Pathogenicity of *Leptosphaeria maculans* Isolates on a *Brassica napus*-*B. juncea* Recombinant Line

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ABSTRACT

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The *Brassica napus*-*B. juncea* recombinant line (MX), resistant to *Leptosphaeria maculans*, was produced by interspecific crosses and bears one gene (*Jlm1*) from the *B. juncea* B genome. We investigated whether this new resistance was race specific by characterizing protection against a large sample of *L. maculans* isolates. The pathogenicity of 119 isolates of *L. maculans* comprising 105 A-group isolates and 14 B-group isolates was studied at the cotyledon stage under controlled conditions using the MX line, the susceptible *B. napus* cultivar Westar, and the resistant *B. juncea* cultivar Picra. All but one of the isolates were pathogenic on 'Westar'. Only 3 of the 105 A-group isolates caused very mild symptoms on 'Picra'.

Two of these strains were isolated from the MX line and the other from *Sinapis arvensis*. The other 102 strains caused hypersensitive-type responses. Most B-group isolates were pathogenic on 'Picra'. There were differences in pathogenicity among A-group isolates tested on the MX line, whereas all B-group isolates were pathogenic on this line. A-group isolates obtained from the MX line were more frequently pathogenic on the MX line than those obtained from *B. napus* cultivars. One isolate from *S. arvensis* infected the MX line. These results suggest that the resistance of the MX line is unlikely to be durable. Thus, the new resistance gene *Jlm1* should probably be used in association with other sources of resistance, in plant breeding schemes, to prevent the breakdown of this resistance.

Additional keywords: blackleg, host-pathogen interactions, *Phoma lingam*.

Most of the *Brassica napus* L. var. *oleifera* (oilseed rape) cultivars currently grown in France are highly susceptible at the seedling stage and moderately susceptible at the adult plant stage to *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. (anamorph: *Phoma lingam* (Tode ex Fr.) Desmaz.), the causal agent of blackleg. Yield losses due to blackleg have been large in Australia (35, 37), Canada (28,29), and some European countries such as France (32), England (12), and Germany (39). Populations of *L. maculans* have been classified into two genetically distinct groups (A and B groups) on the basis of pathogenicity (8,17), isozyme banding patterns (3,5,14,42), and molecular markers (16,18,45). A-group isolates (also called highly virulent, aggressive, or Tox+) and B-group isolates (also called weakly virulent, nonaggressive, or Tox0) are not interfertile (4,41) and may be considered to be different species (13,22,23). A-group isolates are responsible for severe stem canker in oilseed rape, whereas B-group isolates mainly cause pith damage (17). The pathogenicity of *L. maculans* differs between *Brassica* species (31). Mustards with the B genome, including *B. juncea* (L.) Czernj. & Coss., are totally resistant to A-group isolates. This resistance is generally manifested as a hypersensitive reaction (HR)-type response (36,42). In contrast, *B. juncea* is susceptible to B-group isolates used to inoculate cotyledons and stems (11,17,42).

The resistance of *B. juncea* to A-group isolates depends on two or three genes (15,24,30,44). *B. juncea* is currently used in many breeding programs to improve the resistance of *B. napus* cultivars at all growth stages (24,30,34). *B. napus*-*B. juncea* recombinant lines containing a single resistance gene (*Jlm1*) from *B. juncea* have

been obtained (6). They are highly resistant under heavy disease pressure in field trials and may become commercially available in the near future.

Monogenic resistance has proved to be ephemeral in a number of crops (25), so it is of great value to test whether there are virulent isolates of *L. maculans* that can overcome the *Jlm1* resistance gene. The potential durability of the resistance conferred by this gene is unknown. Its resistance spectrum was assessed by investigating differences in the pathogenicity of *L. maculans* populations in the presence of this new source of resistance in *B. napus*.

L. maculans isolates were recovered from various cultivated and wild cruciferous hosts, particularly from plants of the *B. napus*-*B. juncea* recombinant line MX that had mild symptoms. We investigated, under controlled conditions, the pathogenicity of these isolates on the MX line and parental species, *B. napus* and *B. juncea*, at the cotyledon stage. We report that the resistance to *L. maculans* conferred to *B. napus* by a single gene (*Jlm1*) from the *B. juncea* B genome was race specific.

MATERIALS AND METHODS

Plant material. The pathogenicity of the isolates was analyzed using three *Brassica* genotypes: the *B. napus*-*B. juncea* recombinant line MX, the *B. napus* cultivar Westar and the *B. juncea* cultivar Picra. The MX line was selected from interspecific hybrid material obtained in Australia by Roy (33). This recombinant line was selected for normal meiotic behavior ($2n = 38$), stability, and homogeneous blackleg resistance at the cotyledon stage at the Plant Breeding Station, INRA-Rennes, France (6). Segregation ratios were analyzed in various progenies, and resistance was found to be monogenic (6). The actual *B. juncea* resistance donor (*BJ168*) and *B. napus* recipient germ plasm ("Cresus-o-Precose") were not available, so the resistant *B. juncea* 'Picra' and the highly susceptible *B. napus* 'Westar' were used as controls in place of the

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actual parents. The MX line and 'Picra' both carry the *B. juncea* resistance gene (*Jlm1*), because they both have the three specific random amplified polymorphic DNA (RAPD) markers tightly linked to the *Jlm1* resistance gene in the MX line (6).

Isolates of *L. maculans*. We studied 119 isolates of *L. maculans* (Table 1). Single ascospore isolates were obtained from pseudothecia on stem base residues and pycnidiospore-mass isolates were obtained from damaged tissues of various oilseed rape genotypes and the MX line as described by Somda et al. (42). Isolates were classified as A- or B-group isolates according to glucose phosphate isomerase patterns (40). Most of the isolates studied were collected in 1992, 1993, and 1994 from heavily diseased field trials. The infected *B. napus* plant stem residues used as a source of inoculum in the trials originated from various growing areas in France. Under these conditions, the frequency of leaf lesions and stem cankers was much lower on the MX line than on susceptible oilseed rape cultivars during the growing season (6). Nevertheless, a few pseudothecia were recovered in the autumn from stem base residues of the MX line. Single ascospore isolates were produced from these pseudothecia.

Twenty-five single-ascospore isolates of *L. maculans* (R1 to R25) were obtained from the MX line in 1992. Forty single-ascospore

isolates (termed MX) from the MX line and nineteen single-ascospore isolates (termed Co) from oilseed rape cultivars were collected from stem residues in a single field experiment in 1993. Six pycnidiospore-mass isolates were recovered in 1993 and 1994 from rare lesions that developed on the leaves (MX-L), stem (MX-S), collar (MX-C), and pods (MX-P) of the MX line. Twenty-two isolates were collected from *B. napus* in diverse geographic regions (France, United Kingdom, Canada, Poland, and Germany). We also studied seven isolates from leaf lesions on *Raphanus sativus* (radish), *Eruca sativa*, *Sinapis arvensis* (wild mustard), and the synthetic *Raphanobrassica* McNaughton (RBMN) (20).

Cotyledon inoculations. Seeds of each genotype were sown in a soil mixture (1:1:1 sand/loam/compost) in 7 × 7 × 8-cm plastic pots. The seedlings were grown at 15°C in a growth chamber with a 16-h light period (280 µE m⁻² s⁻¹) for 10 days. The cotyledons were slightly wounded in the center of each lobe with a needle, and a 10-µl drop of a pycnidiospore suspension (10⁷ pycnidiospores per ml) was deposited on each wound. There were, therefore, four inoculation sites per plant. Inoculum was produced as described by De March et al. (9) and stored at -20°C until used. Inoculum stored in this way remains viable for at least 2 years. A plastic cover was placed over inoculated plants to create an atmosphere

TABLE 1. List of A- and B-group isolates of *Leptosphaeria maculans* used in this study

Isolate	Type	Location	Host	Group	Year of isolation
A290	Sa ^a	Le Rheu (France)	<i>Brassica napus</i>	A	1985
A314	Pm ^b	Le Rheu (France)	<i>B. napus</i>	A	1985
A447	Pm	Melford (Canada)	<i>B. napus</i>	A	1987
A759	Sa	Delmes (France)	<i>B. napus</i>	A	1990
A764	Sa	Demanges (France)	<i>B. napus</i>	A	1990
A775 and A813	Sa	Le Rheu (France)	<i>B. napus</i>	A	1990
A824	Sa	Demanges (France)	<i>B. napus</i>	A	1990
A827	Sa	Mondonville (France)	<i>B. napus</i>	A	1990
A901	Sa	Braunschweig (Germany)	<i>B. napus</i>	A	1992
A922	Sa	Deux Villes (France)	<i>B. napus</i>	A	1992
A923	Sa	Rigny (France)	<i>B. napus</i>	A	1992
A924	Sa	Mairy (France)	<i>B. napus</i>	A	1992
A925	Sa	Tressange (France)	<i>B. napus</i>	A	1992
A929 and A930	Pm	Edmonton (Canada)	<i>B. napus</i>	A	1993
A931 and A932	Pm	United Kingdom	<i>B. napus</i>	A	1993
Co1 to Co4	Sa	Le Rheu (France)	<i>B. napus</i>	A	1993
Co7 to Co14 (except Co8 and Co10)	Sa	Le Rheu (France)	<i>B. napus</i>	A	1993
Co17 and Co18	Sa	Le Rheu (France)	<i>B. napus</i>	A	1993
Co21 to Co25	Sa	Le Rheu (France)	<i>B. napus</i>	A	1993
Co27 and Co29	Sa	Le Rheu (France)	<i>B. napus</i>	A	1993
R1 to R25	Sa	Le Rheu (France)	MX line	A	1992
MX-1 ^c to MX-3	Sa	Le Rheu (France)	MX line	A	1993
MX1-2 ^d to MX1-14 (except MX1-4)	Sa	Le Rheu (France)	MX line	A	1993
MX2-1 to MX2-8 (except MX2-3)	Sa	Le Rheu (France)	MX line	A	1993
MX3-1 to MX3-8 (except MX3-5)	Sa	Le Rheu (France)	MX line	A	1993
MX4-1 to MX4-9 (except MX4-4 to MX4-6)	Sa	Le Rheu (France)	MX line	A	1993
MX-S ^e	Pm	Le Rheu (France)	MX line	A	1994
MXC1 ^e and MXC2	Pm	Le Rheu (France)	MX line	A	1994
MX-T1 ^e and MX-T2	Pm	Le Rheu (France)	MX line	A	1994
Es1	Pm	Le Rheu (France)	<i>Eruca sativa</i>	A	1994
Rs1	Pm	Le Rheu (France)	<i>Raphanus sativus</i>	A	1994
Sa1	Pm	Le Rheu (France)	<i>Sinapis arvensis</i>	A	1994
B867	Pm	Dijon (France)	<i>B. napus</i>	B	1985
B908	Pm	Lyon (France)	<i>B. napus</i>	B	1985
B980 and B981	Sa	Poznan (Poland)	<i>B. napus</i>	B	1992
MX-4 to MX-8	Sa	Le Rheu (France)	MX line	B	1993
MX-L ^c	Pm	Le Rheu (France)	MX line	B	1993
Es2	Pm	Le Rheu (France)	<i>Eruca sativa</i>	B	1994
Rs2	Pm	Le Rheu (France)	<i>Raphanus sativus</i>	B	1994
Sa2	Pm	Le Rheu (France)	<i>Sinapis arvensis</i>	B	1994
RBMN	Pm	Le Rheu (France)	<i>Raphano brassica</i>	B	1994

^a Sa = single-ascospore isolates.

^b Pm = pycnidiospore-mass isolates.

^c Isolate number 1 originated from the MX line.

^d The first digit designating the plant and the second digit designating the ascospore isolate recovered randomly from pseudothecia on the MX line (MX).

^e Pycnidiospore-mass isolates obtained from leaf (L) stem (S and T) and canker (C) of the MX line.

with 100% relative humidity (RH). The plants were incubated in the dark at 20°C for 24 h in a growth chamber and then placed in an atmosphere with 80 to 90% RH (without plastic cover) with a 16-h photoperiod. The senescence of cotyledons was delayed by removing young leaves as soon as they appeared. We previously demonstrated that this does not affect the plant response (H. Brun, unpublished data).

Assay conditions were standardized so that independent experiments could be compared. We used as a control an avirulent isolate (A314) previously used to select resistant plants among various progenies from *B. napus*-*B. juncea* interspecific crosses produced as part of the plant breeding scheme at the Plant Breeding Station. Resistance was correlated with three RAPD markers closely linked to the resistance gene (6). Two additional tests were also performed to assess the reliability of the results presented here (Table 2). The pathogenicity of one isolate that was avirulent (A314) and two that were virulent (MX4-7 and R2) on the MX line was evaluated on the MX line and 'Picra' (experiment 1) and on 'Westar' and the MX line (experiment 2), and the results were compared with those obtained with 'Westar', 'Picra', and the MX line (experiment 3).

Disease assessment. Fourteen days after inoculation, symptoms on cotyledons were scored using the 0 (no visible reaction) to 9 (total collapse of the tissue) rating scale of Williams and Delwiche (46). Each plant was considered to be a replicate, and each isolate was used to inoculate 16 plants of each genotype (giving 64 inoculation sites per genotype-isolate combination). A disease index (DI) was calculated according to the formula $DI = \sum(N_i \times i)/N_t$, in which N_i is the number of inoculation sites with score i (0 to 9) and N_t is the total number of inoculation sites (i.e., the number of well-developed cotyledon lobes). For each treatment, mean disease indices and standard deviations were calculated.

Pathogenicity is the ability of a pathogen to attack a species and it has two basic components (2). Virulence is a qualitative trait of the isolates and defines the compatibility of the host-pathogen interaction. In this case, incompatibility (i.e., avirulence in the isolate and resistance in the host) was expressed as a HR-like response. Aggressiveness characterizes the ability of virulent isolates to induce more or less severe symptoms. The following pathogenicity classes were defined: $DI < 4$ indicated an incompatible interaction (avirulent isolates), $4 \leq DI < 6$ indicated moderate aggressiveness, and $DI \geq 6$ indicated that the isolate was highly aggressive.

Data analysis. The distributions obtained were not normal and the variances were not homogeneous, so nonparametric tests were used. The three independent experiments were tested using the Wilcoxon test ($\alpha = 0.05$) under PROC NPARIWAY in the Statistical Analysis System (SAS, Institute, Inc., Cary, NC) to compare the distributions of the plant disease indices for each isolate-cultivar combination. The results obtained with the MX line and 'Picra' were compared between experiments 1 and 3. The results obtained with 'Westar' and the MX line were compared between experiments 2 and 3. The Kruskal and Wallis test ($\alpha = 0.05$) was used to compare the various isolates from oilseed rape and the MX line within A and B groups. Isolates were classified into homogeneous

groups by the Noether test (38) using the mean rank from the Kruskal and Wallis test. The distributions of isolates between the two populations recovered from oilseed rape cultivars and from the MX line were compared using the χ^2 test ($\alpha = 0.05$), the mean disease indices being grouped into eight classes, 1 to 2, 2 to 3, and so on, to 8 to 9.

RESULTS

Reproducibility of the test. Isolates (A314, MX4-7, and R2) were assigned to the same pathogenicity classes according to genotype in all experiments (Table 2). For instance, isolate A314 was avirulent on both the MX line and 'Picra', but was virulent and highly aggressive on 'Westar' in the cotyledon tests in this study. The Wilcoxon test was used to compare the distribution of indices. There was no difference between experiments for any isolate-cultivar combination, except for 'Picra' inoculated with isolate R2. However, isolate R2 was avirulent on 'Picra' in both experiments.

Pathogenicity of A-group isolates recovered from *Brassica* genotypes. The Kruskal and Wallis test indicated that there were significant differences between isolates for each genotype. The Noether test classified the isolates into several (six to eight) homogeneous groups that overlapped for each genotype. We attempted to assign the isolates to the three pathogenicity classes.

The 37 A-group isolates from *B. napus* cultivars were avirulent to virulent on the MX line, whereas they were all virulent on 'Westar'. All these isolates caused a HR-type response on *B. juncea* 'Picra' (Table 3).

The 65 A-group isolates from the MX line were also avirulent to virulent on the MX line, and all but one isolate, R15 (DI of 2.9 ± 1.8), were virulent on 'Westar'. However, R15 belonged to the same homogeneous group as the moderately aggressive isolates. All but two isolates (MX-C1 and MX1-13) were avirulent on 'Picra', but MX-C1 and MX1-13 belonged to the same homogeneous group as other avirulent isolates (Table 4). Isolates recovered from a single plant of the MX line differed in pathogenicity on the MX line (e.g., MX2-1 [DI of 1.9 ± 0.9] was avirulent, whereas MX2-4 [DI of 8.8 ± 0.7] was virulent and highly aggressive). They were classified into two different homogeneous groups.

The pathogenicity of isolates recovered from *B. napus* was compared with that of isolates from the MX line. There was no difference between the two populations on 'Westar' and 'Picra', whereas there were large differences ($\chi^2 = 11.23$, $P = 0.0038$) on the MX line (Fig. 1). The proportion of isolates in each DI class differed between the two populations, the proportion of isolates virulent on the MX line being higher among the isolates recovered from this line.

Pathogenicity of isolates in relation to geographic origin. The pathogenicity of French isolates of *L. maculans* collected from various cultivars of *B. napus* grown in several locations differed (Table 3). Only isolates A923 (DI of 6.8 ± 2.0) from Rigny and A759 (DI of 5.5 ± 1.5) from Delmes were virulent on the MX line. The isolates from Le Rheu (France) differed in pathogenicity on the MX line and included both virulent isolates (Co12, Co4, Co18, Co13, Co22, Co17, Co11, and Co24) and one avirulent isolate

TABLE 2. Three experiments in which *Leptosphaeria maculans* isolates avirulent (A314) and virulent (MX4-7 and R2) on the MX line were evaluated for pathogenicity on cotyledons of 'Picra', 'Westar', and the MX line to assess the reliability of the results^a

Isolates	Experiment 1 ^b			Experiment 2 ^c		Experiment 3 ^d	
	MX	'Picra'	'Westar'	MX	'Westar'	MX	'Picra'
A314	1.9 ± 0.8	1.3 ± 0.4	7.7 ± 0.9	1.1 ± 0.2	6.6 ± 1.4	1.6 ± 0.5	1.1 ± 0.2
MX4-7	9.0 ± 0.0	1.8 ± 0.6	9.0 ± 0.0	8.9 ± 0.1	8.5 ± 0.6	8.6 ± 0.6	1.9 ± 0.5
R2	8.5 ± 1.2	1.0 ± 0.1	8.5 ± 0.9	7.5 ± 1.1	8.5 ± 0.6	8.7 ± 0.7	1.8 ± 0.6

^a Disease was recorded 14 days after inoculation using a 0 (no visible symptom) to 9 (total collapse of the tissue) scale. Data are the disease indices (DI) $\pm$ standard deviation. Interaction phenotype: $DI < 4$ = resistant; $4 \leq DI < 6$ = moderately resistant; and $DI \geq 6$ = susceptible.

^b 18 plants inoculated per genotype.

^c 10 plants inoculated per genotype.

^d 16 plants inoculated per genotype.

(A314). All Canadian and English isolates were avirulent on the MX line.

Pathogenicity of B-group isolates recovered from *Brassica* genotypes. All B-group isolates tested were highly aggressive on the MX line and ‘Westar’, irrespective of their origin (Table 5). Most of the B-group isolates tested were virulent on cotyledons of ‘Picra’, but isolate B867 was avirulent and significantly different from the other isolates.

Pathogenicity of A- and B-group *L. maculans* isolates recovered from other crucifers. A-group isolates were collected from other crucifer species. Isolate Sa1, recovered from *S. arvensis*, was highly aggressive on ‘Westar’ and the MX line. Isolate Rs1, from *R. sativus* was avirulent on all three genotypes (Table 3). Isolate Es1, recovered from *E. sativa*, was avirulent on the MX line and ‘Picra’, but was moderately aggressive on ‘Westar’. We also assessed the pathogenicity of four B-group isolates, recovered from wild and other cultivated cruciferous hosts, on these three genotypes (Table 5). Isolate Es2 (from *E. sativa*) was avirulent on ‘Picra’, but the other isolates obtained from *Raphanobrassica* (RBMN), *S. arvensis* (Sa2), and *R. sativus* (Rs2) were all virulent on all the genotypes tested.

TABLE 3. Pathogenicity of A-group isolates of *Leptosphaeria maculans* recovered from *Brassica napus* and other crucifers on cotyledons of the three genotypes

Isolate	Disease index ^a		
	MX	‘Westar’	‘Picra’
Co12	7.0 ± 2.8	8.0 ± 2.0	1.7 ± 0.7
A923	6.8 ± 2.0	9.0 ± 0.0	2.0 ± 1.4
Co4	6.6 ± 2.1	7.0 ± 2.1	1.9 ± 0.8
Co18	6.3 ± 1.8	8.8 ± 0.7	2.2 ± 1.1
Co13	6.1 ± 2.8	9.0 ± 0.0	1.5 ± 0.6
Co22	6.0 ± 1.6	7.9 ± 1.3	2.4 ± 1.2
Co17	5.8 ± 3.0	9.0 ± 0.0	1.6 ± 1.2
Co11	5.6 ± 2.3	7.9 ± 1.9	1.5 ± 0.7
A759	5.5 ± 1.5	9.0 ± 0.0	2.5 ± 1.1
Co24	4.4 ± 2.0	6.3 ± 1.7	1.5 ± 1.0
A447	3.4 ± 1.2	9.0 ± 0.0	1.7 ± 0.7
Co1	3.4 ± 1.7	7.0 ± 2.8	1.3 ± 0.7
Co2	3.3 ± 1.2	7.0 ± 2.2	2.2 ± 1.1
Co14	3.2 ± 1.4	5.7 ± 2.5	0.6 ± 0.7
A925	3.1 ± 1.1	8.8 ± 1.0	0.9 ± 0.8
A764	3.0 ± 1.3	9.0 ± 0.0	1.2 ± 0.5
Co3	3.0 ± 1.4	6.8 ± 2.5	1.7 ± 0.9
A932	2.9 ± 1.3	8.4 ± 2.0	1.5 ± 0.9
A824	2.8 ± 1.0	8.8 ± 1.2	1.0 ± 0.3
Co21	2.8 ± 1.4	8.8 ± 0.9	0.8 ± 1.0
Co25	2.7 ± 1.2	8.4 ± 1.3	1.6 ± 0.8
A290	2.6 ± 1.0	9.0 ± 0.0	0.9 ± 0.4
A813	2.6 ± 1.1	8.9 ± 0.5	1.1 ± 0.4
Co23	2.4 ± 0.7	9.0 ± 0.0	3.1 ± 1.6
A775	2.4 ± 1.1	9.0 ± 0.0	0.9 ± 0.4
A924	2.2 ± 0.9	8.1 ± 1.6	1.0 ± 0.5
Co27	2.2 ± 1.2	4.7 ± 2.3	1.8 ± 0.8
Co29	2.2 ± 1.1	5.3 ± 2.4	0.8 ± 0.8
Co9	2.1 ± 0.6	9.0 ± 0.0	1.7 ± 1.2
A931	2.0 ± 0.9	8.8 ± 0.8	1.1 ± 0.5
A929	2.0 ± 0.9	7.1 ± 2.1	0.9 ± 0.2
Co7	1.9 ± 0.4	9.0 ± 0.0	2.8 ± 1.5
A827	1.8 ± 1.0	7.6 ± 1.6	0.9 ± 0.3
A922	1.8 ± 1.1	6.8 ± 2.1	0.7 ± 0.4
A314	1.5 ± 0.9	6.6 ± 2.3	0.8 ± 0.6
A930	0.2 ± 0.4	5.6 ± 2.3	0.0 ± 0.0
A901	NT ^b	8.9 ± 0.5	1.0 ± 0.5
Sa1	8.1 ± 0.9	9.0 ± 0.0	4.4 ± 0.8
Es1	3.5 ± 1.0	5.3 ± 0.6	3.4 ± 0.7
Rs1	2.0 ± 0.8	2.2 ± 0.6	1.8 ± 0.5

^a Disease was recorded 14 days after inoculation using a 0 (no visible symptom) to 9 (total collapse of the tissue) scale. Data are the disease indices (DI) of 16 replicates of each genotype-isolate combination ± standard deviation. Interaction phenotype: DI < 4 = resistant; 4 ≤ DI < 6 = moderately resistant; and DI ≥ 6 = susceptible.

^b NT = not tested.

TABLE 4. Pathogenicity of A-group isolates of *Leptosphaeria maculans* recovered from the MX line on cotyledons of the three genotypes

Isolate	Disease index ^a		
	MX	‘Westar’	‘Picra’
MX-T2	8.9 ± 0.5	8.5 ± 1.3	2.7 ± 1.3
MX2-4	8.8 ± 0.7^b	8.9 ± 0.4	2.7 ± 1.1
MX-S	8.8 ± 0.7	9.0 ± 0.0	3.0 ± 1.7
MX-C1	8.6 ± 1.2	8.7 ± 1.1	4.1 ± 1.5
MX4-2	8.6 ± 1.1	8.9 ± 1.0	2.2 ± 1.4
MX4-3	8.6 ± 1.0	7.6 ± 1.9	0.7 ± 0.7
MX4-7	8.6 ± 0.9	8.5 ± 0.9	1.7 ± 1.0
R2	8.5 ± 1.5	8.4 ± 1.3	1.6 ± 0.9
R6	8.5 ± 1.5	8.6 ± 1.1	2.3 ± 1.0
MX-C2	8.5 ± 1.1	8.5 ± 1.2	3.4 ± 1.4
MX4-9	8.1 ± 2.0	8.5 ± 1.2	3.2 ± 1.9
R4	8.1 ± 1.7	8.6 ± 1.3	1.9 ± 1.0
R7	8.1 ± 1.6	8.9 ± 0.6	1.9 ± 0.5
R8	8.1 ± 1.5	9.0 ± 0.0	2.5 ± 1.4
R13	7.9 ± 1.8	9.0 ± 0.0	1.4 ± 0.6
MX-1	7.6 ± 1.6	8.6 ± 1.5	2.0 ± 1.3
R9	7.3 ± 2.2	8.7 ± 1.3	2.8 ± 1.3
R14	7.2 ± 2.5	7.5 ± 2.3	1.3 ± 0.9
MX-T1	7.0 ± 2.1	7.9 ± 1.7	1.4 ± 0.4
MX2-2	6.8 ± 2.5	7.7 ± 1.7	2.3 ± 1.4
R18	6.5 ± 1.8	8.7 ± 1.1	1.0 ± 0.6
MX-3	6.3 ± 2.1	7.5 ± 1.9	2.5 ± 1.3
MX4-8	6.0 ± 2.6	6.3 ± 1.4	2.7 ± 1.5
MX2-8	5.9 ± 2.4	8.9 ± 0.5	2.3 ± 1.1
MX2-5	5.9 ± 2.1	8.5 ± 1.4	3.1 ± 1.8
MX1-13	5.8 ± 1.8	8.9 ± 0.4	4.3 ± 1.8
R23	5.8 ± 1.6	9.0 ± 0.0	3.4 ± 1.8
MX1-11	5.3 ± 2.1	8.6 ± 1.1	1.9 ± 1.2
R1	5.2 ± 2.3	6.5 ± 1.9	1.5 ± 0.8
MX1-8	5.2 ± 1.8	8.9 ± 1.1	3.9 ± 1.8
R21	4.9 ± 2.0	7.8 ± 1.7	1.3 ± 0.7
MX3-8	4.7 ± 1.7	9.0 ± 0.0	1.9 ± 1.1
MX1-9	4.5 ± 1.9	8.6 ± 1.0	1.7 ± 1.0
R10	4.4 ± 2.2	9.0 ± 0.0	2.6 ± 1.3
MX1-14	4.4 ± 2.2	7.1 ± 2.0	1.8 ± 1.1
MX3-2	4.4 ± 1.8	8.3 ± 1.1	1.7 ± 1.2
MX-2	4.2 ± 3.7	7.9 ± 2.9	1.3 ± 0.8
R20	4.1 ± 1.4	5.4 ± 1.7	1.3 ± 0.5
MX1-12	3.9 ± 1.5	7.0 ± 2.1	1.1 ± 0.7
MX4-1	3.8 ± 1.3	8.7 ± 1.6	1.4 ± 0.9
MX3-7	3.7 ± 1.8	8.8 ± 1.1	2.7 ± 1.5
R5	3.6 ± 1.8	9.0 ± 0.0	1.5 ± 0.7
MX2-7	3.6 ± 1.5	6.2 ± 1.3	1.5 ± 0.6
MX1-10	3.3 ± 1.6	8.7 ± 1.3	1.5 ± 1.0
MX2-6	3.2 ± 1.5	6.4 ± 1.7	1.4 ± 0.8
R16	3.1 ± 2.2	9.0 ± 0.3	1.6 ± 0.9
R24	3.0 ± 1.4	8.9 ± 1.1	1.6 ± 0.9
MX3-1	2.9 ± 1.7	8.2 ± 1.9	2.0 ± 1.1
MX1-5	2.7 ± 2.6	5.3 ± 1.5	1.3 ± 1.1
R22	2.6 ± 1.2	9.0 ± 0.0	2.2 ± 1.0
MX3-4	2.6 ± 0.9	8.5 ± 1.2	2.4 ± 1.7
R11	2.5 ± 2.0	9.0 ± 0.0	1.6 ± 0.9
R12	2.5 ± 1.5	8.8 ± 0.8	1.7 ± 0.9
R25	2.5 ± 1.2	8.9 ± 0.5	1.1 ± 0.4
MX1-2	2.4 ± 1.6	6.2 ± 1.3	2.3 ± 1.0
MX3-3	2.3 ± 0.8	7.3 ± 2.3	2.0 ± 0.9
MX1-3	2.2 ± 2.0	5.8 ± 1.9	2.5 ± 1.4
R19	2.2 ± 0.8	9.0 ± 0.0	0.7 ± 0.6
R3	2.1 ± 1.0	8.6 ± 1.1	1.4 ± 0.8
MX3-6	2.0 ± 1.2	7.0 ± 2.6	0.8 ± 0.8
R15	1.9 ± 1.7	2.1 ± 1.8	0.9 ± 0.3
MX2-1	1.9 ± 0.9	5.0 ± 1.3	0.9 ± 0.5
MX1-6	1.8 ± 1.5	5.7 ± 1.8	0.9 ± 0.8
MX1-7	1.6 ± 1.6	5.2 ± 1.6	1.6 ± 1.1
R17	1.2 ± 1.0	5.7 ± 1.3	0.3 ± 0.4

^a Disease was recorded 14 days after inoculation using a 0 (no visible symptom) to 9 (total collapse of the tissue) scale. Data are the disease indices (DI) of 16 replicates of each genotype-isolate combination ± standard deviation. Interaction phenotype: DI < 4 = resistant; 4 ≤ DI < 6 = moderately resistant; and DI ≥ 6 = susceptible.

^b Bold values are the values mentioned in the text.

DISCUSSION

Between 1977 and 1983, blackleg was successfully controlled in France and other European countries by exploiting the adult plant resistance of the French *B. napus* cultivar Jet Neuf (31; M. Renard and H. Brun, unpublished data). However, most cultivars currently in use do not have strong enough resistance to blackleg throughout plant development (juvenile and adult stages). The *B. napus*-*B. juncea* recombinant line (MX), selected using the A-group isolate A314, has strong cotyledon resistance to some A-group isolates from oilseed rape cultivars. This line is highly resistant in field conditions to *L. maculans* populations associated with *B. napus* cultivars (6). Assessment of the resistance of the MX line at the cotyledon stage to populations of *L. maculans* from various origins and of the effect of this resistance on the evolution of the pathogen populations may facilitate more effective use of this new source of resistance.

Variation in pathogenicity among B-group isolates. All B-group isolates tested were virulent on the MX line. Thus, the re-

sistance conferred by the *Jlm1* gene in *B. napus* was ineffective against B-group isolates. Resistance to B-group isolates was not used as a selection criterion in the production of the MX line, so this lack of resistance is not surprising. The resistance of 'Picra' to some B-group isolates suggests that *B. juncea* may possess genes conferring resistance to B-group isolates. These putative genes could be exploited to increase the resistance of *B. napus* to this group of *L. maculans* populations. However, we need to know whether this resistance is effective throughout the life of the plant.

Our results for 'Westar' and 'Picra' are consistent with previous data concerning the pathogenicity of *B. juncea*-related B-group isolates (42) and the pathogenicity of this group of isolates on cotyledons of many crucifer species (11,17). We found that other wild cruciferous host species may also act as reservoirs for B-group isolates that cause disease on *Brassica* species.

Variation in the pathogenicity of A-group isolates. The susceptible standard 'Westar' can be used to detect isolates lacking virulence genes effective against *B. napus*. The moderate to high susceptibility of 'Westar' to all but one of the A-group isolates tested was consistent with previous observations (18,21,42). Among the A-group isolates, the avirulent isolate R15 (recovered from the MX line) lacked virulence on 'Westar', even though it was classified in the same homogeneous group as moderately aggressive isolates, probably because of the large number of two-way comparisons performed. *B. juncea* 'Picra' had a HR-type resistance to most of the 105 A-group isolates tested. This strong cotyledon resistance to blackleg is conferred by two or three major genes in *B. juncea* (24,30,44). Our results show that there is extensive genetic diversity within local A-group populations and even between isolates recovered from pseudothecia on the same stubble. Indeed, virulent and avirulent A-group isolates were recovered from the same MX line plant, suggesting that there was variation at the single plant level. The recovery of isolates avirulent on the MX line from pseudothecia produced on this resistant line is surprising. It is possible that some isolates are avirulent at the cotyledon stage but virulent at the adult stage, as suggested by Somda et al. (43) for 'Picra'. Alternatively, there may be genes suppressing the expression of avirulence genes in *L. maculans* isolates. Indeed, Ellingboe (10) demonstrated that some crosses between two isolates of *Magnaporthe grisea*, both of which were virulent on a particular rice cultivar, were found to give some progenies that were avirulent on the same cultivar. They concluded that there was a

Fig. 1. Frequencies in each mean disease index of A-group isolates in populations of *Leptosphaeria maculans* recovered from *Brassica napus* cultivars (black bars) and from the *Brassica napus*-*B. juncea* recombinant line MX (white bars) according to their pathogenicity on *B. napus* 'Westar', *B. juncea* 'Picra', and the MX line.

TABLE 5. Pathogenicity of B-group isolates of *Leptosphaeria maculans* recovered from the MX line, *Brassica napus* cultivars, and other crucifers on cotyledons of the three genotypes

Isolate	Disease index ^a		
	MX	'Westar'	'Picra'
MX-4	9.0 ± 0.0	9.0 ± 0.0	8.5 ± 1.6
MX-5	9.0 ± 0.0	9.0 ± 0.0	6.7 ± 2.4
MX-6	9.0 ± 0.0	9.0 ± 0.0	7.2 ± 3.2
MX-L	8.8 ± 1.1	8.1 ± 1.5	4.6 ± 2.7
MX-7	8.7 ± 1.5	9.0 ± 0.0	7.4 ± 2.5
MX-8	8.4 ± 1.7	7.8 ± 2.6	7.3 ± 2.8
B980	9.0 ± 0.0	7.6 ± 2.2	6.8 ± 3.0
B981	9.0 ± 0.0	9.0 ± 0.0	7.8 ± 2.3
B867	9.0 ± 0.0	8.7 ± 0.8	2.6 ± 1.4
B908	8.9 ± 0.5	NT ^b	4.8 ± 2.1
RBMN	9.0 ± 0.0	9.0 ± 0.0	7.2 ± 2.3
Rs2	9.0 ± 0.0	8.5 ± 0.9	6.8 ± 1.6
Sa2	8.8 ± 0.4	8.7 ± 0.9	5.1 ± 2.4
Es2	7.9 ± 1.2	8.2 ± 0.9	3.7 ± 1.7

^a Disease was recorded 14 days after inoculation using a 0 (no visible symptom) to 9 (total collapse of the tissue) scale. Data are the disease indices (DI) of 16 replicates of each genotype-isolate combination ± standard deviation. Interaction phenotype: DI < 4 = resistant; 4 ≤ DI < 6 = moderately resistant; and DI ≥ 6 = susceptible.

^b NT = not tested.

dominant suppressor of an avirulence gene in one of the virulent parents. Thus, it is of vital importance to study the genetic basis of the avirulence/virulence of *L. maculans* on the MX line.

There were differences in virulence among A-group isolates recovered from *B. napus* and used to inoculate cotyledons of the MX line. *B. napus* isolates from Canada and England were avirulent on the MX line. Virulent isolates were detected in two French locations. Isolates able to attack the MX line were more abundant among those recovered from the MX line at Le Rheu (France) than among those recovered from oilseed rape cultivars. This result suggests that the line exerts a selection pressure on *L. maculans* populations and, therefore, that *L. maculans* populations may adapt and increase on the MX line in the areas where *B. napus* cultivars are grown. Only one of the two or three putative resistance genes has been transferred into *B. napus*. This may account for the resistance spectrum of the MX line being narrower than that of the *B. juncea* cultivar.

We have shown that there are *L. maculans* populations capable of overcoming the resistance conferred by the *Jlm1* gene. The occurrence of such pathotypes suggests that the *Jlm1* gene is race specific and raises concerns about the use of the *Jlm1* resistance gene in France. Such virulent pathotypes are currently present at a low level on *B. napus* cultivars, but may become abundant if cultivars with *Jlm1* resistance are introduced.

Only one isolate recovered from *S. arvensis* was tested, but our results suggest that this species could be infected by isolates virulent on oilseed rape whether or not the *Jlm1* gene is present. *S. arvensis* is widespread in the *B. napus* growing areas of France and is, therefore, a significant source of inoculum for blackleg in oilseed rape crops. In contrast to the results obtained with Canadian isolates (22,27), French *L. maculans* isolates recovered from wild crucifers were similar to A- or B-group isolates. However, the role played by infected wild crucifers in the epidemiology of blackleg in France is unknown. Further studies involving collections of *L. maculans* isolated from various wild crucifers to determine their role in the evolution and maintenance of virulent pathotypes, especially against new resistance genes, would be useful. This information is necessary for the successful management of blackleg resistance in oilseed rape cultivars.

Consequences for breeding strategies. We have demonstrated that the monogenic resistance originating from the *B. juncea* B genome can be overcome in the MX line at the cotyledon stage by virulent isolates of *L. maculans* found in the *B. napus* growing areas of France. The frequency of virulent isolates increases if the MX line is grown in the field. Thus, alternative strategies for breeding *B. napus* with blackleg resistance are required. Resistance gene pyramiding had been successfully used in the wheat-rust pathosystem (26). However, Kousik and Ritchie (19) reported that the improvement in resistance to the pepper bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*), obtained with two genes used simultaneously, is not effective against some isolates. Gene pyramiding was developed to improve cultivar resistance and is well documented in many pathosystems (1,26), but it has yet to be achieved in the *B. napus*-*L. maculans* pathosystem. Therefore, the effect on blackleg control of resistance gene pyramiding in a particular oilseed rape genotype is unpredictable. The diversification of resistance sources may be of value for developing resistant oilseed rape genotypes. Isolates virulent and highly aggressive on the MX line but avirulent on 'Picra' may make it possible to identify the remaining *B. juncea* resistance genes in progeny resulting from the interspecific hybridization of *B. napus* and *B. juncea*. They could also be used to screen *Brassica* genus and other crucifer species for genes conferring complementary resistance. Lines with *Jlm1* resistance or with resistance originating from *B. nigra* are available (7), so it would be of great value to combine these resistance sources. The pyramiding of *Jlm1* and other resistance genes into French *B. napus* cultivars with partial adult plant resistance (e.g., 'Darmor') could improve resistance to *L. maculans* at

all plant growth stages. All these strategies require such genotypes to be tested under various field conditions and over several years before development on a commercial scale, because stem canker, a manifestation of blackleg disease, is economically important. Efficient control of this endemic disease should combine the use of resistant cultivars with agricultural practices to reduce inoculum pressure (e.g., destruction of infected residues after harvesting) and the eradication of wild cruciferous hosts within and around fields containing oilseed rape crops.

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