and 3. This indicates that these RCBC lines could be useful as model crops for many types of research on clubroot. The advantage of RCBC lines as model crops is that a line can be chosen that is similar in growth habit and root and shoot architecture to the crop of interest. Also, these lines are small in stature and set seed within 42 days; therefore, they are suitable for use where space is at a premium (e.g., controlled conditions). Finally, although seed of the RCBC lines is expensive relative to Shanghai pak choy and canola, the lines have been continually available for many years and, therefore, are likely to be consistently available over time. This represents a substantial advantage over commercial canola cultivars that can be removed from the marketplace without notice (1).

Most of the 84 *A. thaliana* lines were susceptible to each pathotype of *P. brassicae* and no line was resistant to all four pathotypes. Lines that did show a differential reaction to pathotype were generally moderately resistant, and none were immune. However, 11 of the 84 lines displayed a resistant reaction (DSI > 33%) to at least one pathotype, and the reaction of these 11 lines was highly consistent between experiments 1 and 2 (r = 0.98 for CI, r = 0.96 for DSI). This consistent response indicates the reproducibility of the experiment and the accuracy of the results. The sequenced genome and the availability of mutants make *Arabidopsis* lines very useful for molecular studies. However, the seed is very small and must be vernalized to ensure adequate germination, and the plant architecture is not similar to most *Brassica* crops; therefore, *Arabidopsis* has some drawbacks as a model crop for many other purposes.

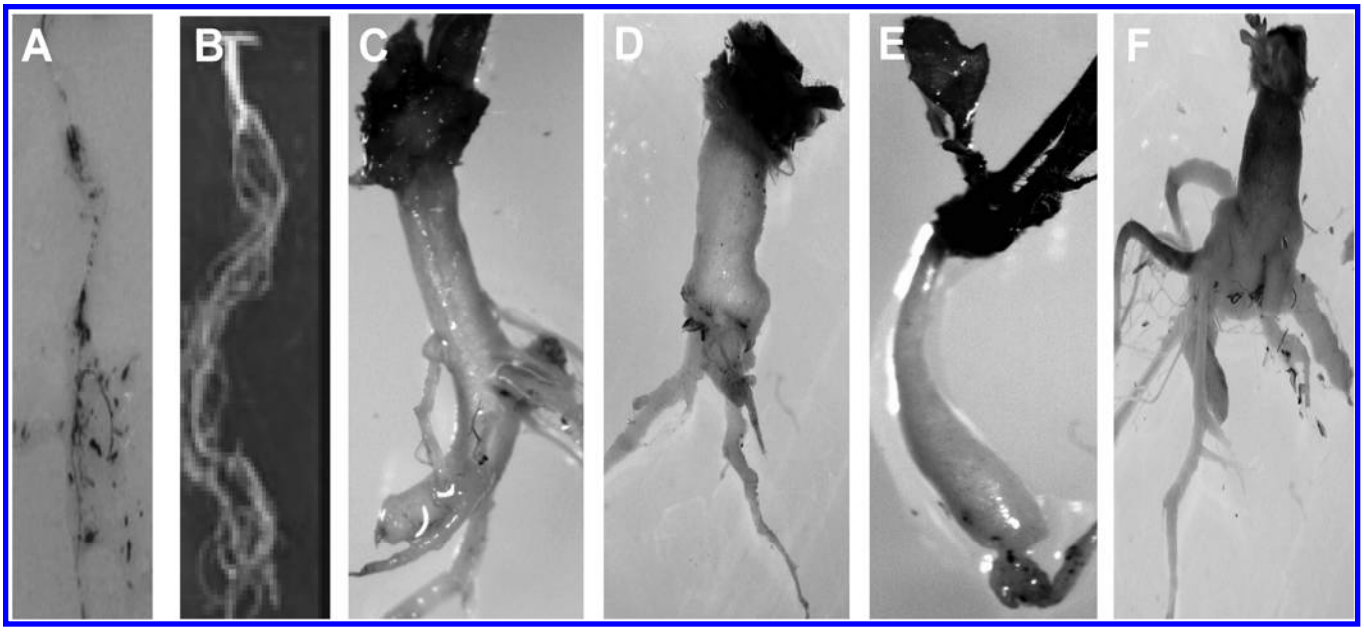

Fig. 2. Roots of **A,** the noninoculated (control); **B,** inoculated resistant line; and **C–F,** range of symptom development in *Arabidopsis* lines.

In previous studies, pathotype 3 consistently produced more severe symptoms of clubroot than pathotype 6 (10,29). Also, canola genotypes with resistance to pathotype 3 or 5 were generally also resistant to other pathotypes (29). In the current trial, pathotypes 3 and 6 produced similar levels of clubroot on the 84 lines of *A. thaliana* but pathotype 6 produced more severe symptoms than pathotype 3 when averaged over the five RCBC lines. Also, the lines that were resistant to pathotype 3 were not necessarily resistant to pathotypes 2, 5, or 6 (Table 3; Figs. 1 and 3).

There was a strong correlation between clubroot severity on RCBC lines grown at a field site where pathotype 6 was dominant (1) and in the controlled environment trials with pathotype 6. This indicates that the pathogen population was uniform and stable. In contrast, the single-spored isolates of pathotypes 3 and 6 did not produce the same pattern of resistance responses as the field collections on several of the *A. thaliana* lines. This supports previous reports of heterogeneity of the pathogen in field collections (4,16,17,25,35). One pathotype may be dominant and easily identified, whereas others may be present at a low frequency and, thus, easily overlooked. However, components of a population that are present at low frequency can be of great interest, especially if they are virulent on cultivars that are resistant to the predominant pathotype (16). The use of specific pathotypes of *P. brassicae* with defined virulence factors, alone and in mixtures, has been recommended to improve the efficiency of selection in resistance breeding (16). Additional research is required to determine the

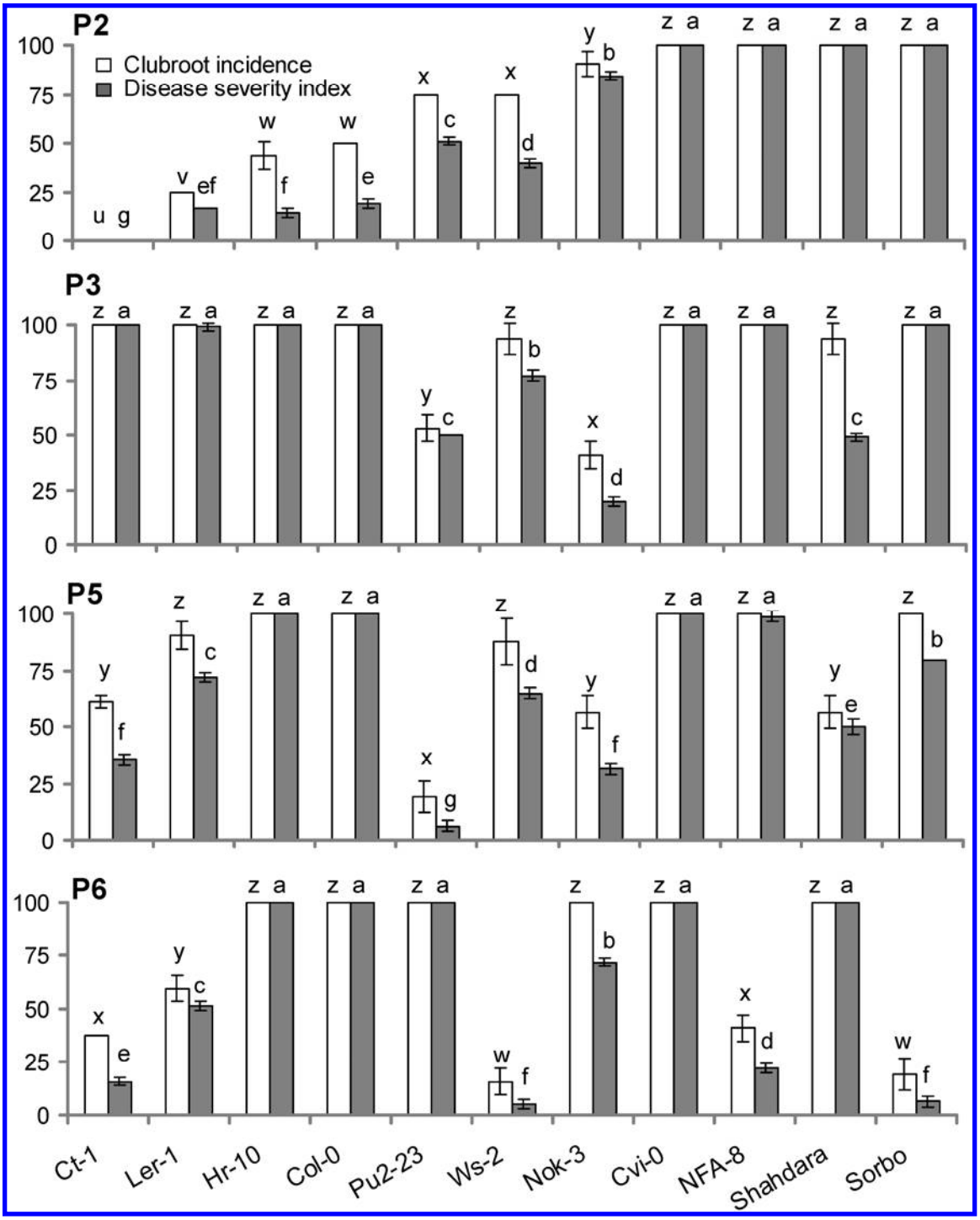

Fig. 3. Mean clubroot incidence and severity of 11 *Arabidopsis* lines to field-collected *Plasmodiophora brassicae* pathotypes 2, 3, 5, and 6 in experiments 1 and 2. Bars with the same letter above do not differ based on Tukey's test at $P < 0.05$. Capped lines represent ± standard error.

reaction pattern of a selected host against a wider range of single-spored isolates.

Inclusion of the susceptible Shanghai pak choy Mei Qing Choy and Chinese cabbage Tsoi-sim in the RCBC trial and Mei Qing Choy in the *Arabidopsis* experiments was very useful. Several sets of inoculations (repetitions in the RCBC experiment, subsets of *Arabidopsis* lines in experiment 1) could be combined with confidence because of the high and consistent levels of clubroot in each inoculated control treatment. Both crops were highly susceptible to each of the *P. brassicae* pathotypes. Previous studies had shown that these cultivars were highly susceptible to pathotype 6 (1,10) but this is the first time that the reaction of these potential model crops has been assessed against a wider range of pathotypes. Seed for Shanghai pak choy Mei Qing Choy and Chinese cabbage Tsoi-sim is readily available commercially and inexpensive; the seed germinates easily, seedlings are vigorous, and the plants grow quickly but are small in stature. We conclude that both Mei Qing Choy and Tsoi-sim have potential for use as susceptible checks in future clubroot studies.

There have been a number of suggestions for how to classify host reaction to *P. brassicae*, based on various cut-off points for DSI (5,8,15,29,31). The cut-off points for DSI include 20% (31), 33% (5,8), and 49% (29). Clubroot reaction in the current study was separated into three categories: (i) resistant = 0 to 33% DSI, (ii) intermediate = 34 to 67% DSI, and (iii) susceptible = 68 to 100% DSI. Although the focus of the current work was on substantial and consistent differences in reaction (resistant versus susceptible) to differentiated pathotypes, the intermediate category of reaction may represent a fruitful area for researchers interested in quantitative resistance and factors that influence the expression of resistance.

Several sets of differential hosts have been proposed and employed in recent years (4,5,15,27,29,33,35). Unfortunately, comparison and identification of the pathotypes described in these different systems is not always straightforward. Substantial variability in the reaction of *P. brassicae* has been observed on the differential lines in each of the three most commonly utilized sets (5,27,35). Also, there is a concern that certain of these differentials

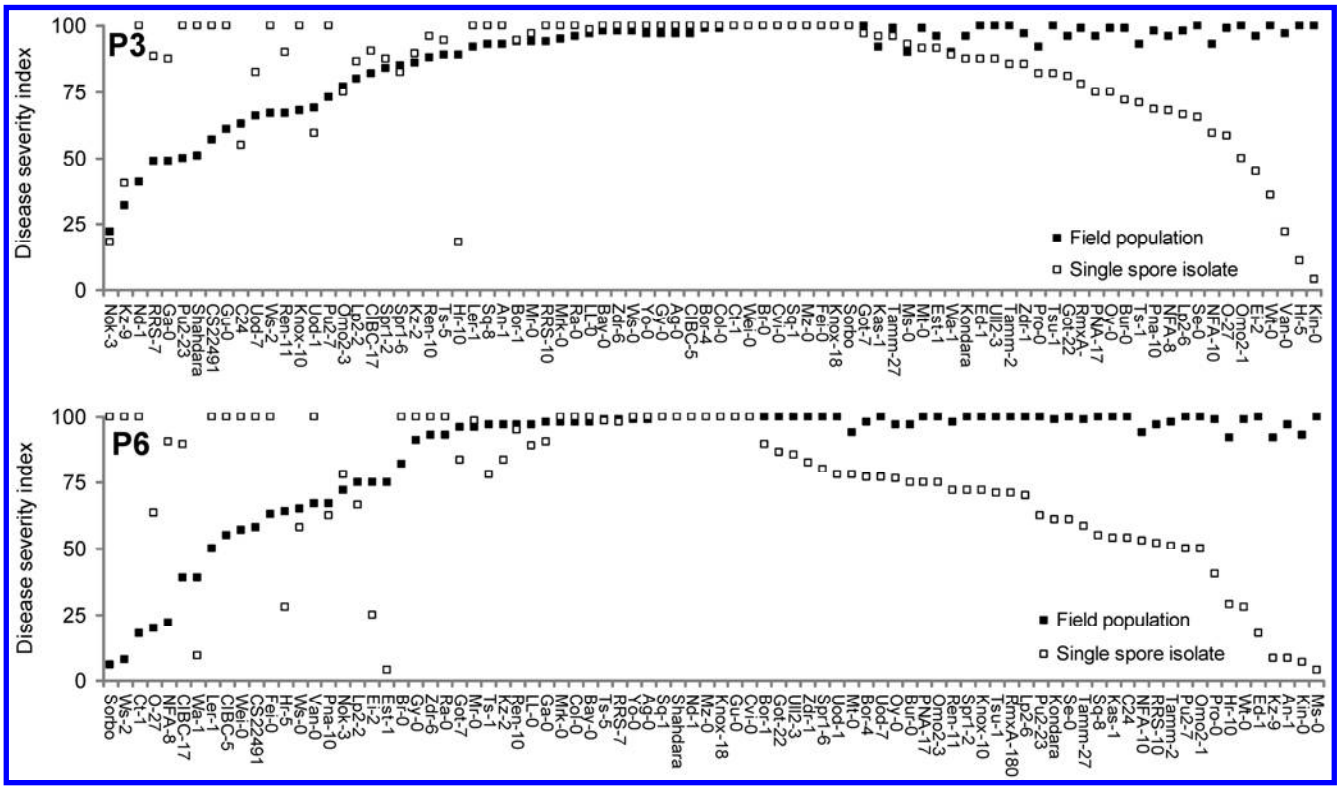

Fig. 4. Clubroot severity (disease severity index) of 84 *Arabidopsis* lines inoculated with a field collected or single-spore isolate of pathotypes **A,** 3 and **B,** 6 of *Plasmodiophora brassicae*.

Table 2. Analysis of variance of clubroot incidence (CI) and disease severity index (DSI) for 84 lines of *Arabidopsis thaliana* inoculated with a field-collected or single-spored isolate of pathotypes 3 and 6 of *Plasmodiophora brassicae*

		CI			DSI		
Source[a]	df	Mean square	*F* value	*P* value	Mean square	*F* value	*P* value
Pathotype 3							
Replication	3	39	1.4	0.24	89	1.5	0.23
Isolate	1	4,934	180	0.0001	2,108	35	0.0001
Line	83	1,049	39	0.0001	1,641	27	0.0001
Isolate × line	83	799	29	0.0001	1,362	22	0.0001
CV (%)			6			9	
Pathotype 6							
Replication	3	24	0.5	0.7	12	0.2	0.91
Isolate	1	12,365	247	0.0001	26,176	387	0.0001
Line	83	1,719	38	0.0001	2,288	34	0.0001
Isolate × line	83	1,852	41	0.0001	2,574	38	0.0001
CV (%)	…	…	8	…	…	10	…

[a] Field versus single-spore isolate of each pathotype; CV = coefficient of variation.

Table 3. Lines of *Arabidopsis*, Rapid Cycling Brassica Collection (RCBC), and Shanghai pak choy that could be used to differentiate the predominant pathotypes of *Plasmodiophora brassicae* in Canada

		Pathotype[a]			
Plant species	**Line**	**2**	**3**	**5**	**6**
Arabidopsis thaliana	Sorbo	S	S	S	R
	Ct-1	R	S	I	R
Brassica carinata	RCBC	R	I	R	S
B. napus	RCBC	R	R	R	R
B. rapa subsp. *chinensis*	Pak choy	S	S	S	S

[a] Pathotype designations correspond to Williams (33). The reaction of RCBC and *Arabidopsis* lines to *P. brassicae* was classified into three categories based on disease severity index value: (i) R (resistant) = 0 to 33% DSI, (ii) I (intermediate) = 34 to 67% DSI, and (iii) S (susceptible) = 68 to 100% DSI.

lack genetic homogeneity (8,27). This likely indicates that these differentials do not reflect the complete range of pathogenic diversity in pathogen populations. An improved differential series in which each of the hosts is genetically uniform would be very useful. Also, these differentials were originally developed to study populations of *P. brassicae* on *Brassica* vegetables and, therefore, may not cover the range of pathogen diversity on canola. This variability has been mentioned as a concern in the Northern Great Plains, where clubroot is an emerging problem on canola (12,13).

Seed of lines chosen for any differential set should be readily and consistently available. There have been problems obtaining seed of some of the lines of the ECD differential set (27,29), especially the seed of the susceptible species in the ECD series, Napa cabbage (*B. rapa* subsp. *Pekinensis* (Lour.) Hanlet) Granaat. It is also helpful if the plants are easy to grow and handle. Scott et al. (25) recommended that the hosts should ideally be commercial cultivars in current use. This approach might be suitable for certain vegetable crops, where some cultivars can remain in production for decades, but would not be practical for canola because cultivars go out of production quickly.

It will take extensive testing to develop a new differential set of hosts for *P. brassicae* pathotypes but the differential response of RCBC and *Arabidopsis* lines might be useful in this process. For example, the lines of *Arabidopsis* and RCBC in Table 3 could be used to identify the four main pathotypes of *P. brassicae* examined in this study. The RBCB line of *B. napus* was resistant and the Shanghai pak choy Mei Qing Choy was susceptible to all the pathotypes. RCBC line *B. carinata* was resistant to pathotype 5 and intermediate to pathotype 3. This is a weaker line in the differential set, and it would be useful to identify a line that had a stronger differential reaction to these two pathotypes. Line Ct-1 was resistant to pathotype 2 but susceptible to pathotype 3, while Sorbo was susceptible to both pathotypes; *B. carinata* was resistant to pathotype 5, while Sorbo was susceptible; and *B. carinata* was susceptible to pathotype 6 but Sorbo and Ct-1 were resistant.

The current study examined the reaction of lines of the RCBC and *A. thaliana* to the predominant pathotypes of *P. brassicae* in Canada. We conclude that many of the RCBC and *Arabidopsis* lines in this study can serve as model plants for research on clubroot, including Mei Qing Choy, which is highly susceptible to the four pathotypes. The RCBC lines, in particular, are readily available and easy to grow. The reaction between the single-spored isolates of pathotypes 3 and 6 differed with reaction to the field collections on several of the 84 *Arabidopsis* lines and warrants further study. Development of a differential system for the pathotypes of *P. brassicae* in the Northern Great Plains may be of value in breeding for resistance and management of clubroot in canola, and the lines identified in this study may have a role in a new system.

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