Sustainable strategies for managing *Brassica napus* (oilseed rape) resistance to *Leptosphaeria maculans* (phoma stem canker)

Sustainable strategies for managing *Brassica napus* (oilseed rape) resistance to *Leptosphaeria maculans* (phoma stem canker)

Under the aegis of a European-Australian workshop held at INRA, Versailles, France, in association with the EU SECURE project (QLK5-CT-2002-01813)

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Phoma stem canker of oliseed rape (*Brassica napus*) caused by *Leptosphaeria maculans* and *L. biglobosa*. From top to bottom: Symptoms on leaves ans stems; world-wide distribution; stem cankers of differing severities; pathogen life cycle in Europe in relation to host resistance. Full details in Fitt *et al.* (pp. 3–15)

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Foreword

Sustainable strategies for managing *Brassica napus* (oilseed rape) resistance to *Leptosphaeria maculans* (phoma stem canker)

The interaction between the fungus *Leptosphaeria maculans* and oilseed rape *(Brassica napus)* is becoming an excellent model system for studying genetics of host–pathogen interactions. *Leptosphaeria maculans* causes phoma stem canker (blackleg) on oilseed rape and other Brassica crops worldwide. Recently, application of molecular techniques has led to increased understanding of the genetics of this hemibiotrophic interaction. The complete sequences of the genomes of *L. maculans* and *B. rapa* (comprising the *Brassica* A genome) will be available soon. This will provide new opportunities to investigate basic metabolic pathways in the host and the pathogen, and detailed knowledge of the disease process.

Worldwide, the major strategy for control of phoma stem canker is the use of cultivars with resistance to *L. maculans*. However, serious epidemics have occurred recently in Australia and Europe when *L. maculans* populations changed such that major gene resistance in oilseed rape was overcome. Thus there is an urgent need to find and deploy sources of resistance to *L. maculans* in a manner that enhances their durability.

This topic was addressed at a workshop (phoma stem canker durable resistance workshop, 13 September 2004) attended by plant pathologists from Australia and Europe, which was held at INRA (Versailles, France) immediately before the second annual meeting of the European Union-funded SECURE (QLK5-CT-2002-01813) project. This special issue of the European Journal of Plant Pathology is based on papers presented at the workshop, with additional contributions from scientists in Canada, Australia and Europe who were not able to attend. To provide an international perspective on each topic, authors of most of the papers are from several countries. We hope that these papers will provide researchers with a synthesis of the recent studies relating to strategies for management of resistance genes to provide effective control of *L. maculans*, and will stimulate further research on this important model system.

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World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*)

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Abstract

Phoma stem canker is an internationally important disease of oilseed rape (*Brassica napus*, canola, rapeseed), causing serious losses in Europe, Australia and North America. UK losses of €56M per season are estimated using national disease survey data and a yield loss formula. Phoma stem canker pathogen populations comprise two main species, *Leptosphaeria maculans*, associated with damaging stem base cankers, and *Leptosphaeria biglobosa*, often associated with less damaging upper stem lesions. Both major gene and quantitative trait loci mediated resistance to *L. maculans* have been identified in *B. napus*, but little is known about resistance to *L. biglobosa*. *Leptosphaeria maculans*, which has spread into areas in North America and eastern Europe where only *L. biglobosa* was previously identified, now poses a threat to large areas of oilseed rape production in Asia. Epidemics are initiated by air-borne ascospores; major gene resistance to initial infection by *L. maculans* operates in the leaf lamina of *B. napus*. It is not clear whether the quantitative trait loci involved in the resistance to the pathogen that can be assessed only at the end of the season operate in the leaf petioles or stems. In countries where serious phoma stem canker epidemics occur, a minimum standard for resistance to *L. maculans* is included in national systems for registration of cultivars. This review provides a background to a series of papers on improving strategies for managing *B. napus* resistance to *L. maculans*, which is a model system for studying genetic interactions between hemibiotrophic pathogens and their hosts.

Introduction

Phoma stem canker (blackleg) is a disease of world-wide importance on oilseed rape (*Brassica napus*, canola, colza, rapeseed, Raps), which can cause serious losses on crops in Europe, Australia and North America (West et al., 2001; Howlett, 2004). The disease is caused by a complex of *Leptosphaeria* species (Mendes-Pereira et al., 2003), the most important of which is *L. maculans*, associated with damaging stem base canker in many countries (West et al., 2001). In Europe and North America, *L. maculans* often co-exists with *L. biglobosa* (West et al., 2002a), which may have evolved from a common ancestor (Gudelj et al., 2004). *Leptosphaeria biglobosa* is associated with upper stem lesions; whilst generally not damaging, they can cause serious losses in countries like Poland with high summer temperatures (Huang et al., 2005).

Basal phoma stem canker (*L. maculans*) can potentially cause total crop loss, for example when highly susceptible Chinese cultivars were grown in Europe (Grezes-Bessett and McCartney, personal communication) or when breakdown of major gene resistance in a susceptible background occurred recently in Australia (Li et al., 2003). This major gene resistance generally operates at the point of entry of *L. maculans* into the plant (cotyledon or leaf), although its effects may last throughout the season because *L. maculans* is a monocyclic pathogen. However, many cultivars grown in countries where *L. maculans* is endemic also have some quantitative background resistance to *L. maculans*, which may operate to impede the progress of the pathogen down the leaf petiole or in the stem tissues, although the genetics is not clearly understood (Rimmer and van den Berg, 1992; Delourme et al., 2004).

Fungicide spray treatments, applied to control stem canker in western Europe in autumn/winter during the leaf spot phase of the disease before the pathogen reaches the stem (West et al., 1999, 2002b), may become impractical if gross margins from growing winter oilseed rape decrease in these countries. Use of fungicide foliar sprays is generally uneconomic outside western Europe, in countries where yields are lower, although fungicides are applied with the seed in Australia and Canada (West et al., 2001). Therefore, for sustainable world-wide production of oilseed rape, strategies need to be developed to manage resistance to *L. maculans* so that it is durable (Rouxel et al., 2003a). This review, which provides the background for a series of papers on developing improved strategies for managing *B. napus* resistance to *L. maculans*, discusses national losses from phoma stem canker, differences between *L. maculans* and *L. biglobosa*, the world-wide spread of *L. maculans*, epidemiology of phoma stem canker in relation to genetics of *B. napus* resistance to *L. maculans* and the role of disease resistance in national systems for registration of oilseed rape cultivars.

National losses from phoma stem canker

Phoma stem canker is now the most serious disease on winter oilseed rape in the UK. Using data from a national (England and Wales) survey, estimates of losses from this disease have increased from *c.* €14M per season in the late 1980s (Fitt et al., 1997) to €56M per season in harvest years 2000–2002 (www.cropmonitor.co.uk) (Figure 1). By contrast, losses from light leaf spot, caused by *Pyrenopeziza brassicae*, the most serious disease of winter oilseed rape in Scotland, have decreased in England and Wales in this period, with estimated losses of *c.* €28M per season in 2000–2002. On a national scale in the UK, both sclerotinia stem rot (*Sclerotinia sclerotiorum*) and dark pod spot (*Alternaria brassicae*) are generally unimportant, with average losses of €2M and €0.4M per season, respectively (Fitt et al., 1997).

These losses are estimated by multiplying England and Wales survey disease incidence data (% plants affected) and appropriate yield loss parameters (Fitt et al., 1997). The survey data are collected by sampling from a stratified series of

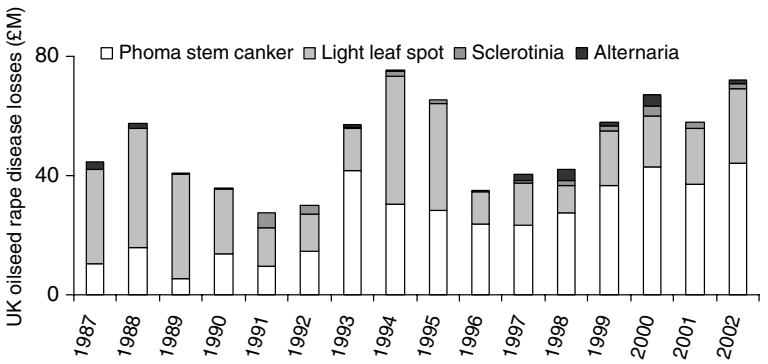

Figure 1. Estimated losses (£1 ≡ €1.4) from diseases (phoma stem canker, light leaf spot, sclerotinia stem rot and alternaria pod spot) in winter oilseed rape in England and Wales, for harvest years 1987–2002, calculated from disease survey data (www.cropmonitor.co.uk) and yield loss coefficients (Fitt et al., 1997).

approximately 100 commercial crops per season (Welham et al., 2004), with numbers of crops sampled proportional to the area of oilseed rape grown in each Defra (UK Department for Environment, Food and Rural Affairs) region. Losses are estimated from data for % plants affected in random samples of 25 plants per crop taken in summer (early July) before harvest. Estimates of yield losses associated with severe epidemics of each of these diseases were based on data from plot experiments with only one disease present, in which fungicides had been used to control this disease. Thus, yield response to fungicide treatment (y) was related to decrease in incidence of phoma stem canker (x) by linear regression ($y = a + bx$). The yield loss coefficient (b) for phoma stem canker was estimated as 0.015 t ha^{-1} for each 1% increase in incidence of the disease. Given the area sown to oilseed rape each season, the national incidence of the disease and the oilseed rape price (estimated as €210 t^{-1}), the yield loss coefficient was used to estimate the loss from phoma stem canker each season to demonstrate trends in the national importance of the disease. Yield loss coefficients relating % yield loss to incidence of phoma stem canker have also been estimated (Zhou et al., 1999); given data for the national average yield in t ha^{-1}, these could also be used to estimate national average yield losses.

Despite the deployment of resistant cultivars, the oilseed rape industry in Australia continues to suffer serious losses from phoma stem canker, as illustrated by losses of €11.3M and €30.1M for the 1998 and 1999 seasons, respectively (Khangura and Barbetti, 2001). In France, losses from phoma stem canker vary between regions and seasons, but generally account for 5 (€36.8M) to 20% (€147M) of the national oilseed rape production (Allard et al., 2002).

Pre-harvest assessments of phoma stem canker can be used, retrospectively, to estimate yield losses from the disease in Europe because most losses are associated with premature death of plants through occlusion of vascular tissues by stem base cankers (West et al., 2001). Although phoma leaf spotting epidemics may be widespread in autumn and winter, such epidemics rarely cause extensive death of plants. If occasional plants are lost, surrounding plants can compensate so that yield is unaffected. By contrast, in Australia, widespread death of seedlings and complete destruction of crops by the disease at any stage from seedling to maturity can occur (Khangura and Barbetti, 2001). In such circumstances, national losses cannot be estimated solely from end-of-season disease surveys and total production of seed.

Differences between *Leptosphaeria maculans* and *L. biglobosa*

Historically, the *L. maculans*/*L. biglobosa* species complex was divided into two groups of isolates, named highly virulent/aggressive and weakly virulent/non-aggressive, from their pathogenicity to oilseed rape stems (Williams and Fitt, 1999). The presence of a non-host specific phytotoxin, sirodesmin PL, in culture filtrates made it possible to divide isolates into Tox^+ (producing sirodesmin PL, highly virulent) and Tox^0 (not producing sirodesmin PL, weakly virulent). Moreover, two different RLFP patterns associated with differences in pathogenicity and pigment production in liquid medium lead to classification of isolates into A (highly virulent, Tox^+) or B (weakly virulent, Tox^0) groups. B-group isolates are a more complex group than A-group isolates. Indeed, B-group isolates were divided into three subgroups; NA1 (NA, non-aggressive), NA2 and NA3 (Koch et al., 1991).

Under *in vitro* conditions, reproducible differences in pseudothecial morphology, the inability to cross A with B-group single ascospore isolates and crossing of opposite mating types of A with A or B with B suggested that the two groups are different species, named *L. maculans* for A-group isolates and *L. biglobosa* for NA1 B-group isolates (Somda et al., 1997; Shoemaker and Brun, 2001). The two species also differ in germination, growth, pigment diffusion, biochemical traits, molecular patterns and pathogenicity. A study, based on the sequence of the internal transcribed spacer region of the ribosomal DNA repeat, established the relationships between seven members of the species complex. These included *L. maculans* 'brassicae' (A-group), *L. biglobosa* 'brassicae' (NA1 B-group, predominant in Europe) and *L. biglobosa* 'canadiensis' (NA2 B-group, predominant in Canada) (Mendes-Pereira et al., 2003).

Whilst typical symptoms caused by *L. maculans* (phoma leaf spot lesions and stem base cankers) are easily identified, it is more difficult to recognise

specific symptoms for *L. biglobosa*. However, *L. biglobosa* leaf lesions generally differ from those of *L. maculans* (Brun et al., 1997; Toscano-Underwood et al., 2001) (Figure 2). Both species are able to survive on stem debris and produce ascospores on unburied debris, but *L. biglobosa* survives longer on unburied debris than on buried debris (Huang et al., 2003a). Under the same conditions, ascospores of *L. maculans* survive longer than those of *L. biglobosa*. Rates of pseudothecial maturation of the two species are similar at 15–20 °C but *L. biglobosa* matures more slowly than *L. maculans* at <10 °C (Toscano-Underwood et al., 2003). In Europe, no yield loss is associated with leaf lesions of either species. On stems, *L. biglobosa* is mainly confined to upper stems (West et al., 2002a), even though both species occur on different stem tissues, including the pith. In France, premature senescence of oilseed rape crops in the absence of phoma stem canker (Brun and Jacques, 1991), associated with a complex of *L. maculans*, *Verticillium longisporum* and *Fusarium* spp., has caused serious yield losses. It is difficult to attribute the losses to specific components of this pathogen complex. More research is also needed to understand effects of *L. biglobosa* on yield and establish relative yield losses caused by *L. maculans* or *L. biglobosa*.

Whilst resistance to *L. maculans*, which may be either major gene or polygenic, has been described (Pilet et al., 2001; Delourme et al., 2004), little is known about resistance to *L. biglobosa*. Nevertheless, some results indicate that genes for resistance to *L. maculans* are not effective against *L. biglobosa*. For example, the genes *Rlm1* in cv. Vivol (Brun et al., 1997) and *Rlm6* in MX lines (not yet commercialised in Europe) both confer resistance to *L. maculans* but not to *L. biglobosa* (Somda et al., 1998; Brun, unpublished results). More research is needed to investigate potential differences in resistance to *L. maculans* in *B. napus* and other crucifers and find sources of resistance to *L. biglobosa*.

World-wide spread of *L. maculans*

L. maculans and *L. biglobosa* have a world-wide distribution, probably due to their transmission in seed of *B. oleracea*, *B. napus*, *B. rapa* and other brassica crops (West et al., 2001). One or other of them is known to occur in Europe (25 countries), Africa (eight countries), Asia (16 countries), North America (Canada, USA, Mexico), central America (five countries), South America (Argentina and Brazil) and Oceania (five countries) (Anon., 2004)

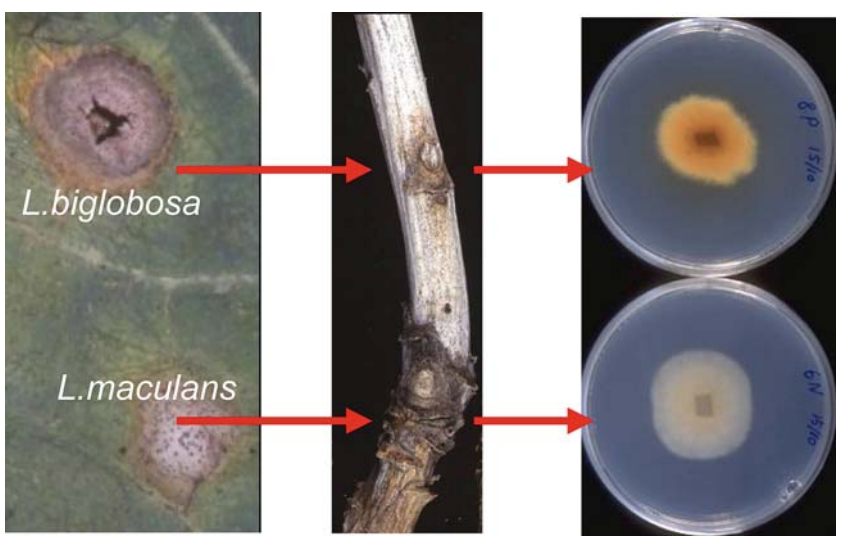

Figure 2. Symptoms of disease on leaves (phoma leaf spot) caused by *L. maculans* (large pale lesions with pycnidia) or *L. biglobosa* (darker lesions, generally smaller) and stems (basal phoma stem canker, *L. maculans* predominant species present; upper stem lesions, *L. biglobosa* predominant species present) of European winter oilseed rape, and cultures of *L. maculans* (no pigment) or *L. biglobosa* (pigment) on potato dextrose agar.

(Figure 3). In most cases, reports do not distinguish between *L. maculans* and *L. biglobosa* or provide information on the brassica crop on which the pathogen was identified. Reports that distinguish between *L. maculans* and *L. biglobosa* are almost entirely based on characteristics of isolates cultured from oilseed rape (*B. napus*).

Leptosphaeria biglobosa 'canadiensis' has been widespread on oilseed rape in Canada since it was first isolated in 1957. *Leptosphaeria maculans* was first isolated from oilseed rape in Saskatchewan in 1975, and subsequently spread to Alberta by 1983 and Manitoba by 1984 (Gugel and Petrie, 1992). Currently, almost all Canadian oilseed rape production is with resistant cultivars. In a survey from 1998 to 2000 in Alberta, Saskatchewan and Manitoba (Keri, Kutcher and Rimmer, unpublished), *L. biglobosa* accounted for 18–48% of the isolates, depending on the year. Both species are widely distributed in the USA (Anon., 2004). *Leptosphaeria maculans* and *L. biglobosa* have recently been reported from Mexico on *B. oleracea* (Moreno-Rico et al., 2001) and Brazil (Fernando and Parks, 2003) and Argentina (Gaetan, 2005) on oilseed rape.

Both *L. maculans* and *L. biglobosa* 'brassicae' occur in France, the UK and Germany, although the relative frequency of the two species differs between locations (West et al., 2001). Until the mid-1990s, phoma stem canker in Poland was almost exclusively associated with *L. biglobosa* (Jedryczka et al., 1994). By 2002, *L. maculans* was widespread on oilseed rape in western Poland, whereas only *L. biglobosa* was found in eastern Poland (Karolewski et al., 2002). Changes in relative frequencies of the two species were also observed in the Czech Republic and Hungary (Szlávik et al., 2003). Thus, there is evidence of an eastward spread of *L. maculans* from western Europe. *Leptosphaeria biglobosa* is established in Russia but *L. maculans* is not (Jedryczka et al., 2002).

Piening et al. (1975) reported severe phoma stem canker on oilseed rape in Kenya from 1972 to 1974 and indicate that the pathogen was present on vegetable brassicas in 1951. From their description of symptoms (severe basal stem cankers), they were probably caused by *L. maculans* and not *L. biglobosa*. *L. maculans*, reported in Natal, South Africa on cabbage crops (Laing, 1986), has probably spread to oilseed rape, introduced into South Africa in 1994 (http://www.arc.agric.za/institutes/ppri/main/news/number60/moth.htm).

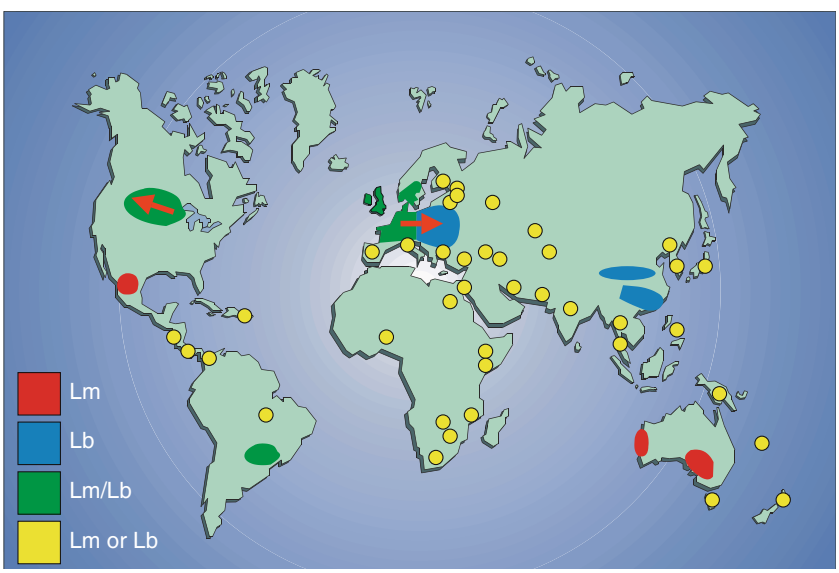

Figure 3. World-wide distribution of *L. maculans* (Lm) and *L. biglobosa* (Lb), showing the direction of spread of *L. maculans* in Canada, where *L. biglobosa* 'canadiensis' was predominant, and eastern Europe (solid arrows), where *L. biglobosa* 'brassicae' was predominant. Areas where populations have been characterised as predominantly *L. maculans* (red), *L. biglobosa* (blue) or a mixture of the two species (green) are indicated by patches. Areas where there have been reports of the pathogens (sometimes only a single report) but the species has not been identified are shown by yellow dots. Based on information in Crop Protection Compendium (Anon., 2004) and other sources available to the authors.

Table 1. Stages in the epidemiology of phoma stem canker (*Leptosphaeria maculans*) in different parts of the world where severe epidemics occur[a]

	Australia	Canada	Europe
Period of ascospore release	Late April–end August	West: May–Aug; Ontario: Sept–Nov, May–Aug	West: Sept–April; East: Sept–Nov, April
Seedling blight (blackleg)	Sporadic outbreaks can severely affect crops (mainly in the west) (June/July)	Occasionally	Uncommon
Phoma leaf lesions	Leaf spots throughout the growing season	West: leaf spots on young or older plants (June, July); Ontario: leaf spots on young winter oilseed rape (Oct–Dec)	West: distinctive leaf spots on young plants, Oct–April; East: little leaf spotting
Phoma stem canker			
Crown canker (stem base)	Most severe phase of disease; can occur at any growth stage	Develops in pre-harvest period (August)	West: most severe phase of disease (May–July); East: rare?
Phoma stem lesions (upper stem)	Observed on stems during and after flowering (Sept–Nov)	Develop in pre-harvest period	Generally more severe in east than west Europe (June/July)
Survival on residues	West: 3–4 years; South–east: 1–3 years	3 years	<2 years

[a]Adapted from West et al. (2001).

Although both *L. maculans* and *L. biglobosa* have been isolated from oilseed rape in Australia (Plummer et al., 1994), the population is almost entirely *L. maculans*. Barrins et al. (2004) found small differences in genetic diversity among isolates according to the cultivar, age of the plants and the region from which they were obtained but populations differing in virulence were not observed. Only *L. biglobosa* has been isolated from oilseed rape in China (West et al., 2000). Since many Chinese cultivars are highly susceptible to *L. maculans* (McCartney and Grezes-Besset, personal communication), this raises the concern that if *L. maculans* isolates are introduced to China considerable damage could result. Furthermore, in China, there are large areas grown to vegetable brassicas. There is a need to improve the resistance to *L. maculans* in Chinese oilseed rape cultivars (*B. napus*) and vegetable brassicas (*B. oleracea, B. rapa*). In the meantime, strict quarantine measures should be employed to ensure that *L. maculans* does not enter China in the next few years. However, two factors relating to crop production practices in China may mitigate the spread and significance of *L. maculans* there. Removal of oilseed rape stem debris from the field after harvest for use as cooking fuel in rural China destroys inoculum. Rotation of oilseed rape with rice involves flooding fields after the oilseed rape harvest, submerging infected residues for long periods. Flooding oilseed rape residues greatly decreased ascospore production after 6 days and almost eliminated it after 10 days (Petrie, 1995).

Epidemiology of phoma stem canker in relation to genetics of *B. napus* resistance to *L. maculans*

Since the oilseed rape growing regions of Europe, Canada and Australia where phoma stem canker causes major economic losses have different growing seasons, types of cultivar resistance, agricultural practices and climates, it is not unexpected that there are differences in the epidemiology of the disease between these areas (West et al., 2001) (Table 1). Wherever phoma stem canker occurs, the air-borne *L. maculans* ascospores are the main source of inoculum (Gladders and Musa, 1980; Salisbury et al., 1995; West et al., 2001) (Figure 4). However, seasonal patterns of ascospore discharge differ between locations and seasons (Khangura and Barbetti, 2001; West et al.,

2002b). Differences in timing of pseudothecial maturity are the main cause of differences in the timing of the start of ascospore discharge (West et al., 1999). Despite this, the main periods of ascospore release in the different countries are predominantly during the late autumn/winter (Gladders and Musa, 1980; West et al., 1999; Salam et al., 2003). In some regions (e.g. Western Australia), ascospore showers often coincide with seedling development (Wherrett et al., 2004). In Western Australia, modelling demonstrated that the dates of both seedling emergence and ascospore development/release are determined by rainfall (Salam et al., 2003).

Maximum yield loss results from ascospore infections that occur at the early seedling stage, when plants are most vulnerable (Barbetti and Khangura, 1999). The role of conidia in the epidemiology of the disease is generally minor in Europe but more important in Western Australia (West et al., 2001). In Australia, there is a good correlation between incidence of cotyledon lesions and subsequent incidence of stem base canker (Li et al., 2005). By contrast, in North America and Europe, cotyledon infection is generally less important (West et al., 2001). In winter oilseed rape, the most damaging stem base cankers are generally associated with phoma leaf spots that developed on leaves three to ten before the onset of rapid stem extension.

Major gene specific resistance to *L. maculans* (Rimmer and van den Berg, 1992; Balesdent et al., 2002) operates when the ascospores infect cotyledons or leaves of seedlings and prevents subsequent spread to the stem and development of cankers (Figure 4). Major gene resistance can be effective for several years under field conditions, provided the corresponding avirulent strains of the pathogen remain prevalent (Rouxel et al., 2003b). However, major gene resistance has broken down in France (Brun et al., 2000; Rouxel et al., 2003b) and Australia (Li et al., 2003; Sprague et al., 2006). Such resistance breakdown is associated with major changes in populations of *L. maculans*. For example, in France, *L. maculans* population changes from avirulence (*AvrLm1*) to virulence (*avrLm1*) to the single dominant *B. napus* resistance gene *Rlm1* between 1990 and 2000 were associated with

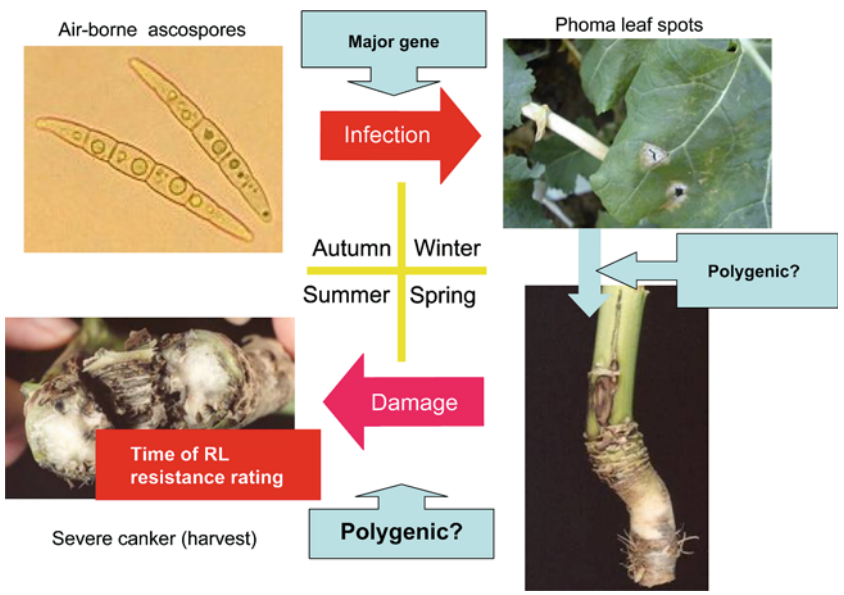

Figure 4. Seasonal cycle of phoma stem canker epidemics in Europe in relation to potential components of oilseed rape (*B. napus*) resistance to *L. maculans*. Epidemics of this monocyclic disease are initiated in autumn (September/October) by air-borne ascospores; the pathogen spreads down the leaf petioles to reach the stem, where stem base cankers or upper stem lesions develop by harvest. A gene-for-gene specific host–pathogen interaction operates at the leaf infection stage but the basis for the background adult plant (quantitative) resistance which operates in the leaf petiole and stem is not known. The UK recommended list (RL) rating for resistance to phoma stem canker (www.hgca.com) is based on assessments of the cross-section of the stem damaged by the pathogen in summer (plants sampled in late June) before harvest (July).

breakdown of this host resistance in commercial crops (Rouxel et al., 2003b). There is also good evidence that the resistance genes *Rlm9*, *Rlm2* and *Rlm4* were rapidly broken down in France after the widespread use of cultivars carrying them (Rouxel et al., 2003b). These studies suggest that a single major gene for resistance operating alone at the leaf infection stage of epidemics is unlikely to be durable.

Cultivars with quantitative resistance, which may operate when the pathogen is spreading down the leaf petiole or into the stem tissues (Figure 4) (West et al., 2001), can be effective in controlling *L. maculans* (Salisbury et al., 1995; Pilet et al., 2001). Use of quantitative resistance in breeding programmes has ensured new cultivars have good background resistance (Delourme et al., 2006). However, quantitative resistance is generally influenced by environmental conditions and its performance can be variable. Quantitatively inherited resistance is likely to be more stable (Pilet et al., 2001) than single gene seedling leaf resistance. Despite this, it is of concern that in Western Australia strains of *L. maculans* may overcome quantitative resistance under glasshouse conditions (Li et al., 2005). As quantitative resistance is controlled by many genetic factors, molecular markers for mapping and characterising quantitative trait loci (QTL) can be used to identify these different genetic backgrounds (Pilet et al., 2001; Delourme et al., 2004).

In Australia, fewer pseudothecia and ascospores were produced on residues from a cultivar with specific resistance from *B. rapa* subsp. *sylvestris* than on residues from cultivars with quantitative resistance (Marcroft et al., 2004). Most ascospores were produced on European winter oilseed rape cultivars with quantitative resistance. Thus the type of resistance deployed may affect reproduction of *L. maculans* and selection for increased virulence.

Importance of resistance to *L. maculans* in national systems for registration of oilseed rape cultivars

In countries where phoma stem canker causes serious epidemics on oilseed rape, there is generally a standard for resistance to *L. maculans* included in the national system for registration of oilseed rape cultivars. For example, in the UK 'recommended list' system for registration of winter oilseed rape cultivars (www.hgca.com) there is an assessment of 'field resistance' to the pathogen (i.e. resistance assessed on adult plants at the end of the season, rather than on cotyledons or leaves of seedlings). Each year the published table of recommended list winter oilseed rape includes 'resistance to stem canker', along with relative gross output, oil content, glucosinolate content and agronomic qualities, such as resistance to lodging and 'resistance to light leaf spot'. The resistance to stem canker or light leaf spot is on a 1 (susceptible) to 9 (resistant) scale, with the minimum standard for resistance to either disease a score of 3. These minimum standards for disease resistance are used in the decision-making progress, alongside other agronomic standards such as minimum lodging resistance, and marketing standards such as low glucosinolate content. Earlier in the selection process, a merit rating for each candidate cultivar is calculated, based on gross output, lodging resistance and resistance to stem canker and light leaf spot.

To assess field resistance to *L. maculans* in the UK, each season a series of 'recommended list' field trials including the candidate cultivars are sown at a range of sites in different parts of the country (www.hgca.com). These include trials where plots are inoculated with winter oilseed rape residues from the previous season with stem canker symptoms, to provide a source of *L. maculans* ascospores to initiate phoma stem canker epidemics a few weeks after sowing in autumn (Huang et al., 2005). In the recommended list trials, contractors assess phoma leaf spot in autumn to confirm that the inoculation has been successful. However, the score (1–9) for field resistance to *L. maculans* is based on assessments of phoma stem canker severity on plants sampled from plots in June, a few weeks before harvest. Currently, 30–50 stems are sampled from each plot in June and the severity of external phoma stem canker at the stem base is assessed (Kenyon et al., 2004) (Figure 5a). Such external assessments may not accurately measure the internal damage to the stem and stems are also cut transversely at the base to record the extent of internal stem blackening (Figure 5b). These external and internal assessments are used to produce an index for stem canker severity, which is inversely related to the resistance rating of the cultivar.