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A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape

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Abstract

Leptosphaeria maculans, the cause of stem canker of oilseed rape (OSR), exhibits gene-for-gene interactions with its host plant. The race structure of *L. maculans* was assessed on the basis of the analysis of 1011 isolates collected in France between 1990 and 2000, with regards to three *AVR* genes, *AvrLm1*, *AvrLm2* and *AvrLm4*. The effect of selection pressure, due to large-scale cropping of *Rlm1* cultivars, on the evolution of races of the fungus was also evaluated. The results revealed a scarcity or complete absence of isolates harbouring *AvrLm2*, whereas isolates harbouring *AvrLm4* were present at a variable level, that was as high as 17.2–31.2% depending on the sample year and location. When obtained from *rlm1* cultivars, isolates harbouring *AvrLm1* always represented more than 83% of the populations until the 1997–1998 growing season. As a consequence, the *Rlm1* cultivars had been highly efficient at controlling the disease and were grown on an estimated 43.7% of the total French acreage in OSR in 1998–1999. However, the increased commercial success of *Rlm1* cultivars was paralleled by a decrease in the proportion of isolates harbouring *AvrLm1* in 1997–1998 and 1998–1999. This resulted in less than 13% of isolates harbouring *AvrLm1* in populations being collected from *rlm1* cultivars in 1999 and 2000, and contributed to the loss of efficiency of the *Rlm1* resistance in the field. The present study is an illustration of one round of a 'boom and bust' cycle that occurred for a pathosystem where it has never been reported before. These data and the high evolutionary potential of *L. maculans* are fully supportive of one pathogen species with a high risk of breaking down resistance genes in OSR and suggest that the development of integrated strategies aiming at maximising the durability of novel resistance is now a priority for this pathosystem.

Abbreviations: IC – infection class; DI – disease incidence; OSR – oilseed rape; *R* gene – race-specific resistance gene; *AVR* gene – avirulence gene.

Introduction

Stem canker of oilseed rape (OSR, *Brassica napus*) caused by the fungal pathogen *Leptosphaeria maculans* (anamorph *Phoma lingam*), is responsible for severe yield losses world-wide (West et al., 2001). During the last 10 years, analyses of populations of the pathogen have identified two main components of what is now considered as a species complex: the A/Tox$^+$ group, regarded as *L. maculans sensu stricto*

Table 1. Resistance genes to *L. maculans* present in cultivars of *B. napus* used in the present study

Rlm phenotype[1]	Name of cultivars
rlm1–rlm2–rlm4	Westar, Lipton, Lirabon, Drakkar, Darmor, Goeland, Shogun, Navajo
Rlm1–rlm2–rlm4	Doublol, Maxol, Vivol, Capitol, Columbus, Cocktail, Cheyenne
Rlm1–rlm2–Rlm4	Quinta (heterogeneous genotype), Cando, Elite
rlm1–Rlm2–rlm4	Glacier, Bristol, Samouraï, Express, Eurol
rlm1–Rlm2–Rlm4	Falcon (heterogeneous presence of *Rlm2*), Synergy
rlm1–rlm2–Rlm4	Jet Neuf, Major, Primor

[1] *Rlmi*, the cultivar is resistant to isolates harbouring the corresponding avirulent allele $AvrLm_i$; *rlmi*, the cultivar is susceptible.

and the B/Tox^0 group whose European component was recently renamed *L. biglobosa* (Rouxel et al., 1994; Balesdent et al., 1998; Shoemaker and Brun, 2001). Renewed assessments of the epidemiology of the pathogen, which took account of the species complex concept, clearly identified *L. maculans sensu stricto* as the main causal agent of the stem canker symptoms responsible for the damaging losses on OSR crops (West et al., 2002). Genetic studies performed on both the plant and the pathogen demonstrated the occurrence of gene-for-gene relationships in the *L. maculans sensu stricto–B. napus* pathosystem. The avirulence gene (*AVR* gene) *AvrLm1* conditions resistance in all cultivars possessing *Rlm1*, including Quinta, Columbus, Vivol, Doublol or Capitol (Balesdent et al., 2001). *AvrLm2*, which is genetically linked to *AvrLm1*, controls resistance in cultivars displaying the *Rlm2* gene, including Glacier or Bristol (Ansan-Melayah et al., 1998; Balesdent et al., 2001). Finally, the *AvrLm4 AVR* gene, which is unlinked to the *AvrLm1–AvrLm2* region, induces a hypersensitive response in cultivars displaying the *Rlm4* resistance gene, such as Jet Neuf (Balesdent et al., 2001). The previously identified races PG2–PG4 (Mengistu et al., 1991) and A1–A6 (Badawy et al., 1991; Kuswinanti et al., 1995) were found to correspond to different combinations of *AvrLm1*, *Avrlm2* and *Avrlm4* alleles in *L. maculans* isolates (Balesdent et al., 2002). In France, a 2-year field assay, conducted in one single experimental location in 1994–1995 and 1995–1996, revealed that 92–95% of *L. maculans* isolates obtained from cultivars lacking *Rlm1* were characterised as possessing *AvrLm1* (Ansan-Melayah et al., 1997). The remaining isolates were virulent to cultivars harbouring *Rlm1*, *Rlm2* and *Rlm4* genes and were characterised as *avrLm1–avrLm2–avrLm4*. No *AvrLm2* isolates were identified. The study by Ansan-Melayah et al. (1997) suggested that specific resistance genes expressed at the cotyledon level can be efficient under field conditions where the corresponding avirulent races of the pathogen are prevalent, such as was found for *Rlm1* where the majority of the *L. maculans* population possessed *AvrLm1*. The expression of the race-specific resistance gene (*R* gene) prevents the pathogen from entering the leaves and therefore prevents the systemic colonisation of the plant which is a prerequisite for the development of stem cankers many months after a successful infection (West et al., 2002). However, the efficacy of resistance of the commercially successful cultivars harbouring *Rlm1* decreased constantly between 1995 and the present day in France, as reported by farmers and advisory bodies. The cause of this decrease in resistance was unclear, and suggested that populations of *L. maculans* were changing to overcome the *Rlm1* resistance gene. Therefore, on the basis of a large-scale survey of populations of *L. maculans* in France between 1990 and 2000, the objective of the present study was to assess race structure of the fungus in France, with regard to the *AVR* genes *AvrLm1*, *AvrLm2* and *AvrLm4*, and to evaluate the effect of the selection pressure due to large-scale cropping of *Rlm1* cultivars on the evolution of races of the fungus.

Materials and methods

Plant materials

The main plant genotypes used, and the *Rlm* genes they harbour are described in Table 1.

Sampling of isolates

Several isolates of *L. maculans* were collected for this study, besides already existing collections such as the International Blackleg of Crucifers (IBCN) collection, Gall 91, Versailles 94–95, Versailles 95–96, Le Rheu 94–95, and IMASCORE collection of

Table 2. Collections of French *L. maculans* isolates collected in 1985 and between 1990 and 2000

Collections	Number of isolates	Sampling month/year	Isolated from (plant part)[1]	Place of sampling[2]	Isolated from: (name of cultivar)	Number of isolates from *Rlm1* cultivars	Reference
IBCN	8	1985 (1 isolate) 1990 (7 isolates)	LL (2 isolates) and unknown	Numerous locations	Primor, Darmor, Unknown (6 out of 8)	Unknown	Volke (1999), Purwantara et al. (2000), Attard et al. (2001 and 2002)
Gall 91	26	1990	SC	Le Rheu, St Pathus	Unknown	Unknown	Gall et al. (1994)
Versailles 94–95	75	12/1994	LL	Versailles	Glacier, Samouraï, Tapidor, Quinta,	18	Ansan-Melayah et al. (1997)
Le Rheu 94–95	34	07/1995	SC	Le Rheu	Unknown	Unknown	Pilet (1999)
IMASCORE	13	04/1994 to 07/1997	Mostly SC	Numerous	Goéland, Bristol, Vivol, Columbus	4	Volke (1999), Attard et al. (2001 and 2002)
Versailles 95–96	196	12/1995	LL	Versailles	Synergy, Quinta	81	Ansan-Melayah et al. (1997)
Phomepi 95–96	53	11/1995 07/1996	C SC	Beziers, Bourges, Epuizay, Nancy, St Pathus	Synergy, Goeland, Eurol, Vivol, Bristol, Falcon, Navajo, Cocktail	6	This study
CETIOM 96–97	103	10–12/1996 03/1997 06–07/1997	hL and dL hL and dL S	Dijon, Nancy, St Pathus, St Florent, Béziers, Surgères	Goéland, Synergy, Bristol, Vivol	30	This study
CETIOM 97–98	93	12/1997 06/1998	hL and dL hC and dC	St Florent	Lipton, Capitol	49	This study
CETIOM 98–99	91	11/1998, 01/1999, 03–06/1999	hS, dS, hC, dC	St Florent	Lipton, Capitol	42	This study
CETIOM 99–00	90	11–12/1999, 02–06/2000	hCo, dCo, hS, dS, hC, dC, dL hS, dS, dC, dL, hL	St Florent	Lipton, Capitol	47	This study
Le Rheu 98–99	70	07/1999	SC	Le Rheu, Prémesques	Unknown	Unknown	This study
Vivol 99	32	07/1999	SC	Le Rheu, La Minière	Vivol	32	This study
Versailles 98–99	40	11/1998, 01 and 03/1999, 06/1999	LL dS, dR, dC	Versailles	Capitol	40	Attard et al. (2001)
Versailles 2000	87	10/2000	LL	Versailles	Drakkar	0	This study
Total	1011						

[1]Plant tissues from which isolates originate are as follows: Co, cotyledons; L, leaves; S, stem; C, crown and R, root. h, healthy; d, diseased (e.g. dC means diseased crown). For all these diseased or seemingly healthy tissues, isolates were single-hyphal-tip isolates purified from colonies growing out of the plant tissue. LL, leaf lesions: isolates were single-pycnidium isolates further purified by isolating single-hyphal-tip; SC, stem canker, isolates are single-ascospore isolates obtained from pseudothecia differentiating on typical stem canker.

[2]Place of sampling, Le Rheu: Brittany, West of France; St Pathus, Northern surroundings of Paris; Versailles: South-Western surroundings of Paris; Béziers: South of France; Nancy: East of France; Epuizay and Bourges: Centre of France; Surgères: West of France; St Florent: Centre of France; Dijon: East of France; Prémesques: North of France; La Mimière: South–Western surroundings of Paris.

European isolates (Table 2). In total, 15 collections of *L. maculans* were analysed in this study comprising of 1011 isolates collected in 1985 and between 1990 and 2000.

Isolation, purification and characterisation of isolates

Single-ascospore isolates were obtained from pseudothecia differentiated on stubble as previously described (Gall et al., 1994; Somda et al., 1996). Single-conidium, single-hyphal-tip isolates were obtained as described by Ansan-Melayah et al. (1997). Hyphal-tip isolates were purified from infected or seemingly uninfected tissues of plants according to West et al. (2002). Prior to pathogenicity analysis, all isolates were assigned to the *L. maculans sensu stricto* species (A/Tox$^+$ group) on the basis of their soluble protein profile (Balesdent et al., 1992), ITS size and restriction profile (Balesdent et al., 1998) or on the basis of their glucose phosphate isomerase patterns (Somda et al., 1996).

The cultures were maintained on V8-agar or Malt-agar plates. Highly sporulating cultures were obtained according to Ansan-Melayah et al. (1995).

Determination of races of L. maculans *following cotyledon-inoculation tests*

Cotyledons of the *B. napus* differential set comprising Westar, Glacier (sometimes replaced by Bristol), Capitol, Doublol, Columbus and Jet Neuf (Balesdent et al., 2001; Table 1) were inoculated with *L. maculans*. Plants were incubated in a growth chamber at 16 °C (night)–24 °C (day) with a 12 h photoperiod. Symptoms were scored 14–27 days after inoculation using the IMASCORE rating scale comprising six infection classes (IC), where IC1–IC3 are classed as resistant and IC4–IC6 are classed as susceptible (Balesdent et al., 2001). At least 10 different plants were inoculated. Isolates originating from the Le Rheu 94–95, Le Rheu 98–99 and Vivol 99 collections were characterized using a similar differential set where Quinta was added and Vivol replaced Doublol.

Disease severity assessment: field experiments

Three series of field experiments evaluated the evolution of disease incidence (DI) on *Rlm1*-harbouring cultivars. A 2-year assay in Versailles in 1994–1995 and 1995–1996 was followed by an additional assay in 1999–2000. A 3-year assay was set up in St Florent from 1997 to 2000, and a 2-year assay was set up in Le Rheu in 1997–1998 and 1998–1999.

Details and results of the 1994–1995 and 1995–1996 field experiments in Versailles were described by Ansan-Melayah et al. (1997). In order to evaluate the evolution of field behaviour of *Rlm1*-harbouring cultivars in Versailles between 1996 and 1999, an additional field assay was set up in 1999–2000. The experimental design was a split-plot with two to four repeats (Jet Neuf and Bristol) or single plots. The cultivars used were Glacier, Bristol, Express, Capitol, Columbus, Falcon, Major and Jet Neuf (Table 1). The field was submitted to natural infection conditions.

In St Florent, the experimental design was a split-plot with 4 repeats and 2 cultivars (Capitol and Lipton). Oilseed rape was sown on 11th September 1997, 17th September 1998 and 8th October 1999, respectively. Contaminated stubble from the previous year experiment was added ca. one week after sowing. The CETIOM 97–98, CETIOM 98–99 and CETIOM 99–00 collections originated from this field experiment.

In Le Rheu, the durability of the Vivol resistance was evaluated according to a published protocol (Brun et al., 2000). The experimental design was a randomised complete block design, with four replicates. Cultivars included were Shogun, Lirabon (only year 2), Samouraï, Glacier (only year 2), Falcon, Capitol, Doublol, Vivol, Quinta, Maxol, Darmor and Columbus (Table 1). Seeds were sown on 9th September 1998 and 5th September 1999, respectively. Infected stubble of cultivar Vivol from various origins was added in order to artificially increase the selection pressure due to isolates able to colonise this *Rlm1*-harbouring cultivar (Brun et al., 2000). Part of the Vivol 99 collection originated from this experimental field.

In all cases, DI was first assessed by measuring the ratio of plants displaying leaf lesions in autumn (occasionally in spring, i.e. April 1998, February 1999 and March 2000 for the St Florent experiments). The severity of stem necrosis was rated ca. one month before harvest using the $G2$ disease index developed by the CETIOM. Sixty plants per plot were uprooted and scored on the basis of external and internal damage, using a six-classes scale, i.e. 1 (no apparent symptoms) to 6 (crown broken). A correction coefficient ($c = 0$, 1, 3, 5, 7, and 9) was attributed to each class (from 0 for class 1, to 9 for class 6), and the $G2$ disease index, ranging from 0 to 9 was calculated as follows: $G2 = \sum_i (n_i \times c_i)/N$, where n_i is the number of plants in class i, c_i is the correction coefficient for

class i, and N is the total amount of plants scored. In the case of the 'Le Rheu' and 'St Florent' experiments, the stem canker severity data were surveyed by variance analysis in a randomised complete block design including the effect of the genotypes, the blocks and the genotype × block interactions per year using the STAT-ITCF software (ITCF, Paris, France) with $\alpha = 0.05$. The genotypes were classified using the Student–Newman–Keuls test (SNK test, $\alpha = 0.05$).

Results

Leaf lesion and stem canker resistance under field conditions

Versailles experiments

As previously described, leaves and crown of cultivars devoid of *Rlm1* were severely diseased in December 1994, June 1995, December 1995, and June 1996 (Ansan-Melayah et al., 1997). Less than 18% of Quinta plants showed leaf lesions, and only 26.4% of Quinta plants showed crown canker in June 1995 (Ansan-Melayah et al., 1997). In July 1996, 96.8% of the *Rlm1*-devoid cultivar Synergy plants exhibited typical crown canker ($G2 = 3.9$), whereas 58.5% of Quinta plants showed typical stem canker ($G2 = 2.5$) (Ansan-Melayah et al., 1997). All cultivars, harbouring *Rlm1* or not, were severely infected in autumn 1999 (65% or more of the plants displayed leaf lesions) and June 2000, with $G2$ ratings as high as 6.5 for cultivar Major (data not shown).

St Florent experiments

15.2% of Lipton plants showed leaf lesions in October 1997, whereas only 0.6% of Capitol plants were diseased. The disease severity three weeks before harvest was very low, with a $G2$ rating always less than 3. However differences were evident between Capitol and Lipton both in terms of the proportion of plants displaying stem lesions (35.6% for Capitol vs. 79.0% for Lipton) and in terms of $G2$ rating ($G2 = 0.86$ for Capitol vs. $G2 = 2.97$ for Lipton) (data not shown).

In autumn 1998 and autumn 1999, the infection level was higher than in 1997–1998. However, the percentage of plants exhibiting leaf lesions was comparable for cultivars Lipton and Capitol (data not shown). The $G2$ rating was evaluated on June 17, 1999, and June 15, 2000. It remained at a low level ($G2 < 2.80$) and, according to the SNK test, the disease severity was not significantly different for Lipton or Capitol, whatever the year considered (data not shown).

Le Rheu experiments

In both years of the experiment (1998–1999 and 1999–2000) disease pressure was high. In the autumn 1998 and autumn 1999, the *Rlm1* cultivars were as diseased as *Rlm1*-devoid cultivars (data not shown). Stem canker severity assessed in June 1999 was very high and showed that the genotypes were separated into two distinct homogeneous groups: susceptible cultivars ($G2 = 6.7$–8.4) encompassing Shogun, Samouraï, Falcon, Quinta, Doublol Capitol, Maxol and Vivol, and resistant cultivars, Darmor ($G2 = 4.2$) and Columbus ($G2 = 4.4$) (data not shown). Stem canker severity was lower in June 2000, and the cultivars were distributed among five homogeneous groups with Darmor and Columbus being the most resistant lines and Lirabon, Doublol, Glacier and Quinta the most susceptible cultivars (data not shown).

Overall structure of race populations in the collections analysed

Five races of *L. maculans* were identified (Table 3). Two of them, the *AvrLm1–avrLm2–avrLm4* and the *avrLm1–avrLm2–avrLm4* races represented more than 90% of the isolates (Table 3). The *AvrLm2* avirulence allele was rare and only found in the form of race *AvrLm1–AvrLm2–AvrLm4* (Table 3). This race was represented in two collections, with isolates originating from three different locations. Five of these putative *AvrLm1–AvrLm2–AvrLm4* isolates were obtained in St Florent in 1997–1998, whereas they were absent in the two subsequent growing seasons. Isolates harbouring *AvrLm4* represented 8% of the isolates in the collection (Table 3). This figure probably does not fully reflect the real occurrence of *AvrLm4* isolates in the entire collection due to the lack of analysis of some of the older collections at a time when *AvrLm4* was not genetically identified and Jet Neuf was not included in the differential set. As a consequence, three collections (Versailles 94–95, Le Rheu 94–95 and Phomepi 95–96) were not analysed for the presence of *AvrLm4*, and only very few isolates from Versailles 95–96 were assessed for the occurrence of *AvrLm4*. Isolates harbouring *AvrLm4* occurred either as race *avrLm1–avrLm2–AvrLm4* or as race *AvrLm1–avrLm2–AvrLm4* with a clear shift from race *AvrLm1–avrLm2–AvrLm4* to race

Table 3. Frequency of occurrence of races of *L. maculans* in different collections of pathogen isolates

Collection	Races of *L. maculans*					Total
	Avrlm1 avrLm2 avrLm4	*AvrLm1 AvrLm2 AvrLm4*	*avrLm1 avrLm2 AvrLm4*	*Avrlm1 avrLm2 AvrLm4*	*avrlm1 avrLm2 avrLm4*	
Versailles 94–95	71[1]	0	nd	nd	4	75
Phomepi 95–96	48	0	nd	nd	5	53
Le Rheu 94–95	28	0	nd	nd	6	34
Sub total	147	0	nd	nd	15	162
IBCN 85–90	5	0	0	1	2	8
Gall 91	23	0	0	0	3	26
IMASCORE 94–97	8	0	0	0	5	13
Versailles 95–96	118	0	0[2]	0	78	196
CETIOM 96–97	78	2	1	6	16	103
CETIOM 97–98	29	5	8	8	43	93
CETIOM 98–99	10	0	2	2	77	91
CETIOM 99–00	2	0	4	0	84	90
Le Rheu 98–99	32	0	5	2	31	70
Vivol 99	0	0	10	0	22	32
Versailles 98–99	0	0	2	0	38	40
Versailles 2000	12	0	17	0	58	87
Sub total	317	7	49	19	457	849
	(37.4%)	(0.8%)	(5.8%)	(2.2%)	(53.8%)	
Total	464	7	49	19	472	1011

[1]Data are number of isolates belonging to each race within each collection.
[2]Only 12 isolates of this collection were tested for the occurrence of *AvrLm4*.

avrLm1–avrLm2–AvrLm4 in the latter collections (Table 3).

Frequency of the AvrLm4 *avirulence allele in populations in France*

AvrLm4-harbouring isolates were not found in the Gall 91 and Versailles 95–96 collections (Table 3), and no *AvrLm4* isolate was present in the 13 French isolates represented in the IMASCORE European collection of isolates, whereas only one *AvrLm4* isolate was present among the eight French isolates of the International Blackleg of Crucifers Network (IBCN) collection. The French isolates of the IBCN collection were isolated in 1985 and 1990, whereas IMASCORE French isolates were obtained in 1994–1997. These collections only represent a quite limited amount of isolates (Table 2, Figure 1), but these data nevertheless suggests the scarcity of *AvrLm4* isolates in France in the early 1990s. From 1996 onward, *AvrLm4* isolates obtained from susceptible, *rlm4*, cultivars were present for each year of sampling, at a ratio that varied between 7.5% of the samples in 1996 and 15.1% of the samples in 2000 (Figure 1). The ratio of *AvrLm4* isolates within the populations seemed to fluctuate from one growing season to the other as indicated by the 3-year experiment performed in St Florent (Table 3). The high ratio of *AvrLm4* isolates in the Versailles 2000 collection could indicate an increase in the ratio of *AvrLm4* isolates within the local population, if compared to their absence in the subsample of the Versailles 95–96 collection (Table 3, Figure 1).

Frequency of the AvrLm1 *avirulence allele in populations in France*

The ratio of isolates harbouring *AvrLm1* was very high in all collections sampled before the 1997–1998 growing season (Table 3). If only samples isolated from *rlm1* plants are considered, in order to avoid the sampling bias due to the selection of *avrLm1* isolates exerted by *Rlm1* plants, 83.3% (1997) to 95.1% (1994) of the isolates were *AvrLm1* isolates before 1998 (Figure 2A). Such a prevalence of *AvrLm1* isolates within natural populations was observed for all locations encompassed within these collections including Le Rheu (West of France), St Florent (Centre of France), Béziers (South), Nancy (East) and St Pathus/Versailles

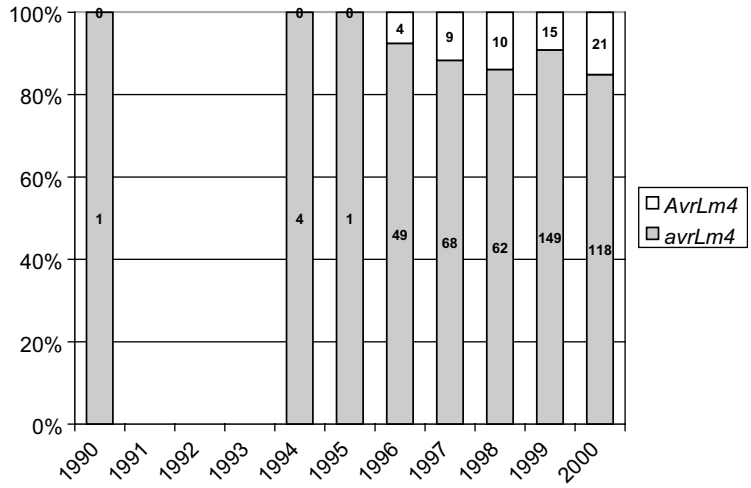

Figure 1. Frequency of isolates harbouring *AvrLm4* or *avrLm4* when obtained from cultivars devoid of the resistance gene *Rlm4*. The number of isolates used to calculate the frequency are shown in each bar.

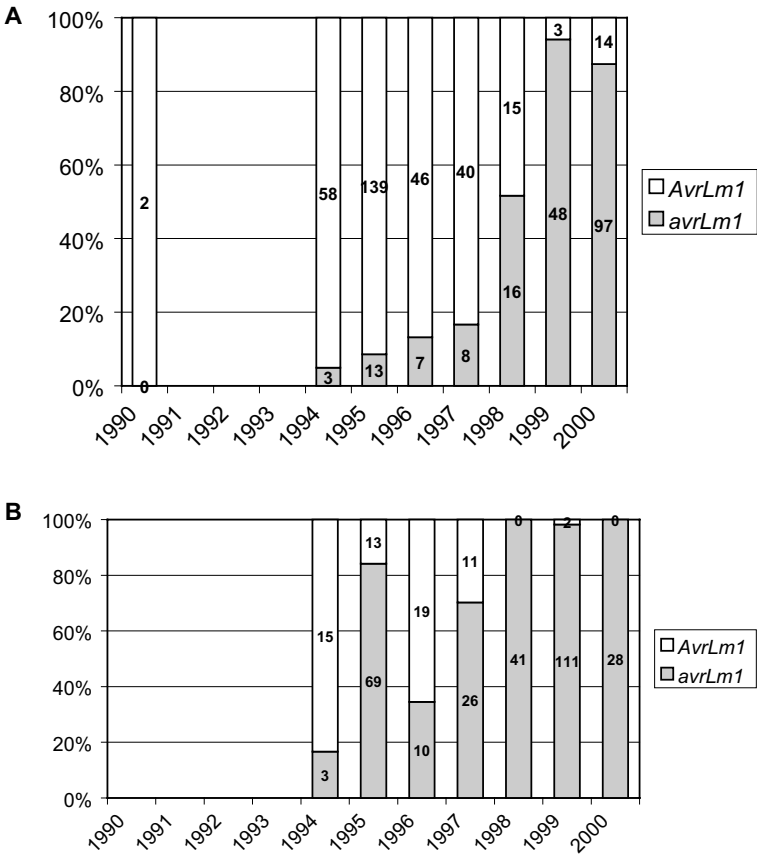

Figure 2. Frequency of isolates obtained between 1990 and 2000 and harbouring *AvrLm1* or *avrLm1* when isolated from (A) cultivars devoid of the major resistance gene *Rlm1*, and (B) cultivars harbouring *Rlm1*. The number of isolates used to calculate the frequency are shown in each bar.

(surroundings of Paris). A dramatic reduction in the percentage of *AvrLm1* isolates was observed in 1998 when 48.4% of isolates were *AvrLm1* and 1999, where only 5.9% of isolates were *AvrLm1* (Figure 2A). A shift in the pathogen population was also observed for the Le Rheu 98–99 collection, although the plant genotype from which the isolates were obtained is unknown. Finally, the year-2000 samplings with only 12.6% of *AvrLm1* in the populations confirmed the shift in frequency of the *AvrLm1* gene in *L. maculans* populations (Figure 2A). All these data indicate that populations of the pathogen now consist of more than 85% of *avrLm1* isolates, virulent on *Rlm1* cultivars (Figure 2A).

It has to be noticed that, even though at a lower ratio than when obtained from *rlm1* cultivars, *AvrLm1* isolates were also isolated from *Rlm1* plants at non-negligible ratios between 1994 and 1997 (Figure 2B). Their ratio was particularly high in 1994 (83.3%) and 1996 (65.5%), but they became rare in the 1998–2000 samplings (Figure 2B). They originated from all parts of infected plants (leaf symptoms or leaf tissues, stem canker, as well as seemingly healthy stem tissues) and from various *Rlm1* cultivars (Quinta, Vivol or Capitol) (data not shown). However, *AvrLm1* isolates were obtained from typical leaf symptoms only from Quinta in the case of the Versailles 94–95 and Versailles 95–96 collections. As previously discussed (Ansan-Melayah et al., 1997), the high number of *AvrLm1* isolates obtained from Quinta is probably attributable to heterogeneity of this cultivar, with 7–10% of plants devoid of *Rlm1*.

Evolution of the acreage of Rlm1 *cultivars and cultural practices in France*

Since 1996, CETIOM carried out mail inquiries in the main OSR production areas. These surveys were used to describe the cultural practices in the different French regions and to follow their evolution along the years. Data collected comprised sowing dates, soil tillage practices or pesticides applications. The number of farmer answers was 4643 for 1995–1996, 2720 for 1996–1997, 1915 for 1998–1999 and 1066 for 2000–2001. The answers represented 10,580–20,140 ha depending on the year of survey. Corrections were carried out according to the Ministry of Agriculture national statistics service (SCEES) data on OSR production. Data analyses were performed using the SAS software. The results were compared with seed production data or with commercial data from seed companies, and provided a good estimate of the frequency of genotype/resistance gene occurrence. Apart from Doublol (registered in 1988) and Maxol (registered in 1991), which were grown on very limited acreages, the first *Rlm1* cultivar to be registered in France was Vivol in 1992. Capitol was registered 1995, and ca. six other *Rlm1* cultivars were registered in the following years. The success of Capitol, and other *Rlm1* cultivars was very great and their acreage rapidly increased between 1996 and 1999, to amount to up to 43.7% of the total acreage grown in OSR in 1999 (Table 4). This overall ratio underpinned regional disparities, with some regions like the Centre of France, where St Florent is located, growing up to 52.5% of its total acreage in *Rlm1* cultivars (Table 4). These genotypes were popular both for their yield and also for their excellent behaviour towards the blackleg disease. However, cropping of *Rlm1* cultivars decreased between 1999 and 2001 (Table 4).

Survey data also showed changes in cultural practices with time. Among them, two can be stressed: (i) sowings after ploughing decreased from 76% in summer 1995 to 65% in summer 2000 (68% and 62% respectively for the Centre of France); and (ii) in the last two inquiries (1999 and 2001) there was a clear

Table 4. Percentage of *Rlm1* cultivars, and Capitol, in relation to the total area grown to oilseed rape between 1996 and 2001 in the whole of France and three specific French regions

	1995–1996	1996–1997	1998–1999	2000–2001
Total area of OSR[1]	870,000 ha	964,000 ha	1,349,000 ha	1,078,000 ha
Whole of France: all *Rlm1* cultivars	19.1%[2]	35.9%	43.7%	20.5%
Whole of France: Capitol only	11.8%	23.6%	26.1%	11.2%
North of France: all *Rlm1* cultivars	23.4%	32.5%	38.4%	12.7%
Centre of France: all *Rlm1* cultivars	19.2%	41.7%	52.5%	15.8%
Brittany: all *Rlm1* cultivars	20.0%	42.9%	38.5%	20.1%

[1]Areas of oilseed rape according to CETIOM (1997, 1998, 1999, 2001).
[2]Values are the ratio of *Rlm1* cultivars (or Capitol only) as compared to the whole acreage of OSR grown this specific season.

decrease in the number and earliness of residue tillage following OSR harvest.

Discussion

This paper describes a 10-year survey of the occurrence and evolution of avirulence alleles *AvrLm1*, *AvrLm2* and *AvrLm4* of *L. maculans* in France. It has nonetheless to be stressed that the oldest collections analysed here only provide indicative data on a global population structure because of the low number of isolates they contained, the absence of data on the cultivar the isolates originated from and the lack of characterisation of the presence of *AvrLm4*.

This study demonstrates the scarcity or complete absence of races harbouring *AvrLm2* in France, as already suggested by Volke (1999), whereas isolates harbouring *AvrLm4*, i.e. races *AvrLm1–avrLm2–AvrLm4* or *avrLm1–avrLm2–AvrLm4* were present at a variable level depending on sample date/cultivar and the location. Until recently, races harbouring *AvrLm4* were thought to be very rare or absent from France, whereas they were present in Germany and the UK (Volke, 1999). Ratios of *AvrLm4* isolates as high as 17.2% (St Florent, 1997–1998), 19.5% (Versailles, 2000) or 31.2% (Le Rheu, La Minière, 1999) could therefore indicate a slow resurgence of a seemingly extinct race. In contrast, such a phenomenon was not observed for races harbouring *AvrLm2* that were known to be present in France, as illustrated by isolate PHW1245, which was genetically demonstrated to be an *AvrLm1–AvrLm2–AvrLm4* isolate, and was isolated in the 1970s (Ansan-Melayah et al., 1998; Balesdent et al., 2002), but are rare, or absent, in the present collections.

As previously established for the Versailles experimental site (Ansan-Melayah et al., 1997), races harbouring *AvrLm1* and mostly race *AvrLm1–avrLm2–avrLm4* were highly prevalent at all locations sampled in France, until 1997–1998. This was consistent with the efficiency of the *Rlm1* cultivars to control the disease and the subsequent commercial success of these. In the present study, a very high efficiency of Quinta or Capitol to protect the crop was noticed for the Versailles 94–95 (see also Ansan-Melayah et al., 1997) and the St Florent 97–98 experiments. In the Versailles 95–96 experiment, Quinta plants were more diseased at stem level than in the previous year. In the 1998–1999 growing season, at Le Rheu and St Florent, and 1999–2000 growing season, at Le Rheu and Versailles, the *Rlm1* resistance gene was insufficient to protect the crop. It was more difficult to conclude for the St Florent 1999–2000 experiment due to the low inoculum pressure after sowing. The loss of resistance efficiency of *Rlm1* is consistent with the presence of a majority (87.4–94.1%) of virulent, *avrLm1*, isolates in the populations of *L. maculans* sampled on *rlm1* in 1999 and 2000. The similar evolution of populations at these three independent locations, corresponding to French regions separated by more than 200 km, as well as contrasted situations with regards to the intensity of growing of OSR, reflected what was observed at the whole country scale, with a loss of efficiency of *Rlm1* cultivars in commercial farms over those years. This resulted in a clear decrease in the commercial success of *Rlm1*-harbouring cultivars in the 2000–2001 growing season (see Table 4).

The 'boom and bust' is defined as a cycle where a new resistant cultivar becomes so popular that it is grown as a homogeneous genotype, enabling the pathogen to overcome the resistance and cause severe epidemics (McDonald and Linde, 2002a,b). The present study is a perfect illustration of one round of such a 'boom and bust' cycle that occurred for a pathosystem where it has never been reported before. It encompasses (i) a novel resistance source, that was initially selected on the basis of its field efficiency (J.P. Despeghel, pers. comm.), and was only later shown to be due to a single gene in the host, *Rlm1* (Ansan-Melayah et al., 1997; 1998); (ii) commercial success of the *Rlm1* cultivars; (iii) rapid increases of the amount of crop with one single resistance gene grown; (iv) a drastic shift from avirulence to virulence in the populations of the pathogen causing; (v) the complete loss of the efficiency of the *Rlm1* resistance in France and the need to uncover and use novel resistance sources and (vi) the decrease of the market shares of *Rlm1* cultivars.

In France, some *avrLm1* isolates were present in natural populations prior to the use of the *Rlm1* resistance gene. For example, the IBCN isolate collected in 1985 at Le Rheu was an *avrLm1* isolate. However, the most striking finding of the present study is the incredible speed with which *avrLm1* isolates became prevalent within the populations. A major shift in the populations of the pathogen occurred in only two growing seasons, 1997–1998 and 1998–1999. In a recent review, McDonald and Linde (2002b) listed 43 plant diseases, of which 36 were due to fungal or oomycete pathogens for which 'accurate records of the time needed to break down resistance genes or to become resistant to

fungicide' are available in the literature. Among these, only two, the rice blast fungus, *Magnaporthe grisea*, and the flax rust fungus, *Melampsora lini*, were shown to be able to overcome new resistance sources in less than 3 years (McDonald and Linde, 2002b). The highest risk of evolution was associated with large effective population sizes, including large overseasoning population, high gene flow, including airborne long-distance dispersion, mixed reproduction system and the uniform monoculture of one *R* gene over large areas. These two fungal species, however mostly display asexual multiplication. As a consequence, the biology of *L. maculans* is indicative of a pathogen species with a high risk of a population adapting to resistance genes. It encompasses (i) a very large population size where the size of the population is maintained in the absence of crops via saprophytic growth on stem debris; (ii) a large-scale obligate sexual recombination on stem debris accompanied by asexual multiplication, whose extent remains unclear to date and (iii) a high gene flow through large-scale dissemination of ascospores (reviewed in Hall, 1992; West et al., 2001). In the case of the *Rlm1* gene aggravating factors explaining the speed of this shift resulted from the conjunction of (i) higher acreages of OSR grown in France (with more than 35% increase in acreage between 1996 and 1999), thus constantly increasing the pathogen population size; (ii) the constantly increasing proportion of *Rlm1* cultivars excluding, or at least reducing the part of *AvrLm1* isolates from subsequent rounds of multiplication; (iii) high disease pressure in the Autumns 1998 and 1999, therefore ensuring the success of *avrLm1* isolates to heavily colonise the plants and increase the chance to find partners for sexual multiplication; and (iv) changes in cultural practices including closer crop rotations and development of 'minimum tillage' practices. For this pathogen, for which the sexual stage taking place on crop residues is of primary importance both for recombination of virulence characters and as the source of primary inoculum, the lack of management of culture residues has direct consequences on evolution of populations.

In accordance with its biology, the data presented here demonstrate the high evolutionary potential of *L. maculans* populations to adapt to novel resistance genes. They corroborate the 3-years breakdown of resistance gene *Jlm1*, also named *Rlm6* (Balesdent et al., 2002) observed in an experimental field using a procedure aiming at maximising the selection pressure exerted on populations of the pathogen by the resistance gene (Brun et al., 2000). Such a rapid loss of resistance is not acceptable for breeders, who spend years obtaining and fixing the resistance trait in commercial cultivars, nor for farmers. The high evolutionary potential of *L. maculans* to break down novel resistance sources clearly suggests the need to monitor the available sources of major gene resistance and use them with care to prevent the pathogen adaptation and the dissemination of novel virulent isolates outside of the regions where novel resistances are used. As in many other pathosystems, the development of integrated strategies aiming at maximising the durability of resistance to *L. maculans* has now become a priority (see the SECURE web site at http://www.secure.rothamsted.ac.uk). In addition to rational use of major resistance, cultural practices, including destroying and burying stem debris, should aim to reduce the size of the pathogen population (CETIOM, 2001; West et al., 2001; McDonald and Linde, 2002a,b).

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