Enhanced Avirulence Management for Durable Control of Blackleg in Europe

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ABSTRACT

Avirulence management has been used for disease control in various crops, particularly against powdery mildew and rusts of cereals. New research is providing information on the population structure of *Leptosphaeria maculans* in different regions and the resistance genes present in the host crop, enabling diversification in canola planting to reduce the risk of epidemics occurring and delay the breakdown of single gene resistance. Integration of avirulence management with cultural practices that reduce disease severity and therefore reduce inoculum production for the following season, is important to enhance the level of control achieved and prolong resistance. This paper summarises recent research relating to this issue with an emphasis on the situation in western Europe, but many of the factors discussed also apply to other regions. The review draws extensively on papers presented at the stem canker durable resistance workshop, September 2004, INRA-Versailles, France.

Key words: Integrated Avirulence Management, Phoma Stem Canker, *Leptosphaeria maculans*, Diversification

INTRODUCTION

Qualitative resistance conferred by single major genes is race specific, can be extremely effective in controlling particular diseases, but can also be broken down rapidly by selection of a virulent pathogen population in just a few seasons (Rouxel et al. 2003; Li et al. 2003; Gladders et al., 2006; Sprague et al., 2006). This is particularly the case if the qualitative resistance is set in a host without a good background of quantitative (polygenic or partial or horizontal) resistance, although there are examples of qualitative resistance being effective over a long period (Eenink, 1976). The probability of breakdown of resistance is affected by the resistance types (qualitative or quantitative), pathogen reproduction (sexual or asexual), pathogen dispersal (airborne spores, rain-splashed spores, seed movement etc.), combination of other resistances (through pyramiding in one host, use of cultivar mixtures, or spatio-temporal rotation), the size of the pathogen population as affected by control methods, environmental conditions and the presence of alternative hosts. In the case of *Leptosphaeria maculans* (blackleg or phoma stem canker of canola or oilseed rape), the pathogen can be expected to have a high evolutionary potential and therefore a high ability to overcome resistance due to its reproductive strategy, effective spore dispersal and large population reservoir (McDonald and Linde, 2002). Aubertot et al. (2006) suggested the term Integrated Avirulence Management (IAM) to describe a strategy to reduce the selection pressure placed on pathogens and simultaneously decrease the general level of disease using cultural, biological or chemical methods in order to reduce further the potential for resistance to be broken. They illustrated this concept using a model to describe the effects of management practices on the control of *L. maculans*.

The idea of combining avirulence management with other forms of control is not new, but generally few other forms of control have been used simultaneously and systematically tested to provide experimental data. Crute (1984) and Wolfe (1984) both described the integrated management of diseases using cultivar resistance group and fungicides. For example, not only could different sources of resistance to powdery mildew be used in winter and spring barley, but also use of specific fungicides only on each crop type would isolate pathogen populations and limit the evolutionary potential of the pathogen. This idea was extended to a scheme in which cultivars would be rotated each season (as a changing cultivar mixture, but the use of different cultivars in adjacent fields could achieve a similar result), and either the fungicide mode of action used, or the cultivar protected by fungicide (other cvs untreated), could also be rotated, (Wolfe, 1984). In cases where different cultivar diversification groups are available, they can be rotated each season, with any one resistance group initially unprotected for a season or two,

followed by protection with a fungicide and finally being omitted from the rotation for one or more further seasons. Mundt et al. (2002) discussed a system combining host resistance, fungicides, host density and host genetic diversity and the effect on disease over different scales and the potential to increase durability.

New perspectives on major gene and quantitative resistance to *L. maculans* in oilseed rape were described by Delourme et al. (2006). This paper summarises our current understanding of the population structure of *L. maculans*, the use of host resistance against this pathogen and describes how IAM could enhance the level of control achieved and prolong the effectiveness of resistance sources.

PATHOGEN POPULATION AND HOST RESISTANCE

Understanding a local pathogen population and the selection pressure it is exposed to is complex as it is affected by races present on wild or other cultivated host plants, spore immigration from distant sources, and spore release from debris of previously cropped and current cultivars. Added to this can be the introduction to a region of new races in contaminated seed, although this is relatively rare. In Europe recently, a survey of *L. maculans* isolates was made to understand what races were present and their relative proportions, in order to improve the deployment of available resistance genes (Table 1; Stachowiak et al., 2006). No isolates had the virulence allele *avrLm6*, while three alleles (*avrLm*2, *avrLm*3, and *avrLm*9) were present in all of the 603 isolates collected. Isolates collected were polymorphic for the avirulence alleles *AvrLm*1, *AvrLm*4, *AvrLm*5 and *AvrLm*7 (the latter found only in Sweden). Only eight races were identified in terms of avirulence alleles present with only two races (Av5-6-7-(8) or Av6-7-(8)) comprising about 90% of the population and four races each comprising less than 1%. An earlier detailed survey in France (Balesdent et al., 2006), produced similar results, with isolates taken from plots of plants lacking any major resistance genes at 20 locations. Virulence alleles *avrLm*2 and *avrLm*9 were present in all isolates and conversely the frequency of *AvrLm*6 and *AvrLm*7 was over 99%. *AvrLm*1, *AvrLm*4, *AvrLm*5 and *AvrLm*8 were polymorphic, while *AvrLm*3 isolates were detected very rarely (<1% of all isolates). The French study tested for nine avirulence alleles rather than eight in the general European survey. In total, only 11 races were found, with Av5-6-7-(8) (virulent on *Rlm*1, *Rlm*2, *Rlm*3, *Rlm*4 and *Rlm*9) present in 65% of isolates. Balesdent et al. (2006) noted that there were differences between sites and in some cases some virulent races, for example those with *avrLm5*, were present despite the fact that the corresponding resistance gene had not been used in commercial oilseed rape crops. The low number of races can be explained by the low number of resistance sources used in most oilseed rape in Europe. In western Europe, cultivars grown extensively in recent years include Capitol (*Rlm*1), Bristol (*Rlm*2, *Rlm*9), Express (*Rlm*2), Mendel (*Rlm*3), Falcon (*Rlm*4), Synergy (*Rlm*4) and Apex (*Rlm*9) (Gladders et al, 2006).

Table 1. Frequency of *L. maculans* races on oilseed rape at different sites in the UK, Germany (D), Sweden (S) or Poland (P)

Race	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**
Nº of isolates	103	100	84	109	147	60	**603**
Nº of races	5	7	4	5	4	5	**8**(total)
Margalef index	0.86	1.30	0.68	0.85	0.60	0.98	**1.09**

Data indicate the AvrLm loci for which the isolate is avirulent. Parentheses indicate that the corresponding locus (*AvrLm*8) has not been characterized. Taken from Stachowiak et al. (2006).

Cultivar resistance scores used to advise growers each season are usually based on trials done in several locations within a particular country or region to account for local differences in the pathogen population. The breeding of resistant cultivars is probably the most common, cost efficient and most effective control method against blackleg of canola and was reviewed by Delourme et al. (2006). Qualitative resistance genes have been identified in canola or deliberately introgressed from related species and research is currently testing a set of isolates to find the number of different major resistance genes present. Additionally, quantitative resistance of some form occurs and this is thought to be race non-specific. Many of the major genes and quantitative trait loci associated with quantitative resistance have been mapped. Possible phenotypic indicators of quantitative resistance include the latent period, sporulation intensity, lesion size, growth rate down the petiole, time from leaf spotting to canker appearance and the rate of canker development. One of these factors thought to be under polygenic control is the period for canker appearance in the stem, for which Zhou et al. (1999) and Sun et al. (2001) showed differing thermal times, from cultivar to cultivar, from the onset of leaf spotting to canker appearance. The earlier appearing cankers were associated with greatest yield loss. Resistance may be modified by environmental conditions such as temperature. Recent work suggests that the *Rlm6*-mediated resistance to *L. maculans* operates effectively at temperatures up to and including 20˚C, but breaks down at 25˚C (Yong-Ju Huang, Unpublished data,). This may explain why infections can be more severe in countries such as Australia. Signalling pathways following inoculation may be another factor. Recent research has shown that pre-treatment of *Brassica napus* leaves with ascospores of *L. biglobosa* or chemical defence activators (acibenzolar-*S*-methyl (ASM) or menadione sodium bisulphate (MSB)) delayed appearance and decreased the area of *L. maculans* phoma leaf spot lesions in both pre-treated (local effect) and untreated (systemic effect) leaves following subsequent inoculation with ascospores of *L. maculans* (Fig 1; Liu et al, 2006). Zadocks (2002) suggested that the speed or extent of induced resistance may be selected for in breeding programmes.

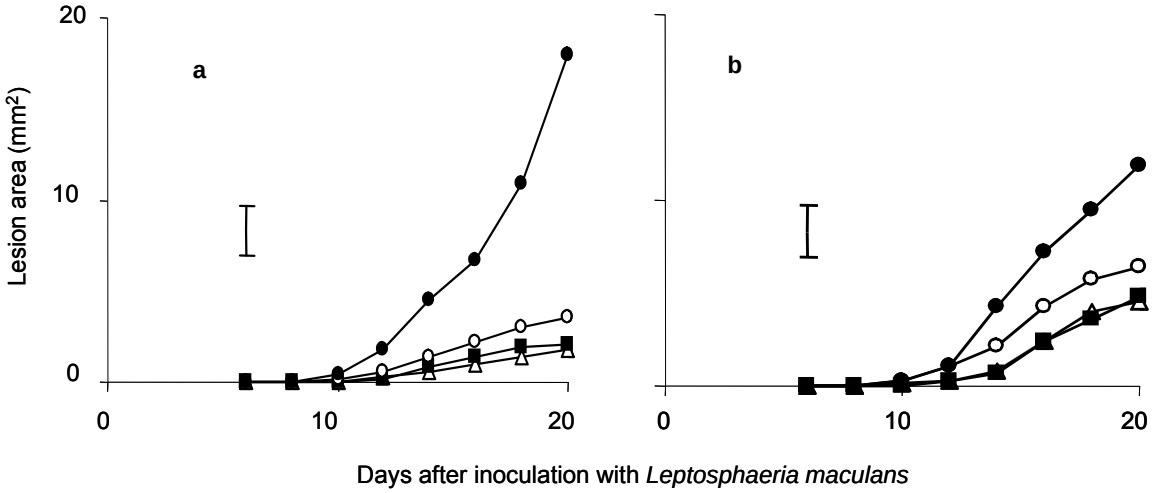

Fig.1. Local (a) and systemic (b) effects on oilseed rape (cv. Madrigal) of inducing treatments (prior inoculation with ascospores of *Leptosphaeria biglobosa* (B) or spraying with chemical defence activators acibenzolar-*S*-methyl (ASM) or menadione sodium bisulphate (MSB)) on development of phoma leaf spot lesions on induced (a) and non-induced (b leaves after challenge inoculation with ascospores of *L. maculans* (A) in a controlled environment experiment. Effects were measured by assessing the areas of lesions up to 20 days after challenge inoculation. Treatments were untreated (control) (●); B + A (○); ASM + A (■); MSB + A (∆). 4 reps of six plants in a randomized block design were incubated at 15ºC with a 16/8 h light/dark regime. Vertical bars indicate mean SEDs (9df). Adapted from Liu et al (2006)

The use of different diversification groups with respect to *L. maculans* on oilseed rape has been advocated recently in France – advice from CETIOM (Centre Technique

Interprofessionnel des Oléagineux Métropolitains) describes four groups: 1 cvs that have very low or low susceptibility, which is thought to be quite stable due to quantitative resistance; group 2 cvs with a similar very low or low susceptibility, based on qualitative and quantitative resistance – these are divided into three sub-groups according to the major genes present (alone or in combination) with the recommendation that cultivation of cvs in the sub-groups is diversified and or rotated (i.e. cvs with the same major genes are not grown in successive years or in nearby fields); group 3 cvs with major gene resistance which has broken down and cultivation is not advised in phoma stem canker affected areas; group 4 cvs that have effective major genes but that have no identified quantitative resistance and so in the short term can be used in rotation with cvs from groups 1 and 2 (Anon, 2004; Gladders et al., 2006). Clearly good farm cultivation records, control of volunteers and stubble management (conflicting with a possible trend towards minimal tillage) will help such diversification schemes. These and other cultural factors are discussed below.

INTEGRATED AVIRULENCE MANAGEMENT

'Field resistance' is a combination of different resistance components: qualitative and quantitative resistances, disease escape and tolerance, each of which may be affected by cultural and environmental factors. Integrated Avirulence Management (Aubertot et al., 2006) is intended to decrease disease severity (increase 'field resistance') and in doing so, reduce selection of a virulent pathogen population and therefore increase the durability of the resistances used for their control (McDonald and Linde, 2002; Mundt et al., 2002; Parlevliet, 2002). IAM not only varies the deployment of resistance genes but also uses a combination of cultural, physical, biological or chemical control methods to reduce the pathogen population.

Resistance deployment strategies include alternating qualitative resistance genes in space and time, or pyramiding resistances into a single cultivar. A factor assisting the spatial deployment of resistances is that in western Europe, areas managed are generally increasing in size, with several traditional farms now managed by a single business and often with whole farms being rotated in terms of crop grown. This also means that cultural practices can be used over larger areas to reduce the exposure of the crop to inoculum using crop rotation to increase separation between inoculum and the crop, stubble management to reduce spore production, tillage to burry crop debris and promote its breakdown. Cultural practices that promote disease escape include an altered sowing date to avoid infections at the most sensitive growth stages, although this is not possible in all regions. Furthermore, disease escape can be enhanced by manipulating plant density (sowing density) and fertilizer regime to increase leaf turn-over in the plant so that leaves may be shed before the fungus has grown down the petiole to reach the stem. New research using isolates genetically modified with reporter genes such as green fluorescent protein (GFP) is enabling the dynamics of pathogen growth in various host tissues to be studied. Since a dense stand of plants produces leaves with longer petioles, there is at least a delay in the pathogen reaching the stem and possibly complete escape if the leaf is shed before stem infection occurs. However, too dense a canopy can lead to tall plants prone to lodging. High autumn nitrogen availability (> 250 kg ha^{-1}) was found to increase canker severity (Aubertot et al., 2004).

Foliar fungicide application can reduce final disease severity substantially both by protecting key leaf layers from infection and reducing the rate of canker development. In many regions it is not economic to apply a fungicide and in locations where chemical control is used, it should be timed to when inoculum is present and aim to protect the earliest leaf layers at risk of infection as these lead to the most severe cankers. A number of schemes have been researched to forecast the release of ascospores or the onset of leaf infections (Penaud et al., 1999; Salam et al., 2003). West et al. (2002) showed that the optimal timing for foliar fungicide applications in England was soon after the onset of significant leaf spotting (*i.e.* > 10 to 20% plants affected) as this prevented new leaf infections and also slowed pathogen growth in existing infections thereby reducing the risk of stem infection. In regions where infections of cotyledons or early true leaves is expected, the strategy of coating fertilizer granules with fungicide, placed beneath the seed at sowing, can give a longer period of protection than seed treatment alone (Barbetti & Khangura, 1999) but is not used in western Europe.

DISCUSSION

Understanding the population structure of *L. maculans* in Europe is helping to direct deployment of resistance sources and the use of diversification schemes. Future monitoring may be assisted by integrating molecular diagnostics with air sampling methods to sample the population of spores present in the air (West et al., 2005). Aubertot et al. (2006) analyse the methods (cultural, physical, and chemical) that can limit the size of *Leptosphaeria maculans* populations in order to improve the efficacy of diversification schemes under Integrated Avirulence Management and the durability of specific resistances in the *L. maculans*/*B. napus* pathosystem. Recently advances have been made in mapping genes or QTLs associated with qualitative and quantitative resistance. This will help with future breeding research and can be coupled with knowledge from recent pathogen surveys to enhance the deployment of resistance genes. Assessment of host resistance may be assisted by new methods under development to relate infection of particular growth stages or plant tissues to final canker severity. Further research is currently attempting to separate components of field resistance especially to identify types of quantitative resistance and disease escape (e.g. the CORDISOR project, West et al., 2004, http://www.cordisor.rothamsted.ac.uk/). A key aspect is technology transfer so that results are passed on to farmers and extension consultants. Gladders et al (2006) compared the various ways in which information is presented in Europe and Australia. In both areas, advice is now often provided at a cost rather than as part of a package by breeders and agrochemical companies but results are also disseminated by levy-funded bodies such as the GRDC (Australia), CETIOM (France) and the HGCA (UK). The Canola Association of Australia and Oilseeds Western Australia also disseminate information throughout the industry. The Internet is now being used more widely to disseminate information e.g. from the HGCA, CETIOM and in the UK a scheme to forecast the onset of phoma leaf spotting is under development (Gladders et al., 2006). These methods will be of increasing importance as the area of oilseed rape production in Europe is currently expanding.

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