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Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe

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Abstract

This paper describes the first large-scale Europe-wide survey of avirulence alleles and races of *Leptosphaeria maculans*. Isolates were collected from the spring rape cultivar Drakkar, with no known genes for resistance against *L. maculans*, at six experimental sites across the main oilseed rape growing regions of Europe, including the UK, Germany, Sweden and Poland. Additionally in Poland isolates were collected from cv. Darmor, which has resistance gene, *Rlm9*. In total, 603 isolates were collected during autumn in 2002 (287 isolates from Germany and the UK) and 2003 (316 isolates from Poland and Sweden). The identity of alleles at eight avirulence loci was determined for these isolates. No isolates had the virulence allele *avrLm6* and three virulence alleles (*avrLm2, avrLm3* and *avrLm9*) were present in all isolates. The isolates were polymorphic for *AvrLm1, AvrLm4*, *AvrLm5* and *AvrLm7* alleles, with virulence alleles at AvrLm1 and AvrLm4 loci and avirulence alleles at AvrLm7 and AvrLm5 loci predominant in populations. Virulent *avrLm7* isolates were found at only one site in Sweden. Approximately 90% of all isolates belonged to one of two races (combinations of avirulence alleles), Av5-6-7 (77% of isolates) or Av6-7 (12%). Eight races were identified, with four races at frequencies less than 1%. The study suggested that *Rlm6* and *Rlm7* are still effective sources of resistance against *L. maculans* in oilseed rape in Europe. The results are comparable to those of a similar survey done in France in autumn 2000 and 2001.

Introduction

Leptosphaeria maculans (Desm.) Ces. et de Not. is an important pathogen of oilseed rape world-wide and causes considerable losses in Australia, north America and western Europe (West et al., 2001; Fitt et al., 2006). Novel sources of resistance to *L. maculans* (phoma stem canker, blackleg), harbouring race-specific resistance genes initially proved to be very effective when introduced into Europe during the 1990s (Rouxel et al., 2003a). However, it became clear that *L. maculans* populations could change under selection, particularly when there was large-scale cropping of cultivars with resistance genes (Howlett, 2004). For example, in France, cultivars with the resistance gene *Rlm1* quickly became popular with growers and were grown over a large proportion of the oilseed rape area. This resulted in rapid adaptation of the *L. maculans* population and breakdown of *Rlm1* resistance (Ansan-Melayah et al., 1997; Rouxel et al., 2003a). Similarly in Australia, breakdown of

a single dominant resistance gene derived from *Brassica rapa* subsp. *sylvestris* was observed after only 2–3 years of widespread commercial cultivation (Li et al., 2003; Sprague et al., 2006).

In Europe, the life cycle of *L. maculans* begins each season when ascospores land on oilseed rape cotyledons and/or leaves in autumn. At present, both polygenic (quantitative trait loci based) resistance to *L. maculans* (Pilet et al., 1998) and at least nine specific resistance genes (*Rlm1–Rlm9*) are known in oilseed rape. *Rlm1-Rlm4, Rlm7* and *Rlm9* are from *Brassica napus* (Ansan-Melayah et al., 1998; Balesdent et al., 2001, 2002; Delourme et al., 2004), *Rlm5* and *Rlm6* from *B. juncea* (Chèvre et al., 1997; Barret et al., 1998; Balesdent et al., 2002, 2005) and *Rlm8* from *B. rapa* (Balesdent et al., 2002). These resistance genes correspond to *L. maculans* avirulence genes *AvrLm1-AvrLm9*. In this paper, we describe the structure of races of field populations of *L. maculans* across the main oilseed rape growing regions of Europe, including the UK, Poland, Germany and Sweden, by comparison to that described in a large-scale survey in France (Balesdent et al., 2006).

Materials and methods

The isolates characterised in this study originated from field experiments established in autumn 2002 at two sites in the UK 75 km apart (Boxworth, Cambridgeshire and Rothamsted, Hertfordshire) and one site in Germany (Teendorf, Lower Saxony), and field experiments established in autumn 2003 at two sites in Poland 450 km apart (Poznan, Wielkopolska and Pulawy, Lublin Region) and one site in Sweden (Svalöv, Skøne). The field plots were sown with the spring cultivar Drakkar as a "trap cultivar". Since this cultivar has no specific resistance genes against *L. maculans*, all isolates in the pathogen population are able to infect plants and cause phoma leaf lesions. One hundred leaves with phoma lesions were collected randomly at each site (Figure 1). An additional trap cultivar, the winter cultivar Darmor (with *Rlm9*), was used in Poland, as plants of cv. Drakkar were killed by the extremely low winter temperatures in this country.

Infected leaves with typical phoma spots were sent to INRA, Versailles or Institute of Plant Genetics, Polish Academy of Sciences, Poznan, where *L. maculans* was cultured from pycnidia on the lesions. Small fragments of leaves with lesions were placed in Petri dishes containing wetted tissue paper and incubated for 2–3 days to induce pycnidia to exude conidia. The conidia produced from these pycnidia were transferred to antibiotic-amended Campbells V8 agar medium (100 mg l^{-1} streptomycin sulphate and 50 mg l^{-1} ampicillin) using a sterile needle. After two or three subcultures, hyphal tips that were free of bacterial and fungal contaminants were transferred to V8 agar medium and kept at ~22 °C for 12–14 days under alternating 12 h white/12 h near-UV light. Conidia of individual isolates of *L. maculans* were suspended in sterile double-distilled water; the suspensions were adjusted to a concentration of 10 conidia ml^{-1} and stored at −20 °C for further analysis.

Leptosphaeria maculans races were identified by inoculating each isolate onto cotyledons of a set of nine differential cultivars/lines (Balesdent et al., 2005), which incorporates eight of the nine known resistance genes (Table 1). In addition to this differential set, cv. Darmor-MX, with the *Rlm6* resistance gene but not characterized at the *Rlm9* locus, was used (Table 1).

Two-week old seedlings of the differential set of cultivars/lines were inoculated with *L. maculans* isolates at the cotyledon stage. The plants were grown in plastic trays in an air conditioned glasshouse maintained with alternating 12 h periods of

Figure 1. Location of oilseed rape experimental sites sampled throughout Europe (1, Rothamsted, UK; 2, Boxworth, UK; 3, Teendorf, Germany; 4, Svalöv, Sweden; 5, Poznan, Poland; 6, Pulawy, Poland).

Table 1. List of the oilseed rape cultivars/lines used in a differential set to differentiate isolates of *Leptosphaeria maculans* in seedling pathogenicity tests

Cultivar	Resistance Gene
Columbus	*Rlm1* + *Rlm3*
Bristol	*Rlm2* + *Rlm9*
22-1-1	*Rlm3*
Jet Neuf	*Rlm4*
150-2-1	*Rlm5*
Darmor MX[a]	*Rlm6*
23-1-1	*Rlm7*
Goéland	*Rlm9*

[a]Not characterized at the *Rlm9* locus.

20–22 °C light/16–18 °C dark. Droplets (10 μl, 10^7 conidia ml^{-1}) of spore suspensions of each of four isolates were placed on half of a cotyledon (i.e. two isolates per cotyledon, two cotyledons per seedling) that had been wounded by puncturing with a sterile needle before inoculation. Each isolate was screened on 12 plants of every differential cultivar/line, with four isolates per seedling. Trays with inoculated plants were covered with a plastic propagator lid, kept in darkness for 48 h and then transferred to a growth chamber with alternating 12 h periods at 24 °C (light)/16 °C (dark) at 70% relative humidity. Host response was scored using a 0–6 scale, where a 1–3 score (small to medium sized necrotic spots) was recorded as a resistant reaction (i.e. the isolate was avirulent on the cultivar) and a 4–6 score (grey–green tissue collapse with or without production of pycnidia) as a susceptible reaction (i.e. the isolate was virulent) (Balesdent et al., 2001).

In total, 603 isolates were studied; 207 isolates from Poland, including 147 from west Poland (Poznan collected in 2003) and 60 from east Poland (Pulawy in 2003), 203 from the UK including 103 from East Anglia (Boxworth in 2002) and 100 from the south east (Rothamsted in 2002), 84 from Germany (Teendorf in 2002) and 109 isolates from Sweden (Svalöv in 2003). Most isolates from Poland (88.9%) were from cv. Darmor (184 isolates; 135 from west and 49 from east Poland) although 23 isolates were from cv. Drakkar (12 from west and 11 from east Poland). The data were analysed by analysis of variance. In addition, two indices were used to analyse diversity of populations. These were the Margalef index, which measures the richness in species (in this case, races) of a population, and the Simpson index of diversity, which also takes into account the evenness of races within each population (Magurran, 1988; Balesdent et al., 2006).

Results

Frequencies of avirulence alleles on a European scale

All isolates were virulent on cv. Bristol (*Rlm2*), line 22-1-1 (*Rlm3*) and cv. Goéland (*Rlm9*). Thus, the avirulence alleles *AvrLm2, AvrLm3* or *AvrLm9* were absent from the 603 isolates tested (Figure 2). In contrast, all isolates were avirulent (possessed the *AvrLm6* allele) on Darmor-MX (*Rlm6*). Similarly, 99.5% of isolates possessed the *AvrLm7* allele, with only three isolates from the Swedish site virulent at the AvrLm7 locus. Three avirulence loci (AvrLm1, AvrLm4 and AvrLm5) were polymorphic, with most isolates avirulent at the AvrLm5 locus (86%), 8.4% of isolates avirulent at the AvrLm1 locus and 2.3% of isolates avirulent at the AvrLm4 locus.

Differences between countries for individual avirulence alleles

Frequencies of alleles *avrLm2, avrLm3, AvrLm6* and a*vrLm9*, which were fixed in the *L. maculans* population, did not differ between the different countries. The remaining avirulence alleles studied were polymorphic. Frequencies of the avirulence alleles ranged from 1.2% (Teendorf, Germany) to 17.6% (Rothamsted, UK) for *AvrLm1*, 0% (Svalöv, Sweden and Poznan, Poland) to 6.8%

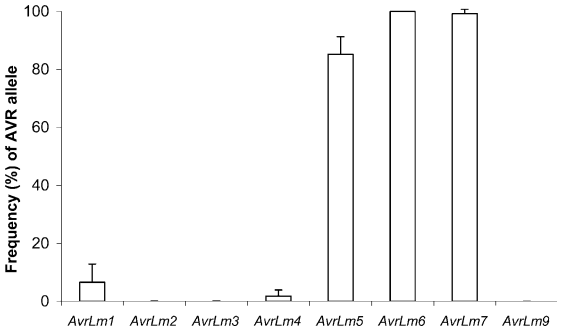

Figure 2. Mean frequency (%) of avirulence alleles in isolates from *Leptosphaeria maculans* populations sampled by isolating from phoma leaf spots on crops in the UK, Germany, Sweden or Poland. Error bars show standard deviation of AVR allele frequencies between experiment sites.

(Rothamsted, UK) for *AvrLm4* and 76.9% (Svalöv, Sweden) to 91.5% (Pulawy, Poland) for *AvrLm5* (Figure 3). Two avirulence loci, AvrLm1 and AvrLm5, were polymorphic at all experiment sites, with significant differences between sites for both alleles ($P = 0.008$ and $P = 0.026$, respectively). Amongst isolates at four sites, the AvrLm4 locus was polymorphic, but the *AvrLm4* allele was absent in isolates from west Poland and Sweden. *AvrLm7* was polymorphic at only one site (Svalöv, Sweden) where *avrLm7* isolates were observed at a low frequency (three isolates).

There were differences between the populations from the two sites sampled in the UK, with approximately twice the frequency of avirulence alleles *AvrLm1* and *AvrLm4* at Rothamsted, Hertfordshire as at Boxworth, Cambridgeshire (Figure 3). The populations of *L. maculans* from the UK and from east Poland contained more avirulence alleles than those from other sites, especially for *AvrLm1* and *AvrLm4*. The proportions of *AvrLm1* and *AvrLm4* alleles in German isolates were less than for the UK sites. In Poland, there were fewer isolates with *AvrLm1* or *AvrLm4* than at UK sites. The mean frequency of the *AvrLm1* allele for two sites in Poland was 9.7%, whereas for the two UK sites it was 14%. For *AvrLm4*, the frequencies were 1.5% for Poland and 4.8% for the UK. When the two sampling sites from Poland were compared, Pulawy (east) had more isolates (12%) with the avirulence allele *AvrLm1* than Poznan (west) (7%). At Poznan, no isolates with the *AvrLm4* allele were found. The frequency of isolates with *AvrLm1* and *AvrLm4* alleles at Pulawy resembled that at Boxworth, UK, with a slightly higher frequency of isolates with *AvrLm1* (12.4% at Pulawy, 10.4% at Boxworth) and an identical frequency of *AvrLm4* (2.9% at each site). The proportion of isolates containing the *AvrLm5* allele ranged from 76.9% in Sweden to 89.7% in Poland. The mean number of avirulence alleles differed between sites ($P = 0.015$). The lowest frequency was at Svalöv, Sweden (2.8%) and the highest at Pulawy, Poland (3.1%).

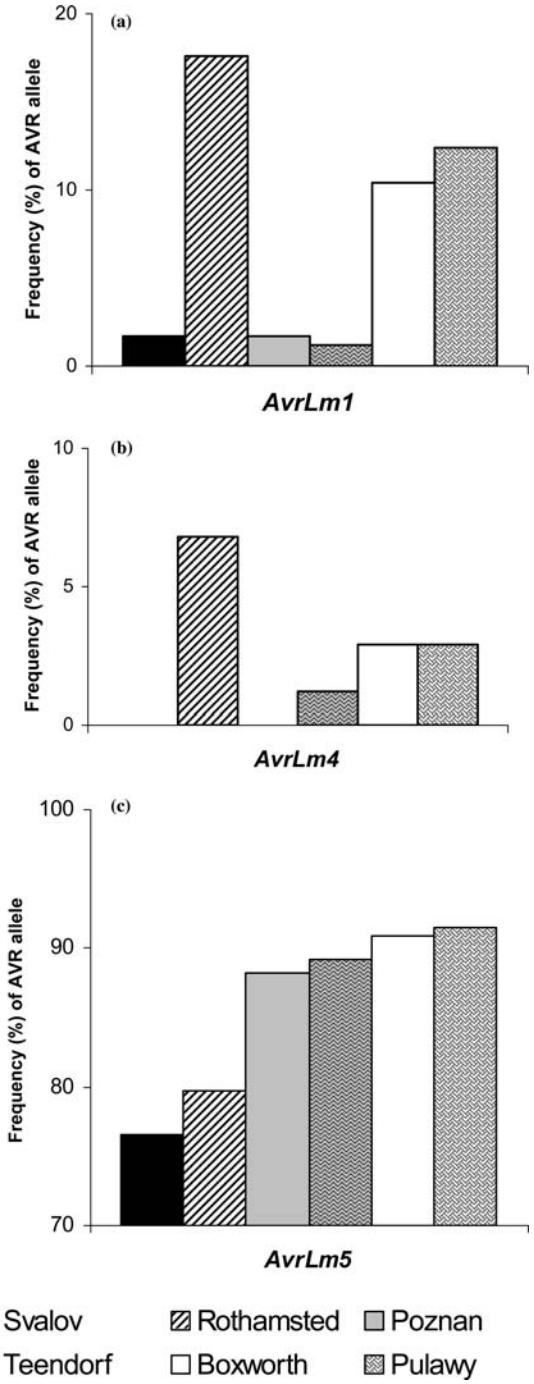

Figure 3. Differences in frequency (%) of avirulence allelles *AvrLm1* (a), *AvrLm4* (b) and *AvrLm5* (c) in field populations of *Leptosphaeria maculans* isolated from phoma leaf spots on leaves of oilseed rape at six sites across Europe.

Race structure on a European scale

Theoretically, nine avirulence genes can produce 512 races, defined as different combinations of virulence/avirulence alleles. However, only eight allele combinations were found in our survey. The most frequent race at all locations (465 isolates out of 603), with the combination of alleles *avrLm1-avrLm2-avrLm3-avrLm4-AvrLm5-AvrLm6-AvrLm7*

-*avrLm9*, was race Av5-6-7-(8) using the terminology proposed by Balesdent et al. (2005). The next most frequent race was Av6-7-(8) (78 isolates). Altogether, the two most frequent races accounted for 90% of all isolates and the other six races had very low frequencies. There were 39 isolates of race Av1-5-6-7-(8), with *AvrLm1*, *AvrLm5, AvrLm6* and *AvrLm7* avirulence alleles. For the three most frequent races, there were differences between sites ($P = 0.003$, 0.044 and 0.047). There were eight isolates of race Av4-5-6-7-(8), five isolates of race Av1-6-7-(8) and three isolates each of races Av4-6-7-(8) and Av6-(8) (only *AvrLm6* allele found). There was only one isolate of the rarest race (frequency 0.2%) with the highest number of avirulence alleles, Av1-4-5-6-7-(8). The only avirulence allele present in all isolates was *AvrLm6* (Figure 2).

Difference in race structure between countries

The race Av5-6-7-(8) was predominant at all sites, with a frequency which ranged from 60% of the population at Rothamsted (UK) to 87% at Teendorf (Germany). The frequency of Av6–7-(8), the second most abundant race, varied from 7% at Pulawy (Poland) to 19% at Svalöv (Sweden). Race Av1-5-6-7-(8) was most frequent at Rothamsted, UK (15%) and present in Germany (1.2%) and Sweden (0.9%) at low frequencies. Other races were present at low frequencies, from 3.9% to 0.5%. Overall, differences between countries for major races were not as important as those for minor races, which were rare or absent at some sites but abundant at others [e.g. race Av1-5-6-7-(8)] (Table 2).

Only the three most frequent races [Av5-6-7-(8), Av6-7-(8), Av1-5-6-7-(8)] were observed at all sites. Races Av4-5-6-7-(8) and Av1-6-7-(8) were both found at four sites, including Boxworth and Rothamsted (UK). Race Av4-5-6-7-(8) was found in Germany and east Poland, but not west Poland or Sweden. In contrast, race Av1-6-7-(8) was present in west Poland and Sweden but not Germany or east Poland. Race Av4-6-7-(8) was found only at Rothamsted and Pulawy. The rarest race, with the highest number of avirulence alleles [Av1-4-5-6-7-(8)], was found only at Rothamsted (UK) (one isolate). The race with the smallest number of avirulence alleles [Av6-(8)] occurred only in Sweden (three isolates).

The most diverse population of isolates of *L. maculans* was at Rothamsted, with seven races including the unique race Av1-4-5-6-7-(8). Consequently, both the Margalef index and the Simpson index of diversity had the highest values at this site (Table 2). There were five races at Boxworth, Svalöv and Pulawy. Six different races were found in Poland; the three most frequent races were at both sites whilst the three rarer ones were present at one site each. The populations of *L. maculans* from west Poland and Germany were least diverse, with only four races.

Table 2. The frequency of *Leptosphaeria maculans* races on oilseed rape at different sites in the UK, Germany (D), Sweden (S) or Poland (PL)

Race [a]	Frequency (%)						
	Boxworth (UK)	Rothamsted (UK)	Teendorf (D)	Svalöv (S)	Poznan (PL)	Pulawy (PL)	Mean
Av5-6-7-(8)	77.9	60.2	86.8	76.0	81.4	80.0	77.1
Av6–7-(8)	8.8	16.3	10.8	19.3	11.6	6.6	12.2
Av1-5-6-7-(8)	9.4	14.7	1.2	0.9	6.5	10.0	7.1
Av4-5-6-7-(8)	2.9	3.9	1.2	0.0	0.0	1.7	1.6
Av1-6-7-(8)	1.0	2.0	0.0	0.9	0.5	0.0	0.7
Av4-6-7-(8)	0.0	2.0	0.0	0.0	0.0	1.7	0.6
Av6-(8)	0.0	0.0	0.0	2.9	0.0	0.0	0.5
Av1-4-5-6-7-(8)	0.0	0.9	0.0	0.0	0.0	0.0	0.2
Number of isolates	103	100	84	109	147	60	100
Number of races	5	7	4	5	4	5	5
Margalef index	0.86	1.30	0.68	0.85	0.60	0.98	1.09
Simpson diversity index	0.38	0.60	0.24	0.39	0.32	0.35	0.39

[a]Race nomenclature according to Balesdent et al. (2005). The numbers indicate the AvrLm locus for which the isolate is avirulent. The number in parentheses indicates that the corresponding locus (AvrLm8) has not been characterized.

Discussion

This study reveals similarities across Europe in the frequency of avirulence alleles and structure of *L. maculans* races. The results of this European survey are comparable to those of a similar survey in France (Balesdent et al., 2006). Both surveys used the same methodology and a similar differential set of oilseed rape genotypes. The French survey was done on *L. maculans* populations in autumn 2000 and 2001, whereas the European survey is of field populations sampled in autumn 2002 (UK, Germany) and 2003 (Poland, Sweden). The observation that no isolates of *L. maculans* possessed the avirulence alleles *AvrLm2, AvrLm3* or *AvrLm9* is consistent with the French survey, which found that virulence alleles *avrLm2* and *avrLm9* were fixed in the *L. maculans* population and more than 99% of isolates (out of 1787) had the *avrLm3* allele. Similarly, all isolates sampled during the European survey were avirulent on Darmor-MX (with *Rlm6*), which is consistent with the French results, where only one isolate was virulent at the avrLm6 locus (Balesdent et al., 2006).

However, there were some differences between the two surveys in proportions of avirulence alleles within the populations. For example, in the European survey *AvrLm1* and *AvrLm4* avirulence alleles were at frequencies of 8% and 2%, respectively, whereas in the French survey the proportions of isolates avirulent on *Rlm1* and *Rlm4* were greater (20% and 9%, respectively). There were differences between the surveys in sampling sites and seasons. The isolates in the French survey were mostly collected in autumn 2000, and another survey in France showed that a rapid decrease in frequency of the *AvrLm1* avirulence allele occurred over three seasons of intensive cultivation of cultivars with the *Rlm1* resistance gene (Rouxel et al., 2003a). It is likely that a similar decrease could have occurred in other European countries. For example in Sweden, the two most common cultivars, Capitol and Cadillac (both with *Rlm1*, MH Balesdent and X Pinochet, France, unpubl.), accounted for 55% of the oilseed rape area in 2002 and could have selected for *avrLm1* isolates.

The contrasting popularities of cultivars with different *Rlm* resistance genes probably explain the different ratios of avirulence alleles at different sites. In France, sites with frequencies of the *AvrLm1* allele $< 10\%$ were in regions with intensive cropping of oilseed rape. In the European survey, the popularity of oilseed rape cultivars with the *Rlm1* resistance gene explains the low frequency of *AvrLm1* allele. The frequency of the *AvrLm5* allele in populations was similar in the French (84%, Balesdent et al., 2006) and European surveys (86%). To our knowledge, the *Rlm5* resistance gene has not been used in commercial cultivars of oilseed rape. The detection of virulent *avrLm5* isolates may be explained by the widespread use of *B. juncea* (source of *Rlm5*) for the production of mustard or as a green manure. On a local scale, this could have selected against *AvrLm5* and increased the frequency of virulent *avrLm5* isolates.

The results from the European and French surveys are also similar for *AvrLm7*. In the European survey, 99% of isolates were avirulent (*AvrLm7*) with only three isolates from Sweden with the *avrLm7* allele. In the French survey, there was only one *avrLm7* isolate out of 1787 isolates sampled (Balesdent et al., 2006). Selection for *avrLm7* might have resulted from extensive cultivation of swedes (*Brassica napus* var. *napobrassica*), of which some genotypes possess the *Rlm7* resistance gene. However, swedes are cultivated on only 150–200 ha in Sweden at Kulla-halvön and Östergötland, 80 and 400 km away from the experimental site at Svalöv. Thus, occurrence of *avrLm7* at Svalöv is unlikely to be associated with cultivation of swede. However, new sources of resistance, including *Rlm7*, are more frequently found in the older oilseed rape cultivars than in new breeding material (Rouxel et al, 2003b). Old cultivars, some of which may possess the *Rlm7* resistance gene, were grown in Sweden for many years before the 1990s. This might explain the occurrence of *avrLm7* isolates in the Swedish *L. maculans* population.

It is significant that in all five countries studied (France, Germany, Poland, Sweden and the UK) several races had the virulence allele *avrLm5*. Thus, isolates with this virulence allele have been detected before the introduction of the corresponding *Rlm* gene into commercial cultivars. This finding highlights the problems associated with the use of new resistance sources with specific, major resistance genes. The widespread use of resistant cultivars or the unregulated introduction of new resistance genes may rapidly result in adaptation

of *L. maculans* to the increased selection and decrease the efficiency of the new resistance (Rouxel et al., 2003a; Howlett, 2004; Sprague et al., 2006). Resistance appears to be more durable if it is polygenic, as in cv. Jet Neuf (Pilet et al., 2001; Delourme et al., 2004). Another strategy for maintaining durability of resistance will be the careful, well-planned deployment of specific resistance genes in a "diversification" programme, where cultivars with known resistance genes are used in rotation (Aubertot et al., 2005).

The *L. maculans* population from Rothamsted (UK) had the greatest diversity, not only in terms of individual avirulence alleles, but also in terms of race structure. A number of oilseed rape cultivars and lines are grown annually on Rothamsted field plots for experimental purposes. Between 1999 and 2002, for example, these included cultivars Apex, Bristol, Lipton, Capitol, Madrigal, Synergy, Jet Neuf, Shannon, Regina, Falcon and Pronto. The selection on the *L. maculans* population imposed by this genetically heterogeneous host background may explain the diversity of avirulence alleles at this site. However, the European survey indicates that, despite different cropping ratios, growing conditions, oilseed rape cultivation practices and cultivars grown in each country, the race composition (for the three most common races) of the *L. maculans* population was similar in different countries.

Analysis of the populations from Poland suggests that wind dispersal of *L. maculans* ascospores and exchange of infected seed have played an important role in dissemination of the pathogen. Before the mid-1990s, a large proportion of the Polish oilseed rape area was sown with local cultivars and epidemics of phoma stem canker were not severe (Jedryczka et al., 1994, 1999a, b). During the mid-1990s, German and French cultivars were widely adopted in Poland and, after 2–3 seasons, a rapid increase in the incidence and severity of stem canker was observed. The Polish *L. maculans* race structure is currently very similar to that in western Europe, which suggests that the initial distribution of *L. maculans* might have been seed-borne. However, most seed production is done within Poland and only seed of new cultivars is likely to be imported. Thus, the Polish population might have been expected to have been the most diverse for number of avirulence genes present. Most isolates from Poland were collected from cv. Darmor, which has *Rlm9*. Therefore, only isolates with the *avrLm9* allele could infect these plants. However, of the 23 Polish isolates collected on cv. Drakkar, all had the *avrLm9* virulence allele, as in other European countries (Balesdent et al., 2005b). Therefore the Polish *L. maculans* population probably does not differ from populations in other countries with respect to the AvrLm9 locus. The increasing importance of phoma stem canker in Poland has also been connected with climate change associated with global warming (West et al., 2005). Widespread release of *L. maculans* ascospores in the autumn produces phoma leaf spots on crops before winter (West et al., 2002) and occurrence of mild winters decreases loss of infected leaves (Huang et al., 2005), thereby allowing the pathogen to colonise the stem and carry over to the next growing season. This hypothesis is examined in a detailed study of the Polish *L. maculans* and *L. biglobosa* populations (Jedryczka et al., 2004).

The little information available on resistance genes in current and historical oilseed rape cultivars (Ansan-Melayah et al., 1995, 1998; Balesdent et al., 2001, 2002; Rouxel et al., 2003b; Delourme et al., 2004, 2006) limits interpretation and understanding of changes and evolution of avirulence genes and race composition in *L. maculans* populations. The European survey suggests that the *Rlm6* and *Rlm7* resistance genes are likely to be effective sources of resistance against *L. maculans* in Europe. The example of the *Rlm1* resistance gene showed that a specific resistance operating at the cotyledon and leaf stage is sufficient to control stem canker epidemics, at least for a few seasons (Ansan-Melayah et al., 1997; Rouxel et al., 2003a; Sprague et al. 2005). Undoubtedly, such specific resistance will be of only short-term value unless combined with quantitative (polygenic) resistance or used in combination with other effective resistance genes. Numerous studies show virulence is achieved or lost through a set of small, highly conserved genetic events (Joosten et al., 1994; Bryan et al., 2000; Attard et al., 2002). Therefore, knowledge of race structure of a pathogen population before and after the introduction of a new resistance gene is essential for the good management of resistance to maintain its durability over several seasons. The recent survey of Balesdent et al. (2006) suggests that a sample of *ca.* 100–200 isolates is sufficient to draw general conclusions

about frequency of avirulence genes and race structure in *L. maculans* populations at a given site. However, this sample size may be too small to detect virulence alleles present at very low frequencies, which is usually the case when a new resistance gene is introduced in an area.

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References

Ansan-Melayah D, Balesdent MH, Buée M and Rouxel T (1995) Genetic characterization of *AvrLm1*, the first avirulence gene of *Leptosphaeria maculans*. Phytopathology 85: 1525–1529

Ansan-Melayah D, Balesdent MH, Delourme R, Pilet ML, Renard M, Tanguy X and Rouxel T (1998) Genes for race-specific resistance against blackleg disease in *Brassica napus* L. Plant Breeding 117: 373–378

Ansan-Melayah D, Rouxel T, Bertrandy J, Letarnec B, Mendes-Pereira E and Balesdent MH (1997) Field efficiency of *Brassica napus* specific resistance correlates with *Leptosphaeria maculans* population structure. European Journal of Plant Pathology 103: 835–841

Attard A, Gout L, Gourgues M, Kuhn ML, Schmit J, Laroche S, Ansan-Melayah D, Billault A, Cattolico L, Balesdent MH and Rouxel T (2002) Analysis of molecular markers genetically linked to the *Leptosphaeria maculans* avirulence gene *AvrLm1* in field populations indicates a highly conserved event leading to virulence on *Rlm1* genotypes. Molecular Plant-Microbe Interactions 15: 672–682

Aubertot JN, West JS, Bousset-Vaslin L, Salam MU, Barbetti MJ and Diggle AJ (2006) Improved resistance management for durable disease control: a case study of phoma stem canker of oilseed rape (*Brassica napus*) European Journal of Plant Pathology 114: 91–106

Balesdent MH, Attard A, Ansan-Melayah D, Delourme M and Renard M (2001) Genetic control and host range of avirulence towards *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*. Phytopathology 91: 70–76

Balesdent MH, Attard A, Kühn ML and Rouxel T (2002) New avirulence genes in the phytopathogenic fungus *Leptosphaeria maculans*. Phytopathology 92: 1122–1133

Balesdent MH, Barbetti MJ, Hua Li, Sivasithamparam K, Gout L and Rouxel T (2005) Analysis of *Leptosphaeria maculans* race structure in a world-wide collection of isolates. Phytopathology 85: 1061–1071

Balesdent MH, Louvard K, Pinochet X and Rouxel T (2006) A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. European Journal of Plant Pathology 114: 53–65

Barret P, Guérif J, Reynoird JP, Delourme R, Eber F, Renard M and Chèvre AM (1998) Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 2 : A 'to and fro' strategy to localise and characterise interspecific introgressions on *B. napus* genome. Theoretical and Applied Genetics 96: 1097–1103

Bryan GT, Wu KS, Farrall L, Jai Y, Hershey HP, McAdams SA, Faulk KN, Donaldson GK, Tarchini R and Valent B (2000) A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12: 2033–2045

Chèvre AM, Barret P, Eber F, Dupuy P, Brun H, Tanguy X and Renard M (1997) Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 1: Identification of molecular markers, chromosomal and genomic origin of the introgression. Theoretical and Applied Genetics 95: 1104–1111

Delourme R, Chèvre A-M, Brun H, Rouxel T, Balesdent MH, Dias JS, Salisbury PA, Renard M and Rimmer SR (2006) Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114: 41–52

Delourme R, Pilet-Nayel ML, Archipiano M, Horvais R, Tanguy X, Rouxel T, Brun H, Renard M and Balesdent MH (2004) A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. Phytopathology 94: 578–583

Fitt BDL, Brun H, Barbetti MJ and Rimmer SR (2006) World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). European Journal of Plant Pathology 114: 3–15

Howlett BJ (2004) Current knowledge of the interaction between *Brassica napus* and *Leptosphaeria maculans*. Canadian Journal of Plant Pathology 26: 245–252

Huang YJ, Fitt BDL, Jedryczka M, Dakowska S, West JS, Gladders P, Steed JM and Li ZQ (2005) Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*L. biglobosa*). European Journal of Plant Pathology 111: 263–277

Jędryczka M, Fitt BDL, Kachlicki P, Lewartowska E, Balesdent MH and Rouxel T (1999a) Comparison between Polish and United Kingdom populations of *Leptosphaeria maculans*, cause of stem canker of oilseed rape. Journal of Plant Pathology and Plant Protection 106: 608–617

Jedryczka M, Lewartowska E and Frencel I (1994) Properties of *Phoma lingam* (Tode ex Fr.) Desm. isolates from Poland.

I. Pathogenicity characterization. Phytopathologia Polonica 7: 71–79

Jedryczka M, Matysiak R and Graham K (2004) Lepto-net and SPEC - new projects to control the stem canker of oilseed rape in Poland. International Organisation for Biological Control Bulletin 27: 125–130

Jedryczka M, Rouxel T and Balesdent MH (1999b) Rep-PCR based genomic fingerprinting of a Polish population of *Leptosphaeria maculans*. European Journal of Plant Pathology 105: 813–823

Joosten MHAJ, Cozijnsen TJ and deWit PJGM (1994) Host resistance to a fungal tomato pathogen lost by a single base-pair change in an avirulence gene. Nature 367: 384–386

Li H, Barbetti MJ and Sivasithamparam K (2003) Breakdown of a *Brassica rapa* subsp. *sylvestris* single dominant blackleg resistance gene in *B. napus* rapeseed by *Leptosphaeria maculans* field isolates in Australia. Plant Disease 87: 752

Magurran AE (1988) Ecological Diversity and its Measurement, Princeton University Press, Princeton, New Jersey

Pilet ML, Delourme R, Foisset N and Renard M (1998) Identification of loci contributing to quantitative field resistance to blackleg disease, causal agent *Leptosphaeria maculans* (Desm.) ces et de Not., in winter rapeseed (*Brassica napus* L.). Theoretical and Applied Genetics 96: 23–30

Pilet ML, Duplan G, Archipiano M, Barret P, Baron C, Horvais R, Tanguy X, Lucas MO, Renard M and Delourme R (2001) Stability of QTL for field resistance to blackleg across two genetic backgrounds. Crop Science 41: 197–205

Rouxel T, Penaud A, Pinochet X, Brun H, Gout L, Delourme R, Schmit J and Balesdent MH (2003a) A ten year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. European Journal of Plant Pathology 109: 871–881

Rouxel T, Willner E, Coudard L and Balesdent MH (2003b) Screening and identification of resistance to *Leptosphaeria maculans* in *Brassica napus* accessions. Euphytica 133: 219–231

Sprague SJ, Balesdent MH, Brun H, Hayden HL, Marcroft SJ, Pinochet X, Rouxel T and Howlett BJ (2006) Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. European Journal of Plant Pathology 114: 33–40

West JS, Fitt BDL, Jedryczka M and Karolewski Z (2005) Evidence for the eastward spread of the aggressive oilseed rape pathogen *Leptosphaeria maculans* in Europe. Plant Protection and Plant Health in Europe: Introduction and Spread of Invasive Species; BCPC Symposium Proceedings, pp. 293–294

West JS, Jędryczka M, Leech PK, Dakowska S, Huang Y-J and Fitt BDL (2002) Biology of *Leptosphaeria maculans* (stem canker) ascospore release in England and Poland. International Organisation for Biological Control Bulletin 25: 21–30

West JS, Kharbanda PD, Barbetti MJ and Fitt BDL (2001) Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathology 50: 10–27