A Comparison of Greenhouse and Field Screening Methods for Blackleg Resistance in Doubled Haploid Lines of *Brassica napus*

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ABSTRACT

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A group of 87 doubled haploid (DH) lines of canola (*Brassica napus*) were screened for blackleg resistance in the greenhouse at three plant growth stages: cotyledon, true leaf, and adult plant. Disease severity data were calculated on 33 DH lines, which were subsequently screened in field experiments over 2 yr along with parental lines and controls. The inoculum level used in field experiments was the same as that used in greenhouse screening. Blackleg disease severity and disease incidence on field-grown lines were highly correlated ($r \geq 0.82$) with greenhouse disease severity data for all three growth stages. In greenhouse experiments, disease severity data for different growth stages were also highly correlated ($r \geq 0.83$). Inoculation of DH seedlings at the cotyledon stage in the greenhouse was a reliable method for evaluating blackleg resistance. Disease severity and disease incidence in field experiments also were highly correlated ($r = 0.996$), suggesting that disease incidence may be used as an estimator of disease severity in field surveys.

Additional keywords: host resistance, *Phoma lingam*, stem canker

Blackleg incited by the virulent form of *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. is one of the more important diseases of plants in the genus *Brassica* in Australia, Europe, and North America (1,2,12–15,17). Major damage from the disease occurs when cankers are produced on the main stem, which leads to prematurity of the crop. In Canada, economic losses from this disease on canola (*B. napus* L.) can exceed 50% (13). The use of genetic resistance is the chief method of controlling the disease and has been utilized effectively in Australia and Europe (17,19,26). Because systemic growth of *L. maculans* is the main pathway for stem canker development (10), genetic resistance is the most preferred method of control, based on efficacy, economics of production, and environmental impact.

In Canada, cultivars of canola with partial resistance have recently been released, but cultivars with high levels of resistance are not yet available (23). In view of the urgent need for cultivars that are highly resistant to blackleg, a breeding program was initiated to transfer blackleg resistance from Australian cultivars to the University of Alberta breeding lines using the doubled haploid method of breeding (3).

An efficient and reliable disease screening procedure is a prerequisite for any resistance breeding program. Both greenhouse and field screening procedures have been used for blackleg, and several inoculation methods have been described (2,12,16,19,25). Gugel et al (8,9) have questioned the reliability of greenhouse screening methods and favored the use of field screening because

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of poor correlations between results from field and greenhouse screening tests. Recently, Rimmer and van den Berg (18) published a list of screening methods used by different workers and recommended screening in growth chambers. Newman and Bailey (17) and Frencel et al (5) have also favored the use of greenhouse seedling tests. Evaluation of seedlings in the greenhouse permits screening of more than one generation per year and can expedite the development of suitable resistant cultivars (2). Poor correlations between seedling and field reactions in some of the earlier studies could have resulted from the inoculation method used, the seedling stage selected for inoculation, or the level of infection in greenhouse or field tests (17).

This paper describes a method of greenhouse blackleg resistance screening to evaluate the response of doubled haploid breeding lines of canola and reports on correlations among various growth stages tested in the greenhouse and between greenhouse and field reactions of these lines to *L. maculans*.

MATERIALS AND METHODS

Two Australian blackleg-resistant *B. napus* cultivars, Maluka and Shiralee, were crossed with susceptible advanced *B. napus* University of Alberta breeding lines 88-51230, 88-53044, and 88-53473. F_1 plants from these crosses were grown in a controlled environment chamber, and doubled haploid (DH) lines were produced by the microspore culture method of Coventry et al (3).

Greenhouse screening. All DH lines and resistant parents were screened using eight single-spored virulent *L. maculans* isolates from collections made in the Canadian provinces of Alberta, Saskatchewan, and Manitoba. These eight isolates were selected on the basis of their virulence and geographic distribution. From each DH line, resistant parent, or control, 64 plants were grown in the greenhouse in 32 plastic pots (6 × 6 cm) containing soilfree growth medium (21). Conditions in the greenhouse were maintained at approximately 21 C and 16-hr photoperiod supplemented with 400W, high-pressure sodium lamps. In each run of the ongoing screening experiment, 15 genotypes were tested. The partially resistant cultivar Profit was included as control. Isolates of *L. maculans* were cultured on V8 agar supplemented with rose bengal (0.4 μg/ml) and incubated at room temperature under a 12-hr photoperiod. Pycnidiospore suspensions from the eight isolates were prepared separately in sterile distilled water from 12- to 14-day-old growing cultures and adjusted to 1×10^6 spores per milliliter.

Seven days after sowing, 64 seedlings were divided into eight groups of eight plants and each group was inoculated with one of the eight isolates. The inoculation technique and evaluation method were modified from those of Delwiche (4). Inoculations were made by placing 10 μl of the spore suspension dispensed from an Eppendorf micropipette on a wound made on each cotyledon by a No. 1 entomological needle. Inoculated seedlings were incubated in a mist chamber (100% RH) for 2 days. The reactions on cotyledonary leaves were recorded 10 days after inoculation on a 0 (resistant) to 4 (susceptible) scale (Table 1). Seedlings were then thinned to one per pot for testing true leaves and stem canker development from systemic infection. All saved plants were reinoculated on the third or fourth true leaf, depending on the plant genotype growth rate, with the respective blackleg isolate used for cotyledon inoculation. The method of true leaf inoculation and incubation was the same as for cotyledon inoculation except that one true leaf on each plant was wounded on each side of the midrib with the tip of the micropipette used for inoculum deposition. Fifteen days after true leaf inoculation, disease symptoms at the inoculation sites were recorded on a 0 (resistant) to 4 (susceptible) scale (Table 1). Plants were scored for stem canker development from systemic infection at plant growth stage 3.2–4.2 (11) on a 0 (resistant) to 6 (susceptible) scale 22 days after true leaf inoculation (Table 1). Plants from resistant lines were then inoculated directly on the stem by making two superficial circular wounds about 2.5 mm in diameter. The two wounds were made 2–3 cm apart, one each on the second and third internode of the main stem, with a multineedle consisting of 33 needles (entomological No. 1); a 10-μl pycnidiospore suspension was then placed at each wound. Plants were evaluated at maturity on a 0–6 scale as described above.

By using this method of greenhouse screening, 87 DH lines were evaluated. Disease severity data were calculated on the 33 DH lines that were subsequently used in field screening experiments. The lines chosen for field study varied in their disease response from fully susceptible to highly resistant. The disease severity data from the eight isolates used were pooled to obtain a mean value for each genotype tested. Disease severity was calculated for all three growth stages using the formula: disease severity (%) = Σ(no. of plants in a disease scale category × disease scale category)/(total no. of plants × maximum disease scale category) × 100.

Field screening. Thirty-three DH lines, resistant parents (Maluka and Shiralee), susceptible parents (agronomically superior University of Alberta lines), and partially resistant and susceptible cultivars Legend, Global, Profit, and Westar were evaluated for blackleg resistance during the 1991 and 1992 growing seasons. Within each year, the screening experiments were conducted as a split-plot randomized block design at Alberta En-

Table 1. Disease severity scales used for recording reactions of *Brassica napus* genotypes to *Leptosphaeria maculans* at various growth stages in the greenhouse and in the field

Plant part	Disease severity	Disease symptoms
Greenhouse		
Cotyledon	0	No visible expression of disease
	1	Necrotrophic hypersensitive response around the wound
	2	Gray-green tissue collapse with distinct margin
	3	Gray-green tissue collapse with diffused margin
	4	Most of the tissue collapsed with pycnidia formation
True leaf	0	No visible expression of disease
	1	Necrotrophic hypersensitive response around the wound
	2	Gray-green tissue collapse with distinct margin
	3	Gray-green tissue collapse with diffused margin
	4	Tissue (1–4 cm) around the wound collapsed with pycnidia formation
Adult plant	0	No visible expression of disease
	1	Lesions on stem at cotyledon leaf attachment sites
	2	Stem less than one-half girdled at cotyledon leaf attachment sites and no pycnidia formation
	3	Stem one-half or more girdled at cotyledon leaf attachment sites and no pycnidia formation
	4	Stem girdled with pycnidia formation
	5	Stem weak and leaves starting to wilt
	6	Plant dead
Field	0	No visible expression of disease
	1	Stem lesions less than one-third of stem diameter and no pycnidia formation
	2	Stem lesions less than one-third of stem diameter and pycnidia formation
	3	Stem lesions one-third to two-thirds of stem diameter and pycnidia formation
	4	Stem mostly girdled but plant not wilted
	5	Stem mostly girdled, extensive tissue damage, plant wilted

vironmental Centre, Vegreville, with four blocks, genotypes as main plot, and blackleg treatment as subplot. Each genotype was planted in four-row plots, each 7 × 0.9 m. Three-row barley plots were planted between the *Brassica* genotypes. One week after seeding, each 7-m plot was divided into 3-m subplots by first cultivating and then planting a 1-m strip of barley in the middle of plots. One of the halves (subplot) of each plot, selected at random, was inoculated with *L. maculans* pycnidiospores at plant growth stage 2.2 (11). The other subplot was not inoculated and served as a check.

For inoculation, spore suspension was prepared from five of the eight isolates used earlier in greenhouse screening. Spore suspensions were prepared at a concentration of 1×10^6 to 1×10^7 spores per milliliter. The surfactant Assist (an oil-based concentrate) was added to the spore suspension at a concentration of 1 ml/L. Inoculum was sprayed on randomly selected subplots at the 2.2 growth stage using a 5-L capacity plastic hand sprayer. All inoculations were done late in the evening to ensure cool and humid conditions conducive for infection.

To evaluate blackleg severity, diseased and healthy plants were recorded from the middle of two rows. Plants from 50-cm borders on both sides of the subplot were not included. Diseased plants were evaluated on a 0 (resistant) to 5 (susceptible) scale (Table 1). Disease incidence (DI) was calculated using the formula: disease incidence (%) = (no. of infected plants in a sample)/(total no. of plants in a sample) × 100. DS for field screening was calculated as in greenhouse screening.

Statistical analysis. Analyses of variance were performed on arcsine-transformed percent disease severity and percent disease incidence data from the field, using the GLM procedure from SAS (20). Sources of variation were: years ($y = 2$), replications ($r = 4$), genotypes ($g = 42$), treatments ($t = 2$), and the interactions. Correlation coefficients were calculated between all possible combinations of DS and DI using the correlation procedure from SAS. Orthogonal contrasts were also performed to compare the mean disease severity values of the different phenotypic groups determined on the basis of greenhouse screening.

RESULTS

The resistance of DH lines in the greenhouse was measured in terms of percent disease severity (Table 2). The genotypes that had low disease severity values at the cotyledon stage maintained

Table 2. Mean values for percent *Leptosphaeria maculans* disease severity and disease incidence of *Brassica napus* genotypes from field and greenhouse experiments in 1991 and 1992

Genotype number	Phenotypic group[a]	Field				Greenhouse		
		Disease severity (%)		Disease incidence (%)		Disease severity (%)		
		Inoculated	Control	Inoculated	Control	Cotyledon	True leaf	Adult plant
1	Global(SC)	23.06	2.96	44.76	8.15	nt[b]	nt	nt
2	DH(R)	2.93	0.95	9.16	3.96	26.2	56.3	16.7
3	DH(DR)	6.76	1.61	18.40	7.19	33.6	35.2	25.0
4	DH(DR)	5.19	0.60	14.59	2.59	38.1	57.8	22.0
5	DH(R)	3.96	1.34	9.15	4.13	25.8	25.0	16.7
6	Legend(SC)	26.95	4.93	47.00	11.59	100.0	88.7	75.8
7	DH(DR)	17.21	2.93	35.11	8.35	36.3	80.5	37.5
8	88-51230(SP)	49.05	7.38	80.05	11.57	nt	nt	nt
9	DH(S)	6.89	0.96	18.46	2.81	92.9	73.4	45.8
10	DH(MS)	8.45	1.26	18.06	3.54	58.6	36.7	25.0
11	DH(R)	2.69	0.34	6.79	0.75	26.2	55.5	18.2
12	DH(MS)	3.48	0.88	8.36	3.80	65.2	39.8	31.3
13	DH(R)	2.69	1.49	8.51	4.55	25.0	25.0	16.7
14	DH(R)	9.05	0.50	17.68	1.80	49.2	28.3	0.0
15	DH(S)	53.96	12.66	82.76	25.54	100.0	100.0	69.8
16	DH(DS)	3.99	0.88	13.18	3.44	59.4	88.9	36.9
17	DH(R)	4.31	1.23	9.85	3.31	26.6	36.7	16.7
18	DH(R)	7.36	0.80	15.30	1.53	25.0	50.0	17.2
19	DH(R)	1.54	0.20	5.06	0.83	37.9	32.0	23.4
20	Maluka(RP)	5.59	1.66	15.10	5.01	35.2	56.3	21.4
21	DH(R)	7.21	1.14	17.69	4.71	34.4	25.0	16.7
22	DH(R)	2.85	0.50	9.25	2.39	43.8	33.6	20.8
23	DH(R)	2.98	1.43	9.33	4.35	51.2	13.7	19.8
24	Westar(SC)	62.51	17.56	87.34	31.86	nt	nt	nt
25	DH(DR)	17.16	4.65	30.89	12.09	28.9	25.8	20.8
26	DH(MS)	5.90	0.78	17.85	2.65	57.4	39.8	18.8
27	DH(R)	8.30	2.43	21.53	8.48	31.6	28.1	16.7
28	DH(DR)	12.95	4.25	33.29	12.04	32.0	48.4	36.5
29	DH(S)	49.13	5.78	76.04	13.70	100.0	100.0	92.2
30	DH(R)	21.45	4.90	40.06	9.65	37.5	25.0	20.8
31	Shiralee(RP)	4.21	1.70	11.16	3.69	32.4	32.8	18.2
32	DH(S)	49.48	9.26	76.83	18.16	100.0	100.0	87.5
33	DH(R)	5.29	1.23	14.58	3.38	25.0	25.0	21.4
34	Profit(SC)	35.91	10.30	56.13	17.60	100.0	100.0	88.0
35	DH(S)	51.00	5.88	78.65	12.61	100.0	100.0	77.6
36	88-53044(SP)	40.69	14.48	62.58	25.40	nt	nt	nt
37	DH(S)	43.61	9.49	70.94	18.94	100.0	97.5	82.8
38	DH(R)	4.85	0.80	13.75	3.40	40.8	10.2	0.0
39	88-53473(SP)	48.77	8.86	76.34	14.96	nt	nt	nt
40	DH(R)	2.62	0.45	8.10	1.75	35.1	10.0	0.0
41	DH(S)	21.86	6.89	39.50	14.25	100.0	92.2	50.5
42	DH(R)	6.15	0.83	16.31	3.11	25.3	25.0	16.7

[a] SC = susceptible control, SP = susceptible parent, RP = resistant parent, DH(R) = doubled haploid (resistant), DH(DR) = doubled haploid (differentially resistant), DH(MS) = doubled haploid (moderately susceptible), DH(DS) = doubled haploid (differentially susceptible), and DH(S) = doubled haploid (susceptible).
[b] Not tested.

Table 3. Analysis of variance for percent *Leptosphaeria maculans* disease severity and disease incidence data from field experiments of *Brassica napus* at Vegreville, Alberta, 1991–1922

Source	df	Disease severity[a]			Disease incidence[a]		
		MS	*F* value	*P* > *F*	MS	*F* value	*P* > *F*
Year	1	901.31	2.34	0.18	396.93	0.42	0.54
Rep/year	6	385.19			945.54		
Genotype	41	1,440.90	45.97	0.00	2,528.21	33.20	0.00
Genotype × year	41	227.37	7.25	0.00	350.15	4.60	0.00
Genotype × rep/year	241	31.35			76.15		
Treatment	1	30,760.14	395.33	0.00	63,801.71	389.28	0.00
Treatment × year	1	36.63	0.47	0.52	33.27	0.20	0.67
Treatment × rep/year	6	77.81			163.89		
Treatment × genotype	41	316.19	12.76	0.00	559.17	8.52	0.00
Treatment × genotype × year	41	36.88	1.49	0.04	93.56	1.45	0.05
Treatment × genotype × rep/year	241	24.77			64.68		

[a] Transformed to arcsine of square root of the percentage

their resistance at the true leaf and 3.2–4.2 plant growth stage (adult plant). Similarly, genotypes that had higher disease severity values at the cotyledon stage were also susceptible at true leaf and adult plant stages. On the basis of their overall disease reaction on different plant parts in the greenhouse, DH lines were grouped into phenotypic classes, viz., resistant (R), differentially resistant (DR), moderately susceptible (MS), differentially susceptible (DS), and susceptible (S); only a few plants were completely susceptible or completely resistant. Percent disease incidence was not calculated in greenhouse experiments because plants were rarely rated at 0 on the scales used for screening. Furthermore, DH lines with lower disease severity in greenhouse tests had lower disease severity and disease incidence values in the field experiments (Table 2). In the inoculated plots of field experiments, DH lines were classified mainly as resistant or susceptible. Most genotypes classified as DH(DR), DH(MS), or DH(DS) on the basis of greenhouse reactions were resistant in the field. DH line No. 9 was an exception, giving a susceptible reaction at the cotyledon stage and a moderately susceptible reaction at the adult plant stage in the greenhouse and a resistant reaction in the field.

The percent disease severity and percent disease incidence data from field experiments were also used to study variation among genotypes and interaction of genotypes with treatments. The analyses of variance for these two characteristics are given in Table 3. The results are consistent for both parameters. *F* values for genotypes were significant, which suggested that genotypes differed from each other and could be grouped into resistant or susceptible classes. The effects of inoculation and control treatments were also significant. The percent disease incidence data varied from 1 to 32 in control plots and from 5 to 87 in treated plots (Table 2). Similarly, percent disease severity varied from 1 to 18 in control plots and from 2 to 62 in inoculated plots. These results suggest that genotypes from different phenotypic classes may show similar field resistance under low disease pressure (control plots had some background disease); under heavy disease pressure, however, genotypes with resistant or differential interaction performed better than susceptible ones (Figs. 1 and 2). Although the treatment × genotype interaction was significant for disease incidence and severity, the genotypes showed the same trend for disease incidence and disease severity in both treatments (Table 3).

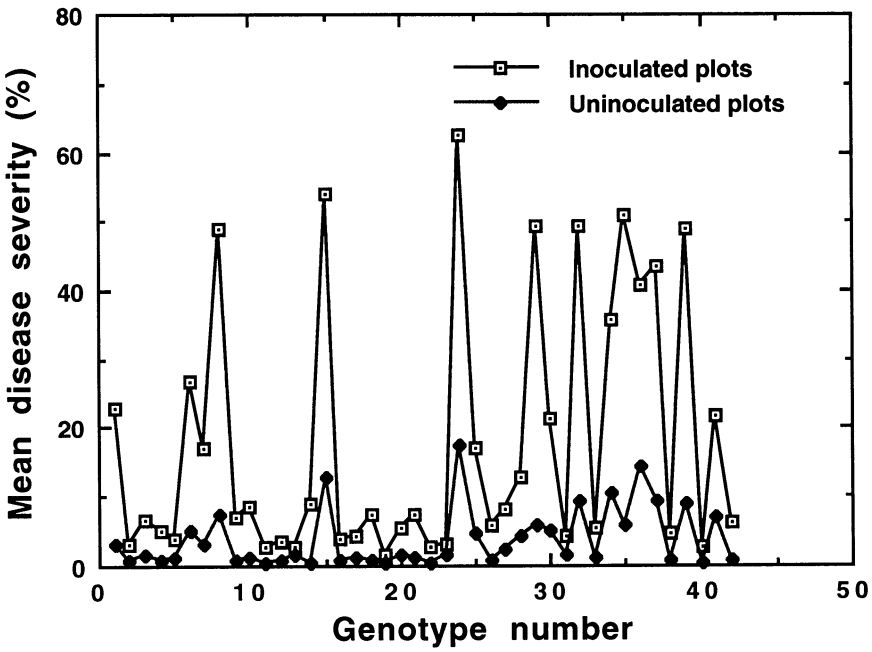

Fig. 1. Mean blackleg disease severity on *Brassica napus* genotypes inoculated or not inoculated with *Leptosphaeria maculans* pycnidiospores in the field, 1991–1992.

The orthogonal contrasts between phenotypic groups DH(R) and susceptible parent, susceptible control, and DH(DR) based on the greenhouse reaction were significant (Table 4). Mean values of breeding lines from these groups indicated that percent disease severity and percent disease incidence were much lower in the DH(R) group but did not differ from the resistant parent group. Similarly, the DH(S) group did not differ from the susceptible control group. These lines had very high values for percent disease severity and percent disease incidence. As DH(DR) and DH(MS/DS) gave resistant field reactions, these groups had very high *F* values when compared with susceptible parent or susceptible control groups and low *F* values when compared with each other or resistant parent group. DH(DR) and DH(MS/DS) groups also differed only slightly from each other.

The coefficients of correlation between greenhouse and field data are presented in Table 5. The disease severity values from field inoculated plots and cotyledon, true leaf, and adult plant stages correlated well. Also, disease incidence values from inoculated plots correlated well with greenhouse data, as did disease severity and disease incidence values from inoculated plots. Within the greenhouse comparisons, once again, coty-

ledon, true leaf, and adult plant stage reactions all correlated well with each other.

DISCUSSION

Newman and Bailey (17) studied the correlations between greenhouse and field evaluations of winter oilseed rape inoculated with *L. maculans* and reported good correlation in some of the experiments and poor or no correlation in others. In their study (17), results from the mature plant test agreed with those from the seedling test, suggesting that there was no real advantage in screening older plants rather than seedlings. Seedlings were inoculated about 17 days after seeding, and inoculations were made on the base of the petiole of the first leaf (17). Helms and Cruickshank (12) inoculated oilseed rape plants at a much earlier stage than did Newman and Bailey (17) and found no correlation with field data when plants were rated on the basis of symptoms developed on cotyledons and hypocotyls. When plants were rated 3 wk after sowing (12), however, the results confirmed the presence of resistance in genotypes tested by Thurling and Venn (24), who used ascospores for inoculation. From these earlier studies, it appears that the relationship of greenhouse and field evaluations depends on the plant stage, plant material, and inoculation method. The *L. maculans* isolates used in the greenhouse and field experiment of a particular study might also influence correlation values if the strains prevalent in the field differed from those utilized in the greenhouse.

The use of doubled haploid lines as test material in the present study has shown that disease reaction of a given genotype (except DH line No. 9) is consistent at any growth stage in the greenhouse as well as in the field. These results are in contrast with those of some of the earlier studies in which test material was selections from open-pollinated populations or in which plants were inoculated at a very early or at a later stage of development (12,17,25). Also, the use of heterogeneous populations in the screening experiments was probably the reason for some of the discrepancies in the results of earlier studies.

The variation in blackleg resistance between different doubled haploid lines indicates that resistance is controlled by either a major gene or tightly linked polygenes that transmit from generation to generation in a block (22). Several resistance mechanisms seem to be operating in the breeding lines studied. The nature of resistance, whether controlled by a major gene or polygenes, is probably nondifferential with large effects (6). Although this type of resistance is rare, it has been reported in oats and cabbage with respect to resistance to Helminthosporium blight and cabbage yellows, respectively (6,7). The presence of nondifferential resistance with large effect in the host-pathogen interaction system under study is further supported by the fact that differential or moderately susceptible lines in the greenhouse were resistant in the field and that DH line No. 9 gave a susceptible reaction at the cotyledon stage and a moderately resistant reaction at the adult plant stage in the greenhouse screening. The changes in environmental conditions from cotyledon to adult plant stage and from greenhouse to field probably are respon-

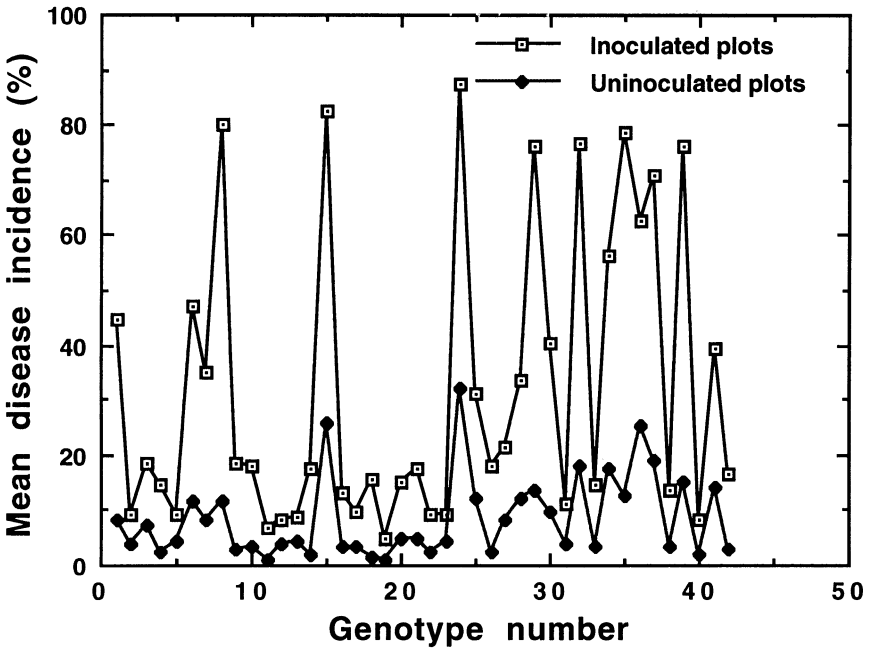

Fig. 2. Mean blackleg disease incidence on *Brassica napus* genotypes inoculated or not inoculated with *Leptosphaeria maculans* pycnidiospores in the field, 1991–1992.

Table 4. Comparisons of the field *Leptosphaeria maculans* disease severity values from different phenotype groups of *Brassica napus* based on greenhouse reaction

Contrasts between phenotypic groups[a]	Mean sum of squares	*F* value	*P* > *F*
DH(R) vs. SP	16,696.55	338.38	0.00
DH(R) vs. RP	0.62	0.01	0.91
DH(R) vs. SC	15,409.65	312.30	0.00
DH(R) vs. DH(DR)	1,311.34	26.58	0.00
DH(S) vs. SP	338.07	6.85	0.01
DH(S) vs. RP	7,645.69	154.95	0.00
DH(S) vs. SC	36.64	0.74	0.39
DH(S) vs. DH(MS/DS)	13,076.29	265.01	0.00
DH(DR) vs. SP	7,836.73	158.82	0.00
DH(DR) vs. RP	431.32	8.74	0.00
DH(DR) vs. SC	5,681.04	115.14	0.00
DH(DR) vs. DH(MS/DS)	685.97	13.90	0.00
DH(MS/DS) vs. SP	11,449.48	232.04	0.00
DH(MS/DS) vs. RP	0.08	0.00	0.97
DH(MS/DS) vs. SC	9,283.66	188.15	0.00
SP vs. SC	466.02	9.44	0.00

[a] SC = susceptible control, SP = susceptible parent, RP = resistant parent, DH(R) = doubled haploid (resistant), DH(DR) = doubled haploid (differentially resistant), DH(MS) = doubled haploid (moderately susceptible), DH(DS) = doubled haploid (differentially susceptible), and DH(S) = doubled haploid (susceptible).

Table 5. Coefficient of correlation (*r*) among various combinations for percent *Leptosphaeria maculans* disease severity and percent disease incidence data from greenhouse and field experiments on *Brassica napus*

Combinations[a]	*r*[b]
DS(f) × DI(f)	0.996
DS(cotyledon) × DS(f)	0.823
DS(true leaf) × DS(f)	0.824
DS(adult plant) × DS(f)	0.820
DS(cotyledon) × DI(f)	0.820
DS(true leaf) × DI(f)	0.824
DS(adult plant) × DI(f)	0.822
DS(cotyledon) × DS(true leaf)	0.856
DS(cotyledon) × DS(adult plant)	0.839
DS(true leaf) × DS(adult plant)	0.903

[a] DS(f) = percent disease severity in field, DI(f) = percent disease incidence in field.
[b] All values are significantly different from zero.

sible for these changes in disease reactions. The microenvironment is better suited for infection at the cotyledon stage than at the adult plant stage or during field screening. The presence of genotype × environment interaction for disease resistance can be explained when resistance is controlled by polygenes. Frencel et al (5) also observed that most resistant and susceptible reaction types were stable during different experiments; however, cultivars of intermediate response were variable for resistance or susceptible reaction in different experiments. Similar observations were made in the greenhouse experiments of the present study, and "intermediate" reaction types were classified as a "differential" or "moderate" reaction type.

High correlation coefficient values in all combinations indicated that, in most cases, the resistance, which could be tested at all three stages, persisted throughout plant development. Keeping in mind the efficiency and effectiveness of the screening methods used, these results favor the use of greenhouse screening. Among the three stages studied in the greenhouse, the cotyledon stage was the most promising; the true leaf stage had a high frequency of missing values because of difficulty in keeping the inoculum droplets on inoculation sites and because leaves senesced due to aging or defense response by the plant during the incubation period. The adult plant stage for testing, although most desirable, required a longer time and more space.

Under field conditions, disease severity is much more difficult to estimate than disease incidence. In our studies, positive correlation values between percent disease severity and percent disease incidence data from field results suggest that for field surveys, disease incidence data can be used to estimate disease severity for a cultivar. Further, resistant DH lines selected from these experiments, depending on their quality parameters and performance in yield trials, can possibly be used directly as cultivars or as elite parents for blackleg resistance in future canola improvement programs.

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