Strategies to prevent spread of *Leptosphaeria maculans* (phoma stem canker) onto oilseed rape crops in China

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Abstract

Field experiments in Europe have shown that Chinese cultivars of winter oilseed rape (*Brassica napus*) are very susceptible to the pathogen *Leptosphaeria maculans* (cause of phoma stem canker). Climatic and agronomic conditions in China are suitable for *L. maculans* since the closely related but less damaging pathogen *L. biglobosa* occurs on the winter oilseed rape crops there. Major gene resistance to *L. maculans* is not durable; when introduced into commercial oilseed rape cultivars it is rapidly rendered ineffective by changes in the pathogen population. The threat to Chinese oilseed rape production from *L. maculans* is illustrated by the way in which *L. maculans* has spread into other areas where previously only *L. biglobosa* was present, such as Canada and Poland. Models have been developed to describe the spread (in space and time) of *L. maculans* across Canada, based on survey data collected over a 15-year period in Alberta province. These models have been used to estimate the potential spread of *L. maculans* across the oilseed rape growing areas of China. 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 as a component of an integrated disease management programme.

Key words: durable disease resistance, global invasive species, PCR-based diagnostics, pest risk analysis, spatio-temporal epidemic spread.

Introduction

Phoma stem canker (blackleg) is an internationally important disease of oilseed rape (*Brassica napus*, canola, rapeseed), causing serious losses in Europe, Australia and North America (Fitt et al., 2006a,b) (worldwide losses estimated at > €300M per season). 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). To date, only the less aggressive *L. biglobosa* has been found in China (West et al., 2000). This suggests that climate and agronomic conditions in China are suitable for the more damaging, closely related species *L. maculans.* Since many Chinese oilseed rape cultivars are highly susceptible to *L. maculans*, this raises the concern that if *L. maculans* isolates are introduced to China considerable damage could result. Furthermore, major gene resistance to *L. maculans* is rapidly rendered ineffective by changes in the pathogen population. China has the largest cultivated brassica area in the world; there are *ca*. 8M ha of oilseed rape (*B. napus*) and *ca.* 1.7M ha of brassica vegetables grown annually, mostly by subsistence farmers. This pathogen has recently spread across Canada (1975-1995), from the USA into Mexico and eastwards across Europe into Poland (Fitt et al., 2006a). If *L. maculans* establishes in China through international trade, then it will considerably impact on cultivated and indigenous brassica species. Crop losses will increase poverty and starvation for subsistence farmers.

Wherever phoma stem canker occurs, the air-borne *L. maculans* ascospores are the main source of inoculum which spreads the disease into nearby crops (West et al., 2001). However, timing of ascospore discharge differs between locations and seasons. Differences in timing of maturity of pseudothecia on oilseed rape crop debris, which are related to weather factors, are the main cause of differences in the timing of the start of ascospore discharge (West et al., 1999). Long-distance spread of *L. maculans* is probably due to its transmission in seed of *B. oleracea, B. napus, B. rapa* and other brassica crops (West et al., 2001). 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 suggest strategies for preventing spread of the pathogen into and within China.

Materials and methods

Surveying spread of L. maculans *across Canada (Alberta province)*

Spring oilseed rape crops (generally sown in May in Alberta) were surveyed for the presence, incidence and severity of *L. maculans* in Alberta province (Canada) from 1983 to 1998 after the discovery and extensive spread of *L. maculans* in the neighbouring Saskatchewan province (McGee and Petrie 1978; Petrie 1978). Samples were taken in July or August when crops were in late flowering or as stubble after harvest. From 1983 to 1986, crops were thoroughly surveyed in the east-central region of Alberta following discovery of the pathogen *L. maculans* near Lloydminster in 1983 (Kharbanda, 1993), suggesting threats of severe epidemics like those in Saskatchewan (previously only the less damaging *L. biglobosa* had been present in Alberta). Plant pathologists examined 25-50 plants per crop sampled from five locations approximately 100m apart along a "W"-shaped path. As *L. maculans* spread westwards and southwards across Alberta over the next 15 years, the extent of the survey was gradually expanded to include the whole of areas within which *L. maculans* was being found. In 1987, crops were sampled to confirm infection by *L. maculans;* 1 crop per 8094 ha in each crop district where oilseed rape was grown was surveyed (Petrie 1978). From 1988 to 1995, crops were randomly chosen within each crop district at a frequency of 1 crop per 2000 ha sown to oilseed rape. Cultivars resistant to *L. maculans* were introduced in Alberta at that time, and so the perceived threat from phoma stem canker disease decreased accordingly. Surveys were continued in districts that still placed a high priority on control of phoma stem canker, where the disease was severe or further spread of the disease was suspected (e.g. the Peace River region in north-east Alberta). Severity of stem cankers caused by *L. maculans* was evaluated throughout the period 1983-1998 using a simple scale (trace – slight – moderate – severe). Disease incidence (% plants affected) data were also collected.

Additional survey data were collected from 1996 to 1998 as part of a larger coordinated oilseed rape disease survey done in Alberta, Saskatchewan and Manitoba provinces of Canada. Each provincial agronomist was asked to identify 6 crops in their area that represented the local range of cultivars, fertiliser inputs and other production practices. The presence or absence of phoma stem cankers on the stem bases or upper stems were recorded for each plant and these data were then used to calculate % plants affected.

Modelling spread of *L. maculans* across Canada and China

Data from surveys in Alberta from 1983 to 1998 were collated to provide 4507 records for modelling spread of *L. maculans* across Canada. The main information used for modelling was the decimal latitude and longitude of each crop sampled and the presence/absence of *L. maculans*. The Peace region data were excluded from the modelling since this region in north-east Alberta is not contiguous with the other main oilseed rape growing areas in central and south Alberta. A simulation model to describe spread of *L. maculans* across Alberta, starting from the Lloydminster area in east-central Alberta, during the period 1983 to 1998 was constructed using these data and the statistical software package GenStat 5 (Payne et al., 1993). This model was then used to predict the westward spread of *L. maculans* across oilseed rape growing regions of China from a point on the east coast (e.g. Shanghai).

Results

Modelling spread of L. maculans across Canada (Alberta province)

Firstly, all the data for the spread of *L. maculans* across Alberta between 1983 and 1998 were organised into spreadsheets and validated to ensure that they had been entered accurately before modelling began. They were then plotted on a map of Alberta to show the spread of *L. maculans* southwards and westwards from east-central Alberta. The annual spread of *L. maculans* from one year to the next was summarised by calculating the mean and standard deviation latitude and longitude for sites with *L. maculans* for each year. Then the mean latitude and mean longitude of these sites were plotted against time (year) to show approximately the rate of spread of *L. maculans* across the province of Alberta. Linear regression of mean latitude and longitude on time (year) gave slopes (degrees/year) of -0.076 (s.e. 0.014) and -0.152 (s.e. 0.039), which describe the movement of *L. maculans* southwards and westwards across the province. These regressions accounted for 67.7% and 49.7%, respectively, of the variance in the data. The range for latitude was much smaller than that for longitude, which probably explains the greater association with time of the latter. The longitudinal rate of spread was used in the simulation model.

To construct the simulation model, the spread of *L. maculans* from one site to another in successive years was estimated by assuming that the pathogen could spread only from the nearest site with *L. maculans* in the previous year. This relationship was described by a logistic regression with 0/1 representing absence/presence of *L. maculans* and log-transformed minimum distance as the dependent variable. The probability (p) that *L. maculans* spread from one infected site to a given site in the next season was related to the logarithm of minimum distance between the two sites as:

$$\text{logit}(p) = a + b \times \log(\text{distance})$$

where $\text{logit}(p) = \log\left(\frac{p}{1-p}\right)$ and a and b are coefficients. Since b is negative and assumed to be the same for all years, the probability of spread decreases with increasing distance of the new site from the site with *L. maculans* the previous season. The coefficient a describes the mean density of sites with *L. maculans* and was allowed to vary from year to year to account for seasonal differences in weather and agronomic conditions.

In the simulation model, the oilseed rape growing area of Alberta was described by a regular grid (51 × 41 points) from 110 to 115 degrees longitude and 51 to 55 degrees latitude in 0.1-degree intervals. Beginning with the single site (Lloydminster) with *L. maculans* in 1983, probabilities of spread for each year were evaluated according to the logistic regression model. Random probabilities ($0<p<1$) that a point (site) on the grid had *L. maculans* were also generated for each point on the grid for each year. If the generated probability was less than the evaluated probability at the site, then it was designated as infected with *L. maculans* for that year. This simulation rapidly spread *L. maculans* in an unstructured way over the grid representing Alberta. Therefore, the simulation model was modified to incorporate additional features based on the estimated rate of longitudinal spread across Alberta (0.152 degrees/year) and on the aggregation (patchiness) of disease development. The aggregation was incorporated by imposing patches over the grid and only allowing a proportion (0.4) of patches to have *L. maculans*.

Modelling spread of L. maculans across China

The simulation model was used to predict the spread of *L. maculans* across the southern (Yangste river) oilseed rape growing area in China. This was represented by a rectangular grid (106 × 46 points) covering the area 100 to 121 degrees of longitude and 24 to 33 degrees of latitude, with 0.2-degree intervals between grid lines. There was assumed to be a single initial source site at the far eastern edge of the grid (near Shanghai). The simulation model predicted that *L. maculans* would spread longitudinally (westward) in 20 years when the rate was set at 0.75 degrees per year plus a small random uniform component. Three scenarios were then chosen to represent different densities of *L. maculans* that were obtained by using different values of the coefficient *a,* taken from the model derived from the Canadian survey data for 1994, 1995 and 1996, respectively. This part determines the density of spread of *L. maculans* (*a*) within each patch, even though the probability of spread of *L. maculans* is a function of the proximity to a site with *L. maculans* in the previous year. A longitudinal boundary was set beyond which it was assumed the annual spread of *L. maculans* was not possible. Finally, the probability of selecting a patch at random within that boundary was set at 0.5 for each year. Rogueing was also incorporated for single site patches when there were sufficient patches with more than 5 sites with *L. maculans*.

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), there is a need to develop strategies to prevent spread of *L. maculans* into China. Provided that strategies adopted to prevent spread of *L. maculans* within China are similar to those used in Canada (where affected crops were destroyed), the simulation model predicts that it will take several years for *L. maculans* to spread across China. This should allow 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 spread of *L. maculans* into China include: 1) Workshops to train research/quarantine scientists in PCR diagnostics to detect *L. maculans* (Liu et al., 2006) in imported seeds. 2) Workshops/manuals to train research/extension scientists to recognise symptoms of phoma stem canker (West et al., 1999, 2001) on wild and cultivated brassicas, and identify *L. maculans* or *L. biglobosa* by PCR or isolation (Williams & Fitt, 1999; West et al., 2002). 3) A survey to describe the distribution of *L. biglobosa* in China and confirm that *L. maculans* is still not present in China. 4) A Pest Risk Analysis for *L. maculans* by Chinese quarantine scientists to determine the risk that it will spread and become established. 5) Workshops with policy-makers about risks from *L. maculans* and strategies to prevent spread into 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 possible *L. maculans* samples 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* (Delourme *et al.,*2006). 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 in Europe, North America or Australia on: 1) Epidemiological differences between *L. maculans* and *L. biglobosa* in survival on debris, infection of leaves and spread to stems (Fitt *et al.,* 2006c). 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 need to introduce durable QTL-mediated resistance to *L. maculans* into Chinese brassica breeding lines. A combination of short-term, medium-term and long-term strategies to prevent spread of *L. maculans* into and within China will involve policy makers, researchers, extension workers and farmers.

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