# Invasion by Leptosphaeria maculans (phoma stem canker on brassicas); from genome to worldwide crop

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**Abstract**

Leptosphaeria maculans and L. biglobosa, which cause phoma stem canker disease, are related pathogens of brassicas that were originally considered as one species but occupy slightly different ecological niches and are now reproductively isolated. Globally, the invasion by the more damaging L. maculans into areas where only L. biglobosa was present occurred in North America in the 1980s and Eastern Europe in the 1990s, whereas there are still areas of the world, such as China, where only the less damaging pathogen L. biglobosa is present. The threat to Chinese oilseed rape production from L. maculans was assessed by using models developed to describe the spread (in space and time) of L. maculans across Alberta, Canada. In addition, the worldwide population of L. biglobosa is much more variable than that of L. maculans. Further evidence is provided by the massive invasion, dated 5-20 million years ago, of the genome of L. maculans by only a few repeated element families. Short-term strategies to prevent occurrence of severe phoma stem canker epidemics in China include training of extension workers to recognise symptoms of the disease and use of PCR-based diagnostics to detect the pathogen on imported seed. Long-term strategies include the introduction of durable QTL-mediated resistance to L. maculans into Chinese oilseed rape cultivars and exploitation of new genomic information about L. maculans and L. biglobosa as a component of an integrated disease management programme.

**Keywords**: blackleg, food security, global invasive species, oilseed rape, pathogen genomics, spatio-temporal epidemic spread

**Introduction**

Phoma stem canker (blackleg) is an internationally important disease of oilseed rape (Brassica napus, canola, rapeseed) and vegetable brassicas (Fitt et al., 2006a). On oilseed rape, it causes serious losses in Europe, Australia and North America; worldwide losses are estimated at > £1000M per growing season at the current UK price of more than £300 per tonne (Fitt et al., 2011). Phoma stem canker pathogen populations comprise two main species, Leptosphaeria maculans, associated with damaging stem base cankers, and L. biglobosa, often associated with less damaging upper stem lesions (Rouxel & Balesdent, 2005). L. maculans and L. biglobosa are related pathogens of brassicas that were originally considered as one species but occupy slightly different ecological niches and are now reproductively isolated (Fitt et al., 2006b). There is evidence that L. maculans should be considered as a global invasive pathogen species (www.issg.org/database); it has spread across Canada (1975-1998), from the USA into Mexico (2001) and eastwards across Europe into Poland (1994-2007) into areas where only the less damaging L. biglobosa was previously present (Fitt et al., 2006a,b). To date, phoma stem canker has not been a serious problem in China and only the less aggressive L. biglobosa has been isolated from brassica crops with phoma stem canker there. However, the two related Leptosphaeria species occupy similar ecological niches, suggesting that climatic and agronomic conditions in China are also suitable for the more damaging, closely related species L. maculans. Since many Chinese oilseed rape cultivars are very susceptible to L. maculans when they are grown in the UK, France or Australia, this raises the concern that considerable damage could result if L. maculans becomes established in China. If L. maculans is introduced into China through international trade and becomes established, then it will considerably impact on both cultivated and indigenous brassica species. Crop losses in oilseed and vegetable brassicas will increase poverty and threaten food security for subsistence farmers.

Further evidence that L. maculans should be considered as a global invasive species is provided by data suggesting that the worldwide population of L. biglobosa is much more variable than that of L. maculans, with some fixed subpopulations that can be related to particular geographic areas (Australia, Canada and the Indo-European continent) (Dilmaghani et al., 2009). Furthermore, there is evidence that there was a massive invasion, dated 5-20 million years ago, of the genome of L. maculans by only a few repeated element families (Rouxel et al., 2011). All these data provide indirect evidence that, in evolutionary terms, L. biglobosa may be older than L. maculans, and that L. maculans may be a recently emerging and invasive species. As the environment and brassica cropping patterns alter in response to climate change, further evolution of these two Leptosphaeria species will occur, including enhanced potential for horizontal gene transfer, with possible increases in the range and severity of phoma stem canker epidemics. To quantify the risk posed by L. maculans to brassicas in China, there is a need to model, both biologically and economically, the risk of entry, establishment and spread of the pathogen (Fitt et al., 2008). Long-distance inter-continental spread of L. maculans may be due to its transmission on seed of B. napus, B. rapa, B. oleracea and other brassica crops by international trade (Chen et al., 2010). However, L. maculans may also be spread in infected stem debris if this is transported over long distances, for example mixed with seed shipments destined for crushing to provide oil for the importing country. Wherever phoma stem canker occurs, the air-borne L. maculans ascospores produced on crop debris are usually the main inoculum which spreads the disease over shorter distances into nearby crops. This paper reports work to quantify the risk to China from L. maculans, based on models fitted to data describing the spread across Canada, and to suggest strategies for preventing entry of the pathogen into and spread within China.

**Modelling potential spread of L. maculans across China**

Assuming that pathogen entry and establishment have occurred, a model developed to describe the spread of L. maculans across Alberta, Canada, where the spread of the pathogen had been surveyed over the period 1983 to 1998, was adapted to predict the westward spread of L. maculans across oilseed rape growing regions of China from a point on the east coast (Shanghai, assuming that L. maculans is introduced into China on imported seed or with crop debris mixed with shipments of seed) (Fitt et al., 2008). This modelling assumes that the rate of westward spread across the winter oilseed rape growing area in the provinces along the Yangtze river valley in central China can be estimated using the model developed for westward spread across a spring oilseed rape growing area in Alberta province, Canada. The simulation model was used to predict the spread of L. maculans across the southern (Yangtze river) oilseed rape growing area in China. There was assumed to be a single initial source site at the far eastern edge of the grid (near Shanghai). Results showed the gradual increase in range (westwards) and density of phoma stem canker caused by L. maculans over a 16 year period.

**New genomic information about L. maculans**

The genome of a European isolate of L. maculans (45.12 Mb, assembled into 76 scaffolds) was recently sequenced and it was shown to have an unusual bipartite structure, with alternating distinct GC-equilibrated and AT-rich blocks of homogenous nucleotide composition (Rouxel et al., 2011). Whereas the majority of the genes are in the GC-equilibrated regions of the genome, the AT-rich blocks comprise one third of the genome and contain effector genes and families of transposable elements (TEs), both of which are affected by Repeat Induced Point mutation. Dating back the time of transposition events strongly suggests that the TEs invaded the L. maculans 5-20 million years ago and in all cases after the separation of the Leptosphaeria genus from other genera in the Pleosporinaea (e.g. Phaeosphaeria spp., Alternaria spp., Pyrenophora spp., Cochliobolus spp.). This, together with the embedding of effectors within TE-rich regions and the lack of orthologous sequences for L. maculans effector genes, strongly suggests a link between genome invasion by TEs and generation of species-specific effector genes that may have been instrumental in the success of L. maculans as a world-wide pathogen of oilseed rape.

## Discussion

Since Chinese cultivars of oilseed rape (B. napus) are extremely susceptible to L. maculans when they are grown in Europe or Australia (Fitt et al., 2006a,b, 2008), there is a need to develop strategies to prevent spread of L. maculans into China. There should be sufficient time for a combination of short-term strategies to prevent spread of L. maculans into China, medium-term strategies to prevent spread within China and long-term strategies to increase the resistance of Chinese cultivars to L. maculans. Short-term strategies to prevent entry and establishment of L. maculans in China include: 1) A Pest Risk Analysis for L. maculans by Chinese quarantine scientists to determine the risk that it will enter China and become established. 2) Workshops to train research/quarantine scientists in PCR diagnostics to detect L. maculans in imported seeds. 3) Workshops/manuals to train research/extension scientists to recognise symptoms of phoma stem canker on wild and cultivated brassicas, and identify L. maculans or L. biglobosa by PCR or isolation of cultures. 4) A survey to describe the distribution of L. biglobosa and confirm that L. maculans is still not present in China. 5) Workshops with policy-makers about risks from L. maculans and strategies to prevent spread in China. 6) Information about the pathogen, disease and strategy publicised throughout China, using TV, radio, newspapers, newsletter, internet and multimedia facilities. 7) A regional coordination mechanism so that samples with possible L. maculans can be passed from community and farmer groups to diagnostic centres, with guaranteed feedback for test results.

Medium-term strategies to prevent spread within China include assessment of Chinese cultivated and wild brassicas for resistance to L. maculans. Use of resistant cultivars is a major potential strategy for plant disease control for subsistence farmers in China. This would decrease crop damage, prevent starvation and poverty, and delay disease spread, especially to natural ecosystems where brassica diversity is rich. There is a need to train Chinese researchers, by placement periods in laboratories in Europe, Canada or Australia, about: 1) Epidemiological differences between L. maculans and L. biglobosa in survival on debris, infection of leaves and spread to stems. 2) Screening Chinese crop cultivars and key biodiversity indicator species of non-crop brassicas for sources of resistance to L. maculans. Chinese researchers can then share this knowledge to help disseminate information to community groups in China. Long-term strategies include the introduction of durable resistance to L. maculans into Chinese brassica breeding lines and exploitation of new genomic information about L. maculans (Rouxel et al., 2011) and L. biglobosa as a component of an integrated disease management programme. By comparison with the costs of a phoma stem canker epidemic in China, the costs of strategies to prevent entry and spread of L. maculans are very small (Fitt et al., 2008) and will make a substantial contribution to the food security of the population.

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