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Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*

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Summary

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• It has frequently been hypothesized that quantitative resistance increases the durability of qualitative (*R*-gene mediated) resistance but supporting experimental evidence is rare. To test this hypothesis, near-isogenic lines with/without the *R*-gene *Rlm6* introduced into two *Brassica napus* cultivars differing in quantitative resistance to *Leptosphaeria maculans* were used in a 5-yr field experiment.
• Recurrent selection of natural fungal populations was done annually on each of the four plant genotypes, using crop residues from each genotype to inoculate separately the four series of field trials for five consecutive cropping seasons. Severity of phoma stem canker was measured on each genotype and frequencies of avirulence alleles in *L. maculans* populations were estimated.
• Recurrent selection of virulent isolates by *Rlm6* in a susceptible background rendered the resistance ineffective by the third cropping season. By contrast, the resistance was still effective after 5 yr of selection by the genotype combining this gene with quantitative resistance. No significant variation in the performance of quantitative resistance alone was noted over the course of the experiment.
• We conclude that quantitative resistance can increase the durability of *Rlm6*. We recommend combining quantitative resistance with *R*-gene mediated resistance to enhance disease control and crop production.

Introduction

Crop protection against pathogens that cause epidemic diseases is a major asset for global food security and sustainable crop production. Using resistant cultivars remains the best method to grow a crop with limited pesticide applications and low production costs. Two main types of resistance are generally described. Quantitative resistance (QR) is usually controlled by multiple genetic factors (quantitative trait loci or QTL) (Lindhout, 2002; Stuthman *et al.*, 2007). It leads to a reduction in symptom severity and/or epidemic progress over time, which can sometimes result in high levels of protection. Quantitative resistance is usually less effective when environmental or plant tissue conditions are favourable to disease (Geiger & Heun, 1989; Zadoks, 1993). By

contrast, *R*-gene mediated resistance is often total and conferred by single dominant *R* gene. *R*-gene mediated resistance is under gene-for-gene recognition mechanisms (Flor, 1955) that trigger hypersensitive response (HR). Both types of resistance coexist according to the pathosystem in a number of crops, as well as in the corresponding wild genetic resources (Stuthman *et al.*, 2007).

Many breeding programs and strategies have been developed to improve cultivar resistance with the objective of resistance durability (Delourme *et al.*, 2006; Rimmer, 2006; Stuthman *et al.*, 2007) because genetic resistance is most useful for growers if it is durable. Johnson (1981) defined durable resistance as 'a resistance that remains effective during its prolonged and widespread use in an environment favourable to the disease'. This definition implies that

resistance durability can only be assessed retrospectively (i.e. after commercial use of resistant genotypes). Therefore, it does not allow predictive inferences, which are important to manage the construction and deployment of resistant cultivars. Resistance type (quantitative resistance vs *R*-gene mediated resistance) is often used as a surrogate – but not entirely adequate – predictor for durability. Indeed, *R*-gene mediated resistance is often isolate-specific and thus exerts a strong selection pressure on pathogen populations that adapt rapidly through selection and multiplication of virulent isolates. Pathogens with high evolutionary potential thus give the highest risk of sudden resistance breakdown (McDonald & Linde, 2002), resulting in a succession of 'boom and bust' cycles (Vanderplank, 1968), which has been observed in various agricultural pathosystems (e.g. Sprague *et al.*, 2006). Quantitative resistance is most often regarded as isolate-non specific and thus postulated to be more durable than *R*-gene mediated resistance (Lindhout, 2002; Stuthman *et al.*, 2007; Poland *et al.*, 2008). However, a number of examples demonstrate that *R*-gene mediated resistance can sometimes be long-lasting (Christ *et al.*, 1987), but also that quantitative resistance can be eroded by increased aggressiveness (i.e. quantitative pathogenicity; see Vanderplank, 1968) in pathogen populations faced for long periods with quantitative resistance (Andrivon *et al.*, 2007; Stuthman *et al.*, 2007). A recurrent concern for plant breeders and plant pathologists is thus to identify the best way to use resistance factors to construct cultivars with the highest resistance level and the best possible intrinsic durability, and deployment strategies for such resistant cultivars in space and time to maximize durability.

The question of resistance durability can be approached as a problem of adaptive response in pathogen populations to selection exerted by resistant hosts (McDonald & Linde, 2002). Strategies to maximize durability should therefore both limit the selection of the more pathogenic variants of the pathogen and reduce pathogen population sizes (Mundt *et al.*, 2002). We postulate that one of the strategies likely to achieve this is to introduce major resistance gene (*R* genes) into cultivars with high levels of quantitative resistance, with a triple expected effect: to enhance the disease control provided by quantitative resistance by using *R* genes to control all avirulent fractions of the pathogen population; to limit selection for virulent isolates, as quantitative resistance slows down the rate of epidemic development and thus decreases the severity of the disease and the effective population size; and to maintain a satisfactory level of protection when the *R* gene is finally overcome.

The expected benefits of combining *R*-gene mediated resistance and quantitative resistance in a single cultivar have been investigated using mathematical models of pathogen evolution (Kiyosawa, 1982; Pietravalle *et al.*, 2006) but rarely confirmed experimentally. A recent paper, using successive artificial reinoculations of known viral isolates under

controlled conditions, showed delayed emergence of virulent variants on hosts combining *R*-gene mediated resistance and quantitative resistance relative to hosts with *R*-gene mediated resistance alone (Palloix *et al.*, 2009). This paper describes work to investigate experimentally the ability of a combination of an *R* gene and quantitative resistance in a single cultivar to increase the durability of the R gene by delaying the selection of virulent isolates in natural fungal populations under field conditions. We also compared the potential for evolution of pathogen populations to render ineffective the resistance conferred by an *R* gene or by quantitative resistance alone. The *Brassica napus–Leptosphaeria maculans* pathosystem and the multiyear recurrent scheme described by Brun *et al.* (2000) were used to test the hypothesis.

Materials and Methods

The pathosystem *Brassica napus–Leptosphaeria maculans*

Leptosphaeria maculans is a heterothallic ascomycete causing phoma stem canker of oilseed rape, a disease of worldwide importance (Fitt *et al.*, 2006). The fungus survives on infected crop residues for several years and produces both sexual and asexual fruiting bodies (pseudothecia and pycnidia, respectively). Ascospores are discharged over several months (mainly in autumn and winter in Europe) from pseudothecia formed on residues, and can spread the pathogen from field to field (West *et al.*, 2001; Fitt *et al.*, 2006). Conidia constitute the secondary inoculum, which contaminates neighbouring plants by rain splash (Travadon *et al.*, 2007). Infection by either ascospores or conidia causes leaf lesions, from which the fungus systemically reaches the stem base where it initiates crown canker (Hammond & Lewis, 1987). The predominance of ascospores produced on residues every year in the primary inoculum explains the high genetic variability observed in most populations of *L. maculans* (Hayden & Howlett, 2005).

Two kinds of resistance are described in *Brassica napus*. There is *R*-gene mediated resistance, caused by a set of *Rlm* genes (Delourme *et al.*, 2006; Huang *et al.*, 2006a). *Leptosphaeria maculans* populations adapt rapidly to such a resistance, so that newly deployed resistant cultivars lose their effectiveness only 3–4 yr after their release (Rouxel *et al.*, 2003; Sprague *et al.*, 2006). By contrast, quantitative resistance operates during the symptomless growth of the pathogen along leaf petioles and in stem tissues (Huang *et al.*, 2009) and cannot be assessed before spring, when it decreases the severity of stem base cankers (Delourme *et al.*, 2006). While both major gene-mediated and quantitative resistance against *L. maculans* may operate in a similar manner at the molecular level (Staal *et al.*, 2008; Persson *et al.*, 2009), in *Brassica napus*, at the phenotype level, they appear

to operate in different tissues at different stages during the process of disease development (Huang *et al.*, 2006a, 2009).

Near-isogenic lines of *Brassica napus* with/without *Rlm6*

A highly effective resistance introgressed from *Brassica juncea* into *B. napus* through interspecific crosses (Chèvre *et al.*, 1997) segregates as a single gene called *Rlm6* and operates against many European isolates of *L. maculans* in both controlled and field conditions (Somda *et al.*, 1996). Nevertheless, a multiyear field experiment has demonstrated that this resistance is not durable when introgressed into a highly susceptible background: recurrent selection of natural *L. maculans* populations on this genotype, called 'MX', leads to a rapid increase in the frequency of virulent isolates (Somda *et al.*, 1999; Brun *et al.*, 2000). The French National Institute for Agricultural Research (INRA) has thus decided not to release for commercial use the improved lines carrying *Rlm6*, but to keep them as research tools. Consequently, a large proportion of *L. maculans* populations in France and the rest of Europe still contain avirulent *AvrLm6* isolates (Balesdent *et al.*, 2006; Stachowiak *et al.*, 2006), which allows work to study the selection exerted on *L. maculans* populations by *Rlm6* in different genetic backgrounds. There is evidence that *Rlm6* operates in *B. napus* against *L. maculans* soon after penetration of leaf stomata by the pathogen, preventing growth from leaf to stem tissues (Huang *et al.*, 2006a).

The 'MX' line (spring type) was used as the progenitor to introduce *Rlm6* into winter-type oilseed rape cultivars Samouraï giving the SamouraïMX line (Chèvre *et al.*, 1997), cv. Darmor, which carries several quantitative trait loci (QTLs) that give it good quantitative resistance to *L. maculans* (Pilet *et al.*, 1998) and the susceptible cv. Eurol, to generate the DarmorMX and EurolMX lines, respectively. 'Eurol' carries the resistance genes *Rlm2* and *Rlm3* and 'Darmor' carries *Rlm9*; *L. maculans* populations in France are 100% virulent against these three genes (Balesdent *et al.*, 2006). 'EurolMX' was obtained by crossing 'SamouraïMX' with 'Eurol', followed by five backcrosses to 'Eurol' and nine selfing generations. Similarly, 'DarmorMX' was obtained by crossing 'SamouraïMX' with 'Darmor', three backcrosses to 'Darmor' and two to four selfing generations. Both 'DarmorMX' and 'EurolMX' were selected using molecular assisted selection on backcross and selfing generations (Chèvre *et al.*, 1997); homozygous lines were confirmed by cotyledon tests before seed multiplication. There is evidence that the QTL associated with quantitative resistance against *L. maculans* that are present in 'Darmor' and not 'Eurol' operate in *B. napus* stem tissues to slow down colonization and stem canker formation (Huang *et al.*, 2009). Therefore, for the purposes of this paper, 'Eurol' and 'Darmor' will be regarded as providing a susceptible background and a quantitative resistant background, respectively, for the *Rlm6*-mediated resistance.

Design of the 5-yr field experiment

Two pairs of near-isogenic lines (NILs), 'Eurol'/'EurolMX' and 'Darmor'/'DarmorMX' were included in all field trials. The susceptible cv. Eurol was useful as a common control, to compare severity of stem canker epidemics between trials. The durability experiment was established in Brittany (western France) during five consecutive cropping seasons (2002/2003 to 2006/2007). It began with an initial trial inoculated with a local pathogen population, which simulates the first year of cultivation of cultivars with a new resistance gene. It was followed by four separate 4-yr field trials (PHO1, PHO2, PHO3 and PHO4), each corresponding to recurrent selection of the *L. maculans* populations by one of the four genotypes (Fig. 1). Each series of trials simulates consecutive years of commercial cultivation of one oilseed rape genotype in adjacent fields. Temperature and rainfall data were recorded daily at the INRA Le Rheu site, within 10 km of the trials.

Inoculum production One oilseed rape stem base including tap root (*c.* 30 cm long) was the unit of inoculum and was described as a residue. Residues were uprooted at random from plots in June before harvest and 40 plants per genotype taken from three central rows of each plot (i.e. 80 plants per genotype per block) were scored for stem canker severity. Residues of each genotype were then stored separately outdoors on permeable canvas sheets during July and August to allow maturation of *L. maculans* pseudothecia and favour ascospore production.

Field plot design and inoculation All four genotypes were sown in early September, in all trials in a randomized block design with four blocks, with 2 m-wide paths between blocks. Each NIL was sown in two adjacent plots per block to ensure that there were enough stem base residues to inoculate the trials in the following cropping season. Individual plots measured 1.5×4 m and included five rows of plants. In the initial trial (2002/2003), all plots were inoculated with two residues m^{-2} of oilseed rape comprising, in equal proportions, residues of susceptible cultivars Samouraï, Shogun, Glacier and Lirabon highly infected in the previous cropping season by a natural local *L. maculans* population.

The PHO1, PHO2, PHO3 and PHO4 series were done from harvest years 2004 to 2007 at sites at least 1 km away from each other to avoid cross-contamination. Each trial was inoculated 2–3 wk after sowing by scattering two residues m^{-2} collected from plots of the appropriate genotype in the corresponding series at the end of the previous season (Fig. 1):
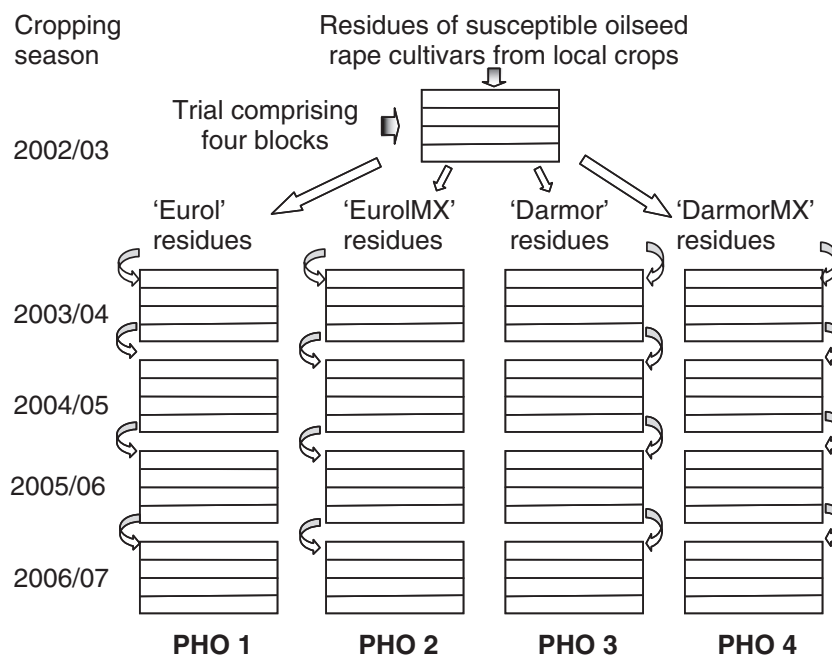
Fig. 1 Diagram illustrating the arrangement of a 5-yr experiment to assess the durability of *Rlm6* (MX) resistance gene to *Leptosphaeria maculans* (phoma stem canker) introduced into winter oilseed rape cultivars either with susceptible ('Eurol') or with quantitative resistance ('Darmor') backgrounds over the period 2002–2007. The experiment started in the autumn of the 2002/2003 cropping season with a field trial infested with highly infected residues of four susceptible cultivars uprooted from nearby crops. In subsequent cropping seasons it was subdivided into PHO1, PHO2, PHO3 and PHO4, each comprising a series of trials, separated each cropping season by *c.* 1 km from each other and done from the 2003/2004 until the 2006/2007 cropping season. Each series was inoculated each autumn with residues of 'Eurol' (PHO1) or 'EurolMX' (PHO2) or 'Darmor' (PHO3) or 'DarmorMX' (PHO4) taken randomly from the specific previous trial in the same series. Every trial was inoculated in the beginning of autumn with two pieces of stem base residue m^{-2} scattered over the soil surface. All lines ('Eurol', 'EurolMX', 'Darmor' and 'DarmorMX') were sown in each trial in a randomized block design comprising four blocks.

PHO1: 'Eurol' residues – evolution of a local pathogen population with little selection by host resistance;
PHO2: 'EurolMX' residues – evolution of the same population under selection by a major gene (*Rlm6*) introduced into a susceptible background;
PHO3: 'Darmor' residues – evolution of that population under selection by quantitative resistance;
PHO4: 'DarmorMX' residues, evolution of the population under joint selection by a major gene (*Rlm6*) and quantitative resistance.

To prevent an increase in isolates virulent against *Rlm6* in local *L. maculans* populations (and hence interference by virulent external inoculum), all remaining residues from plots sown to genotypes with *Rlm6* or from plots inoculated with 'EurolMX' or 'DarmorMX' residues were uprooted at the end of each cropping season and burned before the formation of pseudothecia.

Trials in the same series could be sown on a neighbouring part of the same piece of field but residues from the previous trial were always ploughed in before sowing the following one. All trials were surrounded by crops of winter barley.

Assessment of leaf lesions Disease incidence (% plants with at least one leaf lesion) and severity (number of leaf

lesions per plant scored) were assessed in each trial once in each cropping season, with the date of assessment (normally in November) depending on the development of leaf lesions. Leaf lesions were counted on both pairs of NILs, on all leaves of each individual plant assessed in a sample of 30 plants per block per genotype. To obtain representative samples from each plot, three samples of 10 consecutive plants were taken from sites evenly distributed across each plot. Whenever possible, three blocks were assessed (90 plants in total) but sometimes two (2005: 60 plants in total) or all four blocks (2002: 120 plants in total) were assessed.

Stem canker assessment Stem canker incidence (% plants with stem base canker) and severity (DI, disease index) were assessed 2–3 wk before harvest (mid June). Eighty plants per genotype and per block were uprooted, and scored on a 1–6 scale (Aubertot *et al.*, 2004) for stem canker presence and severity, based on the extent of internal symptoms at the stem base of each plant. Stem canker incidence was calculated as the proportion of plants in classes 2–6 (i.e. including those showing even minute symptoms), and disease severity was assessed as DI, computed as a sum of weighted proportions of plants in each class: $DI = \Sigma_i (ni \times ci)/N$ [ni, number of plants in class

(i = 1….6); ci, weighting coefficient (0 for class 1, 1 for class 2, 3 for class 3, 5 for class 4, 7 for class 5 and 9 for class 6); N, total number of plants scored]. The temperature and rainfall data were fitted to the weather-based model of Evans *et al.* (2008) to predict the development of epidemics, including severity of canker at harvest, on cultivars with or without quantitative resistance to *L. maculans* in each cropping season.

Seedling pathogenicity tests to determine virulence frequencies in *L. maculans* populations

Isolates In autumn 2002, 50 single-ascospore isolates were obtained from the four susceptible cultivars used as inoculum in the initial trial. In the following cropping seasons, 25 single-ascospore isolates per genotype were recovered every autumn (2003–2005) from 'Eurol', 'EurolMX', 'Darmor' or 'DarmorMX' residues used as inoculum. Single ascospores were obtained by placing a small piece of stem tissue bearing pseudothecia on the cover of a Petri dish, over water agar (20%) supplemented with streptomycin sulphate (0.1 g l^{-1}), for 12–18 h. Single ascospores deposited onto the medium were cut out individually, under a binocular microscope, with a very sharp glass needle and transferred to malt agar (20%, 20%) supplemented with streptomycin sulphate (0.1 g l^{-1}). Only one isolate per residue (plant) was assessed for virulence at seven *Avr* loci using cotyledon tests in controlled conditions. Sometimes it proved difficult to obtain residues of 'EurolMX' or 'DarmorMX' with pseudothecia. In 2003, there were only 14 and 10 residues with pseudothecia for 'EurolMX' and 'DarmorMX', respectively, from > 50 residues assessed per genotype. For 'DarmorMX', there were nine and seven isolates collected in the autumn of 2004 and 2005, respectively. Moreover, pseudothecia of other species, mainly *Leptosphaeria biglobosa* (Shoemaker & Brun, 2001) and *Fusarium* spp., and pycnidia (visually similar to pseudothecia) were also present on these 'MX' lines, further reducing the number of isolates of *L. maculans* recovered from these genotypes.

In 2004/2005, the composition of the overall inoculum received by PHO1 and PHO2 trials was assessed. This was the inoculum from residues of either 'Eurol' or 'EurolMX' and from external airborne ascospores. Thus, 'Drakkar' (without any known major resistance genes) was sown in autumn 2004 in PHO1 and in PHO2 trials. Ten leaves of 'Drakkar' with leaf lesions were sampled on 10 separated plants per block from three blocks each of PHO1 and PHO2. One pycnidial isolate per leaf lesion and per plant (i.e. 30 isolates per trial) was transferred to malt agar and included in the seedling pathogenicity tests. In 2004/2005 natural inoculum (i.e. from external airborne ascospores) was also investigated in a trial sown with 'Drakkar' without using any crop residue inoculum. This trial was established

at least 4 km away from any of the four trial series. Isolates were obtained and assessed according to the same procedure.

Seedling pathogenicity tests The differential host set comprised seven genotypes: 'MT29' (*Rlm1, 9*), 'Eurol' (*Rlm2, 3*), line '22.1.1' (*Rlm3*), 'Falcon' (*Rlm4*), line '150.2.1' (*Rlm5*), 'EurolMX' (*Rlm2, 3, 6*), 'Darmor' (*Rlm9*), to identify virulence/avirulence alleles at *Avr* loci (*AvrLm1* to *AvrLm6* and *AvrLm9*) in each isolate. The protocol described in Chèvre *et al.* (2008) was then used for production of inoculum and assessment of avirulence profiles. Frequencies of the avirulence alleles and of different races (combinations of *AvrLm* genes) were calculated.

Statistical analysis

For each series and trial, for data inspection and presentation, the mean number of leaf lesions (severity) obtained per plant, the mean percentage (incidence) of plants with leaf lesions, the mean stem canker severity (DI), and the mean percentage (incidence) of plants with stem canker were calculated across the blocks sampled for each genotype. The data per cropping season and per trial were submitted to ANOVA including the effect of the genotypes and taking account of the blocks. Following ANOVA, the standard error of the difference (SED) between means was output and selected means for the genotypes were compared using the least significant difference (LSD) between means at the 5% level of significance.

A position and parallelism regression analysis was used to examine the trends in three variables (incidence and severity of leaf lesions, and stem DI) over cropping season. For each variable, this assessed the statistical significance of an overall linear trend and whether this trend was the same (i.e. parallel) but differently positioned (shifted) for the background ('Eurol' or 'Darmor') or the MX status (without/with *Rlm6*) or for both these factors (which would result in four parallel lines). Finally, the analysis was used to assess the significance of the trend being different for each or both of the factors, the latter situation resulting in four separate lines. The best (most parsimonious) model is found using *F*-tests to assess the additional variance accounted for by changing from a single line to parallel lines and then, if necessary, from parallel lines to separate lines.

The relationship between (DI) and the number of leaf lesions preceding this for 'Eurol' and 'EurolMX' and taking data from the initial trial (2002/2003) and PHO1 and PHO2 (2003/2004 to 2006/2007) was examined using a non-linear least squares regression, including assessment (*F*-tests) of whether separate curves were statistically significant for the two cultivars. The GENSTAT (11th edition; Lawes Agricultural Trust (Rothamsted Research) VSN International Ltd., Hemel Hempstead, UK) statistical system was used for all statistical analyses.

Results

Effects of quantitative and qualitative resistance on seasonal changes in composition of *L. maculans* populations

The avirulence alleles present in the natural *L. maculans* populations near Rennes, detected on 'Drakkar' in autumn 2004, were similar to those in the population in autumn 2002, at the start of the experiment, except for *Avrlm4* (Table 1). The alleles *AvrLm2*, *AvrLm3* and *AvrLm9* were never observed in *L. maculans* isolates. There was little difference between populations from different sources of debris in the frequencies of *AvrLm4* and *AvrLm5*, except for natural inoculum sampled from 'Drakkar' (Table 1). Isolates carried three or four *AvrLm* alleles out of the seven *AvrLm* alleles that could be detected with this differential host set. The greatest number of races per population was 11, in the initial inoculum (Table 2); the most frequent race was Av5–6.

There was no effect of quantitative resistance on the composition of *L. maculans* populations, with similar frequencies of avirulence/virulence alleles in *L. maculans* populations selected on 'Eurol' or 'Darmor' in PHO1 or PHO3 series. These frequencies fluctuated a little seasonally but were similar to those observed in the initial inoculum and in natural inoculum sampled with 'Drakkar' in 2004. By contrast, and as expected, the qualitative *Rlm6* resistance greatly affected the composition of *L. maculans* populations. Although few pseudothecia were observed on 'EurolMX' residues in autumn 2003 in PHO2, single ascospore isolates obtained from these residues were all virulent (a*vrLm6*) to *Rlm6* and the residues with pseudothecia increased thereafter. Similarly, few single ascospore isolates (7–10) could be recovered from 'DarmorMX' residues in PHO4. Frequencies of avirulence/virulence alleles were otherwise similar in populations collected from 'EurolMX' residues (PHO2) and from 'DarmorMX' residues (PHO4) (Table 1).

Surprisingly, the frequency of the avirulence allele *AvrLm1* had greatly increased at the same time as the frequency of the virulence allele *avrLm6* increased in autumn 2003 in PHO2 and PHO4 compared with PHO1 and PHO3. For example, its frequency increased from 31% in the initial population in autumn 2002 to 92% on 'EurolMX' residues, and remained high thereafter. The number of *L. maculans* races detected ranged from two to eight (Table 2). The most frequent races were Av5–6 in PHO1 and PHO3 and Av1–5 in PHO2 and PHO4. This reflects the selection against *AvrLm6* and for *AvrLm1* by the presence of *Rlm6*. Based on the expected proportion of recombination given a linkage distance between *AvrLm1* and *AvrLm6* of 6 cM (Fudal *et al.*, 2007), χ^2 tests on the observed frequencies of isolates having neither or both *AvrLm1* and *AvrLm6* compared with frequencies of isolates having one or other allele suggested a highly significant difference between observed and expected frequencies in all cases ($P < 0.001$, Table 2), with more isolates having one or the other allele than expected.

Less than 1% of leaf lesions on 'Eurol' were caused by isolates virulent against *Rlm6* in PHO1 and PHO3, whatever the cropping season, the genotype used as inoculum and the severity of stem canker (Table 3). By contrast, almost all leaf lesions on 'Eurol' were caused by virulent isolates in autumn 2003 in PHO2 and PHO4 with residues carrying *Rlm6*, indicating that few isolates came from external inoculum. During the period 2003–2006, the mean number of leaf lesions per plant on 'EurolMX' caused by virulent isolates remained stable in PHO4 compared with PHO2. It is surprising that the proportion of virulent isolates in the *L. maculans* population required to render *Rlm6* resistance ineffective at harvest 2005 was similar in autumn 2004, whether it was assessed by counting numbers of leaf lesions on 'EurolMX'/'Eurol' (34.7%) or by obtaining 23 isolates from leaf lesions on 'Drakkar' sown in PHO2 and analysing them in cotyledon pathogenicity tests (39.1%) (data not shown).

Development of leaf lesions and stem canker in the initial trial (2002/2003)

In the initial trial, there were large numbers of leaf lesions in autumn on 'Eurol' and 'Darmor', with 96% and 98% of plants with at least one leaf lesion and averages of 7.6 and 8.9 leaf lesions per plant, respectively. By contrast, 'EurolMX' and 'DarmorMX' had only 8% and 4% of plants with leaf lesions and averages of 0.1 and 0.04 leaf lesions per plant, respectively. These differences in leaf lesions between lines with *Rlm6* resistance against *L. maculans* and cultivars without it were reflected in differences in the incidence (data not presented) and severity of stem base cankers in June 2003. Stem cankers were severe on 'Eurol' (DI = 7.64) but very slight on 'EurolMX' and 'DarmorMX' (DI = 0.70 and 0.39, respectively). The severity of canker on 'Darmor' was intermediate (DI = 3.67).

Effects of quantitative resistance on seasonal changes in leaf lesions and stem canker ('Darmor' vs 'Eurol')

There was a clear relationship between the severity of stem canker shortly before harvest and the severity of leaf lesions in the previous autumn over the period 2002/2003 to 2006/2007 in 'Eurol' and 'EurolMX', with an increasing number of leaf lesions, up to four per plant, associated with increasing severity of stem canker (Fig 2). Above this threshold of four leaf lesions per plant in November, an increase in severity of phoma leaf lesions did not result in increased severity of stem canker; the relation was best described by an asymptotic exponential curve, with an

Table 1 Percentage of the avirulence allele at each of the four *Avr* loci in *Leptosphaeria maculans* populations sampled in autumn, either by obtaining single-ascospore isolates from basal stem oilseed rape residues of initial inoculum (2002/2003 cropping season) and of genotypes used as inoculum in PHO1 to PHO4, each comprising a series of trials in the following cropping seasons (2003/2004, 2004/2005, 2005/2006) or by obtaining single pycnidial isolates from leaf lesions on cv. 'Drakkar' (2004/2005).

Frequency (%) of avirulence allele in population[1]

AvrLm gene	Initial inoculum[2] 2002/2003	Natural inoculum[3] (Drakkar) 2004/2005	'EuroI' residues (PHO1) 2003/2004	2004/2005	2005/2006	'EuroIMX' residues (PHO2) 2003/2004	2004/2005	2005/2006	'Darmor' residues (PHO3) 2003/2004	2004/2005	2005/2006	'DarmorMX' residues (PHO4) 2003/2004	2004/2005	2005/2006
AvrLm1	31	9	21	24	28	**92**[4]	**96**	**92**	21	24	12	**70**	**89**	**57**
AvrLm4	18	0	11	12	16	23	4	4	21	8	8	20	0	0
AvrLm5	84	46	100	68	100	92	92	76	100	84	84	80	100	100
AvrLm6	**84**	**91**	**100**	**100**	**80**	0	0	0	**92**	**92**	**76**	10	0	0
Total no. of isolates tested	49	22	28	25	25	13	24	25	24	25	25	10	9	7

[1] Allele frequencies in isolates were assessed in controlled conditions on cotyledons of a differential host set able to detect seven avirulence alleles (*AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm5*, *AvrLm6*, *AvrLm9*). The frequencies of avirulent alleles *AvrLm2*, *AvrLm3* and *AvrLm9* were all 0%, so these data are not presented.
[2] Initial inoculum comprised the residues of susceptible oilseed rape cultivars Samouraï, Glacier, Shogun and Lirabon sampled locally after harvest in 2002.
[3] Cultivar Drakkar with no known *R*-genes was sown at least 4 km away from the closest of the trials PHO1 to PHO4, in a separate field trial with no oilseed rape residues present, to assess the frequencies of different alleles in natural inoculum near Rennes, France.
[4] The populations with the greatest frequencies of *AvrLm1* or *AvrLm6* alleles are shown in bold type.

Table 2 Percentage of each race in *Leptosphaeria maculans* populations sampled in autumn, either by obtaining single-ascospore isolates from basal stem oilseed rape residues of initial inoculum (2002/2003 cropping season) and of genotypes used as inoculum in PHO1 to PHO4, each comprising a series of trials in the following cropping seasons (2003/2004, 2004/2005, 2005/2006) or by obtaining single pycnidial isolates from leaf lesions on cv. Drakkar (2004/2005)

Frequency (%) of race in population[1]

L. maculans race	Initial inoculum[2] 2002/2003	Natural inoculum[3] (Drakkar) 2004/2005	'Eurol' residues (PHO1) 2003/2004	2004/2005	2005/2006	'EurolMX' residues (PHO2) 2003/2004	2004/2005	2005/2006	'Darmor' residues (PHO3) 2003/2004	2004/2005	2005/2006	'DarmorMX' residues (PHO4) 2003/2004	2004/2005	2005/2006
Av-[4]	0	0	0	0	0	0	0	0	0	0	4	0	0	0
Av1-	0	5	0	0	0	7	8	24	0	4	0	15	0	0
Av4-	0	0	0	0	4	0	0	0	0	4	4	0	0	0
Av5-	4	0	0	0	0	7	4	8	8	4	4	15	11	43
Av6-	10	41	0	24	0	0	0	0	0	0	4	0	0	0
Av1-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Av1-5-	8	5	0	0	12	57[6]	83	64	0	0	8	31	89	57
Av1-6-	0	0	0	4	0	0	0	0	0	8	0	0	0	0
Av2-5-	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Av4-5-	2	0	0	0	0	0	0	0	4	0	0	0	0	0
Av4-6-	2	0	0	4	0	0	0	0	0	0	0	0	0	0
Av5-6-	**48**	**46**	**68**	**44**	**48**	0	0	0	**56**	**64**	**72**	0	0	0
Av1-4-5-	0	0	0	0	0	21	4	4	0	0	0	0	0	0
Av1-5-6-	8	0	21	16	16	0	0	0	16	8	0	8	0	0
Av4-5-6-	8	0	11	4	16	0	0	0	12	4	0	8	0	0
Av1-4-5-6-	6	0	0	4	0	0	0	0	4	4	0	0	0	0
Weakly aggressive[5]	2	2	0	0	0	7	0	0	4	0	0	23	0	0
Number of races	11	4	3	7	5	5	4	4	7	8	7	6	2	2
Total no. isolates tested	50	22	28	25	25	14	24	25	25	25	25	13	9	7
Significance[7]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[1]Frequencies of races in populations were assessed by testing isolates in controlled conditions on cotyledons of a differential host set able to detect seven avirulence alleles (*AvrLm1, AvrLm2, AvrLm3, AvrLm4, AvrLm5, AvrLm6, AvrLm9*).
[2]Initial inoculum comprised the residues of susceptible oilseed rape cultivars Samouraï, Glacier, Shogun and Lirabon sampled locally after harvest in 2002.
[3]Cultivar Drakkar with no known *R*-genes was sown at least 4 km away from the closest of the trials PHO1 to PHO4, in a separate field trial with no oilseed rape residues present, to assess the frequencies of different alleles in natural inoculum near Rennes.
[4]*Av* is the denomination of *L. maculans* races according to the nomenclature of Balesdent *et al.*, 2006.
[5]Weakly aggressive isolates gave too few symptoms to be able to differentiate between virulent and avirulent isolates whatever the resistant host assessed.
[6]The most frequent race in each trial is in bold type.
[7]*P*-value for χ^2 test comparing observed frequencies of occurrence of Av1 and Av6 alleles in isolate populations to frequencies expected for two genes separated by a linkage distance of 6 cM (Fudal *et al.*, 2007).

Table 3 Seasonal changes in the percentage of leaf lesions caused by virulent (*avrLm6*) isolates of *L. maculans* assessed in autumn as the number of leaf lesions per oilseed rape plant on 'EurolMX' divided by the number on 'Eurol' in each trial

Trial	Genotype used as inoculum	Autumn	No. of leaf lesions per plant on EurolMX	No. of leaf lesions per plant on 'Eurol'	Leaf lesions EurolMX/ [no. of lesions Eurol] ×100[1]
Initial	Susceptible	2002	0.12	7.58	1.55
PHO1	Eurol	2003	0.01	4.46	0.22
		2004	0.00	2.21	0.00
		2005	0.00	15.52	0.00
		2006	–	–	–[2]
PHO2	EurolMX	2003	0.46	0.41	113.11
		2004	1.52	4.38	34.70
		2005	2.34	4.95	47.17
		2006	5.62	9.00	62.48
PHO3	Darmor	2003	0.01	3.94	0.25
		2004	0.01	4.06	0.25
		2005	0.02	4.29	0.35
		2006	–	–	–
PHO4	DarmorMX	2003	1.28	1.36	93.89
		2004	0.29	0.44	65.41
		2005	0.15	2.04	7.37
		2006	0.35	8.96	3.94

Seasonal changes in the percentage of leaf lesions caused by virulent (*avrLm6*) isolates of *Leptosphaeria maculans*.
[1]The mean numbers of leaf lesions per plant assessed once in autumn on at least 60 plants of 'EurolMX' (leaf lesions due to only virulent isolates *avrLm6*) and of 'Eurol' (leaf lesions caused by avirulent *AvrLm6* plus *avrLm6* isolates) in each trial were used to estimate the percentage (%) of leaf lesions caused by virulent isolates.
[2]Not assessed.

asymptote of 4.95 for DI as leaf lesions increased. By contrast, for 'Darmor' there was no relationship between severity of leaf lesions and stem base canker severity (data not presented).

By contrast with autumn 2002, the overall conditions in 2003 were less favourable for the development of leaf lesions and although the incidence of leaf lesions was similar (Fig. 3a), there were fewer lesions per plant on 'Eurol' and 'Darmor' (Fig. 4a) in PHO1 than in 2002. The position and parallelism regression analyses suggested that there was little effect of quantitative resistance on the incidence (Fig. 3) or severity (Fig. 4) of leaf lesions in autumn. The data for 'Eurol' and 'Darmor' were fitted by common lines within MX status (i.e. without/with *Rlm6*) for all four series over the period 2003–2006 for incidence (Fig. 3), and for three of the four series of trials for severity (Fig. 4). There was a trend towards a greater number of leaf lesions on 'Darmor' than 'Eurol' but the difference was significant ($P = 0.005$, *F*-test) only in PHO3 (Fig. 4c). However, the regressions showed a seasonal increase with time in the incidence and severity of leaf lesions on 'Eurol' and 'Darmor' in all four series over the period 2003–2006. For example, in PHO1 the incidence of leaf lesions on 'Eurol' increased from 71% (autumn 2004) to 100% (autumn 2005), and the average number of leaf lesions per plant increased from 2.2 in autumn 2004 to 15.5 in autumn 2005.

By contrast, the regression analyses showed significant ($P < 0.05$, *F*-tests) effects of quantitative resistance on severity of stem canker, with more severe canker on 'Eurol' than on 'Darmor' in all four series (Fig. 5). However, there was no difference between 'Eurol' and 'Darmor' in incidence of stem base canker except in the 2004/2005 cropping season (data not presented). The 2003/2004 cropping season was less favourable for the development of stem canker than the 2002/2003 season, with the severity of stem canker on 'Eurol' in (PHO1) half that in the previous season (Fig. 5a). These differences between the two cropping seasons were confirmed by the predictions for severity of stem cankers made from the weather data using the model of Evans *et al.* (2008) (data not presented). The regression analysis shows a seasonal increase with time in the severity of stem canker on 'Eurol' and 'Darmor' over the period 2003–2006 in (PHO1, Fig. 5a), (PHO2, Fig. 5b) and (PHO3, Fig. 5c). By contrast, there was no increase with time in DI in the series with 'DarmorMX' inoculum, with some canker on 'Eurol' and little on 'Darmor' (PHO4, Fig. 5d). 'Darmor' resistance was more effective against stem canker development when inoculum concentration was low, as in the cropping seasons 2003/2004 and 2004/2005, with DI = 3.63 and DI = 3.29 for 'Eurol' compared with DI = 1.51 and DI = 1.30 for 'Darmor' in PHO1. In addition, 'Darmor' resistance was effective against virulent populations selected
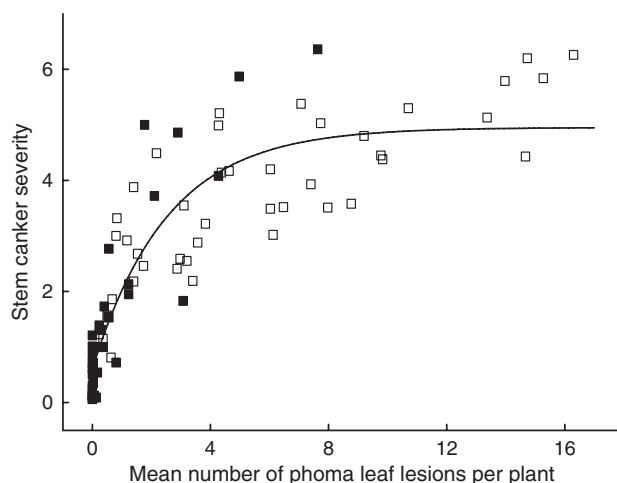
Fig. 2 Relationship between severity of stem canker assessed in June before harvest using a disease index (DI) (y) and severity of leaf lesions assessed the previous autumn (generally November) (x) caused by *Leptosphaeria maculans*, including data for 'Eurol' and 'EurolMX' *Brassica napus* lines from the initial trial in 2002/2003 and two subsequent series of trials PHO1 and PHO2, over the cropping seasons 2003/2004 to 2006/2007 (see Fig. 1). The DI ranges from 0 (all plants healthy) to 9 (all plants dead). The fitted curve is an asymptotic exponential equation $y = 4.95(1 - 0.88\exp(-0.394x))$ (SE 0.240, 0.032, 0.069), $R^2 = 79.9\%$, $s^2 = 0.706$, df = 79. There was no significant difference between the two cultivars (Eurol (open squares) and EurolMX (closed squares)) for the estimated parameters in the asymptotic exponential model ($P > 0.05$, F-tests), so a common model was used. There was no equivalent relationship found with 'Darmor'/'Darmor MX' data, which are not presented.

on 'EurolMX' residues in PHO2, as its DI was less than those of the other lines. Although canker severity was always less on 'Darmor' than on 'Eurol', the production of ascospores on residues of both cultivars caused similar seasonal patterns of disease in their respective series of trials PHO1 (Fig. 5a) and PHO3 (Fig. 5c). All lines assessed developed similar DI in the two series of trials. Moreover, DI on 'Darmor' in PHO3 increased similarly with time to that in PHO1.

Effects of qualitative resistance on seasonal changes in leaf lesions and stem canker ('EurolMX' vs 'Eurol'; 'DarmorMX' vs 'Darmor')

The regression analyses showed that both 'EurolMX' and 'DarmorMX' always developed fewer leaf lesions and less severe stem canker than their recurrent cultivars 'Eurol' and 'Darmor' in PHO1 (Figs 3a,4a,5a) and PHO3 (Figs 3c,4c,5c) series. This was also the case for the initial 2002/2003 trial ($P < 0.05$, LSD test). Moreover, there was no difference in severity of leaf lesions or stem canker between the genotypes with *Rlm6* 'EurolMX' and 'DarmorMX' in PHO1 and PHO3 series. However, a few leaf lesions were observed on these *Rlm6* lines in the 2002/2003

trial and subsequently in the PHO1 and PHO3 series, suggesting that isolates virulent against *Rlm6* existed at a low frequency in the local population. When 'EurolMX' or 'DarmorMX' residues were used as inoculum in the PHO2 and PHO4 series, respectively, there was a low incidence (Fig. 3b,d) and severity (Fig. 4b,d) of leaf lesions on plants assessed in autumn 2003, compared with those inoculated with 'Eurol' residues (PHO1, Figs 3a,4a). However, the DI had increased greatly on 'EurolMX' by the third year (2004/2005) of the PHO2 series, showing that the *Rlm6* resistance in this line was no longer effective. The incidence of plants with leaf lesions on 'Eurol', 'Darmor' and, to a lesser extent, on 'EurolMX' and 'DarmorMX in autumn from 2004 onwards was much greater than in autumn 2003 (Fig. 3b). As a consequence, there was little difference in DI the following summer between 'EurolMX' and 'Eurol' in this series from the 2004/2005 cropping season onwards (Fig. 5b). Although the epidemic was not severe in PHO2 in 2004/2005 because of the use of 'EurolMX' residues as inoculum, the severity of epidemics increased in subsequent seasons.

Effects of combining quantitative and qualitative resistance on seasonal changes in leaf lesions and stem canker ('DarmorMX' vs 'EurolMX')

The combination of quantitative and qualitative resistance in 'DarmorMX' was effective in control of leaf lesions and stem canker. For example, in PHO1 in the autumn of 2005, leaf lesions were abundant on all plants of 'Darmor' whereas only 1.7% of 'DarmorMX' plants had leaf lesions (Fig. 4a). Similarly, in that year, the incidence and severity of stem canker were considerably less on 'DarmorMX' (27% of plants with symptoms, DI = 0.27) than on 'Eurol' (incidence 86%, DI = 5.87) ($P < 0.05$, LSD test, for both incidence and severity). 'DarmorMX' resistance remained effective throughout the PHO4 series with few leaf lesions and little stem canker developing at least until the 5th year of the experiment (Figs 3d,4d,5d). It was difficult to find pseudothecia of *L. maculans* on 'DarmorMX' residues over the 4 yr of its use as inoculum, and most of the pseudothecia found on this line were of *L. biglobosa* (data not shown).

Discussion

This work provides experimental evidence, for an arable crop grown in successive seasons, that the combination of qualitative effective major gene and quantitative polygenic resistance to a pathogen both improves control of the disease and increases durability of the qualitative resistance. When combined with quantitative resistance in 'DarmorMX', the qualitative *Rlm6* resistance provided effective control of phoma stem canker until at least the 5th year, 2 yr longer than when it was deployed in a susceptible
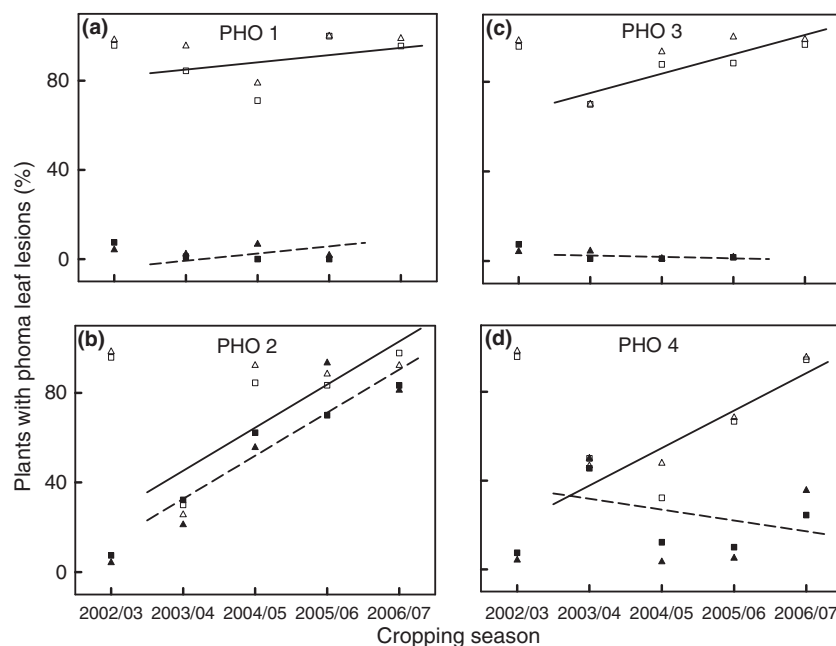
Fig. 3 Seasonal changes in the incidence (%) of winter oilseed rape plants with at least one leaf lesion (*y*) caused by *Leptosphaeria maculans* in autumn (generally November) over the period from 2002 to 2006 (*x*) on two pairs of *Brassica napus* near-isogenic lines (NILs) ('Eurol'/ 'EurolMX' and 'Darmor'/'DarmorMX') without or with the *Rlm6* resistance gene (MX lines) in different genetic backgrounds ('Eurol', suscepti-ble; 'Darmor', quantitative polygenic resistance). Data are from the initial 2002/2003 trial and then from 2003/2004 to 2006/2007, from four series of trials each recurrently inoculated in autumn either with stem residues of 'Eurol' (a, PHO1), 'EurolMX' (b, PHO2), 'Darmor' (c, PHO3) or 'DarmorMX' (d, PHO4) (see Fig. 1). Results for 2002/2003 are shown as means (SED = 3.73, df = 9, LSD (5%) = 8.43). Lines of best fit from a parallel lines regression analysis for 2003/2004 to 2006/2007 using the factors background (Eurol or Darmor) and MX status (with-out/with *Rlm6*) are shown with solid lines for 'Eurol' (open squares) and 'Darmor' (open triangles), and with dashed lines for 'EurolMX' (closed squares) and 'DarmorMX' (closed triangles); (a) two parallel lines, $y = 78.5 + 3.24x$ (SE. 6.17, 1.66) ('Eurol' and 'Darmor'), $y = -7.25 + 3.24x$ (SE 5.45, 1.66) ('EurolMX' and 'DarmorMX'), $R^2 = 94.7\%$, $s^2 = 108.7$, df = 35; (b) two parallel lines $y = 6.79 + 19.22x$ (SE 2.17, 8.30) ('Eurol' and 'Darmor'), $y = -5.79 + 19.22x$ (SE 2.17, 8.30) ('EurolMX' and 'DarmorMX'), $R^2 = 65.8\%$, $s^2 = 277.6$, df = 41; (c) two non-parallel lines $y = 57.7 + 8.64x$ (SE 6.81, 1.87) ('Eurol' and 'Darmor'), $y = 3.72 - 0.64x$ (SE 3.25, 9.68) ('EurolMX' and 'DarmorMX'), $R^2 = 94.8\%$, $s^2 = 102.9$, df = 34; (d) two non-parallel lines $y = 4.0 + 16.88x$ (SE 3.82, 13.9) ('Eurol' and 'Darmor'), $y = 41.5 - 4.88x$ (SE 3.82, 13.9) ('EurolMX' and 'DarmorMX'), $R^2 = 55.8\%$, $s^2 = 429.3$, df = 40.

background 'EurolMX'. This conclusion, based on direct experimental evidence for a field crop attacked by a fungal disease, is consistent with both theoretical predictions (Pietravalle *et al.*, 2006) and glasshouse work with a virus disease (Palloix *et al.*, 2009). Moreover, an effect of quantitative resistance has been suggested by Brun *et al.* (2000) to explain the superior durability of the *R* gene from *B. nigra* compared with that from *B. juncea,* introgressed into *B. napus* lines either with or without quantitative resistance, respectively. The benefit of this combination of *R*-gene mediated resistance and quantitative resistance may have involved a decrease in airborne inoculum concentration because the quantitative resistance limited the number of pseudothecia formed, and a decrease in number of leaf lesions because operation of *Rlm6* eliminated leaf lesions caused by the avirulent fraction of the pathogen population. This interpretation is supported by the small numbers of pseudothecia on residues and of leaf lesions observed on 'DarmorMX' throughout the years in PHO4 whereas no such restriction occurred on 'EurolMX' in PHO2. The

results of this experiment, in which only *c.* 40% of the *L. maculans* population was virulent in the third season when severe epidemics occurred on 'EurolMX' in PHO2, suggests that population size of virulent isolates is more important in determining the effectiveness of qualitative resistance than the frequency of virulent isolates. The considerable decrease (by comparison with 2002/2003) in severity of stem canker on all host genotypes (including the susceptible 'Eurol') in trials inoculated in 2003 and 2004 with inoculum from the 'MX' lines provides good evidence that *R* genes act by reducing pathogen population sizes. The rate of loss in effectiveness of resistance in 'EurolMX' in PHO2 was comparable to that of other major genes in susceptible backgrounds in commercial agricultural crops. Resistance derived from *Brassica rapa* var. *sylvestris* intro-duced into 'Surpass' was ineffective and associated with devastating epidemics within 2 yr after its cultivation over large areas in Australia (Sprague *et al.*, 2006). Resistance conferred by *Rlm1* introduced into several commercial cul-tivars in France was also rendered ineffective after 2–3 yr
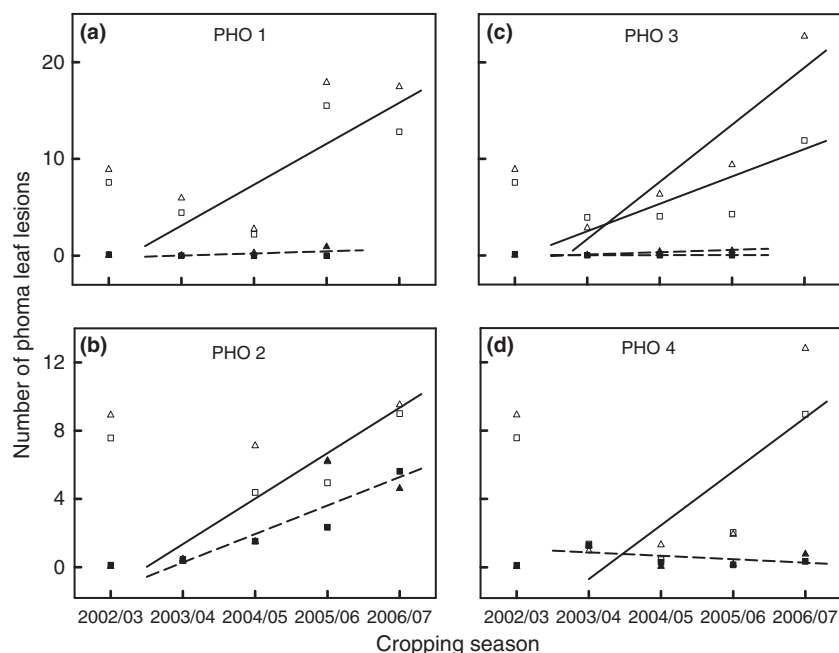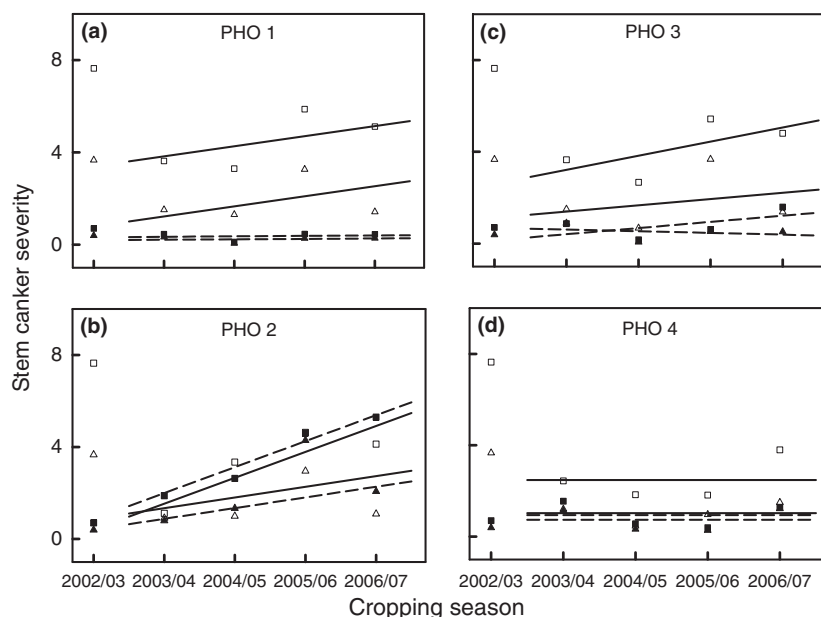
Fig. 4 Seasonal changes in the number of leaf lesions caused by *Leptosphaeria maculans* per plant (*y*) in autumn (generally November) over the period from 2002 to 2006 (*x*) on two pairs of *Brassica napus* near-isogenic lines (NILs) ('Eurol'/'EurolMX' and 'Darmor'/'DarmorMX') without or with the *Rlm6* resistance gene (MX lines) in different genetic backgrounds ('Eurol', susceptible; 'Darmor', quantitative polygenic resistance). Data are from the initial 2002/2003 trial and then from 2003/2004 to 2006/2007 from four series of trials recurrently inoculated in autumn either with stem residues of 'Eurol' (a, PHO1), 'EurolMX' (b, PHO2), 'Darmor' (c, PHO3) or 'DarmorMX' (d, PHO4) (see Fig. 1). Results for 2002/2003 are shown as means (SED 0.384, df = 9, LSD(5%) = 0.869). Lines of best fit from a parallel lines regression analysis for 2003/2004 to 2006/2007 using the factors background (Eurol or Darmor) and MX status (without/with *Rlm6*) are shown with solid lines for 'Eurol' (open squares) and 'Darmor' (open triangles), and with dashed lines for 'EurolMX' (closed squares) and 'DarmorMX' (closed triangles); (a) two non-parallel lines $y = -5.35 + 4.23x$ (SE 2.21, 0.61) ('Eurol' and 'Darmor'), $y = -0.44 + 0.22x$ (SE 3.14, 1.05) ('EurolMX' and 'DarmorMX'), $R^2 = 75.9\%$, $s^2 = 10.82$, df = 34; (b) two non-parallel lines $y = -3.98 + 2.67x$ (SE 1.17, 0.32) ('Eurol' and 'Darmor'), $y = -3.07 + 1.67x$ (SE 1.17, 0.32) ('EurolMX' and 'DarmorMX'), $R^2 = 73.1\%$, $s^2 = 3.02$, df = 40; (c) four non-parallel lines $y = -2.50 + 2.52x$ (SE 2.44, 0.67) ('Eurol'), $y = -11.74 + 6.41x$ (SE 2.44, 0.67) ('Darmor'), $y = 0.0 + 0.01x$ (SE 3.47, 1.17) ('EurolMX'), $y = -0.39 + 0.24x$ (SE 3.47, 1.17) ('DarmorMX'), $R^2 = 84.4\%$, $s^2 = 6.62$, df = 30; (d) two non-parallel lines $y = -6.99 + 3.15x$ (SE 1.38, 0.38) ('Eurol' and 'Darmor'), $y = 1.28 - 0.20x$ (SE 1.38, 0.378) ('EurolMX' and 'DarmorMX') $R^2 = 68.9\%$, $s^2 = 4.22$, df = 40.

(Rouxel *et al.*, 2003). This indicates that the results obtained in small field experiments can adequately reflect the mechanisms acting over large geographical scales during the commercial use of resistant cultivars.

There was no evidence that quantitative background resistance affected the surprising increase in the frequency of isolates avirulent (*AvrLm1*) against *Rlm1* associated with the increase in frequency of isolates that were virulent (*avrLm6*) against *Rlm6*; similar phenomena were observed in both PHO2 and PHO4. One explanation may be that selection for virulence at the *AvrLm6* locus was indirectly linked to selection against virulence at the *AvrLm1* locus (if selection for virulence *avrlm6* occurred in *Avrlm1* isolates, which represented 30% of the initial population). Another explanation might be that there is a greater fitness cost of virulence at both loci (*avrLm1/avrLm6* isolates) than of virulence at only the *AvrLm6* locus (*AvrLm1/avrLm6* isolates). It is likely that selection at the two loci may be linked, since they are located within 6 cM of each other on the *L. maculans* genome (Fudal *et al.*, 2007).

Furthermore, there is evidence of a fitness cost of virulence at the *AvrLm1* locus (Huang *et al.*, in press), as at the *AvrLm4* locus (Huang *et al.*, 2006b). Like evolution for virulence at the *AvrLm1* locus, evolution for virulence at the *AvrLm6* locus often involves deletion of the gene (Fudal *et al.*, 2007, 2009). It is important to understand which mechanism is responsible for the link between *AvrLm1* and *avrLm6*, because either mechanism can be exploited in the management of resistance against *L. maculans* but they require different breeding and cultivar deployment strategies. Negatively linked indirect selection offers the possibility of 'recycling' ineffective resistance genes in successive cycles of selection but requires that different *R* genes are kept separate in different cultivars for them to be effective (Andrivon & de Vallavieille-Pope, 1993). By contrast, fitness costs that favour monovirulent isolates against multivirulent isolates would suggest that pyramiding of several *R* genes in a single cultivar should be an effective strategy to control the disease (Stukenbrock & McDonald, 2008).

Fig. 5 Seasonal changes in the severity of stem canker caused by *Leptosphaeria maculans* assessed in June using a disease index (DI) (*y*) over the period from 2002 to 2006 (*x*) on two pairs of *Brassica napus* near-isogenic lines (NILs) ('Eurol'/'EurolMX' and 'Darmor'/'DarmorMX') without or with the *Rlm6* resistance gene (MX lines) in different genetic backgrounds ('Eurol', susceptible;'Darmor', quantitative polygenic resistance). Data are from the initial 2002/2003 trial and then from 2003/2004 to 2006/2007 from four series of trials recurrently inoculated in autumn either with stem residues of 'Eurol' (a, PHO1), 'EurolMX' (b, PHO2), 'Darmor' (c, PHO3) or 'DarmorMX' (d, PHO4) (see Fig. 1). The DI ranges from 0 (all plants healthy) to 9 (all plants broken down). Results for 2002/2003 are shown as means (SED 0.336, df = 9, LSD (5%) = 0.761). Lines of best fit from a parallel lines regression analysis for 2003/2004 to 2006/2007 using the factors background ('Eurol' or 'Darmor') and MX status (without/with *Rlm6*) are shown with solid lines for 'Eurol' (open squares) and 'Darmor' (open triangles), and with dashed lines for 'EurolMX' (closed squares) and 'DarmorMX' (closed triangles); (a) two pairs of parallel lines $y = 2.95 + 0.44x$ (SE 0.440, 0.115) ('Eurol'), $y = 0.35 + 0.44x$ (SE 0.440, 0.115) ('Darmor'), $y = 0.30 + 0.019x$ (SE 0.440, 0.115) ('EurolMX'), $y = 0.169 + 0.019x$ (SE 0.440, 0.115) ('DarmorMX') $R^2 = 85.3\%$, $s^2 = 0.53$, df = 58; (b) two pairs of parallel lines $y = -0.73 + 1.13x$ (SE 0.566, 0.151) ('Eurol'), $y = -0.061 + 0.47x$ (SE 0.566, 0.151) ('Darmor'), $y = -0.26 + 1.13x$ (SE 0.566, 0.151) ('EurolMX'), $y = 0.41 + 0.47x$ (SE 0.566, 0.151) ('DarmorMX'), $R^2 = 64.3\%$, $s^2 = 0.91$, df = 59; (c) four non-parallel lines $y = 1.99 + 0.62x$ (SE 0.640, 0.172) ('Eurol'), $y = 0.86 + 0.27x$ (SE 0.640, 0.172) ('Darmor'), $y = -0.13 + 0.27x$ (SE 0.640, 0.172) ('EurolMX'), $y = 0.76 - 0.08x$ (SE 0.640, 0.172) ('DarmorMX'), $R^2 = 73.4\%$, $s^2 = 0.79$, df = 57; (d) four parallel lines $y = 2.48$ (SE 0.182) ('Eurol'), $y = 1.03$ (SE 0.182) ('Darmor'), $y = 0.95$ (SE 0.182) ('EurolMX'), $y = 0.74$ ('DarmorMX') (SE 0.182), $R^2 = 46.3\%$, $s^2 = 0.53$, df = 60.

These results suggest that the quantitative resistance in 'Darmor' did not exert selection for specific virulence in the pathogen population, as the frequency of avirulence/virulence alleles in isolates from 'Darmor' residues did not change over the years. It was similar to that of the initial *L. maculans* population and to that recovered from the susceptible cultivar 'Eurol'. This may be explained by the fact that, while qualitative *R*-gene resistance operates in the leaf tissues (Huang *et al.*, 2006a), the quantitative resistance against *L. maculans* operates later in disease development, in leaf petiole and stem tissues (Fitt *et al.*, 2006; Huang *et al.*, 2009). Whereas quantitative resistance is generally considered to be durable (Poland *et al.*, 2008), there is experimental data to show that some pathogens can adapt to gradually erode such polygenic resistance and render it ineffective (Andrivon *et al.*, 2007). However, in our field experiment, the 'Darmor' resistance was still effective after 5 yr of recurrent selection on *L. maculans* populations. Nevertheless, it is important to widen the genetic base of quantitative

resistance against *L. maculans* in oilseed rape, as the quantitative resistance in European oilseed rape is mainly based on 'Jet Neuf' resistance. 'Jet Neuf' was cultivated as the only cultivar in France and the rest of Europe from 1977–1983, without erosion of its resistance (Delourme *et al.*, 2006). This lasting performance of resistance fits well with Johnson's (1981) definition of durable resistance. Nevertheless, 'Jet Neuf' also carries *Rlm4* and there are some avirulent *AvrLm4* isolates currently found in European populations of *L. maculans* (Stachowiak *et al.*, 2006). Therefore, the durability of 'Jet Neuf' resistance may have been derived from a combination of an efficient qualitative major gene with quantitative resistance.

Breeders may be reluctant to put in extra effort required to combine polygenic quantitative resistance with an effective major gene resistance in new cultivars, as there is no immediate benefit in breeding cultivars with only an effective major resistance gene. However, our results clearly show that this strategy benefits yields in the long-term by

extending the durability of resistance, so that new resistance genes can be effectively deployed for longer periods. Such durable resistance to crop diseases provided by combining quantitative and qualitative resistance can help to avoid the devastating 'boom and bust' cycles (Stukenbrock & McDonald, 2008) and hence make an essential contribution to global food security.

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